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#### Supplementary material:

FigureS1.ThedesignoftheexperimentFigureS2.AcornsowingonpredefineddistancebetweentheacornsTableS1.Chemical propertiesofthe analyzed soilTableS2.SoiltextureclassesTableS3.Results of Tukey test forthe differencesbetween the meanvalues of acorn size and growthelements of different oak species

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# Growth characteristics of one-year-old seedlings of three autochthonous oak species in suboptimal growing conditions

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## Abstract

The paper presents the growth characteristics of one-year-old seedlings of three autochthonous oak species (Fagaceae family): Turkey oak (Quercus cerris L.), Hungarian oak (Quercus frainetto Ten.) and downy oak (Quercus pubescens Willd.) in suboptimal growing conditions. The analyzed oak species are widely distributed in Serbia. One of the most important benefits of oak species is wood production (Hungarian and Turkey oak), and they have a promising role for the use in the future regarding the climate change as all species can be encountered on dry sites. Acorns were collected in the autumn of 2017 in natural stands and a sample of 400 acorns per each oak species was immediately sown in a nursery seedbed in the form of a random block system with four replicates. At the end of 2018, the available sample of seedlings was analyzed morphometrically. The seedlings were classified according to the number of shoot growth flushes into one-flush-growth and multi-flush growth seedlings. Turkey Oak had the highest number of seedlings which also recorded higher values in total height, root collar diameter and number of leaves. These results were obtained on alkaline soil with the presence of weeds and rodents and the absence of irrigating. The presence of multi-flush growth was recorded in all three oak species.

Differences between analyzed three oak species exist in these suboptimal conditions. However, in full light conditions, a similar growth pattern was recorded, indicating to the similar adaptability of the species.

### Keywords

Quercus; Seedlings; Light conditions; Alkaline soil; Acorn size; Multi-flush growth; Reforestation

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## **1** Introduction

The knowledge of growth characteristics of one-year-old plants is important from several aspects in forestry. From the aspect of species bioecology, the growth characteristics show the reaction of plants to growing conditions. In the forestry stand conditions that are under certain anthropogenic impact the growth characteristics include a wider norm of reaction in plants and are considered from a silvicultural standpoint (Bobinac 2011). Otherwise, in controlled conditions of cultivation they include a narrower norm of reaction and are predominantly considered from the viewpoint of optimal reaction (Ivetić 2013; Ivetić et al. 2016).

Multi-flush growth during the growing season (polycyclism) is a characteristic trait for genus *Quercus* L. (Bobinac et al. 2012). In Serbia, the reported characteristics of multi-flush growth of one-year-old plants of autochthonous oak species can be examined on the basis of previous research carried out on pedunculate oak (Bobinac 1994, 1999, 2011; Bobinac and Vilotić 1994; Pap et al. 2013), Turkey oak (Bobinac and Vilotić 1997, 1998; Bobinac 1997, 2001, 2002), sessile oak (Krstić et al. 2018) and Hungarian oak (Šušić 2017; Šušić et al. 2019). Downy oak, as a rare and endangered species (Šijačić-Nikolić et al. 2016), whose status is highly unfavorable in Serbia (Banković et al. 2009), and is potentially important for wider use in terms of emerging climate change (Pasta et al. 2016), is almost unexplored in literature in Serbia, when it comes to juvenile phases of development.

The paper presents the results of the comparative research on the growth characteristics of one-year-old seedlings of three autochthonous oak species: Turkey oak (*Quercus cerris* L.), Hungarian oak (*Quercus frainetto* Ten.) and downy oak (*Quercus pubescens* Willd.) in suboptimal growing conditions under full light.

## 2 Materials and methods

Acorns of downy oak (*Quercus pubescens* Willd.), Hungarian oak (*Quercus frainetto* Ten.) and Turkey oak (*Quercus cerrs* L.) were collected in November 2017 in natural stands. Acorns of downy oak were collected in the area of Belgrade in Management Unit Košutnjak, whose wider area is characterized by the zonal community at the southern boundary of Pannonian Plain (*Quercetum cerridis-virgilianae* Jov. et. Vuk. 77). Acorns of Turkey oak and Hungarian oak were collected in the area of Kraljevo, whose wider area is characterized by the zonal forest of Hungarian oak and Turkey oak (*Quercetum frainetto-cerridis* Rudski 1949 *s.l*).

