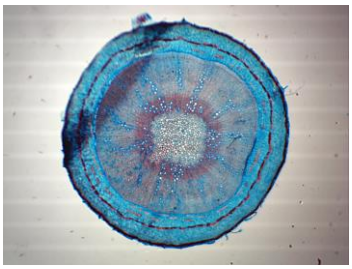

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Variability of morpho-anatomical traits of one-year-old *Quercus* sp. container seedlings

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Abstract

In the paper was investigated the variability of the morphological and anatomical traits of one-year-old seedlings of different species from the genus *Quercus* (*Quercus robur* L.*, Quercus petraea* Matt. Liebl. and *Quercus frainetto* Ten.) cultivated in a plastic container Bosnaplast 18. The acorn was sown in November 2022, while the measurements of the studied elements were performed at the end of the growing season next year. In terms of morphological characteristics, the highest mean values of both researched parameters (root collar diameter and height) were recorded in *Q. robur*, followed by *Q. frainetto*, while the lowest mean values were found in *Q. petraea*. As for anatomical parameters, the following were analyzed: vessels width, the proportion of bark, pith and wood, as well as the participation of early- and latewood zones. Vessels width shows the least average values in *Q. petraea*, while no significant differences were recorded between *Q. robur* and *Q. frainetto*. The highest presence of wood, as well as the early zone within it, was found in *Q. robur*. The proportion of bark is a little bit higher in *Q. frainetto* compared to the other two species, while the pith proportion is significantly lower in *Q. robur* than in the other two species. Based on the obtained results, we can conclude that *Q. robur* seedlings show the best performance, before all from morphological point of view.

Keywords

Bosnaplast 18 container; Morpho-anatomical variability; *Q.robur*; *Q.petraea*; *Q.frainetto*

Contents

1 Introduction

Oaks are a dominant woody plant genus in the northern hemisphere, which occupy a wide range of habitats and are ecologically diverse. Species distributions result from trait composition through environmental filters, as water availability (Cosme et al. 2017). Water is essential for the growth and maintenance of woody plants – in angiosperms, water and nutrient transport is conducted through vessels, but other cell types are also involved in the distribution of water as fibres - mechanical support and parenchyma - metabolite transport and water storage (Zanne et al. 2010; Zieminska et al. 2015). Wood cells do not operate in isolation from one another and are anatomically and functionally integrated – the coupling of vessels and other cells varies across species and can be modified by climate and mechanical stability (Zieminska et al. 2015; Morris et al. 2018). Besides morphological parameters, such as root collar diameter, height, and root development, there are also a few anatomical traits (vessels/tracheid width and number, wood rays height, proportion of wood, bark, and pith) that can give us useful information related to seedling quality (Jokanović et al. 2024). Some papers (Preston et al. 2006; Martinez-Cabrera et al. 2009; Cosme et al. 2017; Olson et al. 2018) found positive relationship between vessel diameter and tree height which affects a future seedling development. However, the risk of cavitation is more often present in case of wider conducts (Niklas and Spatz 2004; Liu et al. 2019). As for participation of different segments (pith, bark, and wood), Jokanović et al. (2024) noted that more powerful developed bark contributes to better protection from pest attack and diseases, while on the other side, thicker wood layer is related to the earlier onset of xylogenesis.

Technology of the nursery production is faced with a big dilemma – bare root or container seedlings. There are a lot of advantages of container seedlings compared to these produced in a conventional manner: higher survival rate; lower physiological shock of seedlings during transplanting which affects faster growth later on; extended period of planting in spring, and earlier onset of planting in autumn, as well; afforestation of degraded sites; production and planting can be performed with mechanized tools and thus reduce overall costs; requires less human work during the planting process (Matić et al. 1996; Ocvirek 1997; Orešković et al. 2006). As for the shortcomings of seedlings produced in containers, one of the main ones is the irregular development and deformation of the root system, which is the case in technologically outdated containers that are often used in nurseries in Serbia (Stilinović 1991).

