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Forest Vegetation of Hardwood Tree Species along the Mirna River in Istria (Croatia)

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ABSTRACT

Background and Purpose: The paper presents the forest vegetation of periodically flooded and wet forests of hardwood tree species along the Mirna River (Istria, Croatia). The main objective was to study the older and less influential stands, and to compare them among themselves and with related syntaxa of the Mediterranean and continental area.

Materials and Methods: The research was conducted on the basis of 33 new phytocoenological relevés and 12 from previous studies, according to the principles of the standard Central European Phytocoenological School.

Results and Conclusions: Based on 45 phytocoenological relevés two main vegetation types were found. In the lowest and periodically flooded habitats grow forests dominated by *Fraxinus angustifolia* and numerous hygrophilous species. In somewhat higher and drier localities, but with a high level of ground waters, grow mixed forests of *Quercus robur*, *Fraxinus angustifolia*, *Ulmus minor* and *Carpinus betulus*, with a greater presence of mesophilous species. The paper analyzes their mutual relationship, phytocoenological affiliation, as well as their position with regard to the related syntaxa of the Mediterranean and continental area. The results suggest isolation and a transitional character of the studied forests, which is a consequence of the biogeographical position in the north Mediterranean, of the ecological conditions, and to a lesser extent of anthropogenic influence.

Keywords: hardwood forests, Mirna River (Istria), floristic composition, differential species, flooded and wet habitats

INTRODUCTION

Mediterranean forests of hardwood trees are very rare nowadays, and one of the best-preserved ones is located on the Istrian Peninsula (Croatia) in the lower course of the Mirna River (better known as the Motovun Forest). The forests along the course of the Mirna River stretch over approximately 1100 ha, and the main tree species are *Quercus robur*, *Fraxinus angustifolia* and *Ulmus minor*, whereas the drier part also holds *Acer campestre* and *Carpinus betulus*. The basic ecological factor for the composition and growth of these forests is the periodically flooding and high groundwater, which reflects on the floristic composition and distribution of syntaxa. In addition, the biogeographical position is specific as it is in the north of the Mediterranean and close to the Dinaric massif, with a strong continental influence.

The first phytocoenological studies [1, 2] presented the entire complex of the Motovun Forest as an autonomous association *Querco robori-Carpinetum betuli "submediterraneum"* within the southeast European alliance of oak-hornbeam forests *Erythronio-Carpinion betuli*. This was subsequently, following corrections to the name of the association, also accepted by other phytocoenologists [3, 4]. Brullo and Spampinato [5] classify them in the association *Querco roboris-Carpinetum betuli* within the alliance *Alno-Quercion roboris*. Finally, Trinajstić [6] defined them descriptively as a new association *Carici pendulae-Quercetum roboris*, also within the alliance *Alno-Quercion roboris*. Vukelić [7] advocates for more intensive pyhtocoenological research because the 2009 forest inventory suggests differences in the composition and structure of stands. According to Vukelić [7], it is not possible to cover the entire forest complex with a single syntaxa.

These different views on nomenclature and syntaxonomy have impelled us to conduct phytocoenological studies of these forest stands in 2016 and 2017. The objective was to survey older and less influential stands, and to compare them among themselves and with related syntaxa of the Mediterranean and continental area. The results of the research should help to define the forest vegetation of this important forest locality, but also of the northern part of the Mediterranean region. When it comes to these issues, the opinions of phytocoenologists are quite varied, which is also evidenced by the overviews of the forest vegetation of Europe or its specific regions [5, 8-12].

MATERIAL AND METHODS

Research Area

The Motovun Forest is situated in the western part of the Istrian Peninsula, in the valley of the Mirna River (43 km long) and its Butoniga tributary (Figure 1). It is some fifteen kilometers away from the sea coast, and only a few more kilometers away from the Dinaric massif Ćićarija (peak Orljak, 1106 m). The terrain altitude ranges between 7 and 17 m, and the forest complexes stretch along 15 km, with the average width of approximately 500 m. Nowadays, the Motovun Forest is known Europe-wide as the habitat of the white and black truffle (*Tuber magnatum* and *Tuber melanosporum*), and the habitat of the Italian agile frog (*Rana latastei*). It is a part of the Natura 2000 ecological network, and 300 ha of the old forest were protected in 1963 as a "special reserve of forest vegetation".

This area is characterized by a moderately warm and humid climate with hot summers. According to the data from the Botoniga weather station (29 m MASL, period 1986-2015) the mean annual temperature is 13.0˚C, and the mean annual precipitation is 1004 mm. For comparison, the mean annual temperature in the area of Abruzzo and Molise in the central part of the Adriatic coast in Italy is higher by up to 4˚C, and precipitation is lower by up to 400 mm [13] than in similar conditions in Istria.

Parent material of the Motovun Forest is composed of Eocene marls and sandstone washed down by torrents from the surrounding hills and deposited into the valley of the Mirna and Butoniga rivers. Periodic floods, ground waters and constant depositing of new detritus cause the creation of different hydromorphic soils. They are characterized by excessive wetting and are in the development stage of pseudogley and gley-pseudogley.

The valley of the Mirna River is surrounded by the zonal vegetation of the downy oak and oriental hornbeam (*Querco pubescenti-Carpinetum orientalis*), with a more pronounced featuring of continental beech and oak-beech forests. Thus in the forests of the Mediterranean region we find species of the Illyrian floristic geo-element such as *Primula vulgaris*, *Lonicera caprifolium*, *Knautia drymeia* subsp. *drymeia* and others species characteristic of the order *Fagetalia* and lower syntaxa.

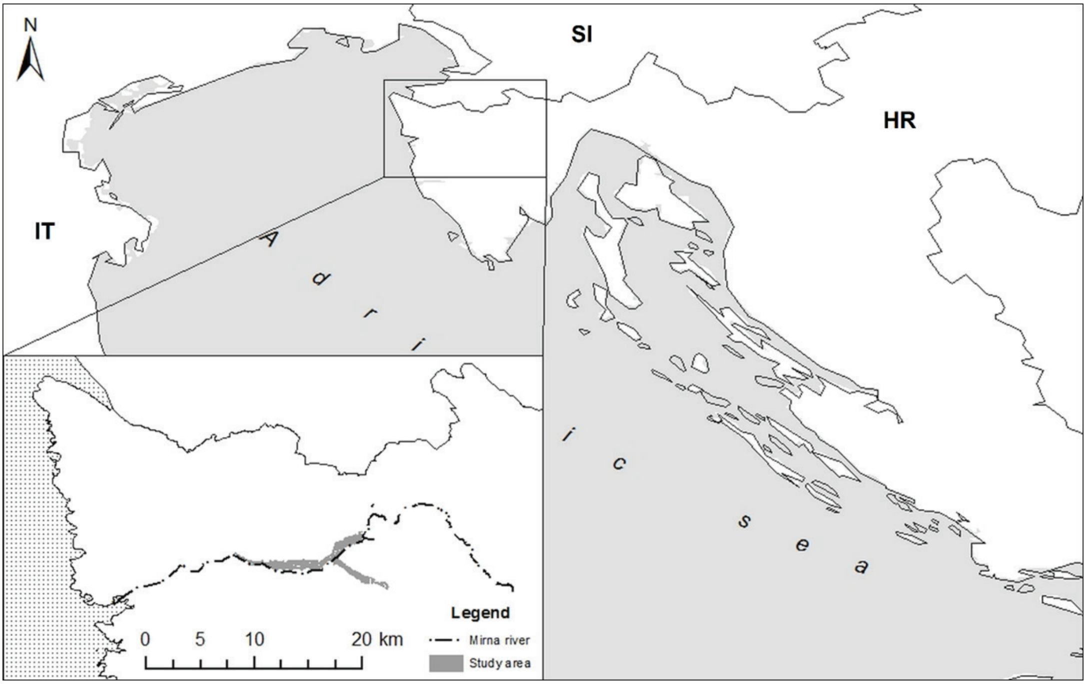

FIGURE 1. Geographical position of the study area.

Data Sampling

Studies of the forest vegetation were conducted following the principles of the standard phytocoenological school [14]. The field phytocoenological survey was conducted on 28 plots in the period from April to mid-July 2016, whereas five unpublished relevés of *Fraxinus angustifolia* stands originate from the ecological vegetation study of hydrological issues of the Motovun Forest [15]. In addition to these relevés, the statistical analysis also includes 12 relevés from previous studies [2]. Positions of new relevés in the WGS84 Coordinate Reference System are listed in the Appendix 1. The surface of the plots was 400 m², inclination 0˚, and the terrain altitude ranged between 10 and 18 m. The plant nomenclature is aligned with the *Flora Croatica Database* [16], and mosses with Atherton *et al.* [17]. Syntaxonomic nomenclature of higher units mostly follows the overviews by Biondi *et al.* [18] for *Fraxinus angustifolia* stands, and Košir *et al.* [19] for mixed hardwood stands. The local syntaxa from previous studies are presented in their original form, whereby a part of the syntaxa is described according to ICPN [20], and a part of them followed the multidimensional distribution of vegetation units [21]. The original names of the syntaxa with their authors and year of publication are listed in the supplementary materials (Appendix 2).

Data Analysis

Vegetation relevés were entered into the TURBOVEG database [22]. Cover-abundance values of the species appearing in several layers were combined in the TURBOVEG program [22], with every plant species being considered with total cover-abundance, regardless of the number of structural layers of the individual relevé in which it appears. Hierarchical clustering was performed in the R package (www.r-project.org) [23]. Bray-Curtis dissimilarity index [24], and square-root transformation of species percentage cover-abundance values were used. In the phytocoenological analysis, we separated our 33 analytical relevés into two types and compared them with the presence class of related phytocoenoses of the Mediterranean and continental part of the southwestern rim of the Pannonian Plain. The diagnostic species were determined using the JUICE 7.0 program [25], based on the analysis of fidelity measure.

The mosses were not recorded in the majority of studies, hence they were not taken into consideration in statistical analyses. Individual species and subspecies were consolidated under a species aggregate.

RESULTS AND DISCUSSION

The statistical analysis clearly classifies the 33 relevés of the Motovun Forest into two clusters or vegetation types. The first type with 23 phytocoenological relevés represents mixed stands of *Quercus robur*, *Fraxinus angustifolia*, *Ulmus minor* and *Carpinus betulus*, whereas the second type with 10 phytocoenological relevés is dominated by *Fraxinus angustifolia*. Both types were in further analyses separately compared to the floristically and chorologically related syntaxa of southern Europe and continental forests of the southwestern part of the Pannonian Plain - western part of the Pannonian sector of the Pannonian-Carpathian floristic province *sensu* [26]. All of the compared syntaxa are listed in Table 1 under their original names, authors, and numbers of relevés.

Forests Dominated by *Fraxinus angustifolia*

The dendrogram on Figure 2 compares the presence classes of 16 *Fraxinus angustifolia* syntaxa in 23 columns. Of the syntaxa of the Mediterranean area, the most significant association is *Carici remotae-Fraxinetum oxycarpae*, while continental forests are represented by the association *Leucojo-Fraxinetum angustifoliae*. The dendrogram clearly separates Mediterranean forests of narrow-leaved ash from continental forests. In the first cluster which encompasses Mediterranean forests of narrow-leaved ash, two sub-clusters are clearly singled out. The studied forests along the Mirna River are classified in the sub-cluster 1a representing the association *Carici remotae-Fraxinetum oxycarpae*. Accordingly, we attached them to this association.

The association *Carici remotae-Fraxinetum oxycarpae* was established in the territory of Abruzzo in Italy [27, 28], and later on also in other parts of the Apennine Peninsula [29-35] and southern Europe [36, 37]. This community occupies lowland localities along river banks or depressions between deposits which are under the influence of the periodically flooding or high groundwater. According to the majority of cited papers, the diagnostically most important species for this association are: *Fraxinus angustifolia*, *Carex remota*, *Ulmus minor*, *Rumex sanguineus*, *Ranunculus lanuginosus*, *Oenanthe pimpinelloides*, *Carex pendula* and *Carex divulsa*.

In the studied forests along the Mirna River, the dominant ash stands stretch over several separate localities in the wettest and lowest parts of the studied area. These are shallower depressions with occasional surface waters. The tree layer is characterized by the complete domination of the narrow-leaved ash and co-domination of the European white elm, while the common oak is individually rarely present, and the common hornbeam is completely missing. In addition to the ash, elm and the mentioned diagnostic species, in the composition of the studied forests sociologically important are the species common in flooded and wet forests (such as *Carex riparia*, *Lycopus europaeus*, *Cardamine pratensis*, *Leucojum aestivum*, *Galium palustre*, *Ranunculus repens*, *Lysimachia nummularia* and others).

In its great area, the association *Carici remotae-Fraxinetum oxycarpae* demonstrates floristic variability, which was also demonstrated in the stands we studied in Croatia. They hold a number of species that have not been reported or are rare in other regions. These are primarily *Crataegus laevigata*, *Cardamine pratensis*, *Deschampsia cespitosa*, *Lysimachia nummularia*, *Alisma plantago-aquatica* and *Lycopus europaeus*. These species were singled out as differential for the new sub-association *crataegetosum laevigatae*, the holotype of which is relevé number 4 in Table 2 (according to Weber *et al.* [20]). Great diagnostic significance is also proper to *Carex riparia*, although it has already been set as a differential species of the sub-association *Carici remotae-Fraxinetum oxycarpae caricetosum cuprinae* [37].

Besides the association *Carici remotae-Fraxinetum oxycarpae*, in southern Europe several similar syntaxa have

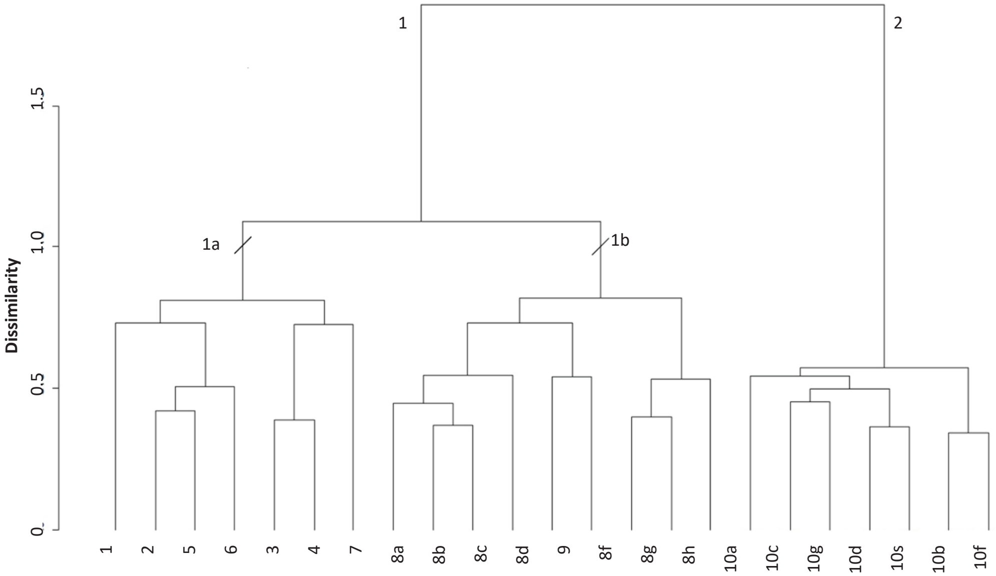

FIGURE 2. Dendrogram of the compared *Fraxinus angustiflolia* syntaxa.

been described with the domination of *Fraxinus angustifolia* [5, 38-43]. Douda *et al.* [10] consider the majority of them to be synonyms for the association *Lithospermo purpureocaerulei-Ulmetum minoris*, which they included into the middle Mediterranean alliance *Populion albae*. The studied forests in Istria fit only partially into such a broadly understood association and its affiliation to the alliance *Populion albae*. They lack important diagnostic species for this association and alliance such as *Bryonia dioica*, *Rubus ulmifolius*, *Iris foetidissima*, *Asparagus acutifolius*, *Celtis australis*, *Rubia peregrina*, *Smilax aspera*, *Oenanthe pimpinelloides*, *Rosa sempervirens*, *Tamus communis*, *Populus alba* and others.

In the previous studies, the community *Carici remotae-Fraxinetum oxycarpae* was classified into different alliances: *Alno-Ulmion*, *Alno-Quercion roboris*, *Alnion incanae*, *Carici remotae-Fraxinion oxycarpae*, *Populion albae* [5, 9, 10, 18, 29, 31, 37]. Our research shows a high coincidence with the alliance *Carici remotae-Fraxinion oxycarpae*, as it is defined in the syntaxonomic overview of the vegetation of Italy [9, 18]. In the cited papers, the species *Fraxinus angustifolia* subsp. *oxycarpa*, *Ulmus minor* subsp. *minor*, *Ranunculus lanuginosus*, *Carex remota*, *Rumex sanguineus*, *Carex pendula* and *C. divulsa* are listed as diagnostic species for it. Without going into the internal differentiation of the species *Fraxinus angustifolia* and *Ulmus minor*, the composition of species corresponds very well to the studied stands along the Mirna River. In the latest review of the vegetation of Europe [12], the *Carici remotae-Fraxinion oxycarpae* is listed as a synonym for the alliance *Lauro nobilis-Fraxinion angustifoliae*. However, the issue of syntaxonomy will continue to be the subject of many discussions.

Mixed Forests Dominated by *Quercus robur, Fraxinus angustifolia, Ulmus minor* and *Carpinus betulus*

Mixed hardwood forests of the studied area are presented with 23 new relevés in Table 3. They grow in drier terrains which are up to 2 m higher than the previous community. They are flooded only in some areas and very rarely, only in extremely wet years in early spring or late fall. The type of soil is pseudogley on alluvial deposits, and such conditions have also reflected on the floristic composition with fewer hygrophytes and more mesophilous species.

The first phytocoenological relevés of such stands were published by Lausi [44] from northeastern Italy along the Po River, and somewhat later by Bertović [2] from the valley of the Mirna River in the Croatian part of Istria. They described them as autonomous associations under different but invalid names, hence Marinček [45] suggested the name *Asparago tenuifolii-Quercetum roboris*. The majority of papers classify the studied forests into the alliance *Erythronio-Carpinion* [1-4, 19]. However, Brullo & Spampinato [5] and Trinajstić [6] classify them into the alliance *Alno-Quercion roboris*. Due to the different nomenclature and syntaxonomic characterization and their status, we surveyed them phytocoenologically in more detail and in further analyses compared them with 10 related syntaxa in 23 synthetic columns (Table 1, Figure 3). The compared syntaxa originate from the north-Adriatic Mediterranean area and southwestern rim of the Pannonian Plain.

The statistical analysis demonstrated the separation of the compared syntaxa into two main clusters: the first one represents drier communities with a significant presence of mesophilous species from alliances within the order *Fagetalia*, and the other one contains communities in periodically flooded and moist habitats with the main subassociation *Genisto elatae-Quercetum roboris caricetosum remotae* (Table 1 numbers 15 a-b, 16 a-f, and 19) within the alliance *Alnion incanae*. In the first cluster, one can distinguish between three sub-clusters: the first one represents *Carpinetum* communities of central and eastern Croatia and the studied area (1a); the second one more humid syntaxa of Slovenia classified in the alliances *Alnion incanae* and *Fraxino angustifoliae-Carpinion betuli* (1b); and the third one the association *Fraxino pannonicae-Quercetum roboris* from northern Italy, which is classified in the alliance *Alno-Quercion roboris* (1c).

The studied forests in the valley of the Mirna River demonstrate affiliation with the sub-cluster 1a, where the dominant forests are oak-hornbeam forests of drier type of central and eastern Croatia. They grow along the course of the Sava River and are connected to the studied forests in Istria, primarily by the great presence of *Carpinus betulus*, *Acer campestre* and other mesophilous species. The floristic and sociological relationships between individual cluster groups were analyzed based on the fidelity of species using the JUICE 7.0 program [25]. We took a total of 216 phytocoenological relevés in the analysis from the main syntaxa representing an individual group (Table 4).

The analysis has shown that the studied forests of the Mediterranean area differ from continental forests in wetter habitats (cluster 2 – *Genisto elatae-Quercetum roboris caricetosum remotae* = *Fraxino pannonicae-Ulmetum glabrae sensu* Douda et al. 2016.) in the lack of a large number of species of wetland and flooded habitats (for instance, *Iris pseudacorus*, *Genista tinctoria*, *Stachys palustris*, *Caltha palustris*, *Carex strigosa*, *Mentha aquatica*, *Carex vesicaria*, *C. elongata*, *Lythrum salicaria*, *Euphorbia palustris*, *Myosotis palustris* etc.). In relation to the Mediterranean hardwood forests (subcluster 1c, column 4 in Table 1) in forests along the Mirna River, there are no southern European species represented in the communities of the alliance *Populion albae*. These species are *Iris foetidissima*, *Rubus ulmifolius*, *Quercus ilex*, *Holcus lanatus*, *Populus alba*, *Cyclamen repandum*, *Hypericum androsaemum*, *Moehringia trinervia*, *Luzula forstery*, and with a smaller presence in the Mediterranean forests there are also *Aristolochia pallida*, *Laurus nobilis*, *Smilax aspera*, *Rosa sempervires*, *Clematis vitalba*, *Asparagus acutifolius* and other species.

The studied stands demonstrate a differential character towards all of the compared syntaxa through a higher presence of the species *Primula vulgaris*, *Vinca minor*, *Lonicera caprifolium*, *Ranunculus lanuginosus*, *Arum italicum*, *Carex pendula*, *Ligustrum vulgare*, *Corylus avellana*, *Ruscus aculeatus*, *Viburnum opulus*, *Aegopodium podagraria*, *Listera ovata*, *Cornus sanguinea*, *Symphytum tuberosum*, and also *Carpinus betulus*, *Acer campestre* and *Ulmus minor*. The majority of these species tend to be distributed in the drier oak-hornbeam and beech forests (alliances *Carpinion betuli*, *Erythronio-Carpinion*, order *Fagetalia*). Hence, in the majority of studies to date, they

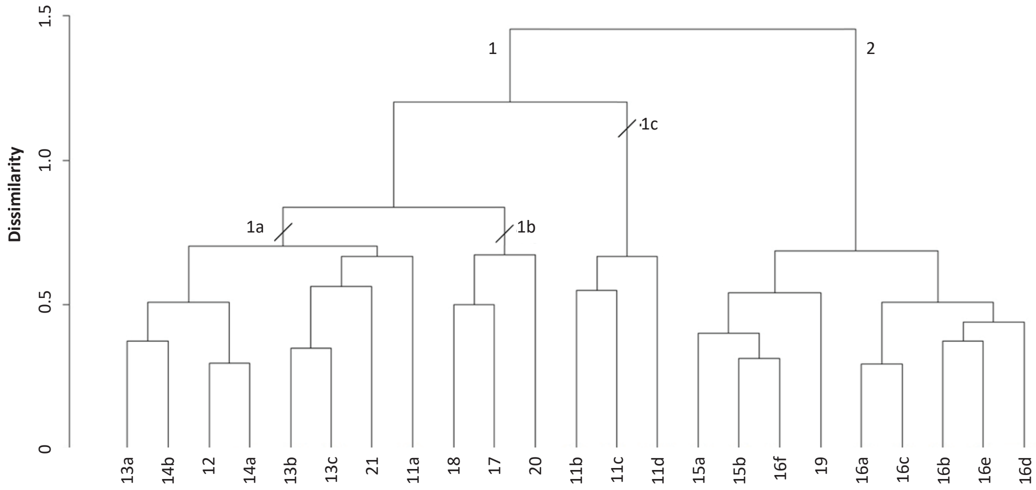

FIGURE 3. Dendrogram of the compared mixed hardwood syntaxa.

TABLE 1. List of literature sources and phytocoenological relevés in the analysis. The numbers of relevés correspond to the numbers in the dendrogram on Figure 2 and Figure 3.

Syntaxa	Source	No of relevés
***Fraxinus angustifolia* forests (Figure 2)**		
Mediterranean area		
1. *Corno-Fraxinetum angustifoliae*	Mercedal & Vilar [37]	6
2. *Viburno lantanae-Ulmetum minoris*	Mercedal & Vilar [37]	39
3. *Ficario ranunculoidis-Fraxinetum angustifoliae*	Mercedal & Vilar [37]	77
4. *Aro italici-Ulmetum minoris*	Mercedal & Vilar [37]	53
5. *Rusco-Fraxinetum angustifoliae*	Gesti et al. [42]	34
6. *Alno glutinosae-Fraxinetum angustifoliae*	Mercedal & Vilar [37]	23
7. *Junco-Fraxinetum oxycarpae*	Mercedal & Vilar [37]	4
8a. *Carici remotae-Fraxinetum oxycarpae populetosum albae*	Pedrotti [27]	5
8b. *Carici remotae-Fraxinetum oxycarpae populetosum albae*	Pedrotti & Cortini Pedrotti [29]	17
8c. *Carici remotae-Fraxinetum oxycarpae populetosum albae*	Pedrotti [30]	8
8d. *Carici remotae-Fraxinetum oxycarpae alnetosum glutinosae*	Gellini *et al.* [31]	8
8e. *Carici remotae-Fraxinetum oxycarpae iridetosum foetidissimae*	Conti & Pirone [33]	10
8f. *Carici remotae-Fraxinetum oxycarpae quercetosum roboris*	Mercedal & Vilar [37]	12
8g. *Carici remotae-Fraxinetum oxycarpae quercetosum pubescentis*	Mercedal & Vilar [37]	9
8h. *Carici remotae-Fraxinetum oxycarpae caricetosum cuprinae*	Mercedal & Vilar [37]	11
9. *Carici remotae-Fraxinetum oxycarpae crataegetosum laevigatae*	this paper	10
Continental area		
10a. *Leucojo-Fraxinetum typicum* and *alnetosum glutinosae*	Glavač [49]	23
10b. *Leucojo-Fraxinetum typicum*	Baričević [50]	5
10c. *Leucojo-Fraxinetum typicum*	Škvorc *et al.* [51]	14
10d. *Leucojo-Fraxinetum alnetosum glutinosae*	Rauš [46]	5
10e. *Leucojo-Fraxinetum alnetosum glutinosae*	Rauš [52]	5
10f. *Leucojo-Fraxinetum alnetosum glutinosae*	Baričević [50]	5
10g. *Leucojo-Fraxinetum alnetosum glutinosae*	Rauš *et al.* [53]	6
Mixed hardwood forests (Figure 3)		
Mediterranean area		
11a. *Fraxino-Quercetum roboris*	Gellini *et al.* [31]	15
11b. *Fraxino-Quercetum roboris*	Manzi [32]	5
11c. *Fraxino-Quercetum roboris*	Brullo & Spampinato [35]	15
11d. *Fraxino-Quercetum roboris*	Pedrotti [27]	5
Continental area		
12. *Querco roboris-Carpinetum*	Glavač [54]	33
13a. *Carpino betuli-Quercetum roboris typicum*	Cestar *et al.* [55]	24
13b. *Carpino betuli-Quercetum roboris typicum*	Rauš [47]	15
13c. *Carpino betuli-Quercetum roboris typicum*	Škvorc *et al.* [51]	21
14a. *Genisto-Quercetum roboris carpinetosum betuli*	Glavač [56]	8
14b. *Genisto-Quercetum roboris carpinetosum betuli*	Baričević [50]	10
15a. *Genisto-Quercetum roboris caricetosum brizoidis*	Rauš [52]	10
15b. *Genisto-Quercetum roboris caricetosum brizoidis*	Baričević [50]	10
16a. *Genisto-Quercetum roboris caricetosum remotae*	Horvat [57]	15
16b. *Genisto-Quercetum roboris caricetosum remotae*	Rauš [46]	5
16c. *Genisto-Quercetum roboris caricetosum remotae*	Rauš [47]	10
16d. *Genisto-Quercetum roboris caricetosum remotae*	Cestar *et al.* [55]	31
16e. *Genisto-Quercetum roboris caricetosum remotae*	Glavač [58]	34
16f. *Genisto-Quercetum roboris caricetosum remotae*	Baričević [50]	10
17. *Pseudostellario europaeae-Carpinetum betuli*	Dakskobler [59]	14
18. *Pseudostellario-Carpinetum betuli leucojetosum aestivi*	Dakskobler [59]	9
19. *Pseudostellario-Quercetum roboris leucojetosum aestivi*	Dakskobler [59]	11
20. *Fraxino-Ulmetum effusae quercetosum roboris*	Košir *et al.* [60]	20
21. *Asparago tenuifolii-Quercetum roboris*	Bertović [2] + this paper	35

TABLE 2. Forests dominated by *Fraxinus angustifolia.*

Number of releve		1	2	3	4	5	6	7	8	9	10	Pres. deg.
Number in Figure 1		24	25	27	28	26	29	32	30	33	31	
Altitude in m		14	14	14	14	14	10	14	11	12	12	
Releve area (00 m²)		4	4	4	4	4	5	5	5	5	5	
Cover in %: tree layer (a)		80	80	85	80	80	60	80	60	70	65	
schrub layer (b)		80	90	40	40	70	50	70	70	40	80	
herb layer (c)		90	70	100	95	100	90	70	100	80	95	
moos layer (d)		1	15	5	1	1	-	-	-	-	-	
Diagnostic species of the association and alliance												
*Fraxinus angustifolia**	a	5	5	5	5	5	3	3	3	4	3	5
Fraxinus angustifolia	b	+	1	1	1	1	2	+	2	2	3	5
*Carex remota**	c	1	3	3	1	2	3	+	2	2	2	5
*Rumex sanguineus**		+	1	+	+	+	2		2	+	2	5
Fraxinus angustifolia		+	+		+		+	+	2	+	+	4
*Ranunculus lanuginosus**		2	+	+			+			+	+	3
Differential species of the subassociation												
Crataegus laevigata	b	2	4	1	1	2	2	+	2	+	2	5
Lysimachia nummularia	c	+	1	2	2	+	+	+	2	2	2	5
Carex riparia		1	2	3	3	3	3	3		2	+	5
Lycopus europaeus		(+)	+	+	+	+	2	2	+	+		5
Cardamine pratensis		+	2	+	+	+			+		+	4
Deschampsia caespitosa		+	+	+	+	+						3
Alisma plantago-aquatica				+	+		+		+	(+)		3
Populetalia albae*												
Ulmus minor	a	2	+	+	+	1			+			3
Populus nigra							2		2			1
Salix alba								2				1
Ulmus minor	b	3	1	+	+	2	3	3	2		3	5
Salix alba										+		1
Carex pendula	c	2	1	1	2	2	2		2	+	2	5
Ulmus minor		+	+				+	2	2		+	3
Salix alba										+		1
Alnetea glutinosae												
Salix cinerea	b							+				1
Valeriana dioica	c		+	1	+							2
Solanum dulcamara								+		+		1
Fagetalia												
Acer campestre	a					+						1
Acer campestre	b	+	+	+	+				+	+	2	4
Lonicera caprifolium		+			+	+						2
Malus sylvestris									+			1
Ranunculus ficaria	c	2	1	+		1			+			3
Circaea lutetiana			+			+			(+)		+	2
Viola reichenbachiana		+		+							+	2
Acer campestre								+		+	+	2
Carex sylvatica								+		+		1

TABLE 2. (continued) - Forests dominated by *Fraxinus angustifolia.*

Querco-Fagetea												
Quercus robur	a					+						1
Ruscus aculeatus	b								(+)	(+)	(+)	2
Quercus robur								+			(+)	1
Hedera helix	c	2	+	+	+	1			+		2	4
Quercus robur								(+)			(+)	1
Rhamno-Prunetea												
Cornus sanguinea	b	1	+	+	+	+	+	2	2	+	2	5
Prunus spinosa		1		1	2	1		2	+	+	+	4
Crataegus monogyna		1	+		+	+	+		+		+	4
Ligustrum vulgare		+	+			+			+		2	3
Euonymus europaea		+	+						+	(+)	(+)	3
Rhamnus catharticus								+		+		1
Clematis vitalba									(+)		(+)	1
Viburnum opulus								+				1
Molinio-Arrhenatheretea												
Leucojum aestivum	c		+	+	+	+						2
Poa trivialis								+	2	+	+	2
Ajuga reptans				+	+		+					2
Lythrum salicaria				+				+	+			2
Juncus effusus									(+)	+		1
Phragmiti-Caricetea elatae												
Galium palustre	c	+	2	1	1		+		2	+	+	4
Mentha aquatica				1	1			+	+			2
Iris pseudacorus					1	+	(+)			+		2
Lysimachia vulgaris				+		+						1
Galio-Urticetea												
Geum urbanum	c	1				+						1
Agrostietea												
Ranunculus repens	c		1	1	+	+	3	+	2	+	+	5
Agrostis stolonifera				+	+							1
Other spp.												
Rubus caesius	b	2	2	2	2	3	2	2	+	+	+	5
Prunus cerasifera								+		+	+	2
Rosa arvensis					+							1
Sorbus torminalis					+							1
Carex otrubae	c			1			(+)			+	+	2
Potentilla erecta				+	+							1
Taraxacum officinale						+			+			1
Galium aparine								2			+	1
Bryophyta	d											
Brachythecium rutabulum		+		+	+	+		+	+	+	+	4
Anomodon viticulosus		+	+	+			+		+	+	+	3
Neckera complanata			+			+			+	+	+	3
Eurhynchium hians		+	2		+							2
Calliergonella cuspidata				1					+	+		2
Hypnum cupressiforme					+			+			+	2
Campylium stellatum			+				+					1
Fissidens taxifolius							+			+		1
Homalothecium sericeum				+					+			1
Brachythecium salebrosum							+	+				1

TABLE 3. Mixed forests dominated by *Quercus robur, Fraxinus angustifolia, Ulmus minor* and *Carpinus betulus*.

Number of relevé		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Number in Figure 1		1	3	5	9	4	6	11	7	8	12	10	2	13	15	17	14	16	22	19	21	18	20	23	
Altitude in m		14	15	15	15	15	16	15	14	14	13	14	14	16	16	16	16	16	17	14	14	16	14	18	
Releve area (00m²)		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	6	4	6	4	4	5	Pres. deg.
Cover in %: - tree layer (a)		100	80	85	90	85	90	70	95	95	90	80	100	90	100	80	90	100	95	90	80	100	85	75	
- schrub layer (b)		70	70	80	70	30	50	60	60	35	80	60	60	70	70	30	40	70	40	70	70	50	80	50	
- herb layer (c)		50	70	60	70	80	60	80	80	90	70	60	70	90	95	90	100	90	95	85	80	80	90	90	
- moos layer (d)		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Diagnostic species of the association																									
Ruscus aculeatus	b	1	1	+	+	1	+	1	1	1	+	+	2	4	3	2	3	3	1	4	4	3	4	1	5
Lonicera caprifolium		-	-	-	-	+	+	+	1	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	3
Carex pendula	c	+	1	1	+	+	3	1	1	3	1	+	2	1	+	1	+	-	1	1	2	1	2	+	5
Primula vulgaris		+	+	1	+	1	+	1	1	1	+	+	1	1	1	1	1	1	2	1	2	+	2	1	5
Lonicera caprifolium		+	-	+	-	-	1	1	1	1	-	-	-	+	-	1	+	-	1	+	+	1	1	1	4
Differential species of the subtypes																									
Prunus spinosa	b	-	-	1	+	+	+	1	2	+	1	1	-	-	+	-	-	-	-	-	-	-	-	-	3
Carex remota	c	+	1	+	2	1	-	2	+	+	1	2	+	-	-	-	-	-	-	-	-	-	+	-	3
Lysimachia nummularia		-	+	-	1	+	+	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	2
Deschampsia caespitosa		-	+	+	1	+	+	+	-	+	+	1	+	-	-	-	-	-	-	-	-	-	-	-	3
Vinca minor		-	-	-	-	-	-	-	+	1	-	-	1	3	4	2	3	3	4	1	1	1	3	3	3
Listera ovata		-	-	-	-	-	-	-	-	-	-	-	-	+	1	+	+	+	+	-	+	-	+	+	2
Symphytum tuberosum		-	-	-	-	-	-	-	-	-	-	-	-	+	1	-	1	1	2	+	-	+	-	+	2
Lamium galeobdolon		-	-	-	-	-	-	-	-	-	-	-	-	2	3	-	2	3	-	1	-	-	1	-	2
Euphorbia amygdaloides		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	1	+	2
Polygonatum multiflorum		-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	2	+	2	2	-	2	-	2
Pulmonaria officinalis		-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	1	2
Carpinion betuli, Erythronio Carpinion																									
Carpinus betulus	a	1	-	-	-	1	1	1	+	+	-	1	1	2	3	+	3	1	2	1	1	3	2	3	5
Acer campestre		3	2	1	+	2	2	1	-	1	-	+	3	3	2	2	2	2	+	1	3	2	1	2	5
Carpinus betulus	b	+	+	-	-	-	+	-	-	-	-	-	1	-	-	+	-	-	+	+	1	+	+	+	3
Acer campestre		+	+	+	1	1	+	+	+	+	-	+	+	+	+	+	+	+	+	1	+	+	+	+	5
Tilia cordata		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	1
Carpinus betulus	c	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	1
Acer campestre		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	1
Helleborus odorus		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	1
Knautia drymeia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	1
Fagetalia																									
Sambucus nigra	b	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Carex sylvatica	c	+	+	+	+	1	2	+	-	+	+	+	1	+	+	+	1	+	1	1	1	1	1	1	5
Viola reichenbachiana		+	+	+	+	1	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-	5
Circaea lutetiana		+	+	-	+	-	+	+	-	1	+	-	+	+	-	-	-	-	-	-	+	-	-	+	3
Brachypodium sylvaticum		-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	+	+	+	2
Ranunculus ficaria		-	+	+	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Allium ursinum		-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	3	-	-	-	-	-	-	1
Euphorbia dulcis		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	1
Lathyrus vernus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Salvia glutinosa		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Mercurialis perennis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1
Populetalia albae																									
Fraxinus angustifolia	a	3	4	4	4	3	4	5	3	2	3	4	2	+	2	2	2	+	2	+	+	2	2	+	5
Ulmus minor		-	1	1	3	1	1	-	1	1	1	1	-	+	-	+	-	-	+	2	+	-	1	1	4
Ulmus minor	b	+	1	1	2	1	+	+	+	+	1	1	+	+	-	-	+	-	+	1	+	+	1	1	5
Fraxinus angustifolia		+	-	+	1	-	+	+	-	1	+	+	+	+	-	-	-	+	-	+	+	+	-	+	4
Ranunculus lanuginosus	c	2	2	1	+	1	1	1	-	1	+	+	-	+	+	+	+	-	-	-	-	-	-	-	3
Rumex sanguineus		-	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Fraxinus angustifolia		-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Ulmus minor		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

TABLE 3. (continued) - Mixed forests dominated by *Quercus robur, Fraxinus angustifolia, Ulmus minor* and *Carpinus betulus*.