The acorns were sown in a seedbed in the nursery of the Faculty of Forestry in Belgrade in suboptimal cultivation conditions. The climate of Belgrade is humid continental with Aridity index of 0.790 (Gocić and Trajković 2013). According to the data from the Republic Hydrometeorological Institute of Serbia

for the period 1981–2010, the average air temperature in the growing season is 19.3°C, while the amount of precipitation during the growing season is 391.9 mm. In the 2018 growing season, the mean air temperature was 2.1°C higher, and the amount of precipitation was 45.8 mm lower compared to the averages from 1981–2010 period (Table 1).

			•		0		
Observation Period	Т [ºC]	T <sub>annual</sub> [ <sup>0</sup> C]	Tgrowing season [ <sup>0</sup> C]	P <sub>annual</sub> [mm]	P <sub>growing</sub> season [mm]		
	Average	15.2	21.4				
2018	Min	-7.8	4.4	572.1	346.1		
	Max	34.7	34.7				
	Average	12.5	19.3				
1981–2010	Min	-18.2	-3.4	690.9	391.9		
	Max	43.6	43.6				

Table 1. Basic climate parameters for the 1981–2010 period and in 2018 in the area of Belgrade.

For the analysis of the soil conditions, a sample of soil at a depth of 0–10 cm and 10–30 cm was taken within each block. The soil in the nursery is loam to clay loam with considerable content of total clay and a relatively high pH value ranging from 7.14 to 7.24 in KCl and 7.49 to 7.67 in H<sub>2</sub>O. The high pH values and the percentage of total clay are pointing out to the soil degradation. According to the results reported by lvkov (1960), for the same site during the 1950s, when the soil was considered to be an eroded eutric cambisol on loess bedrock, the pH value was neutral in the surface layer and at a depth of 10-20 cm it was 6.08 in KCl and and 6.20 in H<sub>2</sub>O. The soil is well secured by available potassium and phosphorus (Tables S1 and S2).

Morphometric characteristics of acorns were measured before sowing for all three species (150 acorns per species). The diameter and length of acorns were measured as indicators of acorn size (Roth et al. 2011). Acorns sowing were done in the form of a random block system with four replicates (Hadživuković 1991). Sowing of 100 acorns, that were previously ocularly assessed as healthy, was carried out in each of the 12 plots (3 types × 4 replicates, Figure S1), so that a total of 400 acorns of each species were sown. The acorn sample of all species was homogenized by removing the largest and smallest acorns, and by sowing at the same distance (Figure S2).

During the growing season, weeding was carried out on three occasions: in the spring at the stage of germination of acorns, mid-year, and just before the end of the growing season, so the seedlings have mostly developed in weedy conditions. The seedlings were not watered so the amount of available water in the soil depended only on the amount of precipitation.

At the end of the 2018 growing season, the number of one-year-old seedlings was determined for each species. The total height and root collar diameter were measured and the total number of leaves counted. On the basis of the number of shoot growth flushes on the seedlings, the height growth type was defined for each seedling (Bobinac 1997). The total height of multi-flush growth seedlings was determined as the sum of all growth flushes on a seedling. The growth flushes determination was based on the number of scars of juvenile buds on the annual axis in the way described by Bobinac (1994, 2001). Root collar diameter was measured just above the cotyledons scars, and the total

number of leaves was obtained by summing the number of leaves from each flush.

The morphometric characteristics of acorns and growth elements of the seedlings were calculated using the standard elements of descriptive statistics. The testing between the means of acorn size parameters between the species and growth elements between the species and between different height growth types of the seedlings was performed using the Tukey test in the statistical program JASP (2018).

# 3 Results

The conducted morphometric analysis of acorn size (Table 2) showed that Turkey oak acorn is larger, i.e. it is both wider and longer, compared to other two oak species. The mean values of acorn diameter and length differ significantly between all the species at the p < 0.01 (Table S3).