In terms of forest species seedlings, smaller containers are more often used according to lower price, easier handling, and greater number of the plants per unit area produced in container types such these (Ivetić 2013). Species with small seed are mainly produced in small containers (Ivetić 2021). On the other side, large containers are more suitable for species with big seed and strong root system (Ocvirek 1997; Topić et al. 2006; Popović et al. 2014). The most important morphological trait which affects seedlings development is root collar diameter (Grossnickle 2012). Larger root collar diameter coincides with larger seedlings which are better adapted to dry habitats compared to seedling with smaller diameter (Andivia et al. 2021). As a result, improved growth is related to more nutrients and water uptake (Villar-Salvador et al. 2012) which protects young plants from drought stress (Grossnickle 2005).

For successful natural regeneration of oak stands, the key stage of development is represented by one-year-old seedlings that are the basis for further growth of the young crop (Šušić et al. 2019).

As for pedunculate oak (*Quercus robur* L.), it is usually located in floodplain forests and one of the greatest problems with this species in Serbia is its infrequency of acorn yield, which makes more difficult the process of natural regeneration of these forests (Rađević et al. 2020). High groundwater level is one of the most significant factors for *Q. robur* survival and development (Letić et al. 2017). Hungarian oak (*Quercus frainetto* Ten.) is an oak species growing in thermophilic deciduous forest in southeastern Europe. It grows in a wide vegetation belt rich in woody species, mostly in habitats with humid continental climatic conditions, frequent summer droughts, highest amount of precipitation in spring, wide temperature range, low winter temperatures (Horvat et al. 1974). According to Annighöfer et al. (2015) sessile oak (*Quercus petraea* Matt. Liebl.) can be found in the most diverse habitats in terms of geological substrate and nutrient content, but optimally uses habitats with moderate growth conditions where most often is endangered of the beech if there is no human activities. Berges et al. (2005) state that extremely dry habitats, as well as habitats with low content of mineral substances, are the least suitable for this species.

The aim of the paper is to establish how, in terms of morphology and anatomy, the one-year-old seedlings of three species of oak (*Q. robur, Q. petraea, Q. frainetto*) produced in the same type of container behave. Based on these results, we can conclude what species of oak, cultivated in this type of container, has the best performances.

2 Material and methods

The only one container type (Bosnaplast 18) was used for planting of seedlings of different *Quercus* species (Table 1). Seeds of the investigated species originate from Srem (*Q. robur*), Fruška Gora (*Q. petraea*) and Lipovica (*Q. frainetto*). It should be noted that seeds were collected at the population level. Container seedlings were produced in the nursery of the PE „Vojvodinašume“ in the area of Sremska Mitrovica. Containers were filled with a mixture of organic substrate Pindstrup and perlite in a volume ratio of 2:1. The filling of the containers was performed manually during sowing at the end of the growing season 2022. Watering was regularly conducted, especially during hot summer days. Plots were located in open air conditions. Combined liquid fertilizer Fitogal - Galenika was used. In order to protect seedlings from pest attack, they were treated with the fungicide Previcur which belongs to a systemic fungicide that is particularly efficient by young individuals. Germination of *Q. robur* seed started in the middle of April and lasted until the end of May. As for seed germination of the other two species, the onset was at the end of April, and it included about 5 weeks. At the end of the growing season, in the last days of October, 30 seedlings per species were lifted and transported to the laboratory in order to perform morphological analyses (total of 90 seedlings). There were measured 30 root collar diameters and 30 total heights per species.

The height was measured with a ruler with an accuracy of 0.1 cm and the distance from the root collar to the terminal bud was determined. Root collar diameter was measured at the level of transition from the root to the aerial part, using a digital calliper, with an accuracy of 0.1 mm (Ivetić 2013).

Table 1. Characteristics of used container.