Alnetalia glutinosae																									
Lycopus europaeus	c	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	1
Cardamine pratensis		+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Querco-Fagetae																									
Quercus robur	a	4	1	1	1	1	+	1	2	3	3	2	4	3	3	3	2	4	3	4	4	3	3	+	5
Pyrus communis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	1
Corylus avellana		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1
Corylus avellana	b	-	+	1	+	1	+	-	-	+	+	+	+	1	1	1	1	1	1	1	2	2	1	+	5
Quercus robur		1	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	-	-	3
Pyrus communis		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hedera helix	c	3	3	3	2	3	2	3	4	4	3	+	3	1	2	3	1	1	3	3	4	3	3	4	5
Quercus robur		+	+	+	+	-	+	-	-	-	-	-	+	+	+	1	-	+	+	-	1	+	+	+	4
Arum italicum		-	-	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	2
Tamus communis		-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	1	-	+	-	2
Anemone nemorosa		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	2	-	-	-	-	-	-	1
Rhamno-Prunetea																									
Crataegus oxyacantha	b	3	2	4	2	1	2	1	+	1	4	3	3	+	1	+	-	+	1	1	2	1	1	3	5
Crataegus monogyna		-	1	+	+	+	+	+	+	+	-	+	+	+	+	+	1	+	+	+	+	-	+	+	5
Cornus sanguinea		-	1	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	2	+	+	-	1	1	4
Ligustrum vulgare		1	+	+	-	1	1	1	2	2	-	-	1	-	-	+	+	+	1	1	1	+	+	1	4
Viburnum opulus		+	+	+	-	-	+	-	+	1	1	+	+	-	-	+	-	+	+	+	+	+	+	-	4
Euonymus europaea		+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	3
Rhamnus cathartica		-	+	-	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	2
Prunus ceracifera		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	1
Ligustrum vulgare	c	-	+	-	-	-	-	-	1	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	1
Rubus plicatus		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Molinio-Arrhenatheretea																									
Ajuga reptans	c	-	+	-	-	-	1	+	-	-	+	+	1	-	-	+	+	-	+	+	+	+	+	1	3
Lythrum salicaria		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-		1
Prunella vulgaris		-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-		1
Other species																									
Rosa arvensis	b	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	1
Cornus mas		-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	1
Eupatorium cannabinum		-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	1
Laurus nobilis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1
Rubus caesius	c	-	-	-	+	-	-	+	1	+	+	1	+	-	-	-	+	-	+	+	-	1	+	+	3
Aegopodium podagraria		-	-	+	-	-	+	-	+	1	+	+	+	-	+	-	+	1	+	-	-	+	-	-	3
Ranunculus repens		-	-	-	1	-	-	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Potentilla erecta		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Galium palustre		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Vicia dumetorum		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	1
Glechoma hederacea		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1
Fragaria vesca		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Ophioglossum vulgatum		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Viola hirta		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1
Polypodium vulgare		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	1
Erigeron annuus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1
Plantago major		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Stellaria nemorum		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Equisetum telmateia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Bryophyta																									
Anomodon viticulosus		+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	4
Brachythecium rutabulum		-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	+	+	+	-	4
Fissidens taxifolius		-	+	-	-	-	-	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	3
Neckera complanata		+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+	+	+	3
Eurhynchium hians		-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+	+	-	2
Homalothecium sericeum		-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	1
Brachythecium salebrosum		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	1
Calliergonella cuspidata		+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	1
Hypnum cupressiforme		-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1
Leucodon sciuroides		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	1
Campylium stellatum		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hygroamblystegium tenax		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	1

TABLE 4. Differential species (marked grey) with frequency (≥30%) and fidelity (*phi* coefficient×100, ≥35) values for the compared mixed hardwood syntaxa: (1) *Genisto eletae-Quercetum roboris*, [57, 46, 47, 58, 50], N-Croatia; (2) *Carpino betuli-Quercetum roboris*, [54, 47, 51, 56, 50], N-Croatia; (3) 23 relevés in the present study + 12 relevés Bertović [2], (4) *Fraxino pannonicae-Quercetum roboris*, [31, 32], Italy.

Group No.	1	2	3	4
No. of relevés	74	87	35	20
Diagnostic species				
Iris pseudacorus	**75**$^{76.5}$	9^{---}	.$^{---}$	.$^{---}$
Genista tinctoria	**70**$^{72.7}$	9^{---}	.$^{---}$	.$^{---}$
Stachys palustris	**58**$^{67.1}$	5^{---}	.$^{---}$	.$^{---}$
Caltha palustris	**52**$^{66.9}$	.$^{---}$	.$^{---}$	.$^{---}$
Carex strigosa	**62**$^{66.8}$	9^{---}	.$^{---}$	.$^{---}$
Lycopus europaeus	**82**$^{64.0}$	19^{---}	22^{---}	.$^{---}$
Mentha aquatica	**52**$^{62.3}$	.$^{---}$	.$^{---}$	5^{---}
Glechoma hederacea	**83**$^{59.5}$	51$^{20.5}$	3^{---}	.$^{---}$
Leucojum aestivum	**43**$^{58.9}$	1^{---}	.$^{---}$	.$^{---}$
Carex vesicaria	**39**$^{56.9}$	.$^{---}$	.$^{---}$	.$^{---}$
Carex elongata	**43**$^{56.8}$	3^{---}	.$^{---}$	.$^{---}$
Galium palustre	**88**$^{54.5}$	18^{---}	11^{---}	50$^{9.6}$
Lysimachia vulgaris	**61**$^{54.4}$	3^{---}	14^{---}	10^{---}
Carex vulpina	**38**$^{52.8}$	.$^{---}$	3^{---}	.$^{---}$
Lythrum salicaria	**49**$^{52.0}$	2^{---}	8^{---}	5^{---}
Euphorbia palustris	**32**$^{51.5}$	.$^{---}$	.$^{---}$	.$^{---}$
Ranunculus repens	**82**$^{50.0}$	16^{---}	31^{---}	30^{---}
Solanum dulcamara	**39**$^{49.5}$	7^{---}	.$^{---}$	.$^{---}$
Lychnis flos-cuculi	**52**$^{49.1}$	3^{---}	.$^{---}$	20$^{1.8}$
Mentha verticillata agg.	**30**$^{45.4}$	3^{---}	.$^{---}$	.$^{---}$
Peucedanum palustre	**30**$^{45.4}$	3^{---}	.$^{---}$	.$^{---}$
Myosotis palustris agg.	**36**$^{45.2}$	9^{---}	.$^{---}$	.$^{---}$
Lysimachia nummularia	**74**$^{40.4}$	41$^{1.0}$	44$^{5.5}$	.$^{---}$
Cardamine pratensis	**44**$^{38.1}$	9^{---}	6^{---}	15^{---}
Cerastium sylvaticum	**43**$^{36.4}$	28$^{14.4}$	3^{---}	.$^{---}$
Oxalis acetosella	.$^{---}$	**48**$^{63.9}$	.$^{---}$	.$^{---}$
Galium odoratum	.$^{---}$	**42**$^{59.1}$	.$^{---}$	.$^{---}$
Dryopteris filix-mas	3^{---}	**42**$^{56.3}$	.$^{---}$	.$^{---}$
Athyrium filix-femina	21$^{2.6}$	**55**$^{53.3}$	.$^{---}$	.$^{---}$
Tilia cordata	.$^{---}$	**38**$^{52.6}$	3^{---}	.$^{---}$
Veronica montana	12^{---}	**68**$^{52.3}$	.$^{---}$	30$^{3.4}$
Galeobdolon luteum	1^{---}	**50**$^{48.9}$	19$^{2.7}$	.$^{---}$
Geum urbanum	19$^{4.9}$	**46**$^{46.1}$	.$^{---}$	.$^{---}$
Carex brizoides	6^{---}	**32**$^{44.1}$	.$^{---}$	.$^{---}$
Anemone nemorosa	.$^{---}$	**33**$^{43.3}$	8^{---}	.$^{---}$
Veronica chamaedrys	6^{---}	**35**$^{42.0}$	6^{---}	.$^{---}$
Primula vulgaris	.$^{---}$	11^{---}	**97**$^{90.9}$	.$^{---}$
Lonicera caprifolium	.$^{---}$	3^{---}	**81**$^{69.3}$	25^{---}
Carex pendula	4^{---}	17^{---}	**97**$^{68.3}$	40^{---}
Vinca minor	.$^{---}$	4^{---}	**53**$^{63.7}$	.$^{---}$
Ranunculus lanuginosus	.$^{---}$	5^{---}	**53**$^{55.0}$	10^{---}

TABLE 4. (continued) - Differential species (marked grey) with frequency (≥30%) and fidelity (*phi* coefficient×100, ≥35) values for the compared mixed hardwood syntaxa: (1) *Genisto eletae-Quercetum roboris*, [57, 46, 47, 58, 50], N-Croatia; (2) *Carpino betuli-Quercetum roboris*, [54, 47, 51, 56, 50], N-Croatia; (3) 23 relevés in the present study + 12 relevés Bertović [2], (4) *Fraxino pannonicae-Quercetum roboris*, [31, 32], Italy.

Group No.	1	2	3	4
No. of relevés	74	87	35	20
Diagnostic species				
Ligustrum vulgare	1^{---}	17^{---}	**89**$^{68.7}$	25^{---}
Corylus avellana	4^{---}	31$^{2.1}$	**83**$^{67.9}$	.$^{---}$
Ruscus aculeatus	.$^{---}$	27^{---}	**97**$^{64.1}$	45$^{3.1}$
Viburnum opulus	22^{---}	19^{---}	**78**$^{60.8}$	.$^{---}$
Aegopodium podagraria	16^{---}	17^{---}	**64**$^{53.8}$	.$^{---}$
Listera ovata	.$^{---}$	16^{---}	**47**$^{50.0}$	.$^{---}$
Cornus sanguinea	14^{---}	33^{---}	**81**$^{46.7}$	35^{---}
Symphytum tuberosum agg.	.$^{---}$	12^{---}	**36**$^{42.3}$	.$^{---}$
Arum italicum	.$^{---}$	.$^{---}$	**28**$^{41.2}$	5^{---}
Iris foetidissima	.$^{---}$	.$^{---}$	.$^{---}$	**65**$^{76.3}$
Rubus ulmifolius	.$^{---}$	.$^{---}$	.$^{---}$	**60**$^{72.8}$
Luzula forsteri	.$^{---}$	2^{---}	.$^{---}$	**60**$^{70.9}$
Moehringia trinervia	8^{---}	18^{---}	.$^{---}$	**65**$^{58.5}$
Myosotis sylvatica	.$^{---}$	.$^{---}$	.$^{---}$	**40**$^{57.7}$
Quercus ilex	.$^{---}$	.$^{---}$	.$^{---}$	**40**$^{57.7}$
Holcus lanatus	.$^{---}$	.$^{---}$	.$^{---}$	**35**$^{53.6}$
Brachypodium sylvaticum	3^{---}	39$^{6.5}$	17^{---}	**75**$^{51.2}$
Cyclamen repandum	.$^{---}$	.$^{---}$	.$^{---}$	**30**$^{49.3}$
Hypericum androsaemum	.$^{---}$	.$^{---}$	.$^{---}$	**30**$^{49.3}$
Populus alba	4^{---}	.$^{---}$	.$^{---}$	**35**$^{49.3}$
Pteridium aquilinum	.$^{---}$	2^{---}	3^{---}	**30**$^{43.6}$
Veronica officinalis	4^{---}	3^{---}	.$^{---}$	**30**$^{41.3}$
Diagnostic species for more syntaxa				
Persicaria hydropiper	**31**$^{28.5}$	**25**$^{18.2}$	.$^{---}$	.$^{---}$
Frangula alnus	**34**$^{26.6}$	**19**$^{3.3}$	14^{---}	.$^{---}$
Agrostis stolonifera	**44**$^{28.9}$	3^{---}	.$^{---}$	**45**$^{30.1}$
Poa trivialis	**44**$^{28.0}$	5^{---}	.$^{---}$	**45**$^{29.1}$
Juncus effusus	**48**$^{25.2}$	18^{---}	3^{---}	**45**$^{21.3}$
Urtica dioica	**43**$^{16.5}$	31$^{1.9}$	.$^{---}$	**45**$^{19.2}$
Carpinus betulus	17^{---}	**100**$^{47.0}$	**89**$^{33.8}$	35^{---}
Euphorbia amygdaloides	.$^{---}$	**28**$^{14.2}$	**36**$^{26.1}$	10^{---}
Polygonatum multiflorum	.$^{---}$	**34**$^{26.9}$	**33**$^{25.3}$	.$^{---}$
Pyrus pyraster	18^{---}	**25**$^{8.6}$	**33**$^{20.9}$	.$^{---}$
Pulmonaria officinalis	1^{---}	**15**$^{5.4}$	**31**$^{34.1}$	.$^{---}$
Crataegus monogyna	36^{---}	**60**$^{4.0}$	**86**$^{34.0}$	45^{---}
Crataegus laevigata	35^{---}	**70**$^{23.9}$	**92**$^{49.1}$	.$^{---}$
Acer campestre	29^{---}	**89**$^{23.5}$	**97**$^{34.5}$	65^{---}
Viola reichenbachiana	12^{---}	**70**$^{15.0}$	**86**$^{34.1}$	60$^{3.6}$
Carex sylvatica	9^{---}	**68**$^{7.9}$	**97**$^{42.9}$	**70**$^{10.6}$
Euonymus europaeus	10^{---}	**62**$^{22.5}$	**50**$^{7.9}$	**50**$^{7.9}$
Hedera helix	9^{---}	49^{---}	**100**$^{44.0}$	**95**$^{38.0}$
Carex flacca	.$^{---}$	.$^{---}$	**28**$^{28.2}$	**20**$^{14.3}$

were classified in the alliance *Erythronio-Carpinion*, and more precisely, according to the latest phytogeographical differentiation of oak-hornbeam forests of southeast Europe [19], they are classified in the sub-alliance *Lonicero caprifoliae-Carpinenion betuli* group *Quercus robur*, within the alliance *Erythronio-Carpinion*. In the studied mixed hardwood forests, of the species characteristic of the alliance abundantly present were *Primula vulgaris* and *Lonicera caprifolium*, while of the differential species of the sub-alliance these are *Anemone nemorosa*, *Vinca minor*, *Euphorbia dulcis*, *Knautia drymeia* and *Lamium galeobdolon*, and of the differential species of the group *Quercus robur* these are *Quercus robur*, *Carex remota* and *Circaea lutetiana* (cf. Košir *et al.* [19], Table 1).

Although in the dendrogram on Figure 3, the continental oak-hornbeam forests and the studied stands in Istria are separated at a relatively low level, they cannot be considered as belonging to the same association. The mixed hardwood forests of the Mediterranean area contain a certain number of species from the warmer climate, but also a higher presence of the narrow-leaved ash, European white elm, and some species associated with a high level of ground waters. They lack a part of continental species that do not penetrate wet habitats of the Mediterranean area, and are frequent in the continental forests of the common oak and common hornbeam (for example, *Galium odoratum*, *Oxalis acetosella*, *Dryopteris filix-mas*, *Tilia cordata* and others). In addition, continental stands are described in the association *Lonicero caprifoliae-Quercetum roboris* [45], which is broadly understood and of heterogeneous composition. This was also confirmed by Douda *et al.* [10], when they divided it into associations *Fraxino panonnicae-Ulmetum glabrae* and *Ficario vernae-Ulmetum campestris*, within the alliance *Alnion incanae*. However, the majority of stands of the association *Lonicero caprifoliae-Quercetum roboris* belong to the snytaxa within the *Carpinion* alliance: stands with the species of Illyrian floristic geo-element will be classified within the alliance *Erythronio-Carpinion*, and those in which these species are lacking in the alliance *Carpinion betuli*. The center of distribution and the largest areas of this association are located in Pannonian Croatia, where they are known under the name *Carpino betuli-Quercetum roboris* [46, 47], whereas in Hungary similar forests have been described as association *Circaeo lutetianae-Quercetum roboris* and others [48].

On the basis of previous vegetation research and this analysis, we have classified mixed forests of hardwood broadleaved trees along the Mirna River in the association *Asparago-tenuifolii-Quercetum roboris*. Its area is in the Mediterranean region of the north-western part of Slovenia and Croatia, and in the eastern part of Venezia Giulia. The diagnostic species of the association are *Ruscus aculeatus*, *Lonicera caprifolium*, *Carex pendula*, *Primula vulgaris* and *Arum italicum*. Although it is of extrazonal character, the cover of the species of the Illyrian floral geoelement is high. The sub-Mediterranean climate benefits their relatively thermophilic character.

The species *Asparagus tenuifolius* is absent from our phytocenological relevés, but is abundantly present in the surrounding zonal forests of the downy oak and the European hop-hornbeam. It does not respond well to wet and occasionally flooded habitats.

Future research of forests in the river valleys of the North Adriatic littoral will show the justifiability of classifying the studied forest stands of hardwood broadleaved trees into the association *Asparago tenuifoli-Quercetum roboris* and the alliance *Erythronio-Carpinion* betuli. Based on our analyses and recent phytocenological trends, their separation into new syntaxa is for now unfounded.

The association *Asparago tenuifolii-Quercetum roboris* was studied in two larger localities, hence its analysis in the hierarchical phytocoenological system is not yet warranted. The study of the stand along the Mirna River can be divided into two subtypes (Table 3): the first subtype of 12 relevés (subtype *Carex remota*, relevés 1-12 in Table 3) is distributed on wetter habitat, shallow depressions and very mild slopes, and according to the drier subtype, the prominent differential species are *Carex remota*, *Deschampsia cespitosa*, *Prunus spinosa*, *Lysimachia nummularia*, *Ranunculus lanuginosus*. In addition to them, there is also an increased presence of other hygrophilous species, especially *Ulmus minor*.

Relevés 13-23 in Table 3 represents a drier subtype (subtype *Vinca minor*) of mixed hardwood stands especially distributed on highest terrains along the Mirna River. The differential species according to the first subtype are *Vinca minor*, *Listera ovata*, *Symphytum tuberosum*, *Galeobdolon luteum*, *Euphorbia amygdaloides*, *Polygonatum multiflorum* and *Pulmonaria officinalis*. To a lesser extent, other species of less humid habitats also have diagnostic significance, and in relation to the previous subtype there is a higher presence of the common oak and common hornbeam.

It should be emphasized that the studied hardwood forests along the Mirna River represent a permanent vegetation stage conditioned by the hydrological regime, primarily by groundwater. For that reason, the presence of the species *Ulmus minor* and *Fraxinus angustifolia* is higher than in other *Carpinetum* communities of southeast Europe.

CONCLUSIONS

The phytocoenological study of periodically flooded and wet forests along the Mirna River in Istria and their comparisons with related syntaxa resulted in defining two associations. *Fraxinus angustifolia* forests with numerous hygrophilous species are classified in the association *Carici remotae-Fraxinetum oxycarpae* with the new sub-association *crataegetosum laevigatae*. The mixed hardwood forests belong to the association *Asparago tenuifolii-Quercetum roboris* distributed in the planar zone in broader river lowlands of the central part of the northern Mediterranean area. Both associations in the studied area lack numerous termophilous species from related syntaxa of southern Europe, as well as many hygrophilous species from related continental flooded and wet forests. The reasons primarily lie in the biogeographical position, ecological conditions and anthropogenic influences in the studied area. It is located at the northern most part of the Mediterranean, deeply retracted into the European continent. It is also marked by Mediterranean and continental climate impacts, especially modified by the 15 km distant Dinaric mountains with numerous species of the Illyrian floristic geo-element. These reasons have caused a certain isolation and transitional character of the area, which, in turn, has reflected on the floristic composition of the studied forests.

Syntaxonomical scheme:
Class: *Querco roboris-Fagetea sylvaticae* Br.-Bl. et Vlieger in Vlieger 1937
Order: *Populetalia albae* Br.-Bl. ex Tchou 1949
Alliance: *Carici remotae-Fraxinion oyycarpae* Pedrotti ex Pedrotti, Biondi, Allegrezza & Casavecchia in Biondi et al. 2014
Ass: *Carici-Fraxinetum oxycarpae* Pedrotti 1970 ex 1992
crataegetosum laevigatae subass. nova hoc. loco
Order: *Fagetalia* Pawlowski in Pawlowski *et al.* 1928
Alliance: *Erythronio-Carpinion betuli* (Horvat 1938) Marinček in Wallnöfer *et al.* 1993
Suballiance: *Lonicero caprifoliae-Carpinenion betuli* Vukelić in Marinček 1994 group *Quercus robur*
Ass: *Asparago tenuifolii-Quercetum roboris* (Lausi 1967) Marinček 1994
Carex remota subtype
Vinca minor subtype

The study results add to the knowledge of the composition and character of flooded and wet forests in this part of Europe, and will also serve to resolve the issues with their syntaxonomy. In addition, they are important in the preservation of these rare and endangered habitat types.

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APPENDIX

Appendix 1.

Table 2. (1) - N 45° 22' 16,5" - E 13° 52' 25,0"; (2) - N 45° 22' 11,9" - E 13° 52' 18,9"; (3) - N 45° 22' 11,3" - E 15° 52' 03,3"; (4) - N 45° 22' 05,6" - E 13° 52' 08,7"; (5) - N 45° 22' 01,9" - E 13° 52' 15,3"; (6) - N 45° 20' 45,4" - E 13° 47' 48,9"; (7) - N 45° 21' 10,8" - E 13° 51' 22,4"; (8) - N 45° 21' 10,8" - E 13° 48' 04,5"; (9) - N 45° 21' 12,3" - E 13° 50' 01,7"; (10) - N 45° 20' 55,9" - E 13° 49' 50,4".

Table 3. (1) - N 45° 22' 16,5'' - E 13° 52' 25,8''; (2) - N 45° 22' 11,9'' - E 13° 52' 18,9''; (3) - N 45° 22' 11,3'' - E 15° 52' 03,3''; (4) - N 45° 22' 05,6'' - E 13° 52' 08,7''; (5) - N 45° 22' 01,9'' - E 13° 52' 15,3''; (6) - N 45° 22' 23,9'' - E 13° 52' 45,7''; (7) - N 45° 22' 17,9'' - E 13° 52' 36,4''; (8) - N 45° 21' 49,4'' - E 13° 51' 15,1''; (9) - N 45° 21' 47,4'' - E 13° 51' 21,8''; (10) - N 45° 21' 53,5'' - E 13° 51' 43,8''; (11) - N 45° 22' 10,2'' - E 13° 52' 51,1''; (12) - N 45° 22' 05,0'' - E 13° 51 48,3''; (13) - N 45° 22' 01,4'' - E 13° 52' 17,8''; (14) - N 45° 22' 03,0'' - E 13° 52' 27,5''; (15) - N 45° 22' 08,0'' - E 13° 52' 36,7''; (16) - N 45° 22' 10,5'' - E 13° 52' 39,1''; (17) - N 45° 22' 12,3'' - E 13° 52' 43,6''; (18) - N 45° 22' 25,6'' - E 13° 53' 14,9''; (19) - N 45° 21' 50,2'' - E 13° 51' 47,3''; (20) - N 45° 21' 55,5'' - E 13° 51' 57,4''; (21) - N 45° 22' 26,0'' - E 13° 53' 01,9''; (22) - N 45° 21' 48,9'' - E 13° 51' 47,5''; (23) - N 45° 22' 36,8'' - E 13° 53' 11,7''.

Appendix 2.

Alnion incanae Pawlowski in Pawlowski *et al.* 1928; *Alno glutinosae-Fraxinetum angustifoliae* Br.-Bl. ex Tchou 1948; *Alno-Quercion roboris* I. Horvat 1938; *Alno-Ulmion* Br.-Bl. et Tüxen ex Tchou 1948; *Aro italici-Ulmetum minoris* Rivas-Martínez in G. López 1976; *Asparago tenuifolii-Quercetum roboris* (Lausi 1967) Marinček 1994; *Carici pendulae-Quercetum roboris* Trinajstić 2008; *Carici remotae-Fraxinetum oxycarpae* Pedrotti 1970 corr. Pedrotti 1992; *Carici remotae-Fraxinion oxycarpae* Pedrotti ex Pedrotti et al. in Biondi et al. 2014; *Carici remotae-Fraxinetum oxycarpae alnetosum glutinosae* Gelini et al. 1986; *Carici remotae-Fraxinetum oxycarpae caricetosum cuprinae* Mercadal *et* Vilar 2013; *Carici remotae-Fraxinetum oxycarpae iridetosum foetidissimae* Conti *et* Pirone 1992; *Carici remotae-Fraxinetum oxycarpae populetosum albae* Mercadal *et* Vilar 2013; *Carici remotae-Fraxinetum oxycarpae quercetosum pubescentis* Mercadal *et* Vilar 2013; *Carici remotae-Fraxinetum oxycarpae quercetosum roboris* Mercadal *et* Vilar 2013; *Carici remotae-Fraxinetum oxycarpae crataegetosum laevigatae* subass. nova hoc loco; *Carpinion betuli* Issler 1926; *Carpino betuli-Quercetum roboris* (Anić 1959) Rauš 1971; *Carpino betuli-Quercetum roboris typicum* Rauš 1975; *Circaeo lutetianae-Quercetum roboris* Borhidi 2003; *Corno sanguineae-Fraxinetum angustifoliae* Lara et Garilleti 1996; *Erythronio-Carpinion betuli* Marinček in Wallnöfer *et al.* 1993; *Fagetalia* Pawlowski in Pawlowski *et al.* 1928; *Ficario ranunculoidis-Fraxinetum angustifoliae* Rivas-Martínez et Costa in Rivas-Martínez *et al.* 1980; *Ficario vernae-Ulmetum campestris* Knapp ex Medwecka-Kornaś 1952; *Fraxino-Quercetum roboris* Gellini *et al.* 1986; *Fraxino-Ulmetum laevis* Slavnić 1952; *Fraxino-Ulmetum effusae quercetosum roboris* Košir *et al.* 2013; *Fraxino pannonicae-Carpinion betuli* Accetto 2006; *Fraxino pannonicae-Ulmetum glabrae* Aszód 1935 corr. Soó 1963; *Genisto elatae-Quercetum roboris* I. Horvat 1938; *Genisto-Quercetum roboris caricetosum brizoidis* I. Horvat 1938; *Genisto-Quercetum roboris caricetosum remotae* I. Horvat 1938; *Genisto-Quercetum roboris carpinetosum betuli* Glavač 1961; *Junco-Fraxinetum oxycarpae* Kárpáti *et* Kárpáti 1961; *Lauro nobilis-Fraxinion angustifoliae* Kárpáti *et* Kárpáti 1961; *Leucojo-Fraxinetum angustifoliae* Glavač 1959; *Leucojo-Fraxinetum angustifoliae typicum* Glavač 1959; *Leucojo-Fraxinetum angustifoliae alnetosum glutinosae* Glavač 1959; *Lithospermo purpureocaerulei-Ulmetum minoris* Bolòs 1956; *Lonicero caprifoliae-Carpinenion betuli* Vukelić in Marinček 1994; *Lonicero caprifoliae-Quercetum roboris* (Rauš) Marinček 1994; *Populetalia albae* Br.-Bl. ex Tchou 1949; *Populion albae* Braun-Blanquet ex Tchou 1948; *Pseudostellario-Carpinetum betuli leucojetosum aestivi* Dakskobler 2016; *Pseudostellario-Quercetum roboris* Accetto 1974; *Querco roboris-Carpinetum betuli* (Soó ex Balacs 1943) I. Horvat et al. 1974; *Querco roboris-Carpinetum* Soó *et* Pócs 1957; *Querco roboris-Carpinetum betuli „submediterraneum“* Bertović 1975; *Querco pubescenti-Carpinetum orientalis* Horvatić 1939; *Querco roboris-Fagetea sylvaticae* Br.-Bl. *et* Vlieger in Vlieger 1937; *Rusco-Fraxinetum angustifoliae* Gesti *et al.* 2003; *Viburno lantane-Ulmetum minoris* Biurrun *et* García-Mijangos in Rivas-Martínez *et al*. 2002.

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Seed Yield and Morphological Variations of Beechnuts in Four European Beech (*Fagus sylvatica* L.) Populations in Croatia

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ABSTRACT

Background and Purpose: Since the last decade, the amount and periodicity of European beech seed crops in Croatia have been disrupted, and thus the regeneration of its forests has become increasingly questionable. The aim of this study was to determine: (1) seed yield variations within and among four European beech populations and its relation with tree crowns projection, (2) variation in seed morphometric traits of the studied populations, and (3) various methods of seed yield estimates.

Materials and Methods: Research was carried out in four populations of registered European beech seed stands. Experimental plots 80×60 m in size were established in 2016. Horizontal crown projections were calculated for 25 dominant beech trees at each plot. Seed yield per tree was estimated based on seed traps, and by visual scoring of seeding intensity. On a sample of twenty trees (five trees per population) total seed crops were collected by large nets covering their whole crown projections. Different methods of seed yield estimates were compared. The amount of seed yield per population (number of beechnuts per ha) was estimated as well. The length, width and mass of beechnuts were measured. Data were analysed to establish differences within and between populations. Relationships between seed yield and horizontal crown projection, as well as among seed morphometric traits were analysed.

Results and Conclusions: The seed trap method overestimated yields when compared with the real yields caught in the large nets. When crown projections were decreased by 25% the seed trap estimation method was improved. A positive correlation between visual scores of seeding intensity and seed yield estimates proved highly unreliable and therefore inaccurate. The results showed high variability of seed yields within and between the populations. The average yields per population varied from 2.4 to 5.9 million seeds·ha^{-1}. A positive and significant correlation between seed yields and crown horizontal projections was shown (R^2=0.6285), indicating that stand density is an important factor for seed production. A positive and statistically significant correlation between beechnut mass and width was obtained (R^2=0.5875).

Keywords: *Fagus sylvatica* L., seed yield, crop estimates, crown projection, seed morphometric traits

INTRODUCTION

European beech (*Fagus sylvatica* L.) is widely distributed across Europe and is one of the dominant forest tree species in Central Europe [1]. In total, beech covers an area of roughly 14 million hectares [2] and, thus, is ecologically and economically one of the most important species in European forestry [3]. In Croatia, beech is the most common tree species as well. It extends over 47% of the total forested area [4], with a proportion of 45% in the total growing stock [5].

Climate change undoubtedly represents one of the greatest dangers on the global level in the 21st century. Most of the current climate projections for Europe predict the increase of mean air temperatures, and increase in frequency, duration and intensity of summer droughts [6-8]. Over the last 140 years mean annual air temperature has increased by approximately 0.8°C [7, 9, 10]. Future projections indicate even more radical changes. Projected emission scenarios as summarised in the IPCC report [7] suggest that the average global air temperature will rise by additional 1.4-5.8°C by the end of the 21st century. The increase of air temperature coupled with a decrease in precipitation during growing seasons will most probably have severe and adverse effects on natural ecosystems, especially in southern and south-eastern parts of Europe [11].

Global change affects the functioning of forest ecosystems that are faced with direct and indirect impacts of a changing climate. Moreover, it is important to know the composition and structure of genetic diversity, especially regarding the so-called "adaptive properties of forest trees" [12]. Due to possible scenarios of global climate change, there is great interest and concern about beech ecosystems. According to Milad *et al.* [13], beech is a species that tolerates moderately dry periods, but if dry periods are prolonged for a number of years, this may have a negative effect on the species. Also, it is certain that drought will have a negative impact on forest productivity and that it will certainly affect the increased mortality of plants [14-16]. Von Wüehlisch [17] stated that natural beech areal reduction will occur and that the most endangered habitats will be those at lower altitudes in the southern and south-eastern parts of the species' distribution area. At the same time, northern and north-eastern parts of the area will become more suitable for beech ecosystems [17, 18].

Historically, beech forests in Croatia have been naturally regenerated. Seed production and its use have been mostly contained in local natural stands [19]. However, Žgela [20] alarmed that there is an increasing need for artificially produced beech seedlings, which requires the collection of sufficient quantities of high-quality seeds. Nowadays, the absence of regular mast crops is one of the big problems of Croatian forestry and the necessity for assisted regeneration of beech stands is seriously raising. The interaction between climate and seed production has been confirmed by several studies [21-24]. Seed production of beech is irregular with a wide variation among years [21-23]. The mast years are strongly affected by annual climatic variability [25] and occur irregularly at intervals of between three and fifteen years [26-29].

Knowledge on dynamics of seed production of European beech in Croatia (as well as in the wider region) is very scarce. It remains largely unknown what the trends are in masting and how climate change, along with other factors, influences crop dynamics. Therefore, a need for research in this field has been recognized. This study represents the very first step in research of seed yield of selected European beech populations in Croatia.

The main objectives of the study were to determine: (1) the correlation between seed yield variations within and between four populations and tree crown projections, (2) variations in seed morphometric traits of the studied populations, and (3) the accuracy of various seed yield estimates.

MATERIALS AND METHODS

The study was carried out in the established experimental plots in four populations of registered European beech seed stands in Forest Administrations Ogulin, Karlovac, Bjelovar and Požega in 2016 (Table 1). The size of each plot was 60x80 m. All beech trees within the plots were numbered and a raster netting marks were placed on every 20 m. The closest dominant beech tree was selected next to each mark (in total, 20 trees were selected in each plot by this method). Additionally, five more trees were selected for observing the accuracy of seed yield estimation.

Tree Measurements

Diameter at breast height (dbh), tree height (h) and crown radiuses were measured on all selected trees. Crown radiuses were measured in four directions (North, South,

TABLE 1. Data of experimental plots and measuring parameters of stands.

	Forest Administration			
	Bjelovar	Ogulin	Požega	Karlovac
Forest office	Veliki Grđevac	Ogulin	Velika	Topusko
Forest management unit	Grđevačka Bilogora	Bukovača	Južni Papuk	Bublen
Forest subcompartment	47b	41c	55a	38b
Register number of forest reproductive material	HR-FSY-SS-222/125	HR-FSY-SS-332/139	HR-FSY-SS-221/188	HR-FSY-SS-223/167
Area of experimental plots (ha)	0.48	0.48	0.48	0.48
Number of beech trees within experimental plots	77	111	138	98
Number of dominant beech trees below which beechnut-collecting traps are placed	20	20	20	20
Average height of dominant beech trees (m)	36	41	37	44
Average diameter at breast height of dominant beech trees (cm)	51.6	53.9	45.3	55.3
Average tree crown projection (m^2)	75.4	76.5	55.9	90.7
Average productive part of dominant beech crown per trees (m^2)	57	57	42	68

East and West) or in more directions in the case of markedly asymmetric crown [49]. Horizontal Crown Projections (HCPs) were calculated on the basis of mean crown radiuses assuming crown circular shape (HCP = mean crown radius^2 × π).

Seed Yield Estimates

Two circular seed traps were placed below opposite (North-South) middle points of the crowns. The surface of a seed trap was 0.5 m^2. Furthermore, large PVC nets were set up at 1 m above ground below five additional trees per plot. These large nets were set up covering total projection area of the tree crowns to collect their whole seed crop. Below two of the five additional trees, seed traps were set up as well, in the same manner as previously explained. The aim was to investigate differences between real seed yield (caught in large nets) and the estimated yield.

Seed yield per tree was estimated by multiplying total mass of the seeds caught in traps with respective HCP. It was noticed that this method overestimated the crops (the estimates were larger than the real amount of seeds caught in large nets) for all trees which were used in this comparison (Table 2). Therefore we tried to improve seed yield estimates by gradually decreasing HCPs used in the calculations (assuming that only a part of crown projection area is productive). The best estimates (values closest to the real seed yields) were obtained with 25% decrease of the HCP (Table 2). Thus, we corrected seed yield estimates of all studied trees so that the total mass of the trapped seeds was multiplied with 75% of a tree's HCP.

Also, seeding intensity of the selected trees was visually scored in September 2016. The trees were visually rated using binoculars and then categorized into six classes [30] due to seeding intensity: 0 - none, 1 - very bad, 2 - poor, 3 - average, 4 - good, 5 -very good.

Seed Measurements

Seeds were collected four times in the interval from September to November. Each time seed collections (per tree) were weighed and seeds were counted. The amount of seeds per population (i.e. the number of beechnuts per ha) was calculated based on the actual number of trees per hectare, the estimated mean yield (kg) per tree and the population mean number of seeds per kilogram.

Random samples of 20 nuts per collection and tree were taken for morphometric analysis. Nut length and width were measured by digital calliper with a precision of 0.01 mm (Figure 1), while the mass of the whole sample was measured by digital scale with a precision of 0.01 g.

Statistical Analysis

All statistical analyses were generated by SAS/STAT software, a free version of SAS University Edition, by SAS Institute Inc., Cary, NC, USA [50]. Descriptive statistics for seed morphometric traits, estimated seed yields and crown projections were performed using the MEANS procedure. Analyses of variance were conducted for seed yields and crown projections using the MIXED procedure to determine variance components due to the effects of populations according to the following linear model (1):

$$y_{ij}=\mu+P_i+\varepsilon_{ij} \qquad \text{(ANOVA model 1)}$$

where: y_{ij} - individual value of a trait; μ - overall mean; P_i - fixed effect of the population i, i = 1,2,3,4; ε_{ij} - random error.

Analyses of variance were also performed for seed morphometric traits using the MIXED procedure to determine variance components due to the effects of populations and

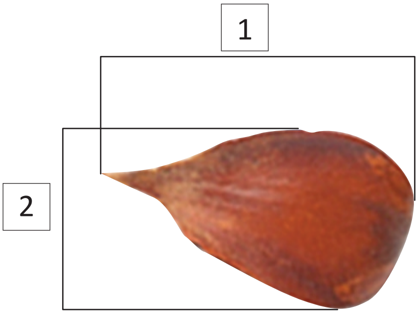

FIGURE 1. Measured seed traits (1 – seed length, 2 – seed width).

TABLE 2. Differences between real seed yields (caught in large nets) and estimated seed yields.