	Table 2	. The morphomet	tric parameters of a	corn size of differ	ent oak species.						
	Quercus pub	bescens L.	Quercus	cerris L.	Quercus frainetto Ten.						
	<i>n</i> = 150 pcs										
	Acorn diameter	Acorn length	Acorn diameter	Acorn length	Acorn diameter	Acorn length					
			[mn	n]							
$\overline{\mathbf{X}}$	10.7	20.0	16.4	30.3	12.2	25.1					
$S_d$	1.0	2.8	1.2	2.8	1.3	2.6					
Min	7.9	13.2	13.5	22.1	9.2	17.6					
Max	13.7	25.7	20.6	40.9	15.3	32.8					
CV%	9.2	13.8	7.1	9.3	10.8	10.5					

Compared to the number of sown acorns in the autumn of 2017, at the end of the growing season 2018, the largest number of plants was recorded in Turkey oak (52%) while only 16% of the plants was found in Hungarian oak (Table 3). Different height growth types were recorded within the available number of seedlings: one-flush growth, two-flush growth, three-flush growth and four-flush growth type.

In all oak species, one-flush growth type seedlings were mostly represented: 76.0% in Hungarian oak, 57.2% in Turkey oak and 55.5% in downy oak. Three-flush growth type seedlings were recorded in Turkey oak and downy oak, and four-flush growth was recorded on only one seedling of downy oak (Table 3). The occurrence of the multi-flush growth during the growing season and the different height-growth types in the same growing conditions are shown on Figures 1 and 2.

The multi-flush growth seedlings have higher values of the analyzed growth elements in all oak species. The data suggest that Turkey oak turned out to be more vigorous compared to Hungarian and downy oak: the largest number of seedlings was recorded in Turkey oak; as well as the highest total height and root collar diameter and the highest percentage of multi-flush growth seedlings (Table 4). The differences are statistically significant regarding the growth elements (Table S3).

Table	3. The perc	entage of diffe	rent height-grov	vth types in the	total number of plants
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	Quercus pubescens L.		Quercus cerris L.		Quercus frainetto T		
	[pcs]	[%]	[pcs]	[%]	[pcs]	[%]	
Number of sown acorns in the autumn of 2017	400	100	400	100	400	100	
Number of seedlings in the autumn of 2018	128	32.0	208	52.0	64	16.0	
One-flush growth type	71	55.5	119	57.2	49	76.6	
Two-flush growth type	47	36.7	85	40.9	15	23.4	
Three-flush growth type	9	7.0	4	1.9	/	/	
Four-flush growth type	1	0.8	/	/	/	/	

Table 4. Growth characteristics of analyzed oak species

			Quercus	pubescen	s L.	Quero	cus cerris L		<i>Quercus frainetto</i> Ten.							
			Drc [mm]	Ht [cm]	LN	Drc [mm]	Ht [cm]	LN	Drc [mm]	Ht [cm]	LN					
		$\overline{\mathbf{X}}$	2.9	10.5	6.6	3.7	13.0	6.3	2.4	8.5	4.4					
		Sd	0.8	3.7	3.9	0.8	4.8	3.3	0.6	1.8	1.7					
Tot	al	Min	0.5	3.2	2.0	0.8	4.7	2.0	1.1	3.9	2.0					
		Max	5.3	27.0	27.0	6.5	34.3	22.0	3.5	14.2	10.					
		CV%	28.6	35.5	58.8	20.7	37.3	52.6	26.0	21.6	37.					
		$\overline{\mathbf{x}}$	2.6	9.0	4.6	3.5	10.9	4.3	2.3	8.2	3.7					
		Sd	0.7	2.9	2.3	0.7	2.5	1.5	0.6	1.7	1.0					
	1F	Min	0.5	3.2	2.0	0.8	4.7	2.0	1.1	3.9	2.0					
		Max	4.2	18.4	16.0	5.1	16.4	12.0	3.5	11.3	6.0					
Эe		CV%	27.5	32.7	49.1	19.5	22.8	34.5	26.6	20.5	26.					
Height-growth type		$\overline{\mathbf{X}}$	3.1	11.4	8.0	3.9	15.7	8.8	2.4	9.5	6.7					
۲h		Sd	0.8	2.5	2.5	0.8	5.5	2.9	0.6	2.0	1.3					
õ	2F	Min	1.6	5.2	4.0	2.1	6.1	4.0	1.6	6.3	5.0					
lt-g			<u>م</u>	)		2	Max	5.3	17.1	16.0	6.5	34.3	20.0	3.4	14.2	10.
<u>ë</u>		CV%	25.6	22.2	31.4	19.3	35.4	33.4	24.9	21.1	19.					
Η̈́		$\overline{\mathbf{X}}$	3.6	16.9	14.5	4.4	19.1	13.3								
		$S_d$	1.0	5.3	5.7	1.4	9.1	6.0								
	3F	Min	1.9	10.8	7.0	3.0	8.5	9.0								
		Max	5.3	27.0	27.0	5.8	30.1	22.0								
		CV%	27.1	31.7	39.0	32.3	47.8	45.0								