Tree label	Horizontal crown projection - HCP (m^2)	Total mass of seeds in traps (kg)	Total mass of seeds in the large net (kg)	Total yield (traps + net) (kg)	Estimated yield (kg)	Difference between estimated and real yield (kg)	HCP reduced by 25% (m^2)	Difference between corrected estimates and real yield (kg)
OG-M2	93.02	0.11	7.39	7.50	10.42	2.91	69.77	0.31
OG-M3	46.31	0.15	5.23	5.38	6.99	1.61	34.73	-0.14
PŽ-M2	80.95	0.24	14.51	14.75	19.43	4.68	60.71	-0.17
PŽ-M3	38.89	0.22	7.09	7.31	8.44	1.13	29.17	-0.98
BJ-M3	129.44	0.22	19.59	19.81	27.83	8.02	97.08	1.07
BJ-M4	83.83	0.11	7.67	7.78	9.31	1.52	62.87	-0.8
KA-M1	61.53	0.13	6.74	6.87	7.91	1.03	46.15	-0.94
KA-M4	92.95	0.14	10.37	10.51	13.01	2.51	69.71	-0.75

seed collecting time according to the following linear model (2):

$$y_{ijk}=\mu+P_i+C(P)_{ij}+\varepsilon_{ijk} \text{ (ANOVA model 2)}$$

where: y_{ijk} - individual value of a trait; μ - overall mean; P_i - fixed effect of the population i, i = 1,2,3,4; $C(P)_{ji}$ - fixed effect of the collecting time j nested within the population i, j = 1,2,3,4; ε_{ijk} - random error.

Tukey-Kramer method of the Tukey HSD test was conducted to determine statistical significance of differences between least square means of the populations and of the collecting times.

Pearson correlation analysis was conducted using the proc CORR to determine relationships between studied traits, as well between seed yield estimates and seed intensity visual scoring.

RESULTS

Seed Yield Estimate and Variation

Differences among real and estimated seed yields were studied on a sample of eight trees. Below crowns of those trees large nets were set up together with seed traps, which allowed their comparison. Initially, differences were too large to prove satisfactory. However, after 25% reduction of HCP values, the values of seed yield estimates become much closer to those of the real crops. Average deviation was 0.65 kg per tree ranging from 0.14 to 1.07 kg (Table 2).

All sampled trees were visualy scored for seeding intensity prior to any seed collecting, with the aim of establishing the relationship between scores and estimated seed crop. Positive correlation (R^2=0.3219) between the crop per square meter of HCP and visual seeding intensity scoring is shown in Figure 2. Despite this positive correlation, it is evident that the method used for visual scoring of a tree crop was highly unreliable, i.e. inaccurate. For example, there was a large amount of yields in score 4, as well as a large overlapping between grades. Therefore, it is necessary to improve the visual scoring method to increase the acuracy of seed yield estimates.

Distributions of yields per tree in studied populations are given in Figure 3. The results showed that the largest mean seed yield per tree was in Karlovac population (7.9 kg), while the smallest in Ogulin population (3.7 kg). The average yield per tree in Bjelovar population was 5.1 kg and in Požega population 4.8 kg. Compared to other populations, Karlovac population had more homogenous distribution of yields per tree with the smallest coefficient of variation (CV=43.36). Analysis of variance showed a statistically significant effect of population (F=4.43, p=0.0063). Tukey-Kramer test revealed statistically significant difference between Karlovac and Ogulin populations (p=0.0042).

Požega population had the largest amount of beechnuts in 1 kg (Figure 4). There were on average 4,787 beecnhuts in Požega population, 4,259 in Ogulin population, 4,100 in Karlovac population, while Bjelovar population had 3,746 nuts per kg.

The largest mean seed yield was in Karlovac population (5.9 million seeds·ha^{-1}). There were 5.2 million seeds·ha^{-1} in Požega population, 3 million seeds·ha^{-1} in Ogulin population and 2.4 million seeds·ha^{-1} in Bjelovar population.

Correlation between Seed Yields and Horizontal Crown Projections

Correlation analysis showed (Figure 5) a positive and statistically significant correlation between tree seed yields and horizontal crown projections (HCPs) (R^2=0.6285). Figure 6 shows differences among populations in distributions of

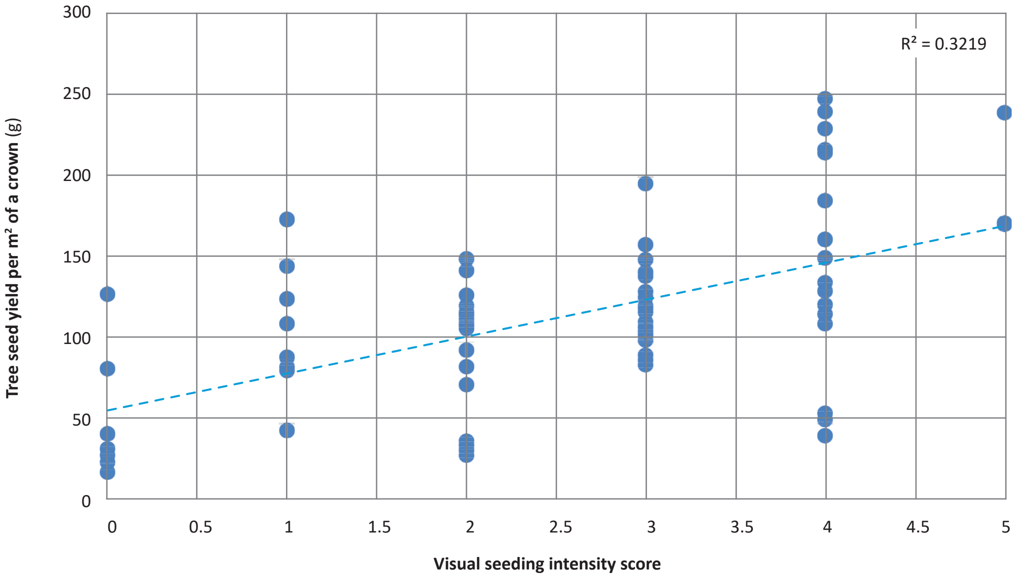

FIGURE 2. Correlation between seed yield per m^2 and visual seeding intensity scores.

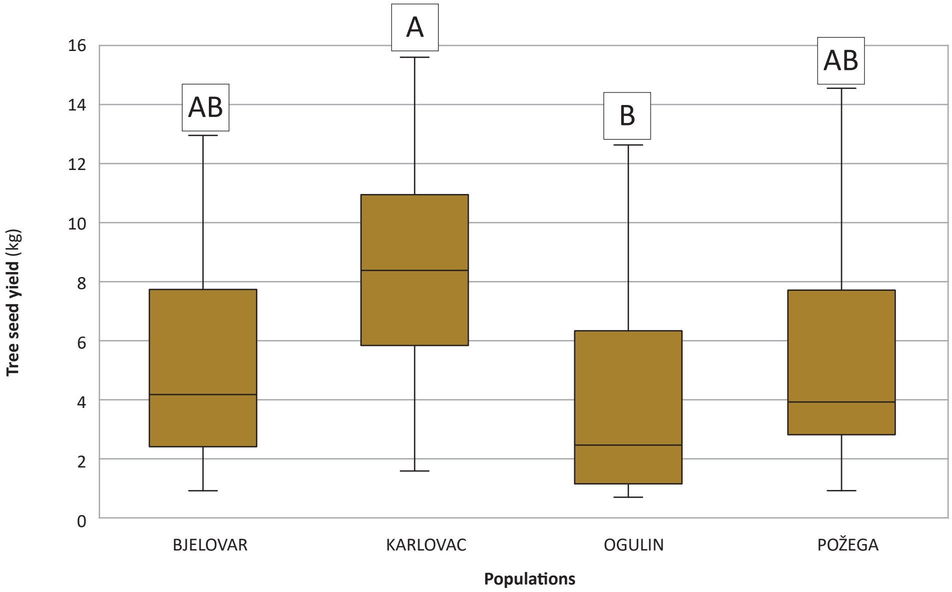

FIGURE 3. Distribution of seed yields per tree in studied populations (the same letters associate populations with no statistically significant differences).

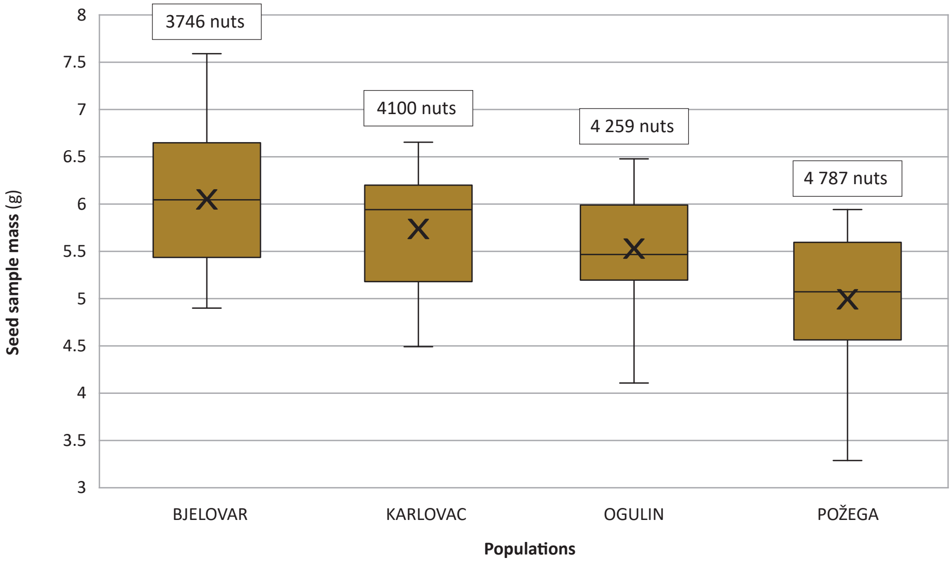

FIGURE 4. Distribution of seed sample (80 seeds) mass per population and the number of seeds in 1 kg.

the HCPs. On average, Karlovac population had the largest HCP (90.7 m^2), while HCPs from Požega population were the smallest (50.46 m^2). Karlovac population on average had largest yields per tree and largest crowns. Average HCP in Bjelovar population was 73.7 m^2 and in Ogulin 63.57 m^2. Although the analysis of variance showed a statistically significant population effect (p=0.0012), the Tukey-Kramer test confirmed significant differences in HCP between Karlovac and Požega (p=0.0007) and between Karlovac and Ogulin (p=0.0397).

Morphological Analysis of Beechnuts

Average beechnut length for all populations was 15.90 mm in the range of 6.48–20.26 mm. The largest mean value of a seed length was in Bjelovar population, which

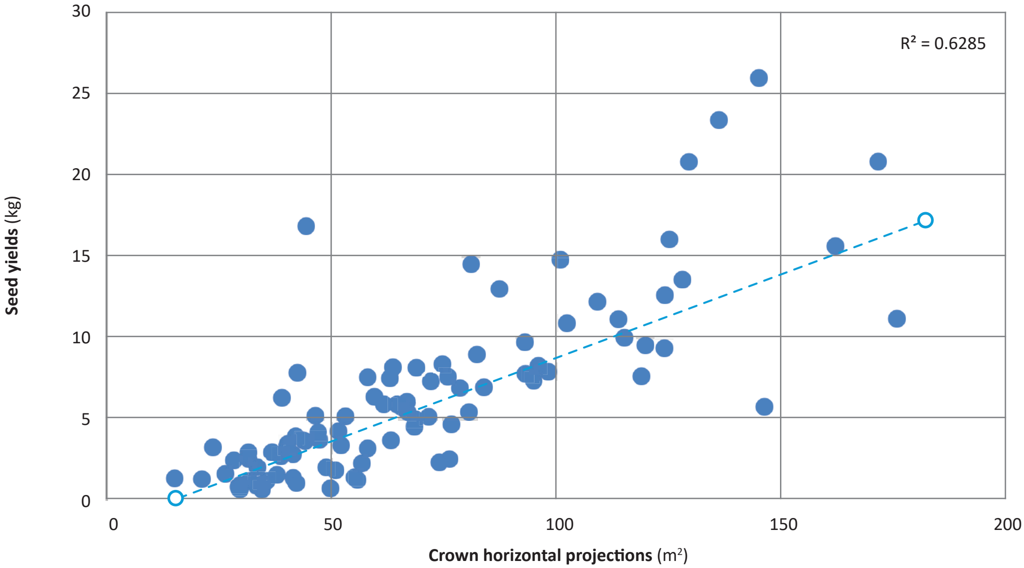

FIGURE 5. Correlation between seed yields (kg) and crown horizontal projections (m^2).

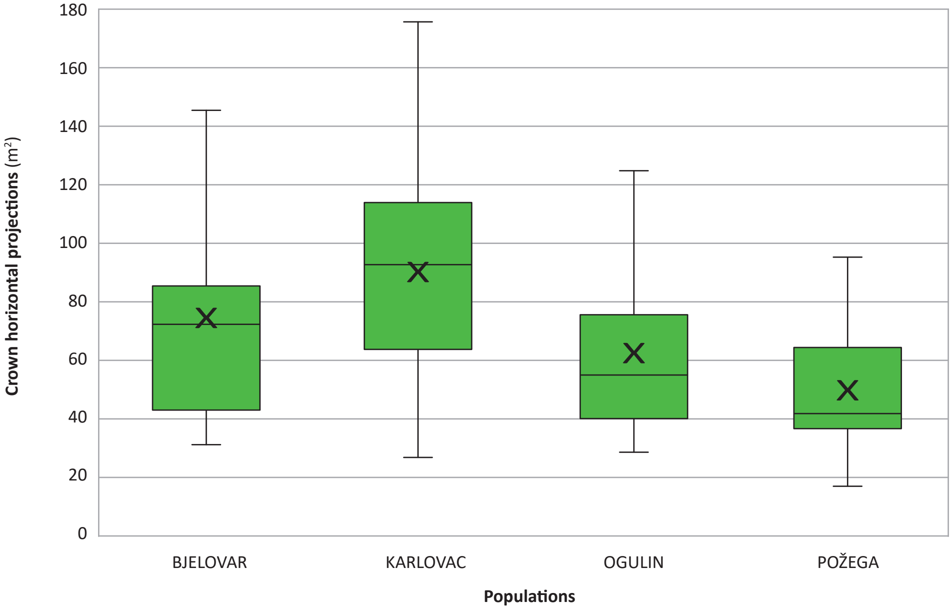

FIGURE 6. Distribution of crown horizontal projections in the studied populations.

is followed by Karlovac and Ogulin populations, while the lowest mean value was in Požega population (Figure 7). Statistically significant differences in a seed length were shown between populations and between collection time within the populations ($p<.0001$). The Tukey-Kramer test indicated that Požega population had significantly smaller seed lenghts than all other populations ($p<.0001$, Figure 7). There was also a significant difference in beechnut length between Bjelovar and Ogulin populations ($p=0.0648$, Figure 7). Regarding collection time, the results showed there were no significant differences in seed length in Bjelovar and Karlovac populations. However, in Ogulin and Požega populations, seeds from the first collection (September) were significantly longer than others (Table 3).

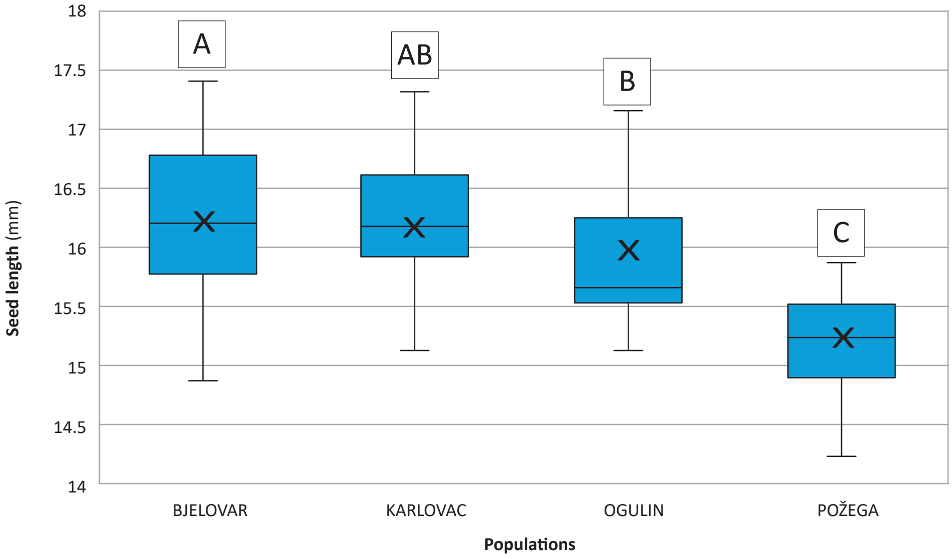

FIGURE 7. Distribution of seed length in the studied populations (the same letters associate populations with no statistically significant differences).

TABLE 3. Tukey-Kramer test for seed length considering the effect of collection (*statistically significant differences, p<0.05).

Effect	Forest Administration (FA)				Adjustment	Adj P
Kolekcija(FA)	BJELOVAR	1	BJELOVAR	2	Tukey	0.9848
Kolekcija(FA)	BJELOVAR	1	BJELOVAR	3	Tukey	0.7271
Kolekcija(FA)	BJELOVAR	1	BJELOVAR	4	Tukey	1.0000
Kolekcija(FA)	BJELOVAR	2	BJELOVAR	3	Tukey	1.0000
Kolekcija(FA)	BJELOVAR	2	BJELOVAR	4	Tukey	0.9210
Kolekcija(FA)	BJELOVAR	3	BJELOVAR	4	Tukey	0.4984
Kolekcija(FA)	KARLOVAC	1	KARLOVAC	2	Tukey	0.8824
Kolekcija(FA)	KARLOVAC	1	KARLOVAC	3	Tukey	0.1486
Kolekcija(FA)	KARLOVAC	1	KARLOVAC	4	Tukey	0.9192
Kolekcija(FA)	KARLOVAC	2	KARLOVAC	3	Tukey	0.9988
Kolekcija(FA)	KARLOVAC	2	KARLOVAC	4	Tukey	1.0000
Kolekcija(FA)	KARLOVAC	3	KARLOVAC	4	Tukey	0.9971
Kolekcija(FA)	OGULIN	1	OGULIN	2	Tukey	<.0001*
Kolekcija(FA)	OGULIN	1	OGULIN	3	Tukey	0.0017*
Kolekcija(FA)	OGULIN	1	OGULIN	4	Tukey	<.0001*
Kolekcija(FA)	OGULIN	2	OGULIN	3	Tukey	1.0000
Kolekcija(FA)	OGULIN	2	OGULIN	4	Tukey	0.9899
Kolekcija(FA)	OGULIN	3	OGULIN	4	Tukey	0.7708
Kolekcija(FA)	POŽEGA	1	POŽEGA	2	Tukey	<.0001*
Kolekcija(FA)	POŽEGA	1	POŽEGA	3	Tukey	0.0001*
Kolekcija(FA)	POŽEGA	1	POŽEGA	4	Tukey	0.0032*
Kolekcija(FA)	POŽEGA	2	POŽEGA	3	Tukey	1.0000
Kolekcija(FA)	POŽEGA	2	POŽEGA	4	Tukey	0.9997
Kolekcija(FA)	POŽEGA	3	POŽEGA	4	Tukey	1.0000

The overall average width of seeds was 8.43 mm in the range of 5.11-13.62 mm. Bjelovar population had highest mean values, while Požega population had the lowest mean value (Figure 8). Analysis of variance did not confirm statistically significant differences regarding collection time, but it was confirmed for the population effect (p=<.001). Tukey-Kramer test showed that Ogulin and Požega populations had significantly narrower seeds than Bjelovar (p=0.0011 and <.0001, respectively; Figure 8) and Karlovac population (p=0.0431 and 0.0002, respectively; Figure 8). Therefore, the populations were divided in two groups according to seed width.

Correlation analysis showed a positive and statistically significant correlation between beechnut sample mass and seed width (R^2=0.5875) (Figure 9). Between seed mass and seed length there was no significant correlation. Also, as the highest values of seed length and seed width were identified in Bjelovar population, the sample mass of 20 seeds was largest in this population as well.

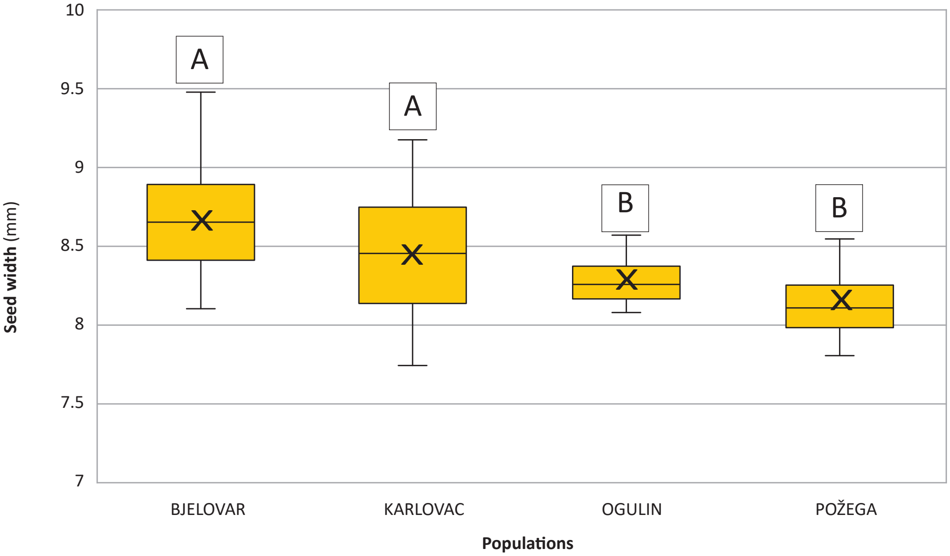

FIGURE 8. Distribution of seed width in the studied populations (the same letters associate populations with no statistically significant differences).

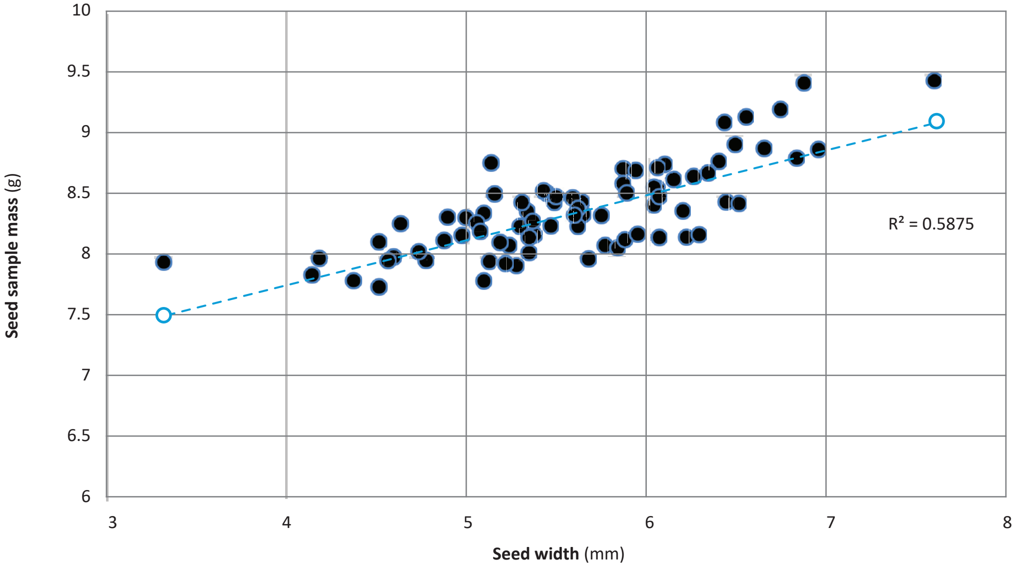

FIGURE 9. Correlation between seed sample (20 seeds) mass and seed width.

DISCUSSION AND CONCLUSIONS

The mast year is a year in which large amounts of seeds are produced over a wider geographical area and when it is possible to naturally regenerate beech stands. In general, there is no consensus on the quantity of seeds that should be produced in a year for it to be considered a mast year [45, 46]. However, there is a quite clear difference between mast years and the diminutive amount of seeds in non-mast years [35]. There are very few records of measured mast quantities. In our research, the average estimated seed yield varied from 2.4 to 5.9 million seeds·ha^{-1}. That amount of seeds may be considered as mast crop and it is comparable with Simak report [47] on mast crops from three stands in southern Sweden from 1974 to 1983 (the mean mast crop was 3.7 million seeds·ha^{-1} per mast year and considerably higher in the liming experiment (5.3 million seeds·ha^{-1})).

Predicting and estimating seed yield is important because seed availability affects stand regeneration. However, comprehensive research of seed production of European beech in Croatia (as well as in wider region of south-eastern Europe) is still lacking.

It is not easy to make an accurate assessment of the seed potential of a particular forest stand. Many factors affect the yield, such as weather conditions, altitude and micro-site which all have an important influence [31]. It was shown that various environmental conditions (both abiotic and biotic) affect successful beechnut production. Best fructification occurred when air temperature was between 15 and 25°C, and relative humidity did not fall below 26–33% [32]. Full mast is usually preceded by warm and dry July (average temperature of at least 15.8°C, and 16 days or more with a maximum temperature exceeding 20°C) [33]. Nevertheless, the mentioned values of climate variables were presented as results observed in Sweden and might differ between beech ecotypes adapted to northern and southern European climate conditions. Generally, research results on *Fagus* species in Europe and North America indicate that masting largely depends on meteorological conditions in the years preceding it [34-36].

Our research indicates that the size of a crown (i.e. crown width) is an another particularly important factor for trees' seed yield [37]. Differences in average HCPs may explain differences in seed yields among the studied populations, at least partially. Within population variations in seed yield are most likely influenced by differences in crown projections among trees. As already confirmed by a study on oak trees [37], the crown size is an important factor forseed productivity. Available spacing has a significant impact on tree growth and development. Thus, widely spaced trees tend to grow larger crowns that are more exposed to sunlight, thus producing larger quantities of seeds. Our research generally acknowledged empirical knowledge that a crop of beechnuts was more abundant at stand margins as compared to conditions below dense stands. Marginal trees gain more heat and light and have better disposition to fructify abundantly than trees inside a dense stand. Of course, our results also indicate that HCPs are definitely not the only cause of differences in seed yields among the populations. For example, Požega population had a relatively good mean crop per tree (not significantlly smaller than Karlovac), despite the fact that this population on average had the smallest crown areas. Apart from the size of crown, factors which conclusively affect fructification are site and stand conditions, health, tree position and climate history [51, 52]. In the long term, tree characteristics and genetics are probably more important than environmental factors in determining actual beechnut production.

Knowing the morphological and biological properties of beech seed from different localities allows us to improve regeneration and viability of beech stands [38]. The results obtained in the study on variability of morphometric characteristics of seed can also be used to preliminary get acquainted with the genetic variability of the studied populations and to improve the production of quality seed and planting material of beech in Croatia. Beechnuts are triangular, and their width and thickness were measured on two sides. Very similar results considering analysed seed traits have been obtained by Drvodelić *et al.* [39] who reported average seed length of 15.42 mm in the range of 14.50-16.33 mm, and average width of 8.14 mm in the range of 7.47-8.97 mm. Gradečki *et al.* [38] reported average seed length of 14.89 mm in the range of 11.31-16.20 mm, and average width of 9.53 mm in the range of 7.63-10.34 mm. If we consider the length and width of the seed as morphological indicators of its variability, we can conclude that within the population variability is very small, while the analysis of variance identified that populations are mutually statistically different. Seed size showed good direct correlation with seed mass. In relation to number of seeds per kg, our results were within the range of other similar reports [40, 41-43]. Our research also indicated high variability of studied nut traits within and among populations. This variability could have been caused by numerous factors, both environmental (e.g. weather, insects) [39] and genetic [44]. European beech is characterized by the possibility of self-pollination, which is an important cause of the occurrence of empty beech seeds [39]. In many forest tree species, controlled self-pollination results in a higher proportion of empty seeds than cross- or wind-pollination [53-56]. Empty seeds of forest trees may also occur as the result of environmental factors, such as limited pollen supply [57] and insect damage [58]. However, factors causing such between-population variability in seed traits are beyond the scope of this study.

Visual surveys of seeding intensity can be conducted relatively simply and quickly by using binoculars. Seed crop estimates before seed fall are an important piece of information for forestry operatives because seed production affects forest regeneration planning. However, such surveys should be satisfactory and accurate, i.e. usable, for at least rough estimates of the yield. As Figure 2 shows, our visual estimate of a crop was highly unreliable. Therefore, to improve this method it should be further modified, most likely by a smaller range of ratings and clearer grades. A good example is given by Nakajima [48], who used more precise seed intensity rates in five classes.

Acknowledgments

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Genetic Diversity of Pedunculate Oak (*Quercus robur* L.) in Clonal Seed Orchards in Croatia, Assessed by Nuclear and Chloroplast Microsatellites

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ABSTRACT

Background and Purpose: Natural stands of pedunculate oak in Croatia have been delineated in seed areas, zones and regions. The current bylaw recommends that the transfer of reproductive material remains limited within zones, but that it is permitted within areas. Clonal seed orchards (CSOs) of pedunculate oak were established to increase genetic quality of seed and to acquire a more regular seed yield than in natural stands. In total 150 plus trees were selected within three seed regions. The selection included a number of favourable traits of tree size and stem quality. Three CSOs were planted with grafted plus-trees. We aimed to establish whether these orchards encompass enough genetic diversity to potentially produce genetically improved and sufficiently diverse reproductive material. We also wanted to assess neutral genetic differentiation between these orchards and compare it with the genetic diversity obtained from chloroplast DNA markers, depicting conserved lineages from recolonization routes. We wanted to investigate spatial genetic structure in the area of our research and provide additional information on the transfer of forest reproductive material.

Materials and Methods: Leaves were collected from all clones in the CSOs. Total genomic DNA was extracted and clones were analysed with eight nuclear and ten chloroplast microsatellite markers. Spatial autocorrelation analysis was performed with nuclear microsatellite data and original plus trees' coordinates, for each CSO separately, to determine whether shared favourable traits among the selected plus trees in smaller distances are the results of relatedness, which the sampling strategy tried to avoid.

Results: We found 28 chloroplast haplotypes belonging to four maternal lineages, and significant differentiation between CSOs, indicating origin from different refuges. Nuclear microsatellites' diversity in the CSOs is quite high and comparable to diversity found within a recent study of Croatian natural populations. Nuclear microsatellites did not show genetic differentiation between CSOs, i.e. between the seed regions and seed zones they represent. No genetic differentiation was found with nuclear microsatellites among haplotypic lineages. We found no genetic structure within the analysed regions.

Conclusions: Lack of differentiation between CSOs found with nuclear microsatellites confirms the permission for transfer of reproductive material between zones within the seed area 1 - Lowland Forests. If original differentiation between chloroplast haplotypic lineages was present after recolonization, it was erased by subsequent gene flow. Lack of genetic structure, with nuclear microsatellites within regions indicates successful sampling strategy.

Keywords: plus trees, clonal seed orchards, seed regions, genetic differentiation, haplotypic lineages, spatial autocorrelation analysis

INTRODUCTION

Pedunculate oak (*Quercus robur* L.) is one of the ecologically and economically most important tree species in Croatia. Based on various ecological and management criteria, as well as research results, natural stands of pedunculate oak in Croatia were delineated in seed management units. According to the current bylaw on forest tree species provenances [1], they are divided into three seed areas, four seed zones and eight seed regions. To increase genetic quality of reproductive material and overcome irregular periodicity of masting in natural stands, a breeding programme for pedunculate oak improvement started in the 1980s [2]. Plus trees were selected in the seed area 1 - Lowland Forests, within three seed regions 1.1.2, 1.2.1, and 1.2.3, which belong to two seed zones, 1.1. and 1.2. (Table 1), based on multiple criteria of tree size and stem quality. Three clonal seed orchards (CSOs) were established with their vegetative copies (grafts).

The knowledge of the species' genetic diversity and the variability of different traits in various environmental conditions becomes especially important in the context of climate change. This particularly refers to the production of well-adapted forest reproductive material. Current research of quantitative traits' genetic variability of pedunculate oak in Croatia has shown that dominant component lies in intrapopulation variability [3-9].

Similar pattern of genetic variability (high intrapopulation and low interpopulation variability) and weak spatial structure of populations in greater distances can also be expected for nuclear microsatellites research on pedunculate oak, as well as sessile oak, when investigated populations belong to large uninterrupted complexes of these species [10, 11]. This was confirmed in recent research of natural pedunculate oak populations in Croatia with nuclear microsatellites, since only a few populations were significantly genetically differentiated and they did not belong to a more uniform complex with other populations [9]. In case of more fragmented populations, especially under strong anthropogenic influence, intrapopulation differentiation becomes higher, although still not as high as intrapopulation variability [12, 13].

Regarding plant species in general, biological factors such as limited gene flow by seed or pollen can lead to genetic differentiation even in continuous populations of species. In such cases, fertilization is not random, but rather conditioned by the distance between individuals [14]. Although the majority of other investigated plant species show pronounced genetic structures in short distances, this is rarely found in forest tree species. In these species the structure is weak or non-existent, sometimes described as almost random [15]. Interestingly, weak genetic structure is noticed in case of forest tree species with different reproductive systems and history, which can be attributed to their joint characteristics: long lifespan and stronger gene flow compared to other plant species. Selection can also contribute to creating genetic structure patterns and enhance or diminish genetic differentiation. Selection caused by diversity of microecologic conditions in the stand can trigger the creation of spatial patterns, while alternatively, in case of inbreeding depression, selection can enhance the dying off of inbred individuals and lead to weakening of genetic structure [16].

Regarding pedunculate oak, there is pronounced asymmetry of gene flow by seed and pollen. Pollen is spread by wind, while seeds are large and heavy and can be carried to larger distances only by animals, humans or along river flows. Research shows that in pedunculate oak gene migration by pollen is approximately two hundred times stronger then migration by seed. This difference is manifested in weaker differentiation of populations when analysed with biparently inherited nuclear markers, while at the same time differentiation by uniparentaly inherited cytoplasmic (in case of broadleaves chloroplast) markers can be substantial. Presumably, limited gene flow by seed primarily causes spatial patterns, but they are subsequently erased by constant and strong gene flow by pollen [15, 17, 18].

Research on correlation of chloroplast markers' diversity with nuclear molecular markers and quantitative traits showed complete incoherence of chloroplast lineages with quantitative traits, and weak concordance with allozyme frequencies [19]. It led to the following conclusions: (1) During the last glacial deciduous oaks were probably limited to three main refuges, which led to genetic differentiation of both nuclear and chloroplast genomes; (2) At the end of the glacial oaks migrated to the north, at which point spatial pattern of chloroplast diversity was established, which has remained highly conserved until today; (3) As oaks progressed in colonization towards central and northern Europe, gene flow by pollen reinstated communication between populations originating from western, or eastern refuges, which led to gradual loss of original differentiation in parts of nuclear genomes. During that process, differentiation in chloroplast genomes remained approximately the same; (4) Local selection pressures influencing the newly established populations gradually led to differentiation of parts of nuclear genomes whose new patterns were completely different from those immediately following the recolonization. During that whole period, chloroplast diversity remains conserved since it is not connected to quantitative traits. All that remains is a weak connection to nuclear markers that are not under strong influence of selection.

The aim of our research is the evaluation of two types of genetic diversities within and among three CSOs, established with grafts of plus trees selected within three legally delineated seed regions, belonging to two seed zones. The first type is genetic diversity of nuclear microsatellite markers, presumably neutral, highly polymorphic, biparently inherited markers that point to gene flow by both seed and pollen. The second type is diversity of maternally inherited chloroplast microsatellite markers, pointing to gene flow only by seed.

Genetic diversity analysis of clones in CSOs will demonstrate whether the selected parental populations, i.e. CSOs, consist of enough diverse and unrelated genotypes to potentially produce genetically diverse and improved seed for filling the natural stands and aiding their regeneration. Genotyping by nuclear microsatellites also lays ground for parentage analysis of crops produced in the CSOs, to determine the true efficacy of the CSOs in producing genetically diverse progeny. Although nuclear microsatellite markers in this study are selectively neutral, high genetic diversity of CSOs for these markers would also point to potentially wider adaptation potential, which is especially important in the context of climate and habitat change [20].

Considering the known positions of original plus trees in the stands, one of the aims is to analyse spatial distribution of nuclear microsatellite genetic diversity within each region. We want to investigate whether there is geographically influenced genetic structure at certain classes of distance, detected by a kinship degree of the selected trees within a region. This way we want to eliminate the possibility that multiple favourable traits shared among geographically close plus trees result from their kinship, which the sampling strategy of 50 m distance between the selected trees tried to avoid [2].

By using chloroplast microsatellites, which reflect the genetic diversity established by postglacial recolonization routes, we will analyse the diversity of haplotypes within and among regions. The results will demonstrate lesser or greater homogeneity of individual regions and possible anthropogenic influence in haplotype diversity of certain regions.

By comparing the analysis with nuclear and chloroplast markers we will try to establish whether the original differentiation by recolonization routes remained detectable in the analysed loci of the nuclear genome.

MATERIALS AND METHODS

Study Area

The leaves for DNA analysis were collected in three CSOs. Detailed information on CSOs, their position and the list of forest districts where plus trees were selected is shown in Table 1, while the position of the CSOs is shown in Figure 1.

DNA Extraction and Microsatellite Analysis

In spring we collected young leaves from one ramet of all clones in the three CSOs. The leaves were kept at $-60^{o}C$ until DNA extraction. We extracted total genomic DNA with GenElute Plant Genomic DNA Miniprep Kit (Sigma®).

We chose eight nuclear microsatellite markers for analysis: ssrQrZAG96, ssrQrZAG108, ssrQrZAG87, ssrQrZAG112 and ssrQrZAG31 from Kampfer *et al.* [21], and ssrQpZAG110, ssrQpZAG9 and ssrQpZAG36 from Steinkellner *et al.* [22]. PCRs were carried out following a slightly changed protocol from Kampfer *et al.* [21].

For chloroplast analysis, we chose 10 microsatellite markers: µdt1, µdt3, µdt4, µcd4, µcd5, µkk3 and µkk4 from Deguilloux *et al.* [23], and ccmp2, ccmp10 and ccmp6 from Weising *et al.* [24]. The PCRs were carried out following a slightly changed protocol from Deguilloux *et al.* [23].

Detailed description of the primers, multiplexes and PCR protocols can be found in Deguilloux *et al.* [23].. The 5' primers were labelled with different fluorescent dyes (WellRED Oligos, Proligo). All PCRs were carried out by PTC-100 thermal cycler (MJ Research®). Fragment separation was performed by capillary electrophoresis on CEQTM 8000, Genetic Analysis System (Beckman & Coulter), and alleles were scored with CEQTM 8000 Fragment Analysis Software.

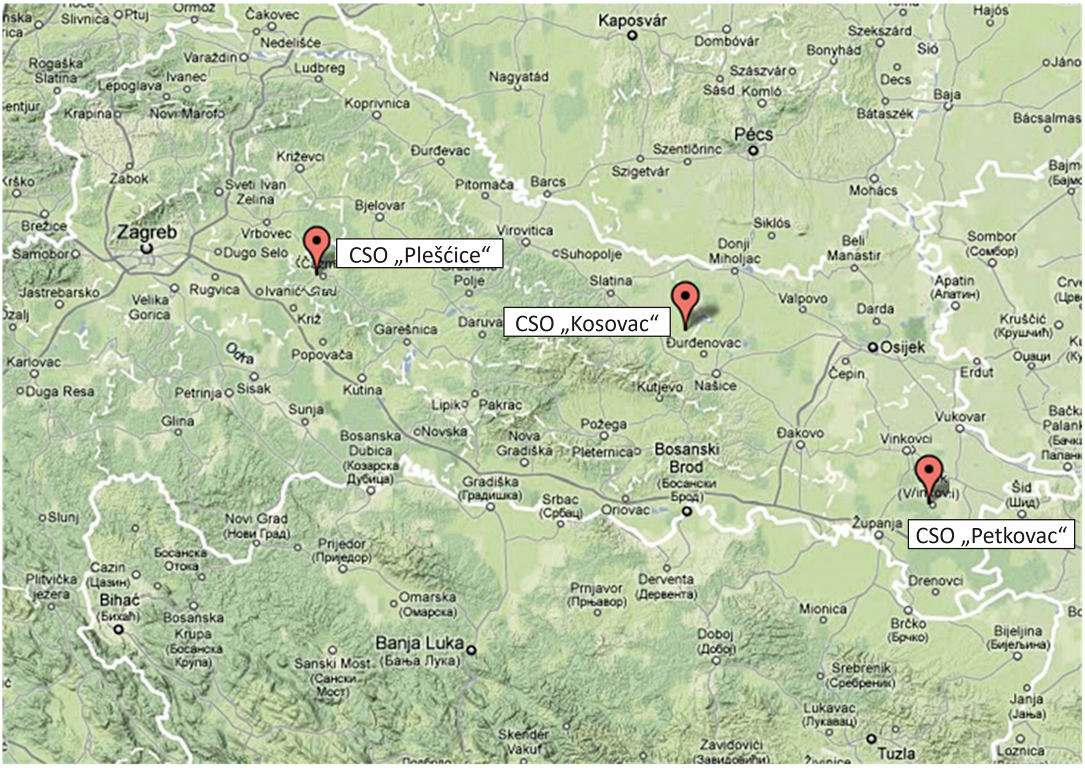

FIGURE 1. Position of Quercus robur clonal seed orchards in Croatia

TABLE 1. Information about Quercus robur clonal seed orchards (CSOs).

CSO	Year	Number of clones	Position	Seed area, zone and region	Selection of plus trees (No of trees in different FD)	
Kosovac	1996	40	FA Našice“ FD „Orahovica“ MU „Obradovačke nizinske šume“	SA 1. Lowland forests SZ 1.1 Podravina and Podunavlje SR 1.1.2. Central Podravina	FD „Slatina“	26
					FD „Donji Miholjac“	9
					FD „Koška“	4
					FD „Đurđenovac“	1
Petkovac	2000	57	FA „Vinkovci“ FD „Otok“ MU „Slavir“	SA 1. Lowland forests SZ 1.2 Posavina, central Croatia and Pokuplje SR 1.2.1. Lower Posavina	FD „Gunja“	19
					FD „Vrbanja“	14
					FD „Otok“	11
					FD „Županja“	9
					FD „Strošinci“	4
Plešćice	2001	53	FA „Bjelovar“ FD „Čazma“ MU „Čazmanske nizinske šume“	SA 1. Lowland forests SZ 1.2 Posavina, central Croatia and Pokuplje SR 1.2.3. Upper Posavina and Pokuplje	FD „Čazma“	15
					FD „Bjelovar“	10
					FD „Garešnica“	10
					FD „Vrbovec“	7
					FD „Grubišno Polje“	5
					FD „Veliki Grđevac“	4
					FD „Daruvar“	2

FA - Forest Administration, FD – Forest District, MU – Management Unit, SA – Seed Area, SZ – Seed Zone, SR – Seed Region

Statistical Analysis

A) Nuclear Microsatellites (nSSRs)

a1) Genetic Diversity and Differentiation

The total number of alleles per locus (Na), the observed HO and HE, expected heterozygosity and Polymorphism Information Content (PIC; [26]) for each microsatellite locus, as well as the average number of alleles (Navg), HO and HE in each region were calculated using PowerMarker v3.25 software [27]. The allelic richness (Nar) as the measure of the number of alleles per locus independent of sample size was calculated by FSTAT v.2.9.3.2 programme package [28], while the number of private alleles (Npr) per region was assessed by MICROSAT [29]. The estimates of Nar, HO and HE among regions were compared using the Kruskal-Wallis test in SAS Release 8.02 (SAS Institute, 2004).

To establish whether the analysed regions belong to Hardy-Weinberg equilibrium we used Wright's F statistics [30]. If there are deviations, the possible reasons are inbreeding within the regions or genetic differentiation between the regions. Entry data are allele frequencies of analysed markers in sampled regions. Potential heterozygote deficiencies, as a result of inbreeding, is described by fixation indices; F_{IS}, on individual level, and F_{ST}, on population (in this case regional) level.

To calculate F_{IS} [31] for each locus in each region and to test congruence between the observed and expected genotypic frequencies under Hardy–Weinberg equilibrium we used GENEPOP v. 3.4 [32]. All probability tests are based on Markov chain method [33, 34], using 10000 steps of repeated memory, 100 data groups and 5000 repetitions per group. To correct it for multiple testing we used sequential Bonferroni adjustments [35, 36] implemented in the SAS Release 8.02 programme (SAS Institute, 2004).

Genetic differentiation among regions was estimated by FST using Weir and Cockerham method [31] as implemented in FSTAT. The significance of FST estimates was assessed by randomization. We calculated Nei's standard genetic distances [37] between pairs of regions with GENDIST programme implemented in PHYLIP ver 3.6b software [38].

a2) Analysis of Molecular Variance (AMOVA)

The proportion-of-shared-alleles distance [39] between pairs of individuals was calculated using MICROSAT and the distance matrix was subjected to the analysis of molecular variance (AMOVA; [40]) using ARLEQUIN version 2.000 [41]. AMOVA was used to partition the total microsatellite diversity into among-region and within-region components. The variance components were statistically tested by non-parametric randomisation tests using 10,000 permutations.

a3) Spatial Autocorrelation Analysis

Spatial autocorrelation analysis demonstrates relationships between specific measures of autocorrelation in different geographical distance classes, by means of correlograms, i.e. distograms. It is also possible to test significance of autocorrelation measure for each individual distance class. Since we analysed nuclear microsatellite markers, we used kinship coefficient for co-dominant markers by Loiselle *et al.* [42], described in detail in SPAGeDi 1.2g programme manual [43].

When plus trees were selected in the stands, their positions were mapped on sub-compartment management maps. Based on those sketches, we transferred their position on digitised management maps in ArcGis 9.2. (ESRI, 2007) programme and retrieved Gauss–Krüger coordinates for spatial analysis.

We performed spatial autocorrelation analysis with SPAGeDi 1.2g programme. Geographical distance between individuals for each region separately was calculated based on Gauss–Krüger coordinates of plus trees. Pairs of plus trees were classified into ten distance classes, containing approximately the same number of trees in each class. Based on nuclear marker analysis we calculated a matrix of kinship coefficient for co-dominant markers. The significance level of average kinship coefficients of individual distance classes was determined by 1000 permutations.

B) Chloroplast Microsatellites (cpSSRs)

b1) Lineages

b1.1) Cluster Analysis (UPGMA)

Genetic distances between haplotypes were calculated in MICROSAT [29] using absolute distance (D_{AD}) metric based on the sum of the number of repeat differences between cpSSR haplotypes (described in detail in Deguilloux *et al.* [23]).

Cluster analysis based on distance matrix was performed using the unweighted pair-group method (UPGMA) a s implemented in NEIGHBOR programme of the PHYLIP ver. 3.6b software [38]. The reliability of the UPGMA topology was assessed via bootstrapping [44] of over 1000 replicates generated by MICROSAT [29] and subsequently used in NEIGHBOR and CONSENSE programmes in PHYLIP.

b1.2) Median – Joining Network of Haplotypes

A Median-Joining (MJ) network [45] was constructed based on cpSSR haplotypes using the Network 4.5.1.6. programme [46]. This method is used in discovering very complex patterns of haplotypes. It uses parsimony criteria for finding median vectors, i.e. consensus sequences or mutually close marker sequences that present a biological equivalent of possible non-sampled or extinct ancestral haplotypes.

b2) Genetic Diversity and Differentiation

Genetic diversity was estimated in each population and in the metapopulation as the number of haplotypes (n_h), the number of haplotypes per the number of individuals (n_h/n), the number of private haplotypes (n_{ph}; i.e. haplotypes found in a single population), the effective number of haplotypes (n_E), haplotype richness (n_{hr}), haplotype diversity (H_E), and the frequency of the most common haplotype (f_h). [47]

Haplotype richness (n_{hr}) as the measure of the number of haplotypes per population independent of sample size was calculated by rarefaction method [47] as implemented in Contrib [48].

An unbiased estimate of the haplotype diversity (H_E) was calculated according to Nei [49].

Genetic differentiation was analysed by three different methods described in Pons and Petit [50, 51], using the Permut programme [52]:

a) Unbiased estimation of G_{ST} [49] according to Pons and Petit [50], considering only haplotype frequencies (unordered haplotypes). Unbiased estimation of G_{ST} involved the calculation of the diversity of each region (h_k), average within-region diversity (h_s), and total diverstiy (h_t)

b) The estimation of N_{ST} [51] for which the genetic similarities between haplotypes (proportion of shared fragments) were taken into consideration (ordered haplotypes). The eof N_{ST} involved the calculation of the diversity of each region (v_k) by taking into account similarities between the haplotypes, average within-region diversity (v_s), and total diversity (v_t).

c) The estimation of R_{ST} [53] for which the genetic distances at individual SSR loci were taken into consideration according to a microsatellite stepwise mutation model (SMM). The estimation of R_{ST} involved calculations similar to those used for the estimation of N_{ST} except that the distance between two haplotypes was the sum (across all loci) of squared difference in the number of repeats [53]. This method was specifically designed for microsatellites by assuming the stepwise mutation model (SMM), while the N_{ST} method makes fewer assumptions about the nature and complexity of mutational patterns detected by fragment length analysis [51].

Detailed calculations of all methods can be found in Deguilloux *et al.* [23].

A comparison of differentiation and diversity measures for ordered (N_{ST} and R_{ST}) vs. unordered (G_{ST}) haplotypes was carried out according to Pons and Petit [51]. Significance was tested on the basis of 1,000 random permutations using the Permut programme [52].

b3) Analysis of Molecular Variance (AMOVA)

The partition of cpSSR diversity within and among regions was studied using the Analysis of Molecular Variance (AMOVA, [40]) as implemented in ARLEQUIN [41], while the significance of AMOVA was tested based on 10,000 permutations of haplotypes among populations. Again, two genetic distance measures between pairs of haplotypes were used based on (1) the number of different alleles (F_{ST}), as well as on (2) the sum of squared size differences (R_{ST}).

AMOVA of Haplotypic Lineages, with nSSRs

Based on the results of cluster analysis the haplotypes were grouped into lineages.

The partition of nSSR diversity within and among lineages (based on cpSSR) was tested using AMOVA in ARLEQUIN [41]. The variance components were tested statistically by non-parametric randomisation tests using 10,000 permutations.

RESULTS

A) Nuclear Microsatellites

a1) Genetic Diversity and Differentiation

Parameters of genetic diversity of analysed nuclear microsatellite loci are shown in Table 2. The values of PIC were in the range of 0.280 to 0.915, with an average of 0.739. Five out of eight markers had PIC values higher than the average, which makes them highly efficient in assessing genetic diversity of these regions. Markers ssrQrZAG96, ssrQrZAG108 and ssrQrZAG112 had lower levels of PIC, of which ssrQrZAG96 was least polymorphic.

The greatest number of alleles was found in markers ssrQrZAG112, ssrQrZAG31 and ssrQrZAG87, and the lowest in ssrQrZAG96, with an average number of 20.25 across all loci.

Expected heterozygosity (H_E) ranged from low 0.288 in ssrQrZAG96 to 0.915 in ssrQrZAG31, with an average of 0.750 across all loci. Observed heterozygosity (H_O) in most loci ranged between the expected values, with an exception of locus ssrQrZAG31, and minor deviations in loci ssrQrZAG108 and ssrQrZAG87. In loci ssrQpZAG9 and ssrQpZAG36 H_O was greater than H_E.

F_{ST} values showed significant differentiation between regions only for loci ssrQrZAG96 and ssrQrZAG108. On average, there was no significant differentiation between regions.

The values of inbreeding coefficient F_{IS} for regions and loci are shown in Table 3. F_{IS} values were significant for the following markers within regions: ssrQrZAG31 values point to significant deviations from Hardy–Weinberg equilibrium (HWE) in all three regions. Values for ssrQrZAG87 and ssrQrZAG112 are also significant for region represented by CSO Kosovac, while ssrQrZAG108 is only significant for CSO Plešćice.

Parameters of genetic diversity of the analysed regions (CSOs) are shown in Table 4. The regions are quiet uniform across all parameters, with most private alleles belonging to CSO Petkovac, and least to CSO Kosovac. Allelic richness (N_{ar}), corrected for different sized samples with rarefaction index, does not show significant differentiation between regions, nor do H_O and H_E.

Genetic distance parameters are shown in Table 5. Nei's standard genetic distances between regions are almost equal, and differentiation between regions is not significant.

a2) Analysis of Molecular Variance (AMOVA)

The results of AMOVA between regions are shown in Table 6. There is no significant differentiation between regions, with 99.96% of diversity lying at the within-region level, meaning that the majority of diversity is caused by differences between individual trees of all three regions.

TABLE 2. Allelic diversity of the microsatellite loci scored in three *Quercus robur* CSOs.

Locus	N_a	H_O	H_E	PIC	$F_{ST}^{\#}$
ssrQpZAG9	20	0.880	0.866	0.852	-0.002
ssrQrZAG31	25	0.260	0.920	0.915	0.000
ssrQpZAG36	18	0.884	0.872	0.860	-0.002
ssrQrZAG87	25	0.757	0.871	0.860	-0.002
ssrQrZAG96	9	0.248	0.288	0.280	0.014^{**}
ssrQrZAG108	17	0.475	0.695	0.678	0.015^{*}
ssrQpZAG110	22	0.589	0.603	0.593	-0.001
ssrQrZAG112	26	0.866	0.881	0.872	-0.002
Mean	20.25	0.620	0.750	0.739	0.001

N_a, the total number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, Polymorphic Information Content
$^{\#***}$ Significant at $P < 0.001$; ** Significant at $P < 0.01$; * Significant at $P < 0.05$; not indicated - non-significant value

TABLE 3. Inbreeding coefficients f across eight microsatellite loci in three CSOs of *Quercus robur.*

Locus	CSO		
	Plešćice	Kosovac	Petkovac
ssrQpZAG9	0.047^{ns}	-0.041^{ns}	-0.033^{ns}
ssrQrZAG31	0.726^{***}	0.753^{***}	0.685^{***}
ssrQpZAG36	-0.040^{ns}	-0.060^{ns}	0.049^{ns}
ssrQrZAG87	0.141^{ns}	0.251^{***}	0.047^{ns}
ssrQrZAG96	0.150^{ns}	0.162^{ns}	0.080^{ns}
ssrQrZAG108	0.400^{***}	0.250^{ns}	0.260^{ns}
ssrQpZAG110	0.062^{ns}	-0.037^{ns}	0.033^{ns}
ssrQrZAG112	0.011^{ns}	0.076^{***}	0.000^{ns}
All loci	0.197^{***}	0.175^{***}	0.124^{***}

Inbreeding coefficients (*f*) were calculated according to Weir and Cockerham (1984)

Significant deviations from Hardy-Weinberg equilibrium: "**" corresponds to significance at the 1% nominal level, "*" significance at the 5% nominal level, and "ns" depicts non-significant values after sequential Bonferroni corrections

TABLE 4. Genetic variation within *Quercus robur* CSOs at eight microsatellite loci

CSO	n	N_{avg}	N_{ar}	N_{pr}	H_O	H_E
Plešćice	50	14.50	12.67	18	0.617	0.755
Kosovac	40	13.25	12.58	12	0.604	0.730
Petkovac	54	15.25	13.06	26	0.635	0.738
p(KW)			0.89		0.98	0.93

n, sample size; N_{avg}, average number of alleles per locus; N_{ar}, allelic richness; N_{pr}, number of private alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; p(KW), probabilities of the Kruskal-Wallis tests among CSOs

TABLE 5. Nei's standard genetic distance (upper diagonal) and pairwise FST values (lower diagonal) among three Quercus robur CSOs.

	CSO (region)	1	2	3
1	Plešćice		0.0466	0.0450
2	Kosovac	0.0004^{ns}		0.0464
3	Petkovac	0.0016^{ns}	0.0013^{ns}	

P-values as obtained by randomizations: "**" corresponds to significance at the 1% nominal level, "*" to significance at the 5% nominal level, and "ns" depicts non-significant values

TABLE 6. AMOVA analysis for the partitioning of microsatellite diversity among and within CSOs.

Source of variation	df	Variance components	Percentage of variation	ϕ_{ST}	P(ϕ)
Among	2	0.00014	0.04	0.0004	0.423^{ns}
Within	141	0.336	99.96		

P(ϕ) - ϕ-statistics probability level after 10,000 permutations; ns – not significant

a3) Spatial Autocorrelation Analysis

Spatial autocorrelation analysis was conducted separately for three regions, based on the analysis with nSSRs and Gauss–Krüger coordinates of original plus trees. The results are shown in Figure 2. Geographical distances were divided in ten classes in which pairs of trees were distributed, according to their mutual geographical distance. Average kinship coefficients for co-dominant markers between all pairs in each class [42] were calculated. Figure 2 shows upper and lower boundaries of 95% confidence intervals. Those intervals represent the boundaries above or bellow which, for a certain geographical distance, pairs of individuals are more or less related then pairs of randomly chosen individuals. The Figure shows that in the studied regions, based on our sample of selected plus trees, we found no geographical genetic structure. There was no class of geographic distance detected, containing pairs of individuals significantly more or less related then randomly chosen pairs of individuals.

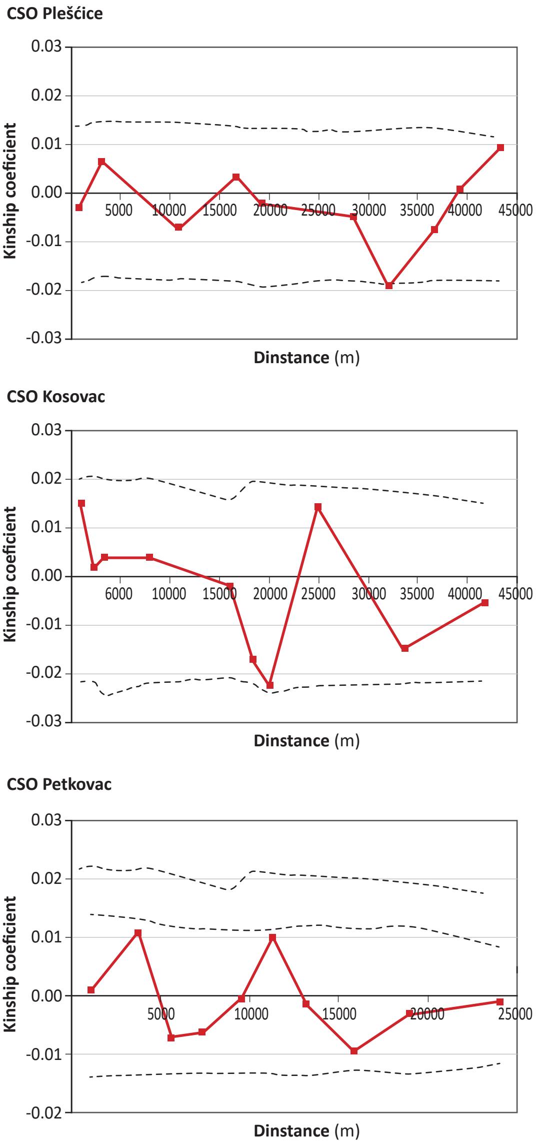

FIGURE 2. Charts of spatial autocorrelation analysis, separately for three CSOs, with 10 distance classes per CSO and eight nSSR loci. Dotted lines represent 95% confidence intervals.

B) Chloroplast Microsatellites (cpSSRs)

b1) Distribution of Haplotypes

Table 7 shows the definition of cpSSR haplotypes found in three regions, based on nine cpSSR markers. Marker µkk3 was monomorphic and was therefore excluded from further analysis. The numbers represent allele size in base pairs (bp). Table 7 also shows numbers of individuals within regions, belonging to different haplotypes. In haplotypes H07, H14, H15, H17 and H18 a specific long allele for µdt4 was found. The authors in [23, 54, 55] did not mention encountering such an allele in their research, but it was definitely found in the inventory of Austrian oak populations [56]. When this allele is included in statistical analysis in its full size, it strongly influences calculations, especially those based on SMM models. In Provan *et al.* [57], the authors also encountered an unusually long allele, sequenced it and determined that it was due to an indel of a sequence close to the microsatellite, so they treated this entire indel as a single mutation step. We suspect that something similar occurred in the sequence of µdt4 marker, so following the example from Provan *et al.* [57] we also corrected the size of this allele, taking the difference of 18bp between allele 148 and 166 as a single mutation step in all further statistical analysis.

TABLE 7. Definition of cpSSR haplotypes and their distribution in the CSOs, based on nine cpSSR markers. The numbers represent allele lengths in base pairs.

cpSSR haplotypes	cpSSR markers									Distribution of haplotypes in the CSOs (number of individuals)			
	μdt3	ccmp10	μcd5	μdt1	ccmp6	μcd4	μdt4	ccmp2	μkk4	Pl	K	P	Σ
H01	126	113	75	84	105	96	147	236	112	1	13	18	32
H02	125	113	75	84	105	96	147	236	112	0	0	23	23
H03	126	112	75	84	105	96	148	236	113	13	0	0	13
H04	127	113	75	84	105	96	147	236	112	2	9	0	11
H05	126	112	75	84	105	95	148	236	113	7	0	0	7
H06	128	113	75	84	105	96	147	236	112	0	6	0	6
H07	126	112	76	85	105	98	166*	237	112	0	3	1	4
H08	125	113	75	84	104	96	147	236	112	0	0	3	3
H09	126	113	75	84	105	97	147	236	112	1	1	1	3
H10	127	113	75	84	105	97	147	236	112	2	1	0	3
H11	126	112	75	84	104	96	148	236	113	2	0	0	2
H12	125	112	74	84	105	96	147	236	112	0	0	1	1
H13	125	112	75	84	105	95	148	236	113	1	0	0	1
H14	125	112	76	85	105	98	166*	237	112	1	0	0	1
H15	126	112	75	85	105	98	167*	237	112	1	0	0	1
H16	126	112	76	84	104	95	148	236	113	1	0	0	1
H17	126	112	76	85	104	98	166*	236	112	1	0	0	1
H18	126	112	76	85	104	98	166*	237	112	1	0	0	1
H19	126	113	75	84	103	96	147	236	112	0	0	1	1
H20	126	113	75	84	104	96	147	236	112	0	0	1	1
H21	126	113	75	84	104	97	147	236	112	1	0	0	1
H22	126	113	75	84	105	95	148	236	113	1	0	0	1
H23	126	114	75	84	105	96	147	236	112	0	0	1	1
H24	127	113	75	84	104	97	147	236	112	1	0	0	1
H25	127	113	75	84	105	96	147	235	112	0	1	0	1
H26	127	113	75	84	105	96	147	236	113	0	1	0	1
H27	127	114	75	84	105	96	147	236	112	0	1	0	1
H28	128	114	75	84	105	96	147	236	112	0	1	0	1
									Σ	37	37	50	124

b2) Lineages

b2.1) Cluster Analysis (UPGMA)

Figure 3 shows UPGMA dendrogram of cpSSR haplotypes based on absolute distances (D_{AD}). Based on bootstrap values it is visible that maternal lineages L3 and L4 create separate branches with strong statistical support, and that lineages L1 and L2 are more related.

The distribution of individuals across the lineages for each region is shown in Table 8. All but three individuals from CSO Petkovac belong to L1, while clones from CSO Kosovac are divided between lineages L1 and L2. Most clones from CSO Plešćice belong to the separate lineage L3, with some clones belonging to L1 and L2. For each CSO there is at least one individual belonging to L4, with the unusually long allele for μdt4.

TABLE 8. The distribution of individuals from CSOs per haplotypic lineages.

Haplotypic lineages	Number / percentage of individuals in CSOs per lineages			Σ
	Plešćice	Kosovac	Petkovac	
1	6 (16.2 %)	15 (40.5 %)	47 (94.0 %)	64
2	2 (5.4 %)	19 (51.4 %)	1 (2.0 %)	26
H12	-	-	1 (2.0 %)	1
3	25 (67.6 %)	-	-	25
4	4 (10.8 %)	3 (8.1 %)	1 (2.0 %)	8
Σ	37	37	50	124

Spatial distribution of individual plus trees marked with the colour of lineage they belong to is shown in Figure 4. Trees belonging to different lineages are not randomly distributed in space, but rather grouped by lineages. For example, all plus trees from CSO Plešćice, belonging to lineages L1 and L2 were selected in Forest District "Garešnica", Management Unit "Međuvođe – Ilovski lug". L2 Trees included in CSO Kosovac are distributed evenly across the entire region's selection area; in the eastern part together with L4 tress, in the middle part with L1 trees, while L1 trees dominate over the western part. Almost all trees selected for CSO Petkovac belong to L1 lineage, with only individual L2 and L4 trees and a single tree with H12 haplotype, which does not belong to any lineages. In two CSOs where there is more than one L4 tree, they were also localized to a smaller area.

b2.2) Median–Joining Network of Haplotypes

Figure 5 shows median–joining network of haplotypes. The sizes of the circles that mark haplotypes are proportional to the numbers of individuals belonging to that haplotype, and the colours in the circles mark the regions (CSOs) and are described in the legend. The size of the circle sector in a certain colour is proportional to the number of individuals of the corresponding region belonging to that haplotype. Lines connecting the haplotypes mark the number of mutation steps leading to the next haplotype. The colours of the lines mark cpSSR markers in which the change of size (bp) in that mutation step occurred, leading to the next haplotype. The colours are also described in the legend. In case when in a certain cpSSR the change in size bigger then 1bp occurred between two haplotypes, a number above the line marks the difference in bp. Haplotypes belonging to lineages defined by UPGMA algorithm are framed by blue dotted lines for comparison, and marked by numbers 1-4. Figure 5

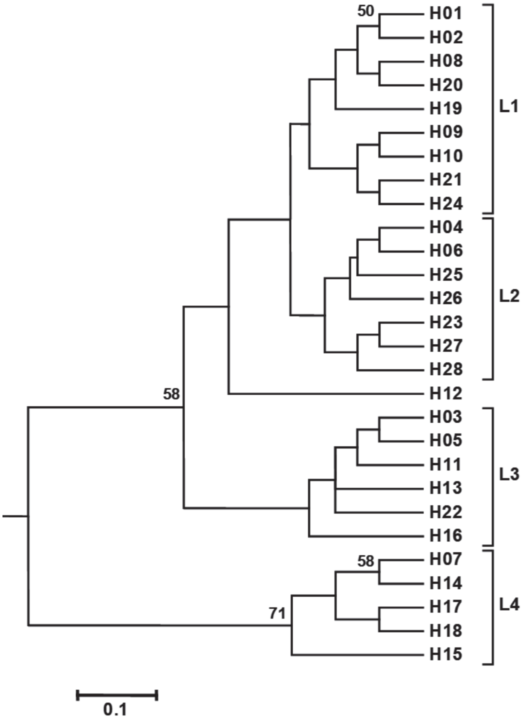

FIGURE 3. UPGMA dendrogram of cpSSR haplotypes (cpSSR1S) based on absolute distance (DAD). Boostrap support values of 1,000 replicates higher than 50% are given above the branches. Putative haplotype lineages (L1 to L4) are indicated on the right.

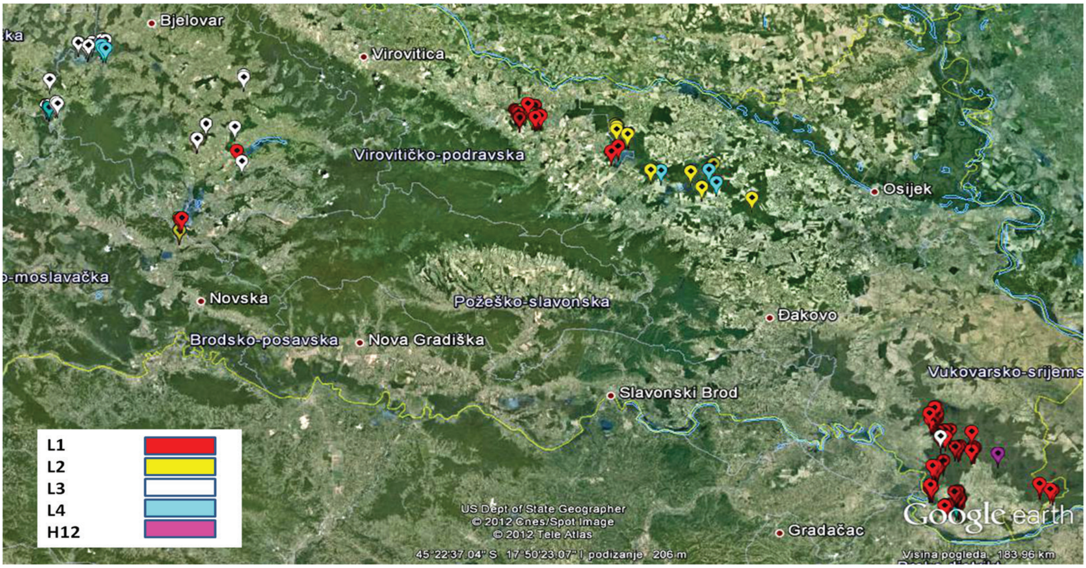

FIGURE 4. Spatial distribution of the selected plus trees in three seed regions, marked by their cpSSR haplotypic lineage. Left group – trees selected for CSO Plešćice (Seed Region Upper Posavina and Pokuplje), middle group - trees selected for CSO Kosovac (Seed Region Central Podravina), right group - trees selected for CSO Petkovac (Seed Region Lower Posavina).

also shows median vectors (mv 1-4), representing biological equivalents of possible non-sampled or extinct ancestral haplotypes, mostly found between groupings of sampled haplotypes.

b3) Genetic Diversity and Differentiation

Parameters of genetic diversity in the studied regions for cpSSRs are shown in Table 9. Greatest haplotype diversity presented as the number of haplotypes per the number of individuals (n_h/n) is observed in CSO Plešćice (0.432), followed by CSO Kosovac (0.270), while CSO Petkovac was most uniform with 0.180 haplotypes per individual. Similar situation is found in other parameters: CSO Plešćice has the greatest number of private haplotypes (n_{ph}), double then other CSOs, as well as the highest effective number of haplotypes (n_E), haplotype richness (n_{hr}) and unbiased estimate of haplotype diversity (H_E). Frequency of the most frequent haplotype was almost equal for CSOs Plešćice and Kosovac and somewhat higher for CSO Petkovac. In the metapopulation, the most frequent haplotype was H01, the overall n_h/n was 0.226 and n_E was 7.719.

Parameters of average within-region diversity (h_S, v_S), total diversity (h_T, v_T) and genetic differentiation (G_{ST}, N_{ST} and R_{ST}) are shown in Table 10, as well as their comparison, considering three different methods for calculating genetic differentiation:

TABLE 9. CpSSR variation in three *Quercus robur* populations.

Population	n	n_h	n_h/n	n_{ph}	n_E	n_{hr}	H_E	f_h
Plešćice	37	16	0.432	12	5.680	15.000	0.847	0.351
Kosovac	37	10	0.270	5	4.548	9.000	0.802	0.351
Petkovac	50	9	0.180	6	2.880	6.425	0.666	0.460
Metapopulation	124	28	0.226	-	7.719	-	0.878	0.258

n - number of individuals; n_h - number of haplotypes; n_h/n - number of haplotypes per number of individuals; n_{ph} - number of private haplotypes; n_E - effective number of haplotypes; n_{hr} - haplotype richness; H_E - unbiased haplotype diversity; f_h - frequency of the most common haplotype (H03 in Plešćice, H01 in Kosovac, H02 in Petkovac, H01 in the metapopulation)

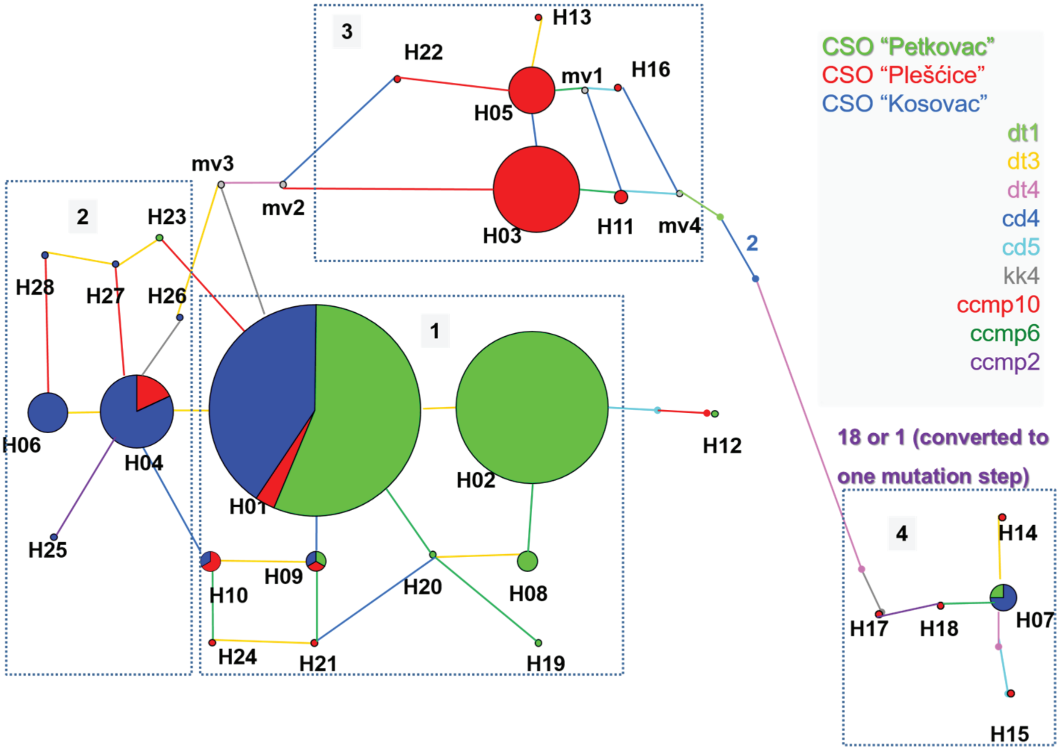

FIGURE 5. Median-joining haplotype network. H01-H28; haplotypes, mv1-mv4; median vectors.

(A) unordered alleles (taking into consideration only the frequencies of haplotypes) – G_{ST};
(B) ordered alleles (taking into consideration genetic similarities between haplotypes, i.e. the proportion of shared alleles) – N_{ST};
(C) differences based on Stepwise Mutation Model (SMM) (taking into consideration genetic distances in individual cpSSR loci) – R_{ST}.

There were no significant differences between methods (A) and (B) and (A) and (C) for the calculation of parameters of average within–region diversity (h_S and v_S) and total diversity (h_T and v_T). However, there are significant differences between N_{ST} and G_{ST}, as well as between R_{ST} and G_{ST}, which points to the existence of significant genetic differentiation between these regions, for cpSSR loci.

a2) Analysis of Molecular Variance (AMOVA)

The results of AMOVA based on cpSSRs are shown in Table 11. Two methods were used for variance components partition: (1) The number of different alleles (F_{ST}) and (2) the sum of squared size differences (R_{ST}).

In both versions, the between-region component of diversity is significant, confirming that the regions are differentiated regarding cpSSR loci. Values obtained by both methods are similar and highly significant.

AMOVA of Haplotypic Lineages, with nSSRs

The results of AMOVA for partition of nSSR diversity in components within and between UPGMA haplotypic lineages are shown in Table 12.

In the within–lineage component lies 99.29% of diversity. The between-lineage component is not statistically significant.

DISCUSSION

Analysis with Nuclear Microsatellites (nSSRs)

Genetic Diversity and Differentiation

Table 13 provides a comparison of H_O and H_E values of nSSR loci from this study with values from various studies of *Quercus robur* natural populations.

The values of genetic diversity parameters for most commonly used markers ssrQpZAG9 and ssrQpZAG36 are similar to those from other studies in Croatia [9] and all around Europe [15, 58-63]. Marker ssrQrZAG87 was also in concordance with other studies, with slightly bigger difference between H_O and H_E than in Lepais *et al.* [64], but lesser than in Neophytou *et al.* [58]. Marker ssrQpZAG110 was lower than in other studies a nd ssrQrZAG112 was higher, but in both markers in this study, H_O and H_E were

TABLE 10. Levels of diversity and differentiation based on (A) unordered alleles, (B) ordered alleles, and (C) stepwise SSR differences.

Parameter	(A) Unordered alleles	(B) Ordered alleles^a	(C) stepwise SSR differences ^{b}
Average within-population diversity	$h_S = 0.772$	$v_S = 0.641^{ns}$	$v_S = 0.633^{ns}$
Total diversity	$h_T = 0.945$	$v_T = 1.009^{ns}$	$v_T = 1.013^{ns}$
Genetic differentiation	$G_{ST} = 0.184$	$N_{ST} = 0.365^{*}$	$R_{ST} = 0.375^{*}$

^{a}Test of the hypothesis that v_S, v_T, and N_{ST} differ from h_S, h_T, and G_{ST}, respectively
^{b}Test of the hypothesis that v_S, v_T, and R_{ST} differ from h_S, h_T, and G_{ST}, respectively
^{ns} not significant (P>0.05), * significant at 0.01<P<0.05), ** significant at P<0.01, *** significant at P<0.001

TABLE 11. AMOVA analysis for the partitioning of cpSSR diversity (A) based on the number of different alleles (FST), and (B) based on squared size differences (RST).