Legend: 1–3F—Height-growth types according to the number of flushes; Drc—root collar diameter; Ht—total height; LN—number of leaves

The growth elements differ significantly between one-flush and multiflush growth seedlings. The only exception is the root collar diameter in Hungarian oak that doesn't differ significantly between these two groups of seedlings. The differences between the values of growth elements are less pronounced when observed between two-flush and three-flush growth seedlings. In downy oak, the three-flush growth seedlings have significantly higher values of total height and number of leaves, and no significant differences were found in root collar diameter values, compared to two-flush growth seedlings. In Turkey oak seedlings, the only significant difference was found for the means of the number of leaves between two-flush and three-flush growth seedlings (Table 5).



Figure 1. The occurence of multi-flush growth in downy oak during the growing season.



Figure 2. Different height-growth types in Turkey oak at the end of the growing season.

Table 5. The results of Tukey test for the mean values of different growth elements of different height growth typesof seedlings

	Quercus pubescens L.							Quercus cerris L.						Quercus frainetto Ten.		
	Drc Ht		lt	LN Drc		rc	Ht		LN		Drc	Ht	LN			
	2F	3F	2F	3F	2F	3F	2F	3F	2F	3F	2F	3F	2F	2F	2F	
1F	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.047	< 0.01	< 0.01	< 0.01	< 0.01	> 0.05	< 0.05	< 0.01	
2F	/	> 0.05	/	< 0.01	/	< 0.01	/	> 0.05	/	> 0.05	/	< 0.01	/	/	/	

# **4** Discussion and Conclusions

The acorn length and diameter of Hungarian oak from different sites in Serbia (Košutnjak, Avala, Loznica, Vrnjačka Banja, according to Jovanović (1957)), and Kraljevo, according to Šušić (2017); Turkey oak from Košutnjak, Avala, Negotin and Vrnjačka Banja (Jovanović 1958), and downy oak, according to the data from the literature (Jovanović 2000) is in compliance with the results shown in Table 2. In the same site conditions, in full daylight in 2016, but under more intensive cultivation, the one-year-old Hungarian oak seedlings had the average values of total height 9.7 cm, the number of leaves 7.9 and root collar diameter 3.7 mm and three flushes were recorded on some of the plants (Šušić et al. 2019). In suboptimal conditions in 2018, a maximum of two flushes was recorded as well as lower values of all growth elements. In Turkey oak, three flushes were recorded, but with lower values of growth elements compared to the one-year-old Turkey oak seedlings in full light conditions on a felling unit (Bobinac 2001). In downy oak, the multi-flush growth is a well-expressed trait that was noted in adult trees (Tomaševski 1952; Bobinac et al. 2000). According to the results shown in this paper, multi-flush growth is expressed in juvenile stages of development as well, and in comparison to Turkey and Hungarian oak, even a seedling with four flushes was found in the growing season in 2018.

In suboptimal growing conditions, characterized by degraded alkaline soil, the presence of rodents and potentially other biotic agents that feed on acorn, the presence of weed and the water availability that depends only on precipitation, differences between the species were recorded in terms of acorn germination and growth characteristics. All three species exhibited multi-flush growth, implying that the light conditions were favorable for the activation of growth flushes during the growing season (Bobinac 2001, 2002). It turned out that Turkey oak was the most vigorous in these growing conditions, compared to Hungarian and downy oak since a higher number of seedlings and higher values of total height and root collar diameter as well as the highest number of seedlings with multi-flush growth were recorded in Turkey oak. This may be related to acorn size in Turkey oak, i.e. to the better initial growth potential for the growth in the first growing season. In these conditions, Hungarian oak was represented by the lowest number of seedlings with lowest values of total height, leaf number and root collar diameter, and less seedlings with multi-flush growth in comparison to downy oak. This cannot be related to acorn size, but rather to degraded alkaline soil conditions and higher demands from Hungarian oak regarding the cultivation measures compared to downy oak.

Differences between analyzed three oak species exist in these suboptimal conditions. However, in full light conditions, a similar growth pattern was recorded, indicating to the similar adaptability of the species.

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