Analysis	Source of variation	df	Variance components	%Total variation	ϕ_{ST}
(A) Based on the number of different alleles (F_{ST})	Among populations	2	0.575	37.11	0.371^{***}
	Within populations	121	0.974	62.89	
(B) Based on squared size differences (R_{ST})	Among populations	2	0.940	38.97	0.390^{***}
	Within populations	121	1.472	61.03	

^{ns} not significant (P>0.05), * significant at 0.01<P<0.05), ** significant at P<0.01, *** significant at P<0.001

TABLE 12. AMOVA analysis for the partitioning of nuclear SSR diversity within and among putative cpSSR haplotype lineages.

Classification	Source of variation	df	Variance components	%Total variation	ϕ_{ST}
UPGMA	Among lineages	3	0.002	0.71	0.007^{ns}
	Within lineages	117	0.337	00.29	

^{ns} not significant (P>0.05), * significant at 0.01<P<0.05), ** significant at P<0.01, *** significant at P<0.001

TABLE 13. Comparison of genetic diversity on nSSR loci between three CSOs from this study and natural populations of Quercus robur from other studies.

Locus		N (number of trees) / P (number of populations)	H_O	H_E
ssrQpZAG9	**3 CSOs Croatia**	**117 / 3 (regions)**	**0.88**	**0.87**
	Morić 2016 [8]	330 / 17		0.87
	Neophytou *et al.* 2010 [57]	48 / 1	0.92	0.85
		48 / 1	0.90	0.86
		48 / 1	0.78	0.82
	Muir and Schlötterer 2005 [58]	103 / 12	0.88	0.88
	Cottrell *et al.* 2003 [60]	387 / 1	0.86	0.87
		58 / 1	0.78	0.89
	Bakker *et al.* 2003 [61]	48 / 1	0.81	0.85
		48 / 1	0.88	0.84
	Buitveld *et al.* 2001 [62]	57 / 1	-	0.86
	Degen *et al.* 1999 [63]	210 / 1	0.88	-
		83 / 1	0.84	-
	Streiff *et al.* 1998 [14]	183 / 1	0.79	0.85
ssrQrZAG31	**3 CSOs Croatia**	**104 / 3 (regions)**	**0.26**	**0.92**
	Muir and Schlötterer 2005 [58]	89 / 12	0.43	0.95
ssrQpZAG36	**3 CSOs Croatia**	**121 / 3 (regions)**	**0.88**	**0.87**
	Muir and Schlötterer 2005 [58]	105 / 12	0.80	0.87
	Cottrell *et al.* 2003 [60]	387 / 1	0.85	0.89
		58 / 1	0.81	0.94
	Bakker *et al.* 2003 [61]	48 / 1	0.67	0.82
		48 / 1	0.92	0.86
	Buitveld *et al.* 2001 [62]	57 / 1	-	0.88
	Degen *et al.* 1999 [63]	213 / 1	0.88	-
		85 / 1	0.88	-
	Streiff *et al.* 1998 [14]	183 / 1	0.79	0.88
ssrQrZAG87	**3 CSOs Croatia**	**144 / 3 (regions)**	**0.76**	**0.87**
	Morić 2016 [8]	330 / 17	-	0.88
	Neophytou *et al.* 2010 [57]	48 / 1	0.43	0.75
		48 / 1	0.34	0.87
		48 / 1	0.50	0.84
	Lepais *et al.* 2006 [64]	30 / 1	0.81	0.85
	Muir and Schlötterer 2005 [58]	101 / 12	0.82	0.89
ssrQrZAG96	**3 CSOs Croatia**	**144 / 3 (regions)**	**0.25**	**0.29**
	Morić 2016 [8]	330 / 17	-	0.42
	Neophytou *et al.* 2010 [57]	48 / 1	0.35	0.33
		48 / 1	0.42	0.39
		48 / 1	0.27	0.27
	Lepais *et al.* 2006 [64]	30 / 1	0.37	0.37
	Muir and Schlötterer 2005 [58]	101 / 12	0.34	0.42
ssrQrZAG108	**3 CSOs Croatia**	**144 / 3 (regions)**	**0.48**	**0.70**
	Muir and Schlötterer 2005 [58]	96 / 12	0.40	0.81
ssrQpZAG110	**3 CSOs Croatia**	**129 / 3**	**0.59**	**0.60**
	Neophytou *et al.* 2010 [57]	48 / 1	0.75	0.74
		48 / 1	0.81	0.84
		48 / 1	0.81	0.74
	Lepais *et al.* 2006 [64]	30 / 1	0.71	0.66
	Muir and Schlötterer 2005 [58]	105 / 12	0.71	0.76
ssrQrZAG112	**3 CSOs Croatia**	**135 / 3 (regions)**	**0.87**	**0.88**
	Morić 2016 [8]	330 / 17	-	0.88
	Neophytou *et al.* 2010 [57]	48 / 1	0.77	0.83
		48 / 1	0.85	0.81
		48 / 1	0.94	0.84
	Lepais *et al.* 2006 [64]	30 / 1	0.74	0.66
	Muir and Schlötterer 2005 [58]	102 / 12	0.63	0.75

almost equal. Marker ssrQrZAG96 had low values for both heterozygosities and PIC (Table 2). This was especially pronounced in this study, although the values were quiet low in other studies as well. This locus also showed significant differentiation between regions in this study (Table 2). The specificity of this marker is that it differentiates between pedunculate and sessile oak based on H_O and H_E on the population level. These values are systematically low for pedunculate oak and high for sessile oak populations from the same area [59]. Marker ssrQrZAG112 shows similar patterns, but vice versa: high for pedunculate and low for sessile oak. In this study ssrQrZAG112 values were very high, but same as the average in other Croatian populations [9]. Since plus trees were all selected in lowland areas, in a typical pedunculate oak habitat, with not much possibility for hybridization with sessile oak, very low ssrQrZAG96 and very high ssrQrZAG112 values are not surprising.

Markers ssrQrZAG31 and ssrQrZAG108 show significantly lower H_O then H_E in this study. Similar result, although not so pronounced, was shown by ssrQrZAG31 in the study of twelve populations from Portugal, Spain, Ireland, Austria, France, Italy, Slovenia and Serbia [59]. In the same study, ssrQrZAG108 had even bigger differences between H_O and H_E then in this study. F_{ST} value for this locus also demonstrates significant differentiation between the regions, although in lesser extent then in a similar study of Bosnian populations [65]. In that research all the loci showed significant differentiation between populations (F_{ST}) and inbreeding coefficients (F_{IS}), which the authors explained by intensive anthropogenic influence and populations' fragmentation throughout history, but they also presumed that the pattern of diversity was partially influenced by different ecological conditions and remnants of historical migration processes. In our study, only two loci are significantly differentiated between regions, while F_{ST} values for other loci, as well as the overall $F_{ST,}$ are not significant (Table 2). Lack of differentiation usually points to effects of balancing evolutionary factors, like gene flow and balancing selection (similar selection pressures acting in all studied populations). It can also result from common descent, in absence of divergent evolutionary factors like random drift or divergent selection (adaptive differentiation due to different selection pressures acting in the studied populations).

According to Greogorius *et al.* [66], generally speaking it is expected that the effect of gene flow, as a balancing factor, is manifested in a greater number of loci, as compared to balancing selection, which usually affects only a few loci. The effect of common descent is also mainly visible in a smaller number of loci, because it is not likely for large parts of the genome to stay intacted through a great number of generations. In this study, regions were analysed with eight nSSR loci, most of which demonstrate lack of differentiation (Table 2). Regions were not significantly differentiated for allelic richness, nor for H_E and H_O. The AMOVA between-region component was very small and not significant (Table 6), Nei's distances between regions were almost equal, and F_{ST} values between pairs of regions were not significant (Table 5). Taking all that into consideration, we can conclude that between these regions gene flow is the prevailing balancing factor, especially because in this whole area, the distribution of pedunculate oak is continuous enough to provide a corridor for gene migration. This is confirmed by recent research of 17 Croatian natural oak populations, with 10 nSSRs [9], where also no differentiation between natural populations from this area was found. Studies of quantitative traits in open pollinated progeny trials from these CSOs show a similar pattern: no significant differentiation between regions [4-6, 8].

Inbreeding coefficients (F_{IS}) show significant deviations from HWE for two loci in CSO Plešćice, three loci for CSO Kosovac and one locus in CSO Petkovac (Table 3). It led to overall significant deviations from HWE in all three regions. F_{IS} values for AG31 [59] are very high for all three CSOs, corresponding to great differences between H_O and H_E in this, but also in other studies [59]. High F_{IS} values point to inbreeding only in case when they are significant in most loci, which was the case in Bosnian populations [65]. Otherwise, high F_{IS} at only certain loci can point to the selection for these loci, selection for genes linked to these loci or the occurrence of null alleles. We used the MICRO-CHECKER programme [67] to check for genotyping mistakes and it showed a possibility that high F_{IS} values for ssrQrZAG31, ssrQrZAG87 and ssrQrZAG108 were caused by null alleles, especially in ssrQrZAG31, where estimated values for null allele frequencies rise up to 35% in all three regions. Therefore, in this locus obviously null alleles were the main reason for significant F_{IS} values. Estimated frequencies for locus ssrQrZAG87 and ssrQrZAG108 are much smaller, but there is reason to suspect that null alleles influenced F_{IS} values for these loci as well.

Another possible reason for significant F_{IS} values only on individual loci is, as mentioned above, the selection on those loci or the genes to which the locus is linked. Locus ssrQrZAG87 is situated in linkage group LG2 of *Quercus robur* map, in one of the genome regions coding for bud burst. Considering its position, it might be possible that high F_{IS} value for this locus in CSO Kosovac comes from the uniformity of phenological forms in that CSO (almost all clones are early leafing [68-70]), as opposed to other two CSOs with a much wider range of leafing forms. However, in this study it is much more likely that null alleles caused those significant F_{IS} values. In other studies, significant F_{IS} values were also often accompanied by significant frequencies of null alleles [15, 58]. Additionally in this study, most F_{IS} values are positive, i.e. there is a slight, although for most loci not significant, homozygote surplus. Considering that for each CSO plus trees were selected on the level of seed region, i.e. a wider range that covers more populations (stands within forest districts and management units), joint analysis of all the trees from a CSO as one population (called region in this study) can lead to the apparent surplus of homozygotes, caused by subpopulation structure, or the so-called Wahlund effect. Overall genetic diversity for nSSR loci encompassed in these three CSOs is similar to the diversity of natural pedunculate oak populations of this area.

Spatial Structure of nSSR Genetic Diversity

Genetic diversity spatial patterns in plants are mostly determined by their reproduction system and life form.

Weakest geographical structure, assessed with biparently-inherited markers, is found in forest tree species, which are mostly outcrossing and have a long lifespan [71]. Considering that oaks belong to that group, studies with nSSRs in oaks have confirmed weak spatial structure of their diversity, usually confined to 30–50 m distances between trees [15, 61, 71-73]. Weak spatial structure in oaks is probably the result of two opposing forces (not considering selection, as an additional factor forming genetic diversity). First, limited natural seed-flow (by gravity and animals) results in groups of related individuals. On the other hand, strong pollen-flow has a reverse balancing effect and disrupts the creation of related groups, resulting from limited seed-flow. Computer simulations [73] confirmed this scenario. In another study [15], it is further confirmed that weak geographic structure in oaks also results from weak survival of inbred individuals, due to inbreeding depression [15, 60]. Considering all that, the results of spatial analysis are as expected, especially since one of the selection criteria was the distance of minimum 50 m between the selected trees. Nonetheless, since there is significant pollen-flow in pedunculate oak, and since the trees were not sampled randomly, but selected based on multiple shared favourable traits, we wanted to confirm that similar phenotypes of trees from closer distance classes are not the result of their relatedness. The results confirmed that pairs of individuals within any distance class are not significantly more related that pairs of randomly sampled individuals (Figure 2).

Chloroplast Microsatellites (cpSSR)

In angiosperms, chloroplast genomes are inherited maternally and represent a useful tool for analysis and differentiation of populations considering postglacial recolonization routes.

A comprehensive research of chloroplast diversity of main European oak species from 2613 populations was conducted with PCR-RFLP analysis. Thirty-two cpRFLP variants were found grouped in six main maternal lineages. Their distribution, along with some results of palynological studies, were used to reconstruct recolonization routes from refuges after the last glacial [17, 18].

Some Croatian populations were also included in the study by Bordács *et al.* [74], and analysed in detail by Slade *et al.* [75]. In the area of Croatia, Bosnia and Herzegovina, Montenegro, Albania and Kosovo they found seven cpRFLP haplotypes, four belonging to Balkan refuge lineage, two to Apennine, and one to Pyrenees or Southern Alpes lineage. In general, total diversity, as well as within-population chloroplast diversity of the Balkan area, is one of the highest in Europe [18].

Chloroplast markers can be of great use in tracking geographical origin of individuals and populations and therefore useful for certification of seed sources, control of wood and timber market, forensics etc., especially for species of great economic value like pedunculate oak [76, 77]. Since cpRFLP analysis is complicated, laborious, demands high quality DNA and sometimes has replicability issues, there was a need for a simpler method. The cpSSR markers were developed and comparatively tested with cpRFLP markers in the same populations [23, 55]. It has been found that the results are abutting, if not more precise with cpSSRs, with resolution increasing with added cpSSR loci.

Total genetic diversity (h_T) of these three regions, assessed with nine cpSSR markers in 124 individuals is very high (h_T=0.95), the same as in the analysis of overall Croatian natural populations [9] (340 individuals from 17 populations). The h_T of French populations with 10 cpSSRs was 0.65, but based only on 30 individuals, targeted to include certain cpRFLP haplotypes. In a study of Grivet *et al.* [54] on 367 individuals from 92 populations from France and the Iberian Peninsula, with six cpSSR markers, eleven haplotypes were found. If we had used only these six markers, we would have gotten 22 haplotypes for our three regions and 124 individuals. This points to great cpSSR diversity of our studied area. Since Croatia is situated on the crossroad of different recolonization routes [75], relatively close to some main refuges (Balkan and Apennine peninsula), it is not surprising to find great and well-preserved haplotypic diversity. The reason for differences between AMOVA partition of diversity between and within regions in this study (within-region diversity greater then between-region) and the study of overall Croatian populations [9] is in the sampling. We had many plus trees sampled in three rather close regions, compared to a smaller number of trees in more separate populations in that study, so it is expected that between population the component in Morić [9] will be higher. Nevertheless, both in this study and in Morić [9], differentiation is highly significant.

Considering a great number of analysed individuals per region and nine analysed cpSSRs, in this study resolution is high, so 28 haplotypes were grouped by UPGMA in four maternal lineages. Comparing our results with populations from study of Slade *et al.* [75], belonging to the same seed regions (population Zdenački Gaj for CSO Plešćice, Slatina and Đurđenovac for CSO Kosovac and Vrbanja and Gunja for CSO Petkovac) we presume that more closely related cpSSR lineages L1 and L2 belong to cpRFLP lineage 5, which is not clearly delineated and originates either from Balkan or Apennine refuge. In both studies (this study and study of Slade *et al.* [75]) most diverse region is the one were trees were selected for CSO Plešćice, were lineages from all three refuges come in contact. The region for CSO Kosovac was somewhat poorer in haplotypes, while CSO Petkovac region was the most uniform. Lineages L3 and L4 are strongly differentiated both from L1 and L2 and between each other, so it is quite possible that they originate from other refuges and belong to cpRFLP lineages 2 (Apennine refuge) or 7 (Pyrenees or Southern Alpes refuge). Lineage L3 is mostly found in northwestern part of Croatia (CSO Plešćice) where, according to Slade *et al.* [75] both cpRFLP 2 and cpRFLP 7 lineage is found, with 7 being a bit more frequent.

In spite of the correction made in this study for the long allele (see Material and Methods), haplotypes of L4 lineage were strongly grouped together, pointing to their different origin from L1 and L2 lineages, as well as L3.

The cpSSR haplotype distribution can be formed not only by recolonization routes, but also by reproductive material transfer. For example, in the CSO Plešćice region in management unit "Međuvođe – Ilovski lug", we found haplotypes of L1 and L2 lineages, common in a more eastern forest complex. Also in forest district "Slatina" (CSO Kosovac) almost all selected trees belong to L1, although this forest district is situated further west from the dominant complex of this lineage. Moreover, in the same region to the east, which is closer to the L1 dominant complex, L2 individuals are prevailing. While it is possible that such distribution is caused by recolonization route admixture or single long-distance dispersal events [78], we cannot exclude the possibility of seed transfer from eastern to further western regions, especially because L1 individuals are mostly found within a complex known for pedunculate oak stem quality, for example in Spačva Basin. Reproductive material from that area was exported to other states for its quality. For example, Slavonian pedunculate oak was intensively planted in Germany at the end of the 19th century and entire stands of that origin are still present in that area, recognizable by stem quality and late flushing. Therefore, it is quite possible that reproductive material from that area was transferred also within Croatia. Based on cpRLFP analysis across Europe, anthropogenic influence on the distribution of genetic diversity of pedunculate oak is stronger than on any other oak species because of its economic value [17, 18].

AMOVA of Haplotypic Lineages, with nSSRs

In an extensive overview of studies of different genomes [79], the authors compared diversities of chloroplast, mitochondrial and nuclear markers in different plant species and found that in most cases differentiation found with cytoplasmic markers does not correspond to the one found with nuclear markers. Also, in oak species, gene flow by pollen is approximately 200 times greater than by seed. This difference is manifested in weak differentiation of populations by nuclear, biparentally inherited markers, as compared to maternally inherited cytoplasmic markers. It is presumed that primarily nuclear diversity corresponded to cytoplasmic markers, but was later erased by strong gene flow by pollen and rearranged by selection pressures.

According to Kremer *et al.* [19], parallel analysis with nuclear and cytoplasmic markers eventually shows weak concordance when diversity of nuclear markers is not strongly influenced by selection. With this in mind, we wanted to compare cpSSR diversity with presumably neutral nSSR loci. The assessment of genetic differentiation is hugely influenced by the choice of markers and by sampling [79]. In our study, considering the small number of sampled regions and many sampled trees per region, differentiation for nSSR was virtually non-existing. As mentioned before, there is a corridor for gene flow between these regions, which obviously led to balancing genetic diversity of nSSRs in the entire studied area. Considering weaker possibilities of seed transfer between regions compared to pollen, there was significant differentiation for cpSSRs. AMOVA for partitioning nSSR diversity between UPGMA chloroplast lineages showed that only about 1% is explained by between-lineage components and it was not significant. Obviously, strong gene flow by pollen erased recolonization patterns of nuclear diversity.

CONCLUSIONS

Molecular analysis with nuclear and chloroplast microsatellite markers of plus trees selected in three regions (upper Posavina, lower Posavina and Pokuplje, and central Podravina) within two seed zones (first two regions – seed zone Posavina, central Croatia and Pokuplje, third region – seed zone Podravina and Podunavlje) and included in three clonal seed orchards of pedunculate oak led to the following findings:

1. Analysis with eight nSSRs shows homogeneity of presumably neutral genetic diversity in the entire studied area, with no genetic differentiation between regions. The regions are not differentiated by genetic parameters, and AMOVA between–region component was very small and not significant. The results point to the existence of strong gene flow between regions, as prevailing balancing factor in this area.
2. Some nSSR loci (AG31, ssrQrZAG108 and AG87) displayed deviations from Hardy-Weinberg equilibrium in some or all CSOs. The most probable cause of increased homozygosity in these loci are null-alleles. Additional reason for positive F_{IS} values for these and other loci is probably the sampling method. The analysis was conducted on the level of seed regions, which are themselves composed of more populations, which probably resulted in substructure of regions, i.e. Wahlund effect.
3. Spatial autocorrelation analysis, with nSSRs and Gauss-Krüger coordinates of original plus trees showed that for no class of mutual distance between the selected plus trees within regions pairs of trees were more related then randomly selected pairs of trees. This points to good sampling strategy and successful avoidance of selecting related genotypes.
4. Molecular analysis with nine polymorphic cpSSRs resulted in 28 haplotypes, belonging to four maternal lineages, by UPGMA algorithm. Lineages L1 and L2 are more related and lineages L3 and L4 are each grouped separately, with greater support. With these markers, significant differentiation was found between regions. Greatest haplotypic diversity was found in CSO Plešćice, smaller in CSO Kosovac and the smallest in CSO Petkovac. Probable cause is the distribution and admixture of recolonization lines from different refuges, which is more pronounced in western parts of Croatia, and possible reproductive material transfer. Analysis with these markers provides good basis for tracking the origins of reproductive material.

These findings lead to the following conclusions and recommendations.

The results of this study with nSSRs show no differentiation between regions in this area and are in concordance with previous studies on quantitative traits [3-9] and a recent study of overall Croatian populations with nSSRs [9]. CpSSR analysis showed geographic structure of haplotypes, but it is connected to recolonization routes and according to another research [19] has no connection with adaptively important traits. Moreover, in this research, smaller differentiation for cpSSRs was observed between the regions belonging to other seed zones (CSO Kosovac and CSO Petkovac) than between those belonging to the same zone (CSO Plešćice and CSO Petkovac). Therefore, the change implemented in current Croatian bylaw on forest tree species provenances (NN 147/11, 96/12, 115/14 and 114/15), by which the transfer of forest reproductive material of pedunculate oak is permitted within the entire seed area, and not just within the seed zone, is justified. This is especially true in the light of climate change, when needs for changes in policy of forest reproductive material transfer are discussed on much larger scales, on the level of whole Europe [80].

We conclude that genetic diversity levels encompassed in these CSOs are comparable to those in natural populations [9] and provide a good basis for the production of quality and genetically diverse progeny, if other conditions for optimal reproduction are achieved. For now, the clones in these CSOs still have not reached their full size and the clones are not equally contributing to the crops [25]. Genotyping with nSSRs also provides the foundation for molecular analysis of the progeny, which would provide a very precise estimation of genetic diversity produced in the CSOs in the given year, and level of contamination with pollen from outside sources. The results from such analysis could then be compared to simpler and much cheaper estimations, based on flowering and fruiting, to find the most appropriate method for assessing genetic diversity of crops. Along with flowering and fruiting observations, it is necessary to regularly observe climatic and other environmental conditions in the CSOs, so that the observed trends could be correlated to various factors and possibly explained. The clones in some of these orchards are grouped by phenology, which creates substructures and prevents random reproduction [69, 70]. According to our results, phenologically compatible clones from all three orchards could be combined in a separate orchard, in order to achieve maximum synchrony and enhance genetic diversity of the crops.

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Correlation of Fruit Size with Morphophysiological Properties and Germination Rate of the Seeds of Service Tree (*Sorbus domestica* L.)

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ABSTRACT

Background and Purpose: The current study aims to evaluate the effect of the fruit size of service tree (*Sorbus domestica* L.) on physio-morphological properties of seeds and the seed germination process.

Materials and Methods: The fruit samples varying in size and divided on the basis of weight into small (5-10 g), medium (11-15 g) and large (16-20 g) were collected from the area of Vukomeričke gorice (45°34′45″N 16°00′11″E), Zagreb County, Croatia.

Results: In all fruits, a significant, positive and very high correlation was found between the fruit length and width (r=0.92). There was a negative and significant correlation between the fruit weight and the fruit shape index (r=-0.47). The number of filled seeds in fruit was significantly higher in large fruits (2.62 seeds) than in medium and small fruits (1.81 and 1.46, respectively). Seeds from large fruits had significantly greater length of radicle (3.89 mm) than those from medium and small fruits (3.27 and 2.88, respectively) after 120 days of stratification at 3°C. The mean germination time of seeds for all fruits in total was 107 days. The seeds with the highest weight had significantly lower rate of moisture absorption. After stratification the seeds of large fruits had the highest germination percentage (83.7%).

Conclusions: The findings obtained will likely impact the propagation of this plant species in the future, because the results of the study favour the usage of seeds obtained from large fruits since they showed highest germination rate.

Keywords: Fruit morphology, radicle emergence, seed germination, stratification, *Sorbus domestica* L.

INTRODUCTION

Service tree (*Sorbus domestica* L.) is a rare wild tree with deciduous nature, 15-20 (rarely 30) m tall with broad, roundish ovoid crown that has the diameter of up to 100 cm (Figure 1). Service tree grows slowly (except in the juvenile period), reaches the age of 200-300 years and prefers warm and mild climate. It does not require any specific soil properties, but it grows well on deep and fertile soils. It can withstand drought, but is sensitive to late spring frosts [1]. In a forest ecosystem, the ecological significance of this tree is great due to the forage (fruits) which it offers to wildlife species. Furthermore, its timber is valuable due to its excellent aesthetic and technical characteristics [2].

It is hard to determine the exact boundaries of natural expansion of service tree because it has been planted and expanded sub-spontaneously since ancient times. It is distributed either patchy or densely in southern and central Europe, northern Africa, Crimea and Asia Minor. However, the Balkan Peninsula, the Apennine Peninsula and southern France are considered as the main area of its cultivation. Nevertheless, *S. domestica* is very rare and threatened in

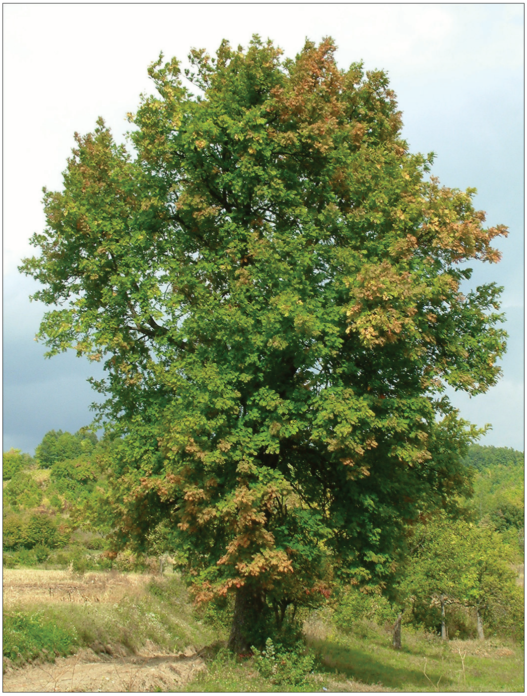

FIGURE 1. Service tree (*S. domestica* L.) in Croatian agro-climatic conditions.

many European countries [3]. Croatia is one of the countries where the environmental conditions favor the cultivation of service tree. This species is extensively found in the Mediterranean area, as well as in the continental part of Croatia [4].

Service tree fruit is edible, apple- or pear-shaped, with a diameter of about 1.5 to 3 cm, yellow-reddish or yellowish with brown spots. In the wild, service tree propagates itself by seed, especially within its natural range [5]. It flowers in March while its fruit ripens from August to September [6]. According to Drvodelić *et al.* [7], the good yield of service tree in Croatia happens in intervals of every four years. Whereas, according to Kausch-Blecken von Schmeling [8], service tree yields highly every two to three years. The reason behind such differences can be justified with a fact that when service tree yields highly it spends a lot of energy for the growth of fruits and seeds and therefore it is not strange that there are big intervals between the years of high yields. Service tree produces more seeds when it is cross-pollinated. Allogamy is possible if the distance between the trees is not more than 800 m [9].

Fresh seeds from the species of genus *Sorbus* L. do not germinate easily and need low temperatures under moist conditions (stratification) [10]. Numerous studies mentioned that fresh *Sorbus* spp. seeds do not germinate immediately after maturation because they need longer period with cold stratification [11-14]. Moreover, as per rules of International Seed Testing Association (ISTA) [15], before the testing of seed germination it is necessary to keep the seeds in cold stratification for 4 months at a temperature ranging from 3 to 5°C. However, Winkler [16] found that the seeds of service tree and wild service tree need stratification conditions, that they germinate well in darkness and should be mixed with appropriate substrates, as well as kept in refrigerator at a temperature from 0°C to 4°C. Temperatures lower than 0°C are not favorable because they freeze down the water that further stops seeds to swell. Moreover, the seeds should be stratified at the beginning of December. Kausch-Blecken von Schmeling [8] found that it is very important to rinse the mesocarp of fruits properly to avoid the occurrence of mold during the storage of seeds. Likewise, Meier-Dinkel [9] suggested that two and a half months before sowing, the seeds should be placed on moist sand in a covered jar and kept in the refrigerator at 4°C where some seeds can germinate at the end of stratification. After stratification, the seeds should be kept on a warm place (20°C) where after a few days they start to germinate. However, Pipinis *et al.* [17] suggested a 3-month period of stratification at 3-5°C since this is essential for breaking dormancy in the seeds of *S. domestica*. Service trees have double dormant seeds due to undeveloped seed embryo and an impermeable seed coat [6].

Surprisingly, very little work has been done so far regarding the morphological studies of service tree fruit and the impact of the fruit size on the physiological properties of seeds. Henceforth, the current study was conducted to determine the morphological characteristics of service tree fruits of different weight and their correlations with physical properties of seeds. In addition, the aim was also to test the laboratory seed germination of fruits of various weight after 120 days of stratification.

MATERIALS AND METHODS

The fruits of service tree were collected in the area of Vukomeričke gorice (45°34′45″N 16°00′11″E), Zagreb County, Croatia, in autumn when fruit ripens. A total of 150 fruits were collected from 10 trees for the analysis and were grouped on the basis of weight as small (5-10 g), medium (11-15 g) and large (16-20 g). The length (mm) and width (mm) of fruits were measured using digital caliper, while fruit weight was measured with laboratory balance (accuracy 0.01 g). The seeds were manually extracted from fruits with the help of a little knife and then the number of filled seeds in every fruit was recorded. After the seeds were air-dried, the weight of each seed (309 seeds from 150 fruits) was measured. The seeds were then stratified. The seeds were moistened and placed on an absorbing filter paper before they were used for stratification. Every seed was coded for recognition of its origin and placed on moistened filter paper covered with another layer of moistened filter paper to avoid draining. Stratification was carried out at a temperature of 3°C and lasted in total for 120 days in accordance with rules of ISTA for overcoming seed dormancy from species of the genus *Sorbus*. The moistening of the filter paper was continued as per need during stratification time. After 120 days, at the end of stratification period, the weight of each seed was measured to calculate the percentage of moisture absorption. The daily monitoring of seeds was performed in order to record the time of seed shell bursting and radicle emergence (Figure 2) up to the length of 2 mm during entire stratification period. The increase in length after radicle cracking was measured on a daily basis using digital caliper. The radicle length after 120 days of stratification is described in detail in the current study.

The statistical analysis was performed with statistical package SAS 9.2 using ANOVA and correlation analysis. The means were compared using LSD test at p≤0.05.

RESULTS AND DISCUSSION

The descriptive statistical data of some morphological characteristics of service tree fruits with different weight is shown in Table 1. Statistically significant differences were determined in length (mm) and width (mm) of the fruits between groups defined by weight (p≤0.0001). Small fruits showed statistically significant difference (p≤0.0001) having the highest fruit shape index (0.98) in comparison with medium (0.94) and large (0.91) fruits. Therefore, it can be concluded that small fruits had a more round shape. A number of filled seeds in *S. domestica* L. fruits were statistically significant (p≤0.0001) and higher in large fruits (2.62 seeds) in comparison with medium (1.81 seeds) and small (1.46 seeds) fruits (Figure 3).

FIGURE 2. Germination of service tree (*S. domestica* L.) seeds.

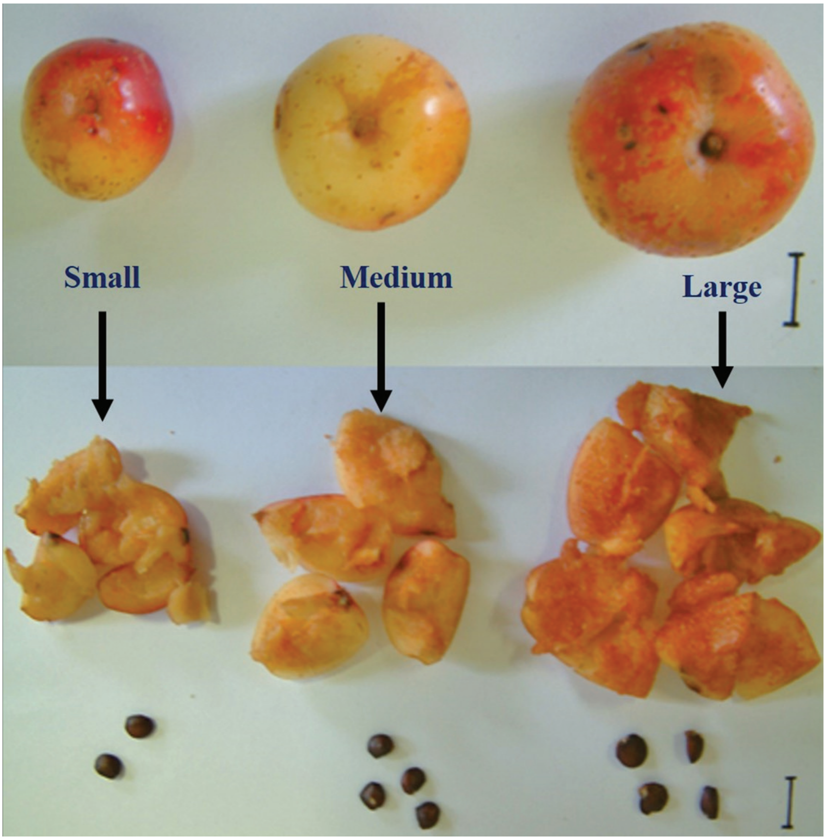

FIGURE 3. Different sizes of fruits of service tree (*S. domestica* L.) showing the variations in the number of obtained seeds.

TABLE 1. Descriptive statistics of some morphological traits of service tree (*S. domestica* L.) fruits of different sizes.

Fruit size	**Fruit length** (mm)	**Fruit width** (mm)	**Fruit shape index** (FL/FW)	**Fruit weight** (g)	**The number of filled seeds per fruit**
Small (5-10 g)	23.19±1.90 a	23.81±1.86 a	0.98±0.05 a	7.67±1.61 a	1.46±0.65 a
Medium (11-15 g)	27.45±1.38 b	29.31±0.81 b	0.94±0.05 b	13.52±0.88 b	1.81±0.90 a
Large (16-20 g)	29.93±1.65 c	32.67±1.71 c	0.91±0.05 b	18.83±2.81 c	2.62±1.18 b

Means followed by different letters in columns indicate that there is statistically significant difference based (LSD test at p≤0.05)

Regarding all fruits in total, a positive and very high correlation was found between fruit length and width (r=0.92), fruit length and weight (r=0.93) and fruit width and weight (r=0.98). Statistically significant (p≤0.0001) correlations were found between the number of filled seeds per fruit and fruit length (r=0.42), fruit width (r=0.50), fruit shape index (r=-0.36) and fruit weight (r=0.50). There was a negative and statistically significant correlation between fruit weight and fruit shape index (r=-0.47) (Table 2).

Within small fruits, a positive and very high correlation was obtained between fruit length and width (r=0.80), fruit length and weight (r=0.87) and fruit width and weight (r=0.96). Statistically significant (p≤0.05) negative correlation was found between the fruit shape index and the number of filled seeds in fruits (r=-0.29) (Table 3).

Within medium fruits, a positive and high correlation was obtained between fruit width and weight (r=0.75) with statistically significant difference at p≤0.001. Statistically significant (p≤0.01) correlation was also found between fruit length and weight (r=0.48) and between fruit width and the number of filled seeds per fruit (p≤0.05, r=0.41) (Table 3).

In the case of large fruits, a positive and very high correlation was obtained between fruit width and weight (r=0.94). Statistically significant (p≤0.001) difference was found between fruit length and width (r=0.53) and between fruit length and weight (r=0.68). Less positive, but statistically significant (p≤0.05) correlation was obtained between the number of filled seeds in fruit and fruit width (r=0.30) and fruit weight (r=0.23) (Table 3).

TABLE 2. Correlation coefficients for the morphological traits of service tree (*S. domestica* L.) fruits (all sizes combined together).

Variables	**Fruit length** (mm)	**Fruit width** (mm)	**Fruit shape index** (FL/FW)	**Fruit weight** (g)
Fruit width (mm)	0.92****			
Fruit weight (g)	0.93****	0.98****	-0.47****	
The number of filled seeds per fruit	0.42****	0.50****	-0.36****	0.50****

**** - correlation significant at p≤0.000

TABLE 3. Correlation coefficients for some morphological traits evaluated on service tree (*Sorbus domestica* L.) fruits of diferent sizes.

Variables	**Fruit length** (mm)	**Fruit width** (mm)	**Fruit weight** (g)	**Fruit shape index** (FL/FW)
Small fruit				
Fruit width (mm)	0.80***	NS	NS	NS
Fruit weight (g)	0.87***	0.96***	NS	NS
The number of filled seeds per fruit	NS	NS	NS	-0.29*
Medium fruit				
Fruit weights (g)	0.48**	0.75***	NS	NS
The number of filled seeds per fruit	NS	0.41*	NS	NS
Large fruit				
Fruit width (mm)	0.53***	NS	NS	NS
Fruit weight (g)	0.68***	0.94***	NS	NS
The number of filled seeds per fruit	NS	0.30*	0.23*	NS

NS, *,**, *** - correlation nonsignificant or significant at p≤0.05, p≤0.01 and p≤0.001, respectively

There are several reports available worldwide mentioning the fruit morphological characteristics of service tree but with little variations. Ballian *et al*. [18] stated that the service tree fruit length was between 17.5 and 35.1 mm, and its width between 15.5 and 24.9 mm. Whereas, according to Miko and Gažo [19], the average fruit width was between 21.4 and 27.2 mm which varied between 17 and 39 mm, and the average length between 23.1 and 32.3 mm which varied between 20 and 37 mm. Furthermore, the fruit weight was between 7.4 and 21.0 g which varied between 4.3 and 22.3 g. Similarly, Májovský [20] when mentioning the botanic description of service tree reported that fruit width was between 21.4 and 27.7 mm, fruit length between 15.0 and 30.0 mm and fruit weight between 20 to 30 g. However, according to Brindza *et al*. [21], average fruit length was between 19.84 and 36.29 mm and width between 18.9 and 32.58 mm. while the average weight was from 4.91 to 18.64 g. The reasons behind such variations might be, as Kárpáti [22] found in his studies, due to the fact that service tree fruits with diameter bigger than 1.5 cm are the result of selection. It is interesting to mention a research conducted by Végvári [23] in Hungary who reported that service tree fruits that have different color (yellow, red, brown) also differ in shape. Whereas, according to the findings of Drvodelić *et al*. [24], who conducted extensive studies on service tree in different parts of Croatia, the average length of fruit was between 22.05 mm (Ogulin) and 25.66 mm (Novi Vinodolski), average width between 24.55 mm (Ogulin) and 27.30 mm (Novi Vinodolski), and average weight of fruit from 8.75 g (Ogulin) to 11.23 g (Novi Vinodolski). In addition, the fruit weight in 2003 was between 3.42 and 17.24 g or on average 7.92 g, in 2004 between 4.65 and 12.20 g or on average 8.37 g, in 2005 between 7.14 and 11.36 g or on average 9.29 g, in 2006 between 6.69 and 10.49 g or on average 8.72 g, and in 2007 between 8.35 and 12.98 g or on average 10.01 g. He further stated that in comparison with the sites from which the samples were taken in Republic of Croatia during five years of research (2003-2007), the average fruit weight of service tree increased from western to eastern parts of the country.

As per findings of Ballian *et al*. [18], the differences between trees are mostly contributed by fruit length of service tree and the genotypes that have large fruits and thus should be used for propagation. According to Drvodelić [7], the service tree fruit's width and length features are more similar between geographically closer sites, such as Nova Kapela and Ogulin, or Rab and Novi Vinodolski in Croatia. He found that the average index of length/width of fruits for all five years he studied was 0.92 for Novi Vinodolski site, 0.97 for Ogulin site, 0.99 for Rab site and 1.03 for Nova Kapela site. He also found that the highest range of length/width index was achieved in year 2006 (0.79-1.61), while in other years that range was more or less equal.

Our findings are more or less similar in comparison to the above-mentioned studies, since the average length of small fruits was 23.19 mm, of medium 27.45 mm and of large 29.93 mm. Similarly, the average width of small fruits was 23.81 mm, of medium it was 29.31 mm and of large 32.67 mm. Moreover, the fruit shape index varied from 0.91 to 0.98, which coincides with the previous studies conducted by Drvodelić *et al*. [24] and Drvodelić [7]. The findings obtained in our study regarding the average fruit weight between 7.67 and 18.83 g are different than the results by Miko and Gažo [19] who reported higher fruit weight of service tree in Slovakia than in Croatia. Similarly, the fruit weight in the study reported by Májovský [20] is much higher than in our research. In addition, Drvodelić *et al*. [24] reported positive and high correlation between fruit length and width (r=0.73), fruit length and weight (r=0.87) and fruit width and weight (r=0.93) that corresponds with the results obtained in the current study.

Regarding the number of seeds in fruits of service tree in different sites of Croatia, Oršanić *et al*. [25] reported that in 2003 it was from 1.35 (Rab) to 2.70 seeds (Nova Kapela) and on average 1.94 seeds. In 2004 the number of filled seeds in fruit were between 1.45 (Novi Vinodolski) and 2.26 seeds (Nova Kapela) and on average 1.74 seeds. On the contrary, Drvodelić *et al*. [24] reported that the average number of filled seeds in fruits of service tree in different parts of Croatia was from 1.80 (Ogulin) to 3.53 (Novi Vinodolski). In addition, Drvodelić [7] reported that the number of filled seeds in service tree fruit (2003-2007) increases from western to eastern parts of Croatia.

Regarding the findings obtained in the current study, the number of filled seeds in fruits ranged from 1.46 for small fruits to 2.62 for large fruits. On average, there were 1.96 seeds in fruit, which is in accordance with research conducted by Oršanić *et al.* [25].

Frehner and Fürst [26] reported that the average number of service tree seeds in 1 kg varied between 40,000 and 55,000 seeds. In our case, the average number of air-dried seeds in 1 kg ranged between the examined groups from 25,126 seeds to 35,336 seeds, which is lower than what was reported by Frehner and Fürst [26].

Statistically significant difference between large fruits and between medium and small fruits ($p \leq 0.001$) was obtained in terms of length of seed radicle after 120 days of stratification. Seeds from large fruits had significantly higher length of radicle (3.89 mm) than the seeds from small (2.88 mm) and medium (3.27 mm) fruits. Considering the percentage of moisture absorption after 120 days of stratification, no significant difference was found between seeds from the examined groups ($p=0.2896$). The weight of one air-dried seed significantly differed between the examined groups ($p \leq 0.001$). It was noticed that seed weight increased with fruit weight. No statistically significant difference was found in mean germination time (MGT) between the examined groups, even though the seeds from large fruits germinated four days earlier than the seeds from medium fruits, and three days earlier than the seeds from small fruits. Mean germination time for seeds regarding all fruits in total was in average 107 days (Table 4).

For seeds from all fruits negative significant correlation ($r=-0.66$) was obtained between length of radicle after 120 days of stratification and the time needed for the beginning of germination. Other correlations were also statistically significant ($p \leq 0.001$). Negative and weak correlation was found between the percentage of moisture absorption after 120 days of stratification and the time needed for the beginning of germination ($r=-0.40$) and seed weight in air-dried condition ($r=-0.22$). It can be concluded that by increasing seed weight in air-dried condition, the percentage of moisture absorption was statistically and significantly decreasing. The correlation

TABLE 4. Descriptive statistics of some morphological and physiological traits of seeds from service tree (*S. domestica* L.) fruits of different sizes.

Fruit size	**Length of radicle after 120 days** (mm)	**Absorbed moisture after 120 days** (%)	**Air-dried seed weight** (g)	**Time to begin germination** (days)
Small (5-10 g)	2.88±0.81 a	43.01±7.99 a	0.0283±0.0078 a	107.66±13.31 a
Medium (11-15 g)	3.27±0.85 a	46.03±28.07 a	0.0365±0.0092 b	108.72±9.79 a
Large (16-20 g)	3.89±1.48 b	42.64±6.84 a	0.0398±0.0090 c	104.85±10.73 a

Means followed by different letters in columns indicate that there is statistically significant difference (LSD test at p≤0.05)

between radicle length after 120 days of stratification and seed weight in air-dried condition was positive and small (r=0.26) (Table 5).

In the case of seeds from small fruits a positive and statistically significant (p≤0.05) correlation was obtained between radicle length after 120 days of stratification and the percentage of moisture absorption (r=0.46). Negative and significant correlation (r=-0.48) was obtained between radicle length after 120 days of stratification and the time needed for the beginning of seed germination, as well as between the percentage of moisture absorption and seed weight in air-dried condition (r=-0.32). Negative and weak (r=-0.39) but statistically significant (p≤0.01) correlation was obtained also between the percentage of moisture absorption and the time needed for the beginning of seed germination (Table 6).

For seeds from medium and large fruits a negative and significant (r=-0.49) correlation was obtained between the length of radicle after 120 days of stratification and the time needed for seed germination, as well as between the percentage of moisture absorption after 120 days of stratification and the time needed for the beginning of seed germination (r=-0.33). The difference between the above-mentioned parameters was significant at p≤0.01. Although negative and small, but statistically significant (p≤0.001), the correlation was obtained between the percentage of moisture absorption after 120 days of stratification and seed weight in air-dried condition (r=-0.32) (Table 6).

For seeds from large fruits, negative and high correlation was obtained between radicle length after 120 days of stratification and the time needed for seed germination

TABLE 5. Correlation coefficients for the morphological and physiological traits of seeds from service tree (*Sorbus domestica* L.) fruits (all sizes combined together).

Variables	**Length of radicle after 120 days** (mm)	**Absorbed moisture after 120 days** (%)
Time for germination (days)	-0.66***	-0.40***
Air-dried seed weight (g)	0.26***	-0.22***

*** - correlation significant at p ≤ 0.001, respectively

TABLE 6. Correlation coefficients for some morphological and physiological traits of seeds from service tree (*S. domestica* L.) fruits of different sizes.

Variables	**Length of radicle after 120 days** (mm)	**Absorbed moisture after 120 days** (%)
	Small fruit	
Absorbed moisture after 120 days (%)	0.46*	-
Time for germination (days)	-0.48*	-0.39**
Air-dried seed weight (g)	NS	-0.32*
	Medium fruit	
Time for germination (days)	-0.49**	-0.33**
Air-dried seed weight	NS	-0.32***
	Large fruit	
Absorbed moisture after 120 days (%)	0.29**	-
Time for germination (days)	-0.79***	-0.45***
Air-dried seed weight (g)	NS	-0.26**

NS, *,**, *** - correlation nonsignificant or significant at p≤0.05, p≤0.01 and p≤0.001, respectively (r=-0.79). Correlation between the percentage of moisture absorption and the time needed for seed germination was negative and significant (r=-0.45). The difference between the above-mentioned parameters was significant at $p \leq 0.001$. There is positive and small, but statistically significant ($p \leq 0.01$) correlation between radicle length after 120 days of stratification and the percentage of moisture absorption after 120 days of stratification (r=0.29), and negative and small correlation between the percentage of moisture absorption after 120 days of stratification and seed weight in air-dried condition (r=-0.26) (Table 6).

After 120 days of stratification the seeds from the group of large fruits (16-20 g) had the highest percentage of germination (83.7%). With the help of Chi-squared test statistically significant difference ($p \leq 0.001$) was found between the examined groups in germination of seeds from fruits with different weight, wherein seed germination in the group of large fruits was significantly higher than seed germination in groups of medium (59.4%) and small (56.2%) fruits. It can be concluded that seed germination of service tree in controlled conditions increased from the group of small to the group of large fruits (Figure 4).

According to Lenartowicz [27], the seeds from the species of the genus *Sorbus* L. have problems with germination, which could indicate relatively high dormancy of embryo. It is one of the mechanisms by which embryos created by self-pollination and by inbreeding could be eliminated. According to Paganová [28], service tree seeds' low temperature and high humidity stratification needs to be longer than 8 weeks to germinate (depending on year). Piagnani and Bassi [29] reported that for overcoming seed dormancy from the species of the genius *Sorbus* L. cold and moist stratification is needed in the period of 3 to 9 months. On the other hand, Drvodelić [7] found that only 105 days of stratification (15 days less than as suggested by ISTA [15]) was needed for overcoming double seed dormancy from four species of the genus *Sorbus* L. in Croatia. It can completely coincide with the results from the current study where mean germination time regarding all fruits in total was 107 days. Powell and Matthews [30] reported the methodology according to which the appearance of radicle was defined as RE (radicle emergence) and mean germination time (MGT). As per findings of Drvodelić [7], the average service tree seed germination rate at the end of stratification period in 2003 was 9.3%, in 2004 15.4%, in 2005 31.2%, in 2006 53.7% and in 2007 only 3.8%. Average service tree seed germination at the end of stratification period (120 days) for all five years of research (2003-2007) was 22.5%. He also reported that it is important to mention that in the years with the highest yield (2003 and 2007) the highest seed germination rate was not achieved at the end of stratification time. That means that the quality of yield does not have effect on the time needed for overcoming seed dormancy in this species.

In our study we obtained much higher seed germination rate after 120 days of stratification, wherein seed from large fruits had germination percentage of even 83.7%.

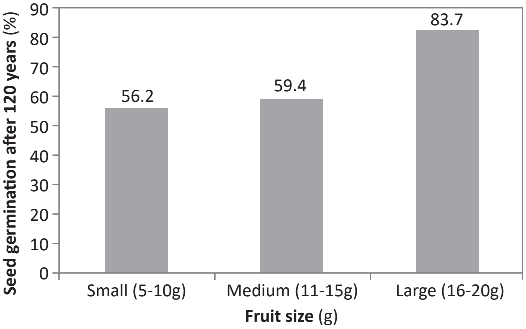

FIGURE 4. Seed germination (%) of service tree (*S. domestica* L.) fruits of different sizes after 120 days stratification (statistically significant difference was found among the examined groups in the germination of seeds from fruits of different size using chi-squared test ($p \leq 0.001$)).

CONCLUSIONS

The findings obtained in this study will likely impact the propagation of service tree in the future. The obtained results suggest using the seeds obtained from large fruits because they had higher number of filled seeds per fruit and showed highest germination rate, as well as earlier germination in comparison to the seeds from medium and small fruits. After 120 days of stratification, the seeds from large fruits (16-20 g) had the highest percentage of germination (83.7%). Although no statistically significant difference was found in mean germination time (MGT) between the examined groups defined by weight, the seeds from larger fruits have germinated four days earlier than seeds from medium fruits and three days earlier than seeds from small fruits. This means that only large fruits should be used for propagation of service tree.

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Scotch Pine Regeneration in Magnesite Pollution Conditions in South Ural, Russia

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ABSTRACT

Background and Purpose: Technogenic load is considered to be one of the major factors which destabilize forest ecosystems. The impact of chronic aero technogenic pollution near large industrial centers severely affects the plants in these areas; thus, plants cannot fulfill their aesthetic and air-cleaning role. Therefore, many studies have been carried out on technogenic pollution and particularly on magnesite pollution. The aim of the study is to investigate characteristics of the Scotch pine natural regeneration process under the forest canopy in magnesite pollution conditions.

Materials and Methods: The research was carried in an aero-technogenic emission zone of Kombinat Magnezit, in South Ural, Russia. The purpose is to assess the soil suitability for reforestation in various degrees (PS-2: High pollution zone, PS-5: Average pollution zone, PS-3: Low pollution zone, PS-4: Very weak pollution zone, and PS-K: Control area) of magnesite pollution. Experimental Plots (PS) are in similar conditions regarding forest vegetation. The major factors that can influence the natural regeneration, such as the quantity of seeds produced by the parents trees, stand density (the number of trees per 1 ha), the composition of the upper horizon of the soil and height, its density, live ground cover (LGC) phytomass and pH of the upper root-inhabited soil layer have been measured.

Results: The results of this study showed that in PS-K conditions a large number of seeds, thin forest litter, small phytomass of alive ground cover, and slightly acidic pH of the root zone of the soil can be found. The dense forest laying, generally composed of fresh and weakly decomposed layers, inhibits seed germination from reaching the mineral soil layer, which results in mortality.

Conclusions: It was evaluated that the success of natural regeneration is effected by the stand density and the occurrence of undergrowth of Scots pine. Soil remediation can positively affect the natural rejuvenation process. Moreover, seed supplementation can play a major role in successful natural rejuvenation.

Keywords: aero-technogenic, magnesite pollution, soil toxicity, weed, seeds, natural regeneration

INTRODUCTION

Natural regeneration is a biological prerequisite of forest longevity as well as the most important indicator of reforestation condition, stability, adaptation extent and reproductive capability. Therefore, the study of forest regeneration in different environments is an important research subject and has a great scientific and practical importance.

Technogenic load is currently considered to be one of the most powerful factors for destabilizing forest ecosystems of boreal zone [1]. Therefore, many studies have been carried out on technogenic pollution [1-3] and particularly on magnesite pollution [4], elevated phytomass [5], the morphological and chemical composition of leaves of pilot cultures (*Betula pendula* Roth) [6], reproduction [7, 8], and the quality of seeds and seed posterities [9, 10] of pilot cultures (*Pinus silvestris* L). These studies suggested the need for reforestation by cultivating pollution-resistant species. Besides, a large number of studies have focused on natural regeneration processes in aero-technogenic pollution conditions [11-15], noting both negative and positive influences of pollutants. Factors such as the total loss or reduction of macrostrobile-forming trees, the increase of the interval between years with high and low potential seed formation on macrostrobile-forming trees, soil toxicity (pH above 8.5 units), the composition of the top soil horizon and size (poorly decomposed litter and high thickness of the horizon A0), as well as increases or decreases in aero-technogenic pollution reduce the recovery of Scotch pine or completely stop this natural process. On the contrary, the decrease in maternal forest stand competition, the improvement of temperature and light modes due to crown thinning and tree drop, a decrease in the coenotic role of the moss-lichen cover and the addition of necessary chemical elements to the soil from pollutant emissions can positively effect natural regeneration. The restoration and the regeneration of Scotch pine stands under magnesite conditions have not been studied much. The aim of this study is to determine characteristics of the Scotch pine natural regeneration process under the forest canopy in magnesite pollution conditions.

MATERIALS AND METHODS

The research was conducted in an aero-technogenic emission zone of Kombinat Magnezit (South Ural, Russia). Kombinat Magnezit is the largest enterprise in Russia for production of fireproof materials. It is located in the city of Satka (Figure 1). The main air pollutant in this area is magnesite dust, which is highly alkaline (average pH is 10) and mostly comprises magnesium oxide, which breaks down to form $Mg(OH)_2$. High-alkaline magnesite dust and gases (carbon and sulphur oxides) in combined emissions affect the surrounding biology.

Forest stands of Scotch pine cultures planted during the early 1980s were selected to investigate the natural regeneration process. The purpose of the VNIILM Ural Forest Experimental Station is to assess the soil suitability for reforestation in various degrees of magnesite pollution. Experimental plots (PS) are in similar conditions regarding forest vegetation and are located on the northeast aspect. PS-2 was located 1 km from the emission source in a high pollution zone (Satka district), PS-5 was in an average pollution zone (3 km from the emission source), PS-3 was in a low pollution zone (5 km from the emission source Satka district), PS-4 was in a very weak pollution zone (10 km from the emission source-Berdyaush district) and PS-K was located 20 km from the combine and represented the background

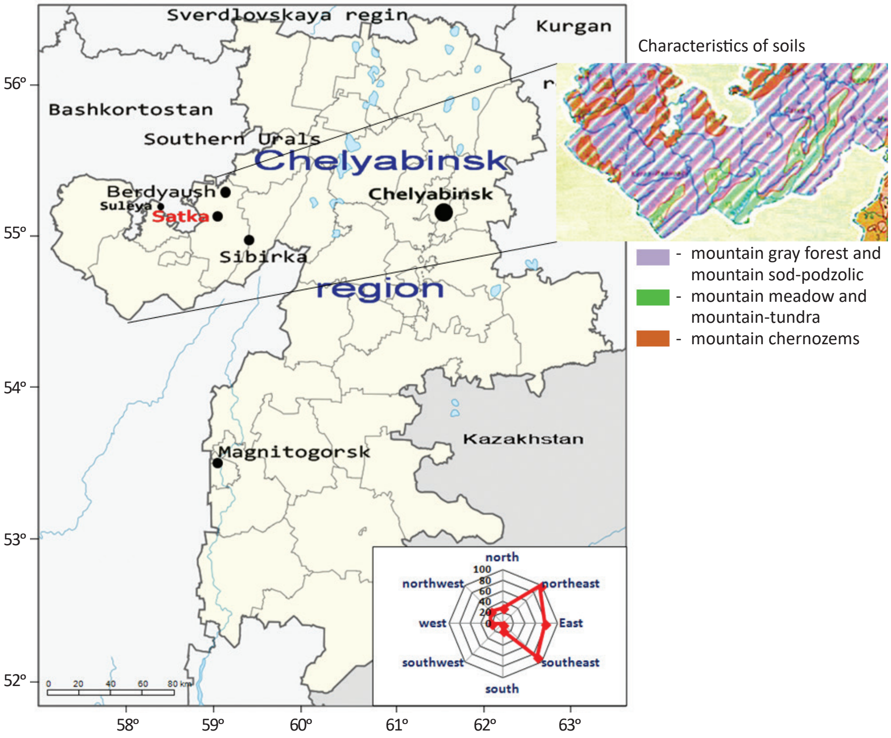

FIGURE 1. The location of the study area, soil types and transforming winds (South Ural, Russia)

condition (Sibirka district). Detailed descriptions of the PSs, pollution levels and the vegetative and reproductive system conditions of the primary forest species have been provided in previous works [7, 9, 16-18]. Although emissions have been considerably reduced in recent years due to modern raw material conversion technologies and cleaning systems, the reduction in pollution level has not been achieved yet [19].

Standard techniques were used to determine the PS sub-growth [20, 21]. The total area of each experimental plot is 1 hectare. In experimental plots, 1×1 m^2 transects were created between 5 m interval in the amount of 50-70 pieces on one experimental plot (Figure 2). At each study site, we counted the number of instances of pine, whose age from 1 to 14 years old and then determined the frequency with which such species of pine occur in the area of 1 ha. The occurrence indicator was calculated by using the regeneration occurrence in relation the total number of platforms [22]. This indicator was used to estimate the natural regeneration and the following evaluation criteria were defined: unsatisfactory (25-49%), satisfactory (50-75%) and good regeneration (>75%) [23].

Previous studies identified the major factors that affect the process of natural regeneration under the forest canopy [21, 22, 24-26]. Forest litter thickness on the same registration platform was measured as the sub-growth density. For live ground cover (LGC) selection at each PS, six 1×1 m registration platforms were placed on a diagonal transect. The LGC phytomass was only identified in dry conditions.

At each PS, the number of seeds carrying trees and parent trees were counted. Seed carrying trees were defined as those with five or more developed macrostrobiles. Upon seed carrying tree identification, the number of macrostrobiles was counted and 5-40 specimens were selected for analysis. Macrostrobile seeds were drilled on their central axes and seed scales were separated to assess the number of full-granular seeds [27]. The quantity of seeds on each tree and in each PS were calculated and converted to a number of seeds per hectare.

RESULTS AND DISCUSSION

The results revealed that the number of macrostrobile-forming trees in the forest changed due to aero-technogenic pollution (Figure 3). In areas with strong, moderate and weak effects of the magnesite pollution, the macro strobil forming trees were 11.19-28.56% less than the background conditions. The smallest quantity of trees with macrostrobiles was found in the strong pollution zone (PS-2), where perennating trees prevail.

The factors that influence the process of natural regeneration under the forest canopy [7, 16, 19, 22, 28] are presented in Table 1. Data from a previous study of seedling density and pine sub-growth in an area of magnesite pollution are shown in Table 2.

The results of our study revealed that in background conditions (PS-K) a large number of seeds, thin forest litter, small phytomass of alive ground cover, and slightly acidic pH

FIGURE 2. Experimental plot

TABLE 1. Factors that effect the process of natural regeneration under the forest canopy.

Pilot site / Distance (km) from the pollution source	**Stand density** (1000 trees·ha^{-1})	**Number of seeds** (1000 seeds·ha^{-1})	**Forest canopy thickness** (mm)	**Phytomass of LGC** (kg·ha^{-1})	**pH** (water) **of top root-inhabited soil layer (0-20 cm)**
PS-2/1	0.99	445.84 ± 1.2	1.07 ± 0.10	1663.53 ± 273.00	9.02 ± 0,05
PS-5/3	4.42	1391.22 ± 3.9	2.39 ± 0.21	697.89 ± 177.26	8.36 ± 0.05
PS-3/5	1.83	797.38 ± 7.6	4.05 ± 0.30	355.53 ± 57.09	7.63 ± 0.03
PS-4/10	2.39	1563.46 ± 2.07	2.95 ± 0.25	649.22 ± 162.17	7.40 ± 0.05
PS-K/20	2.42	3445.96 ± 1.4	1.80 ± 0.28	681.47 ± 144.47	5.88 ± 0.06

TABLE 2. Seedling density and pine sub-growth in magnesite pollution conditions.

Pilot site	**Distance from the emission source** (km)	**Average quantity of sub-growth** (1000 specimens·ha^{-1})					**Total sub-growth, excluding seedlings** (1000 specimens·ha^{-1})
		Seedling (2 years younger)	**<0.1 m (3-4 years)**	**0.1-0.5 m (5-7 years)**	**0.6-1 m (8-10 years)**	**1.1-2 m (11-14 years)**	
PS-2	1	0	3.00 ± 0.72	2.33 ± 0.90	0	0	5.33
PS-5	3	1.97 ± 0.12	19.86 ± 2.80	13.52 ± 3.98	2.68 ± 0.92	0.99 ± 0.38	37.05
PS-3	5	0	3.8 ± 0.55	0.6 ± 0.34	0.2 ± 0.14	0	4.60
PS-4	10	0.67 ± 0.29	5.33 ± 2.10	0	0.33 ± 0.23	0	5.66
PS-K	20	0	0	0	0	0	0

of the root zone of the soil can be found. The dense forest laying, generally composed of fresh and weakly decomposed layers, inhibits seed germination from reaching the mineral soil layer, which results in mortality.

At the very weak pollution level site (PS-4), the sub-growth density was 5.66 thousand specimens/ha. The regeneration process is considered successful with regard to future sub-growth preservation. The lack of sub-growth in age categories of 5-7 years and 11-14 years is significant and can be attributed to the seed carrying frequency. The limiting factor at this site was the forest litter thickness.

At the weak pollution level site (PS-3), the sub-growth density was 4.6 thousand specimens/ha. The regeneration process is considered successful with regard to the future sub-growth preservation. The forest litter thickness and the small quantity of seeds produced by the forest stand impacted the regeneration process at this site.

The highest sub-growth density (37.05 thousand specimens·ha^{-1}) was observed at the average pollution level site (PS-5). All age categories of sub-growth were observed and the regeneration is considered successful.

The sub-growth density at the strong pollution level site (PS-2) was 5.33 thousand specimens/ha. The process of regeneration was negatively influenced by the small quantity of seeds, highly-alkaline reaction of the root-inhabited soil layer and the LGC phytomass. At this site, regeneration was possible only where there was no vegetation cover and there were only two age categories of sub-development (i.e. 3-4 years and 5-7 years). The regeneration process can also be considered successful at this site.

TABLE 3. The occurrence of pine sub-growth in magnesite pollution and background conditions.

Pilot site	**Distance from the emission source** (km)	**Average number of reliable sub-growth** (1000 specimens·ha^{-1})					**Occurrence** (%)
		Seedling (2 years younger)	**<0.1 m (3-4 years)**	**0.1-0.5 m (5-7 years)**	**0.6-1 m (8-10 years)**	**1.1-2 m (11-14 years)**	
PS-2	1	0	13.3	20.0	0	0	26.7
PS-5	3	12.7	39.4	29.6	14.8	5.6	62.0
PS-3	5	0	26.0	6.0	2.0	0	28.0
PS-4	10	3.3	10.0	0	3.3	0	13.3
PS-K	20	0	0	0	0	0	0

A decrease in the sub-growth density with a corresponding increase of age variable characterises each PS. The low quantity (or absence) of seedlings in each PS is associated with the low harvests in 2011 and 2012. The high number of 3-4 year old sub-growth specimens coincides with 2009 and 2010, in which high yields were achieved.

The occurrence of pine sub-growth in magnesite pollution and background conditions is provided in Table 3. Occurrence coefficient indicators characterise the natural regeneration process as satisfactory at the average pollution level in experimental plot PS-5 and unsatisfactory in all other experimantal plots. This result can be explained by the stand densities and seed amounts.

Scotch pine, a widely distributed and important forest-forming tree, is characterized by high sensitivity to technogenic polluting agents [29]. Sazonova and Olchev [30] emphasized that comparisons of the responses of *Picea obovata* Ledeb. and *Pinus sylvestris* L. trees to industrial pollution showed that a relationship between tree vitality status shown by visual traits and by physiological criteria was more evident for *P. obovata* than *P. sylvestris* trees. Thus, spruce may be less resistant to pollution than pine trees [31]. Silver birch and Scotch pine trees were more severely impacted by dust pollution compared to Sukachyov's larch trees. Soil remediation is required in strongly affected areas. The use of 12 cm thick peat layers in the soil mitigates the pollution impact on plant growth [32].

Air pollution resulted in increased defoliation and decreased three growth. Trees closer to the source of emissions are most severely damaged. With increasing pollution, the lifespan of Scotch pine needles is reduced by 40% in comparison with the same species at the control site [32].

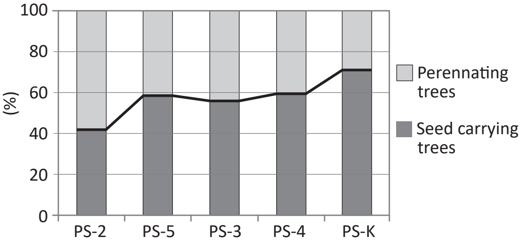

FIGURE 3. The share of seed carrying trees on the pilot sites (PS).

CONCLUSIONS

Our research showed that in background conditions natural regeneration of Scotch pine is completely absent. However, the quantities of pine sub-growth at all levels of magnesite pollution were sufficient for natural regeneration and the process was successful with regard to the future sub-growth preservation. Furthermore, the average pollution level in experimental plot PS-5 had the largest amount of sub-growth and was the only site with a satisfactory occurrence indicator. The relationship between soil and plants, under the influence of the accumulated aerial technogenic impacts of emissions in polluted areas, should be examined comprehensively for a long time.

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Soil Measurements in the Context of Planning Harvesting Operations and Variable Climatic Conditions

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ABSTRACT

Background and Purpose: Terrain trafficability and vehicle mobility during timber extraction are highly influenced by terrain characteristics, as well as by soil bearing capacity. Insufficient planning, too heavy vehicles, excessive traffic, poor soil knowledge, together with bad weather conditions, cause severe disturbance to forest soil. Damage to the forest ecosystem arises due to felling and timber extraction operations, regardless of the technical means used in this process. Traffic intensity plays an essential role in soil compaction because deformations can increase with the number of passes, which may lead to excessive soil disturbance.

Materials and Methods: The research was conducted in Zalesina, a hilly and pre-mountainous part of Gorski kotar, Croatia, in two management units (MUs) comprising of 569.64 ha of selective beech and fir forests. Physical and mechanical properties of forest soil, essential for vehicle mobility and terrain trafficability during timber extraction are based on measuring the following soil parameters: 1) soil penetration resistance, 2) soil shear strength, and 3) current soil moisture. Measurements were made during one calendar year, on five sampling plots (three in MU "Belevine" and two in MU "Kupjački vrh") chosen on the prevailing soil types in both MUs.

Results: The highest values of cone index and shear strength were recorded in July which was the warmest and driest month with only 7 rainy days. Current soil moisture was the lowest in July at ϕ=15.26% vol, while the lowest values of cone index were from October to January with precipitation at its climax from September to January with cumulative 1232 mm of rain and 846 cm of snow. Lower values of penetration resistance were in MU "Kupjački vrh" at 0.96 MPa, which is related to overall terrain structure of high karst and surface roughness, making the sampling of plots difficult. By comparing cone index values (CI_{15}) and the mean values between 5 and 25 cm of depth (CI_{5-25}), the variation coefficient indicates a lower variability of CI_{5-25} values on four out of five sampling plots. However, t-test showed no statistically significant difference between these soil parameters.

Conclusions: Data regarding soil bearing capacity in a map form as an additional layer to other maps of stand characteristics would ensure better opportunities for planners or operators to reduce and avoid rutting and soil compaction. Measured data on penetration resistance, soil shear strength and current soil moisture have not only given in a simple and a fast manner the in situ stand conditions, but they have also shown their dependence on climatic conditions.

Keywords: digital penetrometer, cone index, soil shear strength, precipitation, soil moisture content

INTRODUCTION

The planning of harvesting operations usually starts with a thematically prepared maps and a pre-planned marking of stand borders and/or marking of trees. A planner besides marking cutting area borders, environmentally delicate areas and information on stand inventory needs to evaluate the best driving routes for vehicles on either temporary forest roads or trails [1], or on the existing primary and secondary forest traffic infrastructure using Geographic Information

System (GIS). One of the most significant applications of LiDAR-derived terrain models has been in facilitating the mapping of areas of anticipated high moisture and potentially high susceptibility to soil damage by vehicles [2]. Terrain trafficability and vehicle mobility during timber extraction are highly influenced by terrain characteristics, as well as by soil bearing capacity. Insufficient planning, too heavy vehicles, excessive traffic, poor soil knowledge, together with bad weather conditions, cause severe disturbance to forest soil. Damage to the forest ecosystem arises due to felling and timber extraction operations, regardless of the technical means used in this process [3] and traffic intensity plays an essential role in soil compaction because deformations can increase with the number of passes, which may lead to excessive soil disturbance [4, 5]. Therefore, one of the most important issues in ground-based timber extraction in forestry is to minimise the soil damage caused by heavy forestry machines [6].

Soil bearing capacity is the ability of the ground to withstand external forces (wheel or track movement), and is determined by compaction, rutting and surface disturbance under external load. In forestry, soil bearing capacity is defined as the maximum permissible tire pressure of the vehicle without damaging the soil [7], depending on the type and texture of the soil, humus layer and skeletal particles (permanent parameters of soil), and the only variable parameter – soil moisture [8]. Soil bearing capacity is expressed in kPa (MPa, $N \cdot cm^{-2}$), but there is no prescribed measurement method, due to the difficulty of accurate and straightforward procedures caused by the natural condition of forest soil, rapid changes of its moisture and the influence of the vegetation roots [9]. In terra-mechanics and wheel – soil interaction, Mohr-Coulomb's failure criterion is the most often applied one, where soil strength is quantified as the maximum permitted strain in the soil before its deformation (cracking, crushing or flowing).

Poršinsky [10] states that the share of sand and clay particles in the soil composition affects the boundary of the shear strength of soil. During vehicle movement on deformable ground, the transfer of force is relevant during load and deformation of the soil in the tangential direction, provided there is a friction between the ground and the tire. As tires of forest vehicles have mandatory treads, tangential stress is ensured in case of tread penetration into the ground. In this case, the most important feature of soil deformation is the strain because force can be achieved due to the shear strength of the soil. In general, it can be concluded that soil with the highest shear strength is over-consolidated clay or well-compacted sand, and soil with the least shear strength are poorly graded sands [10-14].

In forestry, soil bearing capacity is usually determined by the penetration of the cone into the ground and is defined as the ratio of force required to press the standardised cone, as well as varying ground resistance to penetration depending on its depth. The ground penetration curve contains data on the estimation of soil strength depending on the depth of the cone penetration, caused by the horizon condition of certain soil types. In practical application, a standardised value of the cone penetration measurement [15] is used in a 15 cm ground depth called the cone index (CI). The same standard recommends the use of a cone index when assessing terrain trafficability and the vehicles' mobility as a parameter used in modelling vehicle traction performance. However, an altered definition of the cone index can be found in literature, where the cone index is the average value of the penetration resistance measurement in the range from 0 to 15 cm of soil depth [16]. Horvat [12] recommends repeated measurements on forest soils due to their inhomogeneous structure, and considers penetrometer measurements as suitable for fast comparative mensuration, mainly when performed with the same penetrometer.

The first forest ground bearing capacity classification based on cone index (CI), shear strength and deformation modulus (E value), as each of these can be measured at once in the field using portable equipment, was made by the EcoWood project, which gave special attention to environmentally efficient timber harvesting operations on sensitive soils [17]. Poršinsky [10] and Poršinsky *et al.* [18], based on measurements of multiple parameters of forest soil bearing capacity in Croatia, expressed doubts about the range of the values representing high ground bearing capacity (H-GBC) at >500 kPa for cone index value; >60 kPa of shear strength. In accordance with the above-mentioned suspicion, Poršinsky *et al.* [8] state that Bygdén's (2012) guideline for ground bearing capacity in forestry is more suitable for the conditions of Croatian forestry, where CI values range between 700 and 1000 kPa, which is categorised as medium hard soil (may soften after rain), and CI above 1000 kPa, which represents hard soil with a good bearing capacity.

Saarilahti [19] recommends that, during soil defrosting, constraints in the production system should be identified due to increased moisture and reduced soil bearing capacity, so major soil damage is reduced. Shoop *et al.* [20] state that the snow cover reduces soil damage, but also the systems' productivity. As the basic features of the snow cover, the authors state its thickness, temperature and density. The vehicle's mobility stops at a snow thickness of 30% of the wheel diameter, while chains on wheels enlarge mobility up to 50% of wheel diameter [21].

Access to soil data on the European level is possible via European Soil Data Centre (ESDAC). ESDAC gives topsoil physical properties for Europe (based on LUCAS topsoil data) for ca 20,000 samples across EU. Some layers for soil properties have been created based on the data from the European Soil Database in combination with data from the Harmonized World Soil Database (HWSD) and Soil-Terrain Database (SOTER) in 10×10 km resolution. The available layers include total available water content, depth available to roots, clay content, silt content, sandy content, organic carbon, bulk density, and coarse fragments [22]. On the national level, an existing Pedological Base Map of Croatia in 1:300,000 scale [23] is still in use.

Talbot *et al.* [2] state that the development of remote and proximal sensing technology and techniques will provide a previously inconceivable amount of data. Authors continue that the machine-mounted sensors that unceasingly collect vast amounts of data will especially give the forest operation researcher a large and continually increasing basis from which to extract useful information. These data can, with the application of sensible analytical approaches, provide significant opportunities for decision support, as well as for operation monitoring and evaluation. Besides the advantage of the availability and use of high-resolution data, prominent future development of GIS applications in forest operations could be an entirely integrated 4D spatial dataset and Virtual Reality (VR) systems [24].

This article deals with measuring soil bearing capacity through measurements of cone index, shear strength and soil moisture content to plan harvesting operations in the right period and gain more knowledge on soil properties specific for operational forestry purposes.

MATERIALS AND METHODS

Study Area

The research was conducted in Zalesina, a hilly and pre-mountainous part of Gorski kotar, Croatia, in two management units comprising of 569.64 ha of selective beech and fir forests. The average growing stock is 446 $m^3 \cdot ha^{-1}$, with an annual increment of 6.83 $m^3 \cdot ha^{-1}$ and a total allowable cut in the period from 2010 until 2019 at 30,565 m^3.

According to Köppen climate classification, the research area belongs to the Cfsbx climatic type [25]. The essential features of the Cfsbx climatic type are cool summers with the mean temperature of the hottest month below 22°C, while the average monthly cool summer temperature ranges from -3°C to +18°C. In the research area chilled air masses are retained due to its geographic position in a wet and closed plateau, leading to a low average annual temperature of just 6.8°C. Zalesina is abundant with precipitation, with annual average values up to 2074 mm in 136 days of precipitation and with two yearly minimums, one at the end of winter – in March with average precipitation of 140 mm, and the other one in summer – in July with average precipitation of 143 mm [26]. Climate elements affect harvesting operations, which may be best reflected by the data on the highest daily precipitation. Thus, in one day up to 154 mm of rain may fall (data from September 1951) in the form of strong showers, which is the same amount of precipitation in June. Therefore, soil protection from erosion and torrents is necessary. A significant share of annual precipitation is significant for late autumn and winter, which consists of 98 days with snow, while the snowfall lasts on average 188 days (from the end of October to the end of June). There is also a significant proportion of snowfall in annual precipitation, up to 26%, so that in spring the melting of snow significantly soaks the forest soil.

In the two adjacent management units (MUs) "Belevine" and "Kupjački vrh", both with natural fir and beech forests, managed in selective cuts, variations in terrain conditions are evident. The relief of MU "Belevine" is mildly undulating and fan-like striated with many beds of water streams with their steep sides affecting timber extraction routes. MU does not contain stoniness, rockiness or similar ground obstacles. Terrain slope on 55.73% of the MU is between 0–20% [27]. Terrain conditions and harvesting system in use resulted in a skid trail network for timber extraction by a skidder. In the MU "Kupjački vrh", karst phenomena and cliffs are common, with a centrally located peak at 997 m and with hillsides descending in all directions. More than 50% of the MU has terrain slopes higher than 34%, and 65.87% of MU is categorised as having uneven to very rough terrain regarding stoniness and rockiness [28]. This all resulted in a built skid road network without which skidders' mobility in this MU would be unattainable [29].

Measuring Equipment

Physical and mechanical properties of forest soil essential for vehicle mobility and terrain trafficability during timber extraction are based on measuring the following soil parameters: 1) soil penetration resistance, 2) soil shear strength, and 3) current soil moisture. Measurements were made during one calendar year, on five sampling plots (three in MU "Belevine" and two in MU "Kupjački vrh") chosen on the prevailing soil types in both MUs (Figure 1):

- BEL 1 – dystric colluvial brown soil
- BEL 2 – dystric brow soil (typically brown podzolic)
- BEL 3 – deep dystric brown soil
- KUP 1 – shallow rendzina
- KUP 2 – shallow and medium deep rendzina

A digital penetrometer Eijkelkamp Penetrologger was used to measure penetration with a cone surface range of 2 cm^2 and the angle of cone at 30° according to the standard ASAE S.313.3 at a penetration speed of 2 $s \cdot cm^{-1}$. The measurements were performed on a monthly basis in 15 repetitions per plot at a depth of maximal 80 cm. Soil bearing capacity was defined by cone index as a derivative of soil penetration resistance at 15 cm depth [15].

For measuring soil shear strength, Eijkelkamp Field inspection vane tester was used with 20×40 mm wing dimension, a measuring range of 0 to 130 kP, and a reading accuracy of 2 kPa. The measurements were performed on a monthly basis in 15 repetitions per plot at a depth of 15 cm.

The current soil moisture was measured by a Fieldscout TDR 300 soil moisture meter at a depth of 15 cm. Measurements were made in repetitions of 15 on all five surface plots, with each of the 15 measurements being the average of 10 measurements so that 150 current moisture data were measured on each plot.

From the State Meteorological and Hydrological Service data on average daily temperatures (°C); average daily wind power ($m \cdot s^{-1}$); daily height of total snow (cm); daily precipitation (mm); and daily insolation (hours) were taken for the analysis and better understanding of the soil bearing capacity dynamics during the year.

RESULTS

According to the national hydro-meteorological data, there were no discrepancies in temperature for autumn and winter in the measuring period, but the amount of precipitation varied. Spring was characterised as warm to very warm (higher values by 0.8-1.5°C) and precipitation was normal (101%) to dry (67%). Summer was extremely warm (temperatures higher by 1.7°C) with normal amount of precipitation at 101%.

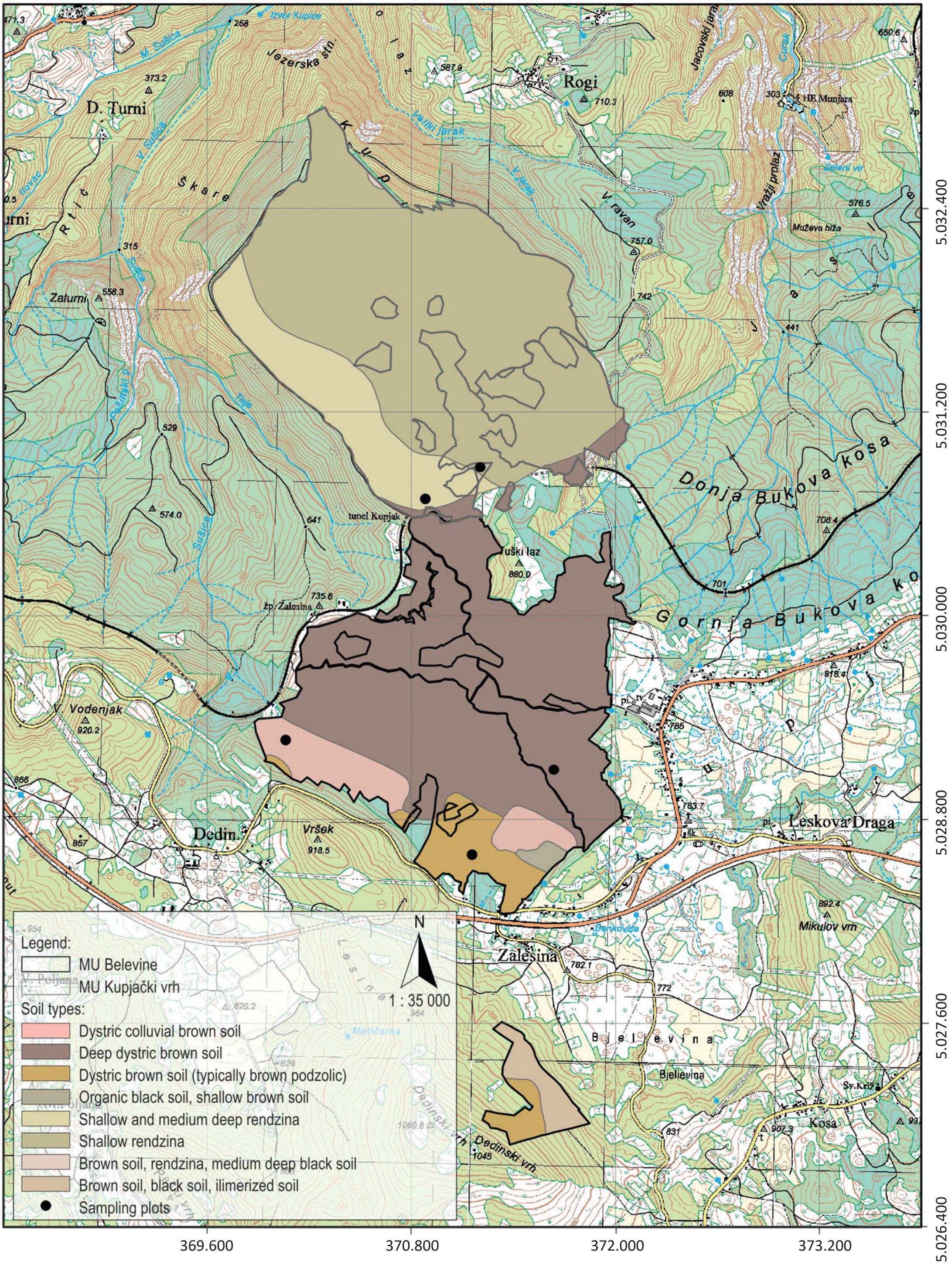

FIGURE 1. Study area

Soil Moisture Content

By a repeated ANOVA analysis made in software Statistica 13.1, a statistically significant difference between sampling plots and the period of measurements were determined (Table 1).

Tukey Post Hoc test (Table 2) showed statistically significant difference between some sampling plots with MS=26.747 and df=70.

Figure 1 shows difference in values regarding the time of measurements (month) and location (sampling plot). The highest average monthly moisture values were recorded in October, and the lowest values in July. There were no field measurements in December due to high snow which prevented approach to the sampling plots.

Figure 3 comprises the climatic data (precipitation amount and snow) together with soil moisture data during twelve months of measurements. The peak of soil moisture content was from September to November on average ϕ=39.10%, which correlates to the high amount of precipitation above 272 mm.

TABLE 1. Repeated ANOVA of current soil moisture measurements (* symbolises significant difference).

Effect	SS	DF	MS	F	p
Intercept	752758.7*	1*	752758.7*	28143.40*	0.00*
Sampling plot	7561.5*	4*	1890.4*	70.68*	0.00*
Error	1872.3	70	26.7		
Period of measurements	60162.4*	11*	5469.3*	329.96*	0.00*
Sampling plot * Period of measurements	13605.0*	44*	309.2*	18.65*	0.00*
Error	12763.3	770	16.6		

TABLE 2. Tukey Post Hoc test of soil moisture content (* symbolises significant difference).

Sampling plot	BEL 1 29.420	BEL 2 31.379	BEL 3 23.302	KUP 1 29.702	KUP 2 30.800
BEL 1		0.005405*	0.000125*	0.985381	0.095375
BEL 2	0.005405*		0.000125*	0.024281*	0.825289
BEL 3	0.000125*	0.000125*		0.000125*	0.000125*
KUP 1	0.985381	0.024281*	0.000125*		0.270384
KUP 2	0.095375	0.825289	0.000125*	0.270384	

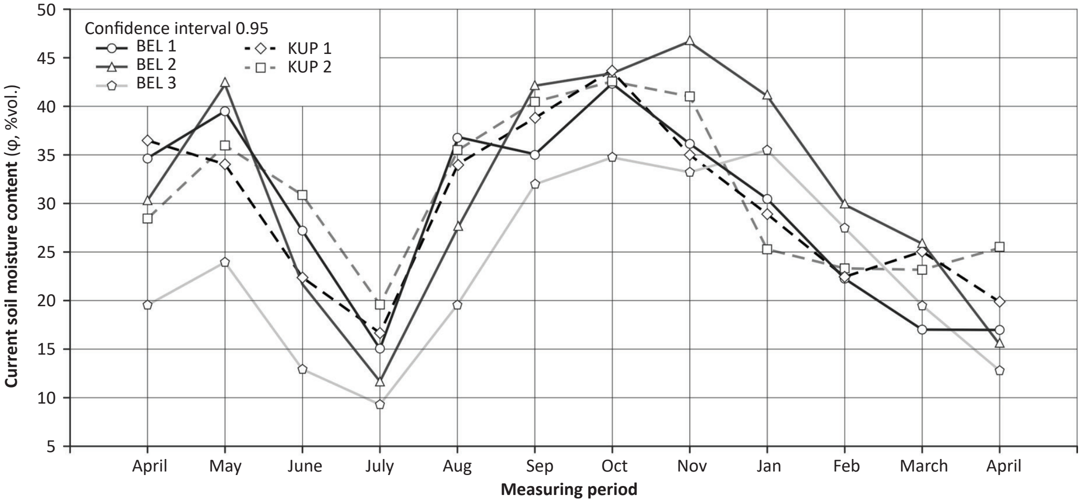

FIGURE 2. Average values of current soil moisture content on sampling plots

Precipitation showed more considerable influence on soil moisture content than snow, whose climax was in January and which remained present in the forest stand till April.

Penetration Resistance

By repeated ANOVA analysis of soil penetration resistance, a statistically significant difference between sampling plots and the period of measurements was determined (Table 3).

Tukey Post Hoc test showed statistically significant differences between all five sampling plots at MS=0.20571 and df=2170.

Figure 4 represents average values of soil penetration resistance per sampling plot in a given period of measurements. The peak of measurements was in July at 3 MPa, and the lowest value was measured at KUP 2 sampling plot in October – 0.62 MPa. The lowest average measurements for all five sampling plots were recorded in January at 0.86 KPa. According to Bygdén's ground bearing capacity classification, all sampling plots (except KUP 2 in October) regardless the month, can be classified as medium hard to hard soils.

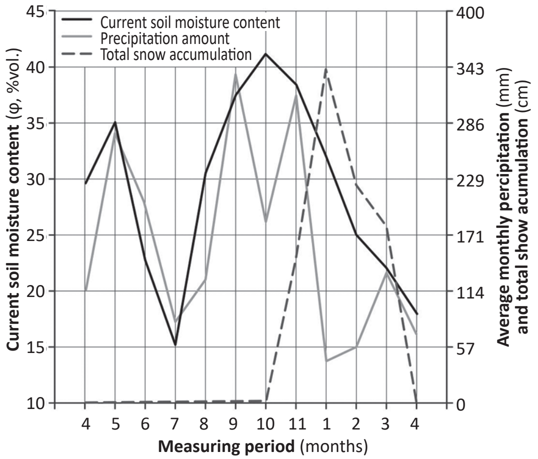

FIGURE 3. Average current moisture content and precipitation

Average values of soil penetration resistance per plot and the period of measurements but depending of soil depth are given in Figure 5. By increasing soil depth, penetration resistance increases (Figure 5), and correlation coefficients show strong relationship (0.82-0.99) between these two variables. The highest average values were recorded in July and August from 1.62 MPa to 3 MPa from the surface up to 30 cm depth. The lowest average values at the soil surface were in February and March, 0.35 MPa and 0.30 MPa respectively. At 15 cm and 30 cm depth minimal values were recorded in October and January, 0.91 MPa and 1.14 MPa respectively. The period of measurements and soil depth show lower influence on soil penetration resistance for sampling plots in MU "Kupjački vrh". On KUP 1 sampling plot the difference in soil penetration resistance between 10 cm and 30 cm depth ranges up to 0.45 Ma, while on KUP 2 sampling plot the difference is smaller and ranges up to 0.25 MPa. On both sampling plots, the highest jump in penetration resistance is at first 10 cm of soil depth. On sampling plots in MU "Belevine" in a month of measurements there has been higher influence on penetration resistance ranging up to 1.0 MPa.

By comparing the values of cone index at a depth of 15 cm (CI_{15}) and mean values between 5 and 25 cm of depth ($CI_{5–25}$), the coefficient of variation (CV) indicates a lower variability of $CI_{5–25}$ values at four sampling plots from 14.56% to 22.84%. At KUP 2 sampling plot variation coefficients of both cone indexes were the same. T-test, however, showed no statistically significant difference between the mean values of the cone index at a depth of 15 cm and 5 to 25 cm.

Shear Strength

By repeated ANOVA analysis of shear strength a statistically significant difference between sites and the period of measurements was recorded (Table 4). Tukey Post

TABLE 3. ANOVA of soil penetration resistance (* symbolises significant difference).

Effect	SS	DF	MS	F	p
Intercept	34988.12*	1*	34988.12*	170085.9*	0.00*
Sampling plot	571.62*	4*	142.90*	694.7*	0.00*
Depth of measurements	2131.69*	30*	71.06*	345.4*	0.00*
Sampling plot * Depth of measurements	162.75*	120*	1.36*	6.6*	0.00*
Error	446.39	2170	0.21		
Period of measurements	2749.35*	11*	249.94*	1549.0*	0.00*
Period of measurements * Sampling plot	1561.96*	44*	35.50*	220.0*	0.00*
Period of measurements * Depth of measurements	123.55*	330*	0.37*	2.3*	0.00*
Period of measurements * Sampling plot * Depth of measurements	319.98*	1320*	0.24*	1.5*	0.00*
Error	3851.53	23870	0.16		

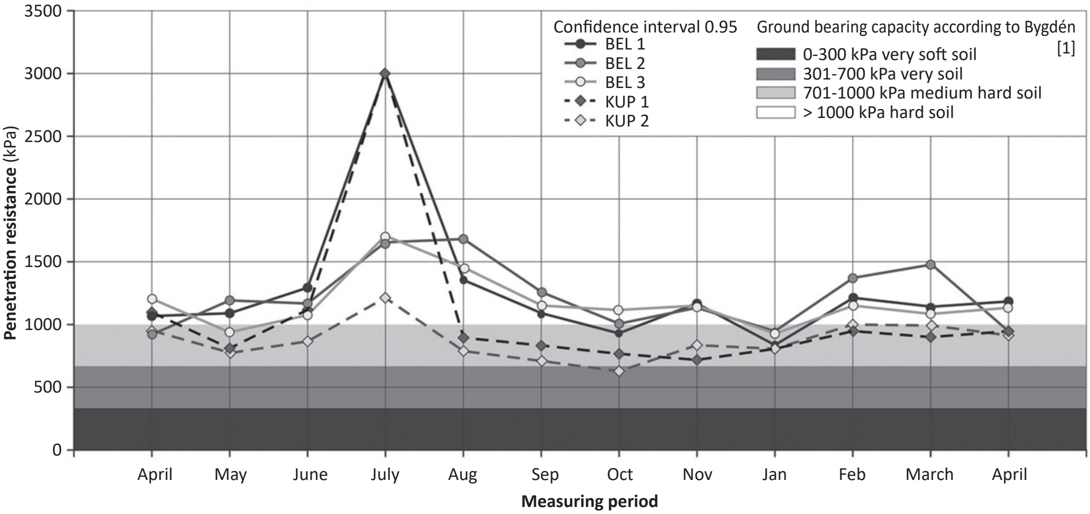

FIGURE 4. Soil penetration resistance per sampling plot in different months.

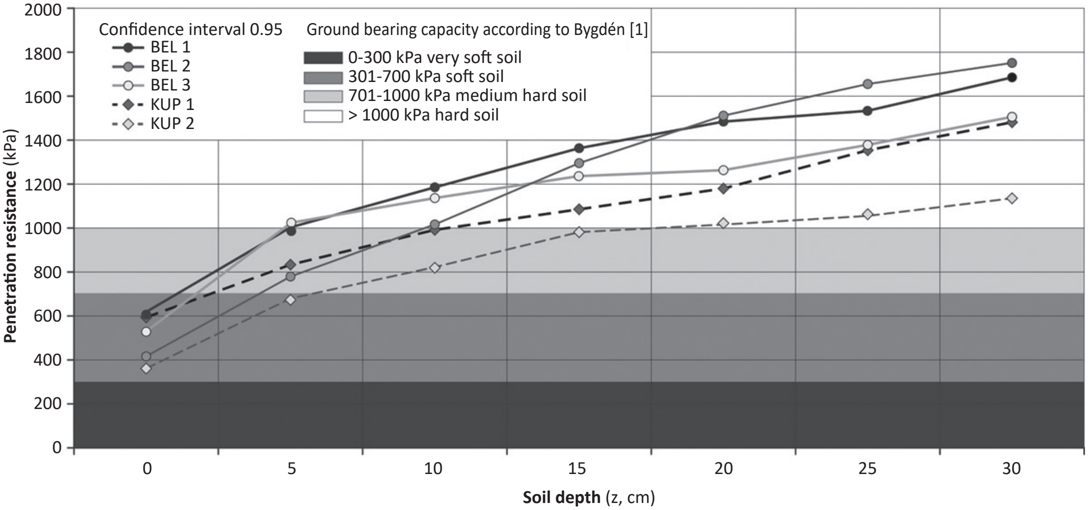

FIGURE 5. Differences in penetration resistance and soil depth.

Hoc test showed statistically significant difference between some sampling plots (Figure 6) at MS=234.07 and df=70, while there were no differences between sampling plots BEL 1 and BEL 2, nor between BEL 3 and KUP 1.

The highest values of soil shear strength at 15 cm depth were recorded in July at τ=130 kPa, while the lowest values were recorded in May at τmax=61 kPa. If monthly mean values are compared to all five sampling plots, data deviation is visible, except in the summer months (July and August) when variation is at its minimum. The lowest mean values of the soil shear strength were measured on sampling plot KUP 1 (τ=62 kPa), and the absolute minimum was measured in October (τ=32 kPa).

When comparing cone index and shear strength to soil moisture (Figure 7) on all sampling plots during one-year period and in 15 cm depth, a consistency is evident. The years' peak of cone index (CI=2109 kPa) and shear strength values (τ=129 kPa) in July was followed by a soil moisture minimum (ϕ=15.26%), after which soil moisture content started to rise steadily, only to reach its high point in October at ϕ=41.21%. In this period both the cone index and shear strength were descending and in October reached 891 kPa and 87 kPa, respectively. After October soil moisture content plunged steeply only to be ϕ=18.05% in April. Cone index and shear strength fluctuated in this period from 997±120 kPa and 100±7 kPa, respectively.

TABLE 4. ANOVA of soil shear strength (* symbolises significant difference).

Effect	SS	DF	MS	F	p
Intercept	7480225*	1*	7480225*	31956.63*	0.00*
Sampling plot	40736*	4*	10184*	43.51*	0.00*
Error	16385	70	234		
Period of measurements	300733*	11*	27339*	109.83*	0.00*
Sampling plot * Period of measurements	130620*	44*	2969*	11.93*	0.00*
Error	191680	770	249		

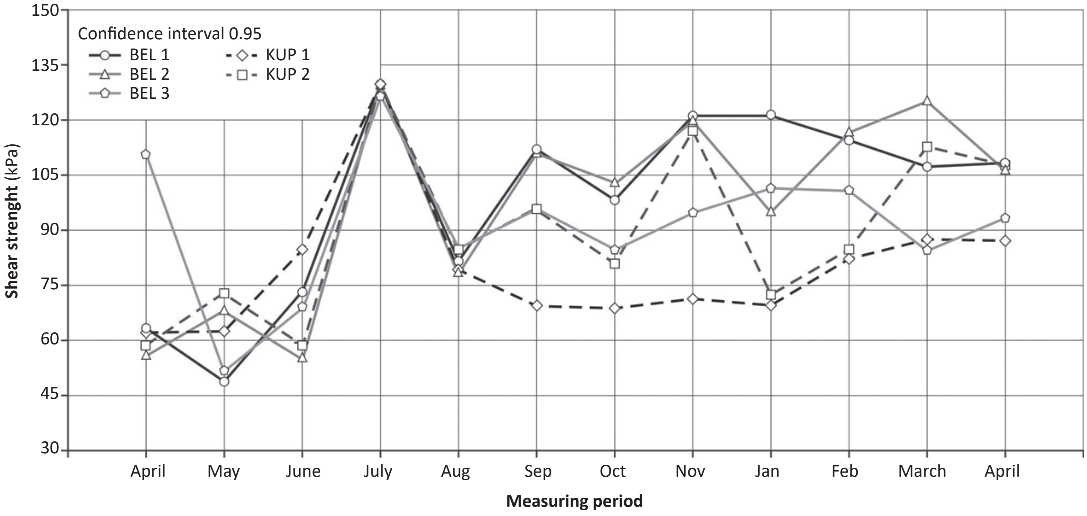

FIGURE 6. Soil shear strength during one-year period.

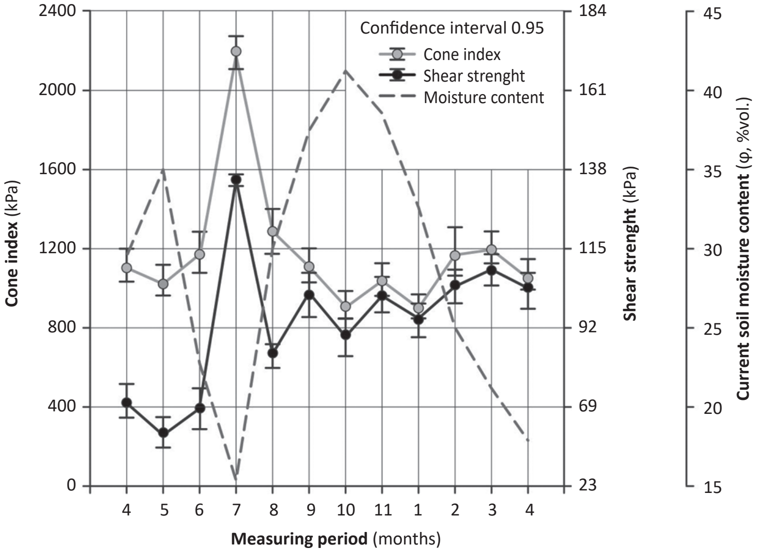

FIGURE 7. Soil measurements throughout the year.

DISCUSSION

The correlation between soil moisture, precipitation and temperature data has been discussed widely [30, 31] and projects such as Global Soil Wetness Project (GSWP) reproduced the seasonal cycle of soil moisture [32]. To retrieve soil moisture, the effects of vegetation, surface roughness, and heterogeneous land cover must be taken into account [33], and in the territory of the Republic of Croatia, these data specific to forestry operations are still missing. There have been several studies on soil penetration resistance having water content or moisture content (MC) as an input variable [34, 35]. The highest values of cone index and shear strength were recorded in July which was the warmest (above average) and driest (within average) month with only 7 rainy days. Current soil moisture was the lowest in July at ϕ=15.26% vol. Climate characteristics in Croatia have shown a trend of decreasing precipitation and increasing temperatures during all seasons in the 20th century [36]. The lowest values of cone index were recorded from October to January with precipitation at its climax from September to January with cumulative 1232 mm of rain and 846 cm of snow. Even though soil moisture is a versatile parameter, data recorded by previous research and models combining cone index and soil moisture by Freitag [37] and Hinze's [38] are non-linear models which all give dependence between these parameters. Data analysis of penetration resistance showed statistically significant differences between five sampling plots, with their average at 1.12 MPa, which according to Bygdén [1] are hard soils with good bearing capacity. Lower values of penetration resistance were recorded in MU "Kupjački vrh" at 0.96 MPa, which is related to the overall terrain structure of high karst and surface roughness [28] in which the placement of sampling plots was difficult. Two chosen areas of KUP 1 and KUP 2 sampling plots were based on a search of deep soil exceptions so that measurements with digital penetrometer, vane tester and soil moisture meter could be achieved.

By comparing cone index values (CI_{15}) and the mean values between 5 and 25 cm of depth (CI_{5-25}), the variation coefficient indicates a lower variability of CI_{5-25} values on four out of five sampling plots which correlate with a research by Pandur [39]. However, t-test showed no statistically significant difference between these soil parameters.

Even though soil penetration resistance was measured up to 80 cm of depth, the majority of changes in penetration resistance were in top 10 to 15 cm of soil after which curves stabilised and showed little to no difference depending on soil depth. Measurements up to 30 cm of soil depth are sufficient for operational forestry purposes [40-42] even though Jansson and Johansson [43] found that traffic-increased bulk density of a silt loam podzol was down to 40–50 cm of soil depth.

Vane tester in a simple way measures the unconfined shear strength of soil – soil cohesion [16], and gives the maximal strength that must be used to provoke a free movement of the soil. Formerly established dependence between penetration resistance and soil shear strength introduced by Meek [44] has shown its influence during field measurements (Figure 7). The lowest values of soil shear strength were recorded from April to June on average as τ=66 kPa, which according to Ward *et al.* [17] places these soils to high ground capacity category (H-GBC). Due to expressed doubts regarding the applicability of the mentioned categorisation in Croatian forestry, at least 80 kPa should be considered as medium hard soils, and values above 100 kPa as hard soils.

CONCLUSIONS

Understanding terrain factors is critical for planning forest operations. Various stand conditions affect the type of machines, harvesting system and the activity itself regarding eco-efficiency, productivity, and revenue. Data regarding soil bearing capacity in a map form as an additional layer to other maps of stand characteristics would ensure better opportunities for planners or operators to reduce and avoid rutting and soil compaction. The measured data on penetration resistance, soil shear strength and current soil moisture have not only given in a simple and fast manner the in situ stand conditions, but have also shown their dependence on climatic conditions. In the absence of these data on the national level, particularly intended for forestry operations in which top 300 mm of soil are crucial, future research challenges should be addressed regarding: 1) the development of unified and straightforward methods for describing forest soil bearing capacity, 2) linking forest soil parameters to long-term monitoring of climatic elements, 3) connecting forest soil-bearing capacity to eco-indicators (occurrence of hygrophytes in forest phytocenoses), and 4) quantifying forest soil bearing capacity classification suitable for a wide range of conditions on the national level. Gaining knowledge on versatile soil bearing capacity throughout the calendar year would support route planning for primary and secondary traffic infrastructure network, as well as defining sensitive parts of logging areas and landing sites, and alarming machine operators to equip vehicles with wider tyres, chains or bogie tracks in specific conditions.

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Fuel Consumption of Forwarders in Lowland Forests of Pedunculate Oak

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ABSTRACT

Background and Purpose: Fuel consumption in timber harvesting operations is significant for both economic and environmental reasons. In economic sense, one significant part of timber harvesting costs is reduced to fuel costs, and in environmental sense 80% to 95% of exhaust emissions and soot particles in forest machinery are in fact associated with fuel consumption.

Materials and Methods: The research object was a 6-wheel Valmet 840.2 forwarder and research was conducted in Forest Administration Vinkovci (lowland part of Croatia) during seeding felling in a stand of pedunculate oak and narrow-leafed ash. For the purpose of measuring fuel consumption on the researched forwarder a differential flow device was installed together with FMS (Fleet Management System) which was used for data transfer.

Results: Fuel consumption is expressed in six different ways concerning: cycle, extraction distance of 100 m, time (hour), load mass (tons), gross load volume (m^3) and product of mass and transporting distance (tkm). Fuel consumption amounted to 0.56 $l{\cdot}tkm^{-1}$ during the extracting of logs and 0.78 $l{\cdot}tkm^{-1}$ during the extraction of energy wood. The results also show an increasing trend of fuel consumption expressed per ton of transported load with the increase of travelled distance during the extraction.

Conclusions: Fuel consumption expressed in $l{\cdot}tkm^{-1}$ is the most accurate fuel consumption indicator because it allows a realistic comparison of different types of machines with different loads (t, m^3) at different extracting distances. The higher fuel consumption of an unloaded forwarder compared to a loaded forwarder can be explained with an increased wheel slip of an unloaded forwarder due to reduced traction between wheels and the soil both in the extraction of logs and energy wood.

Keywords: wood extraction, fleet management system, fuel flowmeter, even-aged forests, fuel consumption, energy wood

INTRODUCTION

Fuel consumption in timber harvesting operations is of economic (costs) and ecological (harmful exhaust gases) importance. Fuel consumption by volume of produced wood may vary depending on ground and operating conditions, driver skills, engine load under working conditions, engine speed, type and technical characteristics of the machine [1].

Determining the influence of certain factors is restricted by the interaction between individual factors. The influence of the machine on fuel consumption is reflected in the technical characteristics of the propulsion engine, the morphological characteristics of the machine, and the way the machine is maintained. The driver influences the fuel consumption by the way of driving, training and caring for the machine's operation [2].

Fuel consumption is mostly influenced by working conditions during timber harvesting operations, which usually include the slope of the terrain, extraction distance, load size and the variety of wood assortments [1].

Rational organisation of timber extraction operations consists of a series of factors that can affect the reduction of fuel consumption and thus the other costs of exploitation as well.

The simplest way to measure fuel consumption of machinery and vehicles is the volumetric method of replenishing tank at the end of the work cycle, work operation or the end of the working day. The filling up of the tank is carried out by using a measuring gauge or by using a measuring gun for accurate measurements [2].

Nordfjell *et al.* [1] state that fuel consumption is traditionally measured by flowmeters [2-4], while new technologies, especially those based on CAN (Controller Area Network) bus technique, enable cheaper and easier measurements because every modern machine already has fuel measuring device as a part of the propulsion engine [5]. The CAN bus technique makes it easier to analyse fuel consumption at a certain moment, which is crucial when defining the most important factors of fuel consumption [6].

Fuel costs reach 10% of the total cost of machine felling and processing in CTL (Cut To Length) method in Canadian forestry [7], while Johansson [8] states that the share of fuel in total costs in Sweden reaches 20% since fuel prices are much higher than in North America. Fuel prices increased considerably at the beginning of this millennium, so at present fuel share in total costs is even higher [6].

Nordfjell *et al.* [1] estimate that fuel consumption of an unloaded forwarder is within the range of 0.23 to 0.38 litres per 100 meters, while fuel consumption of a loaded forwarder is 10% higher in comparison to the unloaded forwarder. According to the results of their research, they state that the average fuel consumption of a forwarder is 13.3 $l \cdot h^{-1}$ when working in the final cut stand, or 10.5 $l \cdot h^{-1}$ in the thinning stand.

Holzleitner *et al.* [9] present the research results of 18 forwarders (6 models) for the period from 2004 to 2008 in Austrian state forests where the average fuel consumption was 11.1 $l \cdot h^{-1}$. Rieppo and Örn [5] report the average fuel consumption of 10.5 $l \cdot h^{-1}$, while Nordfjell *et al.* [1] report that 61-62% of the fuel is consumed during loading and driving of a loaded forwarder. The results of both papers are based on the average values of a large number of researched forwarders in which detailed analyses of the influence of ground surface and fuel system on fuel consumption are neglected [6]. Đuka *et al.* [10] give calculated data on the energy consumption of forwarders according to Athanassiadis [11], who states that in Sweden the average estimated fuel consumption of forwarders is 0.935 $l \cdot m^{-3}$ of roundwood without bark. Ackerman *et al.* [12] state that fuel consumption amounts to 0.38 $l \cdot m^{-3}$ or 13.45 $l \cdot h^{-1}$ when extracting wood assortments from pine stands in South Africa. Suvinen [6] states that steep terrain, and especially driving on uphill slopes when loaded, will naturally increase fuel consumption substantially.

Based on the results obtained by comprehensive field research, Nordfjell *et al.* [1] provide an equation for the calculation of fuel consumption of a 20 to 21-ton forwarder while extracting technical roundwood and pulpwood without bark in conditions of good soil bearing capacity:

$$Q = 0.288 + (0.00638/V) \cdot L \qquad (1)$$

where: Q is fuel consumption ($l \cdot m^{-3}$), V is load volume (m^3), and L is average extraction distance (m).

Löfroth and Rådström [13] point to a reduction of forwarder's fuel consumption in primary timber transport in Sweden, where fuel consumption decreased from 2.5 $l \cdot m^{-3}$ to 1.7 $l \cdot m^{-3}$ between 1985 and 2005. This reduction is attributed to the cooperation between machine manufacturers, users of these machines, and researchers. Machines and working methods of the increasing efficiency have quickly been introduced on a wide scale. As future guidelines for reducing fuel consumption, they introduce: the application of more efficient diesel engines, the increase in the efficiency of the hydraulic system whose efficiency is still under 60%, the incorporation of the CTI (Central Tire Inflation) system, which has a beneficial effect on fuel consumption and soil damages, energy recovery from lowering individual hydraulic crane boom due to gravity, both optimization of export trails and marking of logs in the forest with the help of the GPS devices in order to avoid the search and, of course, further development and improvement of the electric-hybrid forwarder.

A forwarder with an electric-hybrid transmission, compared to the classic hydrostatic-mechanical transmission, has lower fuel consumption of 20 to 50%. Such forwarder is also lighter because of a simpler transmission that does not have a bogie axle. Its mass is 9500 kg, while its carrying capacity is 12,000 kg [14, 15].

The aim of this research was to show the fuel consumption of a Valmet 840.2 forwarder in the extraction of logs and energy wood during seeding felling in a lowland stand of pedunculate oak, narrow-leafed ash and hornbeam.

MATERIALS AND METHODS

Study Area

The research was conducted in Forest Administration Vinkovci, Forest Office Otok, management unit Slavir, sub-compartment 143a (N 45°03'52.2", E 18°53'35.4") during seeding felling in a 134 year-old stand of pedunculate oak, narrow-leafed ash and common hornbeam. The main characteristics of the researched forest stand are flat terrain, 79 m altitude, and average extraction distance 1125 m. A total of 249 trees with the total volume of 536 m^3 were assigned for felling.

In the researched department of 4.91 hectares, felling and processing were made with a chainsaw. Felling was directed to avoid overlapping and hitting the remaining trees. Forest residues were transported by a forwarder, which means that the extraction of energy wood took place after the extraction of logs. Felling and extracting were time-separated. The timber was processed by a buck-to-quality method according to the valid norms, while the crowns were cut in a way to make the loading and transportation of energy wood easier. Average length of logs was 3.5 m (min 2.0 m, max 7.4 m), average diameter 36 cm (min 20 cm, max 66 cm) and average volume 0.4 m^3 (min 0.1 m^3, max 1.6 m^3). The main characteristic of energy wood loads is low density of the load which consists of crown branches of felled trees with a lot of air space between the branches. Energy wood loads are much longer than loads of logs, but because of low density, the average weight of energy wood load is approximately 30% lower than the average weight of a load of logs.

During the extraction of the logs, the ground was covered with a 10 cm thick snow that was melting with every day. The snow has completely melted until the last day of the extraction of energy wood.

The research object was a 6-wheel Valmet 840.2 forwarder shown in Figure 1, whose technical features are shown in Table 1.

For the purpose of measuring fuel consumption on the researched forwarder Valmet 840.2, a differential flow device was installed (Figure 2). This device was installed on the fuel supply line that supplies fuel from the fuel tank to the high-pressure fuel pump on the propulsion engine. Since the pump always supplies more fuel than the engine consumes, a certain amount of fuel is always returned to the tank. To measure the exact amount of used fuel, the return quantity also passes through the measuring device, so a differential correction was made.

The advantage of this measuring device is precise measurement of fuel consumption, and one of the disadvantages is its sensitivity to impurities that may appear in the fuel [16].

With newer engine types with built-in sensors, the installation of this meter may result in a drop in pressure in the fuel supply system, as well as in difficulties in the propulsion engine operation. Instead of this type of meter, for fuel consumption measurement, a metering probe installed in the fuel tank can be used. The main disadvantage of the measuring probe is lower precision than with the differential fuel consumption meter.

FMS (Fleet Management System) was used for data transfer. Its role is to record specific parameters on the vehicle and wirelessly send the recorded data via GPRS or SMS through the monitoring centre to the end user. The end user has insight into vehicle parameters by connecting to the internet using a computer or a mobile phone. The standard parameters that are monitored on almost every vehicle are the current position of the vehicle, fuel consumption, engine speed, etc. The basic component of the FMS is a mobile unit

TABLE 1. Main technical characteristics of Valmet 840.2 forwarder.

Valmet 840.2	
Drive equation	6 x 6
Mass (kg) – declared/weighted*	13,800/16,100
Load carrying capacity (kg)	11,000
Length (mm)	9007
Width (mm)	2640
Height (mm)	3800
Ground clearance (mm)	680
Engine type	Valmet 620 DWRE
Power (kW) at 2 200 rpm	124
Euro emission standard	Euro 2
Torque (Nm)	670 Nm at 1400 rpm
Transmission	Hydrostatic-mechanical
Length of bunk area (mm)	4200
Hydraulic crane type	CRF 7
Maximum crane reach (m)	9.1
Tires: front	600/65 x 34
Tires: rear	600/55 x 26.5

*Declared - listed in the prospectus for the basic model;, weighted – a real mass of the researched forwarder with additional equipment (bigger/stronger hydraulic crane, front pushing board, etc.)

FIGURE 1. Valmet 840.2 forwarder.

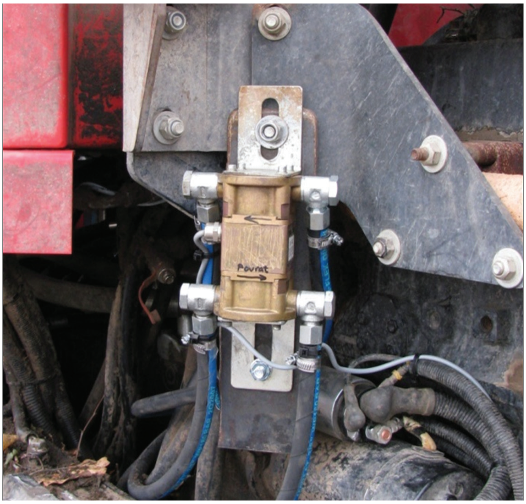

FIGURE 2. Differential fuel EltraTec flowmeter and its technical characteristics (Type: EltraTec – FFS/I; Power supply: 10-30 V; Electricity consumption: 40 mA; Accuracy: ±3%; Protection: IP 65; Minimum fuel flow: 67 ml·min^{-1}; Maximum fuel flow: 3.3 l·min^{-1}).

that is installed in the vehicle and at any time that allows position monitoring via a GPS device (navigation component) which is its integral part. For vehicle communication with the control centre, the mobile unit also has a built-in GPRS modem that represents the communication component. The biggest advantage of the mobile unit is that it can be connected to different measuring transducers which can be used to monitor the operation of the machine. The measuring transducers that were connected to the mobile unit in this study were a flowmeter for measuring fuel consumption and a tachometer for measuring the speed of the propulsion engine. The mobile unit was also connected to forwarder's computer and to one of the solenoid valves of the hydraulic installation through which the hydraulic crane is operated.

Research Methods

In the data obtained from the control centre system, containing the fuel consumption along with the coordinates of the forwarder's position, detection of hydraulic crane operation and the engine speed is shown in dependency of time. By comparing all recorded parameters using the ArcGIS program package the transition (fix) points that represent the transition between individual components of the cycle were determined. Each cycle is divided into four basic components: driving unloaded, loading, driving loaded and unloading. Based on the transition points, fuel consumption was calculated for each of the individual cycle components and finally the total fuel consumption was calculated for each cycle (l·cycle^{-1}).

With the help of the transition points, through calculation, time duration of each of the components of the extraction was obtained, which provided data for the calculation of the total duration of each cycle and hourly fuel consumption (l·h^{-1}).

Using ArcGIS program package, based on the recorded coordinates of the position of the forwarder, the length of the travelled distance was obtained and the fuel consumption was expressed by consumed fuel per 100 m of the travelled distance (l·100 m^{-1}).

During the extraction of logs, the numbers on identification plates of the assortments were noted down on the landing site. Identification plates were put on the front of every log in the forest stand after processing and before extraction. Based on these numbers gross volume (with bark) of each piece of roundwood in each cycle of the forwarder was obtained. The length of the logs was measured with the measuring tape and the mean diameter in the centre of the log with the calliper. Fuel consumption in each cycle of the forwarder was related to the total gross volume of logs, and on this basis, fuel consumption per cubic meter of extracted timber (l·m^{-3}) was obtained.

During the departure of the forwarder from the stand to the forest road, which is also a landing site, a portable measuring platform which is described by Bosner *et al.* [17] for measuring the axle load of the forwarder was installed on stable and flat terrain next to the forest road and near the landing site. For each cycle, the measuring platform was used during the extraction of logs and energy wood. By measuring axle load of an empty and a full forwarder, the mass of the load in each cycle was obtained. With the ratio of the consumed fuel to the load mass in each cycle, fuel consumption per ton of extracted wood (l·t^{-1}) was obtained.

The most accurate way to express fuel consumption is through the ratio of consumed fuel to the travelled distance and to the mass of the extracted wood (l·tkm^{-1}), because consumption is expressed in such a way that it can be compared to other vehicles, regardless of the working conditions. Fuel consumption expressed in l·tkm^{-1} allows a realistic comparison of different types of machines with different loads (t, m^{3}) at different extracting distances.

Statistical analysis was carried out in the StatSoft STATISTICA 8 software package. To make conclusions about the significance of the difference between the two independent data sets, the Student's t-test was used with a level of 5% significance ($p<0.05$). In the study of stochastic dependence between satisfying correlated variables, regression analysis was used. The selection of the equalisation curve was carried out by the coefficient of the correlation (R), t-variable (t-Stat), the probability value (p-value) and regression coefficients.

RESULTS

The fuel consumption of the Valmet 840.2 forwarder was measured during the transport of forest residues and while extracting logs and energy wood from sub-compartment 143a (management unit Slavir) during seeding felling in the stand of pedunculate oak, narrow-leafed ash and hornbeam (Figure 3).

Table 2 shows the average values of fuel consumption for 28 cycles of extracting logs and 24 cycles of extracting energy wood. The fuel consumption is expressed in six different ways concerning: cycle, extraction distance of 100 m, time (hour), load mass (tons), gross load volume (m^{3}) and product of mass, and transporting distance (tkm).

TABLE 2. Fuel consumption of a Valmet 840.2 forwarder.

	Cycles – N	Load mass	Load volume	Total travelled distance	Fuel consumption – Q					
		kg	m³	m	l·cycle^{-1}	l·100m^{-1}	l·h^{-1}	l·t^{-1}	l·m³	l·tkm^{-1}
Logs	28	12317^{a}	13.37	2272^{a}	15.61^{a}	0.69^{a}	17.36^{a}	1.29^{a}	1.18	0.56^{a}
En. wood	24	8468^{b}		2235^{a}	14.81^{a}	0.66^{a}	15.17^{b}	1.74^{b}		0.78^{b}

$^{a\ b}$ values in the same column marked with the same letter do not represent statistically significant difference according to t–test ($p<0.05$)

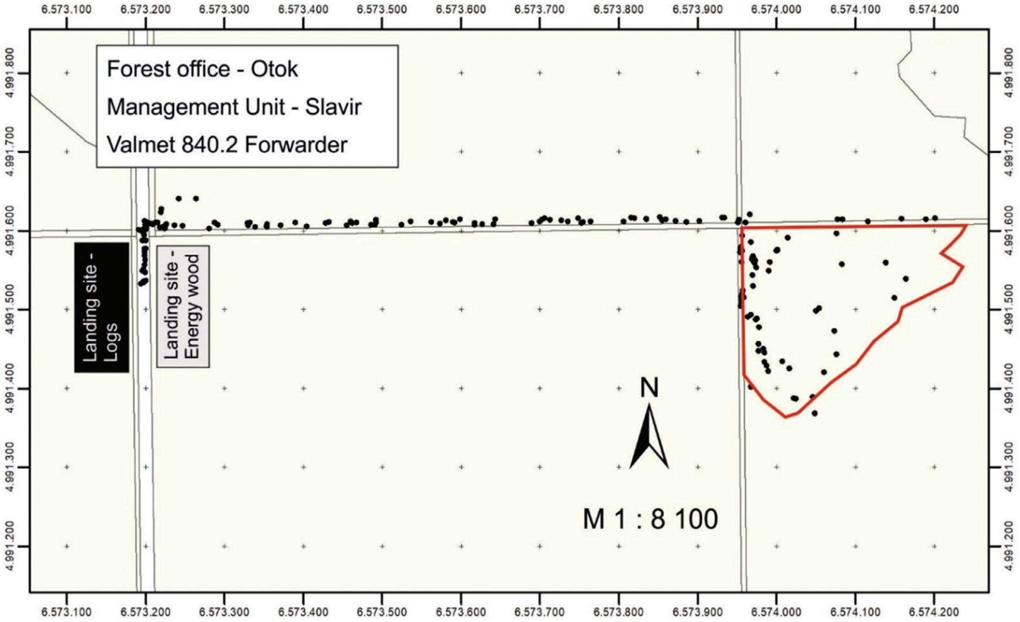

FIGURE 3. Sub-compartment 143a with positions of landing sites for roundwood, long-meter firewood and energy wood.

In the case of fuel consumption per cycle, higher consumption was measured during the extraction of logs (15.61 l·cycle^{-1}) than when extracting energy wood (14.81 l·cycle^{-1}), but according to statistical analysis using t-test, there is no significant difference in consumption per cycle between these two variants with a probability of 95%.

Regarding fuel consumption per distance of 100 m, there is no significant difference between the consumption during the extraction of logs and energy wood, but consumption is also higher during the extraction of logs (0.69 l·100 m^{-1}) than during the extraction of energy wood (0.66 l·100 m^{-1}).

Calculated hourly fuel consumption is higher while extracting logs (17.36 l·h^{-1}) and statistically differs from hourly consumption while extracting energy wood (15.17 l·h^{-1}).

The reason for higher fuel consumption while extracting logs can be attributed to the higher mass (and also the density) of the load, which averaged in 12,317 kg and which is statistically different from the average mass of energy wood (8468 kg), since the extraction distance for logs (2272 m) and energy wood (2335 m) is not statistically different.

Fuel consumption per metric tons of transported load is higher when extracting energy wood (1.74 l·t^{-1}) and statistically differs from fuel consumption when extracting logs (1.29 l·t^{-1}). The reason for the higher fuel consumption during the extraction of energy wood expressed in this way is the lower average mass of energy wood load as compared to the load mass of logs.

Fuel consumption per gross volume of the load when extracting roundwood is 1.18 l·m^{-3}. According to the fuel consumption expressed in l·tkm^{-1}, the value of 0.56 l·tkm^{-1} was achieved while extracting logs from sub-compartment 143a, while fuel consumption during the extraction of energy wood from the same sub-compartment was 0.78 l·tkm^{-1}. These two values statistically differ.

The diagrams in Figure 4 show an increasing trend of fuel consumption expressed per ton of transported load with the increase of travelled distance during the extraction of logs (Figure 4a) and energy wood (Figure 4b). The increasing trend is the same while extracting both the logs and energy wood, but the amount of fuel consumption is higher when it comes to energy wood extraction because of the reduced load mass. Due to large dispersion of fuel consumption data expressed per tons of transported load, low coefficients of determination were obtained (in the case of extracting the logs R^2=0.13 and of extracting energy wood R^2=0.06). As expected, there is no dependence between fuel consumption and extraction distance.

According to Table 3, the biggest share in forwarders' fuel consumption is when driving unloaded. In both of the stated components the share of spent time is the same (driving unloaded: logs – 16.8% and energy wood – 17.8%; driving loaded: logs – 19.5% and energy wood – 20.9%), so the share of fuel consumption in these components is similar. The shares of travelled distance in the mentioned components are similar as well. Together they make over 80% of the total travelled distance of an average cycle. In this case, the travelled distance has a greater impact on the total fuel consumption than the time duration of the components of wood extraction cycle.

In the case of a loading component, the share of fuel consumption while loading energy wood (27.2%) was higher than while loading logs (17.2%). While loading energy wood, the share of time spent is also higher (42.3%) as compared

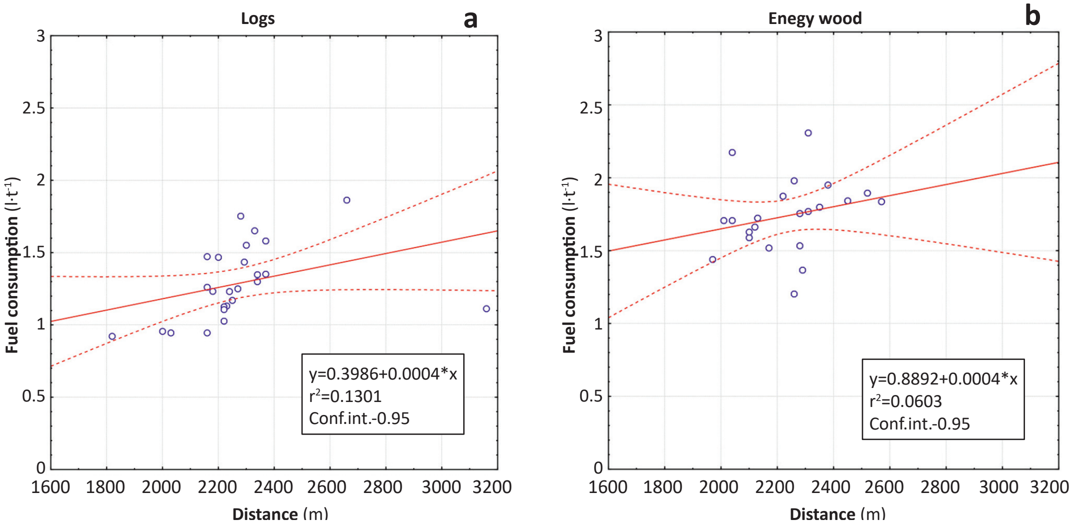

FIGURE 4. Dependence of fuel consumption per load mass on the total driving distance.

TABLE 3. The shares of fuel consumption by components of the working cycle.

	Share of time (%)		**Share of fuel consumption** (%)		**Share of driven distance** (%)		**N hydraulic crane engagement**	
	Logs	En. wood	Logs	En. wood	Logs	En. wood	Logs	En. wood
Driving - unloaded	16.8	17.8	30.2	32.3	41.2	42.5		
Loading	29.0	42.3	17.2	27.2	7.7	9.8	99	163
Driving - loaded	19.5	20.9	28.2	28.2	42.5	44.4		
Unloading	34.7	19.0	24.4	12.3	8.6	3.4	163	78

to time spent while loading logs (29%). The reason for the higher fuel consumption and the amount of time spent when loading energy wood is the higher number of electro-valve engagements for the displacement of the hydraulic crane (163) or the higher workload of the hydraulic crane as compared to loading logs where on average 99 engagements were detected. The reason for the increased number of hydraulic crane engagements while unloading logs is of an organizational nature. Logs were subsequently measured at the landing site in order to control the measurements previously done in the stand. Thus, it can be assumed that the number of hydraulic crane engagements (163) was in fact three times higher than it would be in normal working circumstances, which would amount to around 55 engagements. In that case, the workload of the hydraulic crane would be lower both while loading and unloading logs as compared to loading and unloading energy wood.

DISCUSSION WITH CONCLUSIONS

The reasons for the increased fuel consumption of the researched forwarder, compared to the results of previous studies, are unfavourable conditions during extraction (snow melting) (Figure 5) which led to increased wheel slip (15.81% - measured in the stand), and a large share of driving on wet trails (during extraction of logs and energy wood 74% and 75.6% respectively of the total travelled distance on a trail). The higher fuel consumption of an unloaded forwarder as compared to a loaded forwarder (Table 3) can be explained with an increased wheel slip of an unloaded forwarder due to a reduced traction between wheels and the soil in both the extraction of logs and energy wood.

According to Nordfjell *et al.* [1], the parameters of calculation are based on the load volume in cubic meters and the average extraction distance in meters. The calculated fuel consumption of the investigated forwarder was 0.77 $l \cdot m^{-3}$, while the measured consumption was 1.18 $l \cdot m^{-3}$. The reason for such significant deviation is due to the greater average extraction distance (1136 m), while Nordfjell *et al.* [1] conclude that the term is suitable for an average extraction distance of about 400 m.

The diagrams in Figure 6 show an increasing trend of fuel consumption expressed per ton of transported load with the increase of travelled distance and the decrease of productivity during the extraction of logs (Figure 6a) and energy wood (Figure 6b). The increasing trend is the same while extracting both the logs and energy wood, but the amount of fuel consumption is higher when it comes to energy wood extraction because of the reduced productivity. Lower productivity is a consequence of the reduced load mass during energy wood extraction. Equations of fuel consumption dependence per load mass on the total driving distance and productivity are noted in diagrams in Figure 6a for extracting logs and Figure 6b for extracting energy wood.

Fuel consumption expressed in l·tkm^{-1} is the most accurate fuel consumption indicator because it allows a realistic comparison of different types of machines with different loads (t, m^3) at different extracting distances. This claim has been confirmed by the results of this research.

FIGURE 5. The state of the trail during extraction with the Valmet 840.2 forwarder.

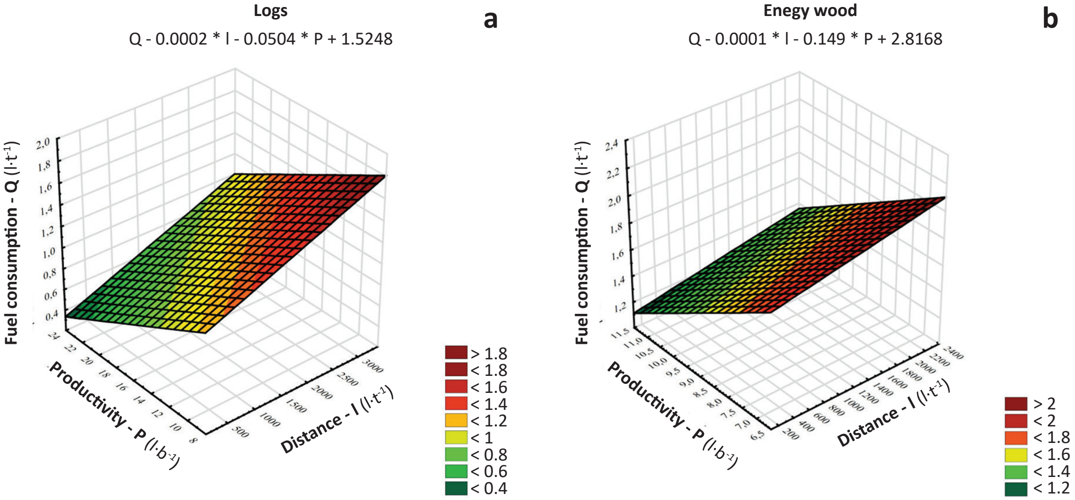

FIGURE 6. Dependence of fuel consumption per load mass on the total driving distance and productivity.

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Monitoring the Health Status of Trees in Maksimir Forest Park Using Remote Sensing Methods

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ABSTRACT

Background and Purpose: In Zagreb, the forests have assumed the characteristics of forest parks, of which the most famous and the most visited is Maksimir Park. The health condition of many trees has deteriorated due to environmental changes and the forest's age. The tree health status can be monitored by direct observation in the field using the terrestrial method, or by remote sensing methods. Recent advances in technology have made it possible to use high-resolution satellite imagery to monitor the damage.

Materials and Methods: In order to estimate the health status of trees, the research area - Maksimir Forest Park - was visually interpreted by means of the WorldView 2 (WV2) satellite image, color composite (8, 5, 3). Such color composite was also used to delineate the strata. After delineation, a systematic sample of 25x25 m was laid over the stratum to help interpret the tree health status. Differences in tree damage were observed by comparing the WV2 images and other satellite images (Google Earth images from 2014 and 2016) recorded at different points in time. Field research was then conducted in order to inventory the current situation.

Results: The main results of the research in Maksimir Forest Park include the calculation of damage indicators by tree species, as well as thematic maps with the spatial distribution of the mean damage (SO) and damage index (IO) for those tree species which are most represented in a particular stratum. Mean and significant damage of the most represented tree species and overall is also presented spatially in the form of thematic maps. A comparison of the results of WV2 satellite imagery and satellite images taken from Google Earth denoted a change in the tree health status, which confirms that satellite imagery can serve to inventory and track the condition in an area over a number of years. Field investigations and the assessment of tree health status confirmed the results obtained by satellite imagery interpretation.

Conclusions: Since quality forest monitoring is based on systematic collection of forest data in any area or at any point in time, the obtained results not only represent the current health status, but also provide a basis for monitoring and predicting the future conditions.

Keywords: WorldView 2 satellite image, visual interpretation, monitoring, the health status of trees, urban forest, GIS

INTRODUCTION

With the rapid development and expansion of urban areas, as well as with a growing share of the population living in such areas, the preservation and protection of green spaces is increasingly gaining in importance. Zagreb is among the very few European capitals with well preserved natural forests which are incorporated into the urban tissue of the city itself [1]. Such forests play an exceptional role in the life of a society which is being intensively urbanized [2]. In particular, this applies to recreational activities [3-5], the reduction of air pollution [6, 7], microclimate regulation [8] and erosion reduction [9]. In Zagreb, forests have assumed the characteristics of forest parks, of which the most famous and the most visited is Maksimir Park. In addition to being a widely used recreational area, it is also an ecological stronghold that offers a variety of benefits. It was the first public park in southeastern Europe and one of the first such parks in the world [10]. Since its foundation the forest has undergone numerous changes, but it still remains a unique set of natural forest elements. In order to retain the stability of the forest ecosystem it is necessary to perform various regeneration and tending operations and maintain the forest in an optimal condition so that it continues to provide all of its non-market functions. The health condition of many trees has deteriorated due to environmental changes and the forest's age. The tree health status can be monitored by direct observation in the field using the terrestrial method [11, 12], or by remote sensing methods, most commonly by interpreting color infrared (CIR) aerial images [13, 14]. Recent advances in technology have made it possible to use high-resolution satellite imagery to monitor the damage. Remote sensing methodology reduces the amount of necessary field work and cuts down on expenditures [15]. This paper shows how visual interpretation of the health status of individual trees is performed on the WorldView 2 satellite image.

MATERIALS AND METHODS

The survey was conducted in the Maksimir Forest Park on an area of 168.40 hectares. In order to estimate the health status of trees, the research area - Maksimir Forest Park - was visually interpreted by means of the WorldView 2 (WV2) satellite image, color composite (8, 5, 3). The combination of bands 8, 5, 3 was chosen so as to clearly distinguish tree species, vegetation types, physiological conditions, developmental stages etc. Damage indicators were determined with the color composite structured in this manner. Such color composite was also used to delineate the strata (Figure 1). The strata were differentiated using visible differences in image details, such as tree species, mixture ratio, stand closure, crown size, the degree of damage etc. The stage of tree damage was assessed according to a determined percentage of assimilation organ absence (leaves), the percentage of yellow leaves and the percentage of branch decline. After delineation, a systematic sample of 25x25 m was laid over the stratum to help interpret the tree health status (Figure 2). At each point of a grid (2982 points), 2 nearest trees (crowns) were estimated and damage indicators (damage - O, mean damage - SO, damage index - IO, mean damage$_1$ - SO$_1$) [16] were calculated for individual tree species, and for all the interpreted species together.

The interpretation results were used to construct thematic maps of the spatial distribution of damage [17]. The thematic maps of the spatial distribution of damage have made it possible to distinguish the most damaged strata in relation to certain tree species. Satellite images available on Google Earth were also used to monitor the health status of trees in Maksimir forest area. Differences in tree damage were observed by comparing the WV2 images and other satellite images (Google Earth) recorded at different points in time (from 2014 and 2016). This way it was possible to identify all the changes that occurred during a certain period of time. Areas with more severe tree damage were determined by using thematic maps and the selected strata and by comparing the satellite images. Stratum 28 containing the largest number of damaged oak trees was delineated in the study area. The changes observed on the trees in the images are marked with circles (Figure 3, 4, 5). Field research was then conducted in order to inventory the current situation.

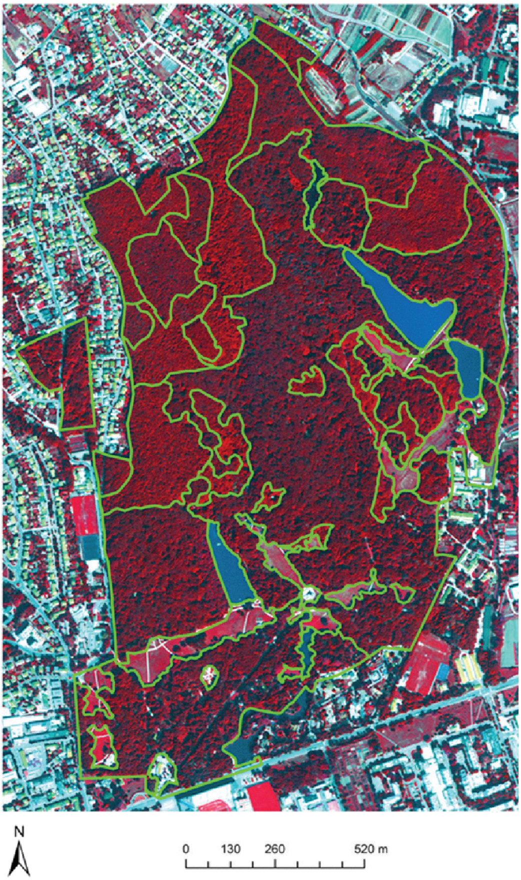

FIGURE 1. The research area - Maksimir Forest Park (with delineated strata).

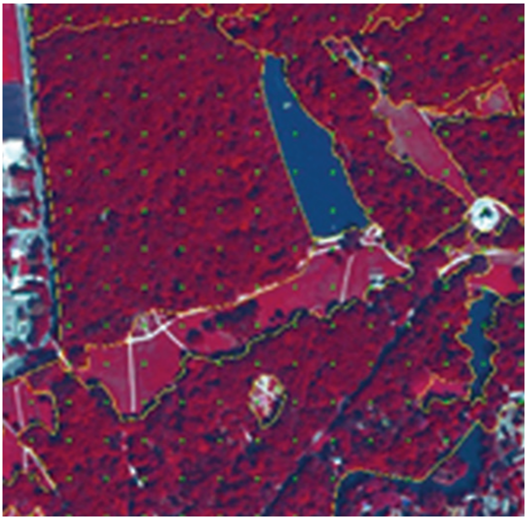

FIGURE 2. The clip of color composite (8, 5, 3) with a systematic sample.

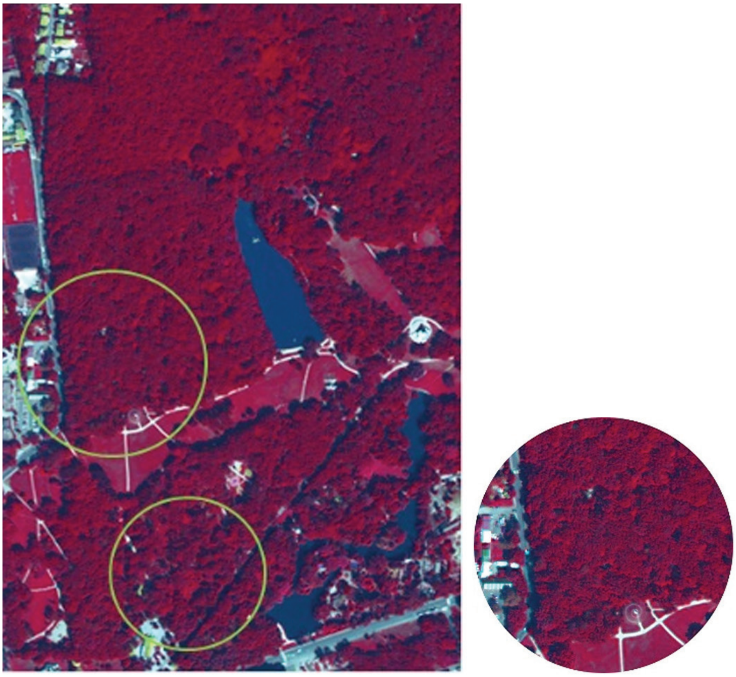

FIGURE 3. WorldView 2 satellite image from 2011 with marked areas of significant damage to oak and a fragment from the Stratum 28.

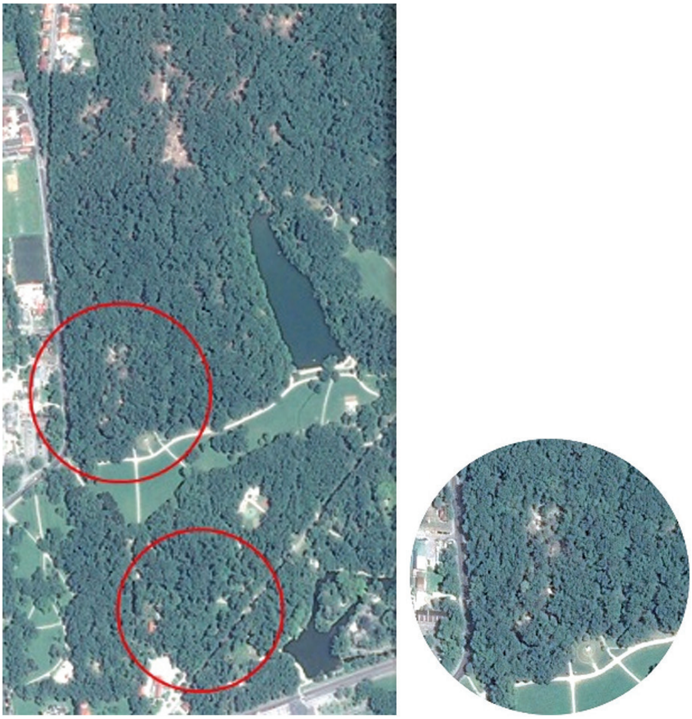

FIGURE 4. Satellite image (Google Earth) from 2014 with marked areas of significant oak damage and a fragment from Stratum 28.

FIGURE 5. Satellite image (Google Earth) from 2016 with marked areas of significant oak damage and Stratum 28 fragment.

In addition to surveying the selected areas, the position was identified in the field with a GPS device. Photo-documentation was recorded for subsequent comparison with the results obtained by the interpretation of the recording. The field survey consisted of 6 locations (Figure 6), which were marked as critical with regard to the damage and health condition of the observed oaks.

RESULTS AND DISCUSSION

The main results of the research in Maksimir Forest Park include the calculation of damage indicators by tree species, as well as thematic maps with the spatial distribution of the mean damage (SO) and damage index (IO) for those tree species that are most represented in a particular stratum. Data were also obtained for the surfaces of individual strata. A total of 5,064 individual trees were photo-interpreted in the systematic 25x25 m sample (2982 points) in the recorded area. The most represented tree species in the sample was oak (4,403 trees), followed by hornbeam (404 trees) and black locust (76 trees). In the entire recorded area, the highest mean damage (SO) and the highest damage index (IO) were recorded for oak. Taking into account all the interpreted trees in the recorded area, mean damage can be estimated at 39.76%, and significant damage (IO) at 70.69% (Table 1). In the sample of 70.69% of the trees classified as 2.1 and higher, mean damage (SO_1) amounts to 50.16% and can be classified as 2.2 degree of damage. Hence, the stands in Maksimir area can be assigned to the category of moderately to severely damaged stands. Mean and significant damage of the most represented tree species (oak) and overall is also presented spatially in the form of thematic maps (Figure 7).

Validation of the tree condition in the field produced the results that represent the current health status of the investigated area. Field investigation, conducted in May 2017, comprised areas of significant oak damage in Maksimir Forest Park. Photo-documentation made on this occasion showed that in Location 1 the majority of badly damaged trees were removed and the area was restored by planting seedlings. Area 1 is located in Stratum 28, in which interpretation comprised the largest number of damaged oak trees. In Area 3, located in Stratum 31, the damaged and diseased trees were replaced with new, young trees (Figure 8).

This can be confirmed by the available literature. Namely, Glavaš [18] states that about 1,000 trees in Maksimir Forest Park were destroyed by a storm. The restoration of the affected areas began in 2014, when new

FIGURE 6. Field investigation in digital orthophoto (GPS tracking route).

trees were planted [19]. A comparison of the results of WV2 satellite imagery and satellite images taken from Google Earth (from 2014 and 2016) denoted a change in the tree health status, which confirms that satellite imagery can serve to inventory and track the condition in an area over a number of years. Field investigations and the assessment of tree health status confirmed the results obtained by satellite imagery interpretation, but also indicated that the future condition of stands can be predicted on the basis of the images [16, 20]. Images taken in different years are a good starting point for predicting the condition in the field for a certain period, which is a prerequisite for good planning.

TABLE 1. Damage indicators by tree species (O - damage; IO - damage index (significant damage); SO - mean damage; SO_1 - mean damage of significantly damaged trees).

Tree species	Damage indicators			
	O	IO	SO	SO_1
	%			
Oak sp.	99.68	79.33	43.58	50.42
Common Hornbeam	37.62	0.00	9.70	
Black locust	85.53	51.32	24.54	34.74
Common beech	48.48	0.00	11.06	
Spruce sp.	80.65	41.94	32.82	59.81
Pine sp.	100.00	64.71	31.84	39.66
Other*	56.63	15.66	14.43	32.50
Total	93.36	70.69	39.76	50.16

*(maple, fruit trees, lime tree, alder, poplar...)

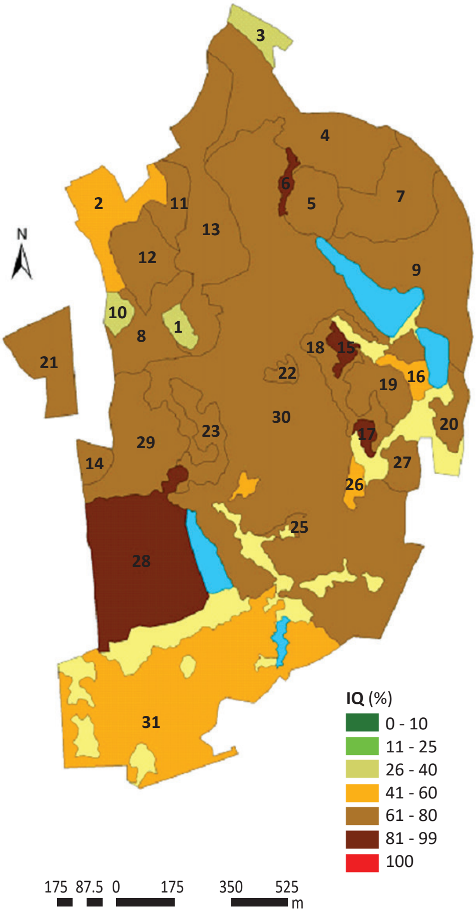

FIGURE 7. Total Damage Index for all tree species (IO - significant damage) - Maksimir Forest Park.

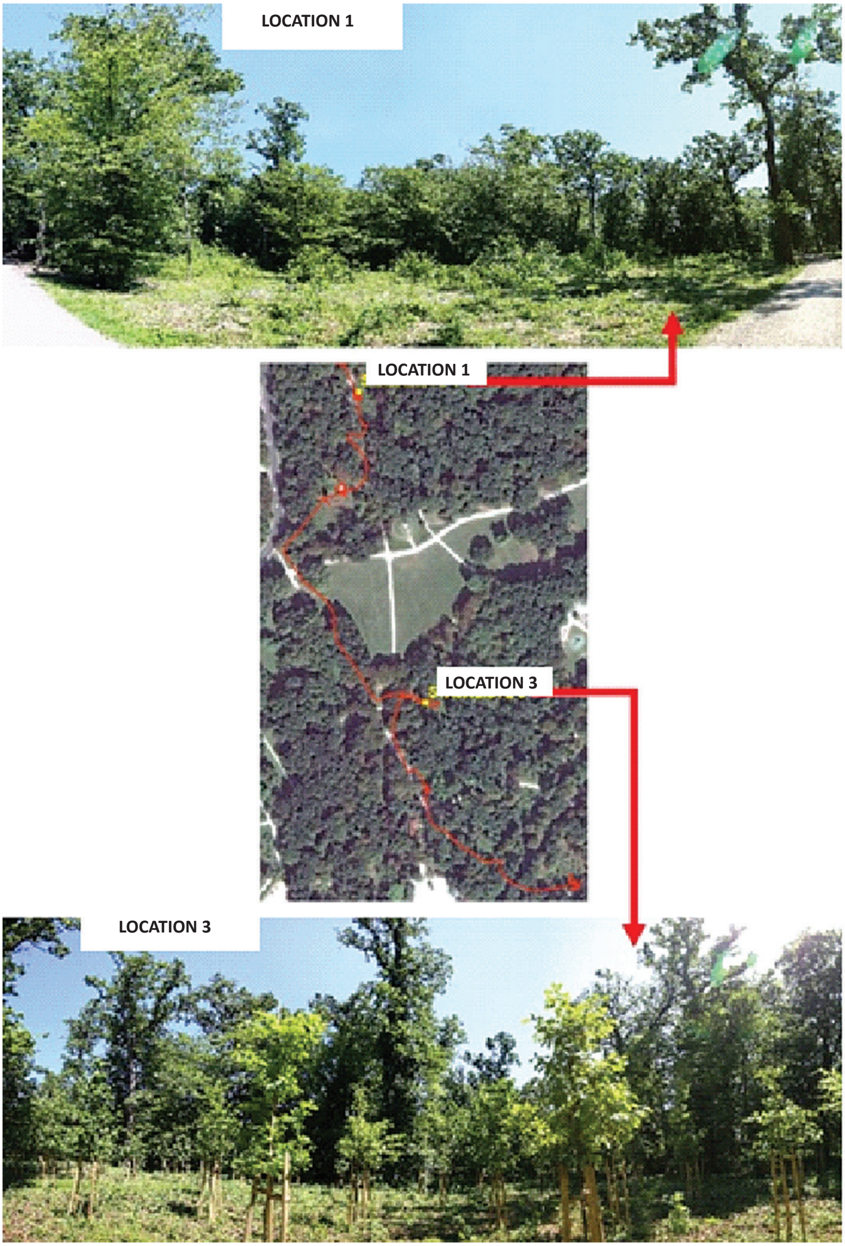

FIGURE 8. Locations 1 and 3.

CONCLUSIONS

A survey conducted by means of the WorldView 2 satellite image showed the current health status of the most common tree species in Maksimir Forest Park. Monitoring tree health by interpreting satellite images enables rapid and economic data acquisition. Since quality forest monitoring is based on a systematic collection of forest data in an area or at any point in time, the obtained results not only represent the current health status, but also provide a basis for monitoring and predicting the future conditions. In urban environment, it is of particular importance to make timely predictions of the changes in tree health status so that diseased trees can be removed and replaced with new ones.

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5. MATOŠEVIĆ D 2013 Box Tree Moth (*Cydalima perspectalis*, Lepidoptera; Crambidae), New Invasive Insect Pest in Croatia. *South-East Eur For* 4 (2): (early view). URL: http://www.seefor.eu/36-vol4-no2-matosevic.html (20 December 2013)

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6. ANČIĆ M, PERNAR R, BAJIĆ M, SELETKOVIĆ A, KOLIĆ J 2013 Detecting mistletoe infestation on Silver fir using hyperspectral images. *iForest* (early view). DOI: http://dx.doi.org/10.3832/ifor1035-006

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8. VAN LAAR A, AKÇA A 2007 Forest Mensuration. Springer, Berlin, Germany, 383 p

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  14. FAO 2012 State of the World's Forests 2012. Food and Agriculture Organization of the United Nations, Rome, Italy, 47 p. URL: http://www.fao.org/docrep/016/i3010e/i3010e.pdf (12 December 2013)
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  15. THE MINISTRY OF AGRICULTURE, FORESTRY AND WATER MANAGEMENT 2006 Regulation on forest management (*in Croatian*). *Official Gazette* 111/06, Zagreb, Croatia. URL: http://narodne-novine.nn.hr/clanci/sluzbeni/128205.html (10 December 2013)

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