Traits	Bosnaplast 18
Form	hexagonal
Cell volume (cm3)	220
Depth (mm)	180
Density (n/m2)	440
Material	hard plastic
Hole	bottom
Cell wall	slick
Dimension (mm)	320 x 215 x180
Number of cells per container	33

Among anatomical traits were measured: wood proportion (%), bark proportion (%), pith proportion (%), and vessels width (μm). Anatomical analysis was conducted on permanent anatomical preparations made on transversal samples which were obtained by previous softening of the whole material, firstly kept in boiling water, and then in the mixture of water, glycerol and ethanol in the same volume proportions. The samples were cut using sliding microtome „Reichert" in transversal segments 20-25 microns thick. Pith width was calculated as the mean value of two perpendicular measurements. Bark width was measured from the both sides of the sample as a mean value. Wood width was measured between pith and bark from the both sides of the sample as a mean value. The percentage of each of these segments was calculated in relation to the total diameter of the sample. The transition from early- to latewood is determined based on the colour difference between the two zones at the transition border. The vessels width was calculated in two perpendicular directions and based on the average value obtained, taking into account completely formed vessels of circular or elliptical shape. It should be noted that the proportion of the wood (early and late zones), bark and pith were carried out with 45 replications, while the calculation of vessels width included 1.350 replications.

The numerical data was processed using descriptive and univariate statistical methods. For each mean value, standard deviation (SD) and coefficient of variation (CV) were determined. The significance of differences among mean values was determined by analysis of variance (ANOVA) and the post hoc Tukey's HSD (honestly significant difference) test. The analyses were preceded by assessing the normality of the data using Shapiro-Wilk's test. Outliers were excluded. The variables departed from the normality (i.e., tracheid width) were subjected to Box-Cox transformation (Box and Cox, 1964). All statistical analyses were performed in Statgraphics Centurion v. XVI.I (2009; Statpoint Technologies, Inc., Warrenton, VA, USA).

3 Results and discussion

The results of descriptive statistics obtained for seedlings of three autochthonous *Quercus* species in Serbia are for the analysis of morphological traits presented in Table 2 and Fig. 1, and for the anatomical analysis in Table 3 and Fig. 2. The analysis of variance (ANOVA) revealed that the means of all morpho-anatomical traits were statistically different between the studied species ($p < 0.05$) forming two or three homogeneous groups depending on the analyzed trait (Tables 2 and 3).

Table 2. Descriptive statistics (mean, SD – standard deviation, CV – coefficient of variation) and analysis of variance (ANOVA) for seedling morphological traits of three oak species in Serbia.

Trait[1]	*Quercus frainetto*			*Q. petraea*			*Q. robur*			ANOVA [3]	
	Mean [2]	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	F	*p*
H (cm)	15.40 b	1.14	7.38	13.02 c	0.83	6.39	17.96 a	1.16	6.48	164.27	0.0000
RCD (mm)	3.65 b	0.45	12.45	3.00 c	0.53	17.69	4.37 a	0.49	11.23	58.62	0.0000

[1] H – height; RCD – root collar diameter; [2]Means with different letters within a row are statistically different at the 95% confidence level.[3] Boldfaced numbers denote $p < 0.05$.

Table 3. Descriptive statistics (mean, SD – standard deviation, CV – coefficient of variation) and analysis of variance (ANOVA) for seedling anatomical traits of three oak species in Serbia.

Trait[1]	*Quercus frainetto*			*Q. petraea*			*Q. robur*			ANOVA [3]	
	Mean [2]	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	F	*p*
Wood (%)	18.70 b	2.71	14.50	22.46 b	3.08	13.70	43.63 a	7.49	17.16	111.53	0.0000
Latewood (%)	53.33 a	4.86	9.11	59.16 a	11.06	18.70	39.44 b	8.88	22.51	20.56	0.0000
Bark (%)	25.54 a	3.64	14.27	22.95 ab	4.75	20.68	21.29 b	2.27	10.65	5.02	0.0111
Earlywood (%)	46.67 b	4.86	10.41	40.84 b	11.06	27.09	60.56 a	8.88	14.66	20.56	0.0000
Pith (%)	55.76 a	4.65	8.34	55.05 a	5.39	9.79	35.08 b	6.58	18.76	66.05	0.0000
VW (μm)	7.15 a	1.08	15.04	5.39 c	1.01	18.66	6.72 b	1.08	16.07	339.56	0.0000

[1] Means with different letters within a row are statistically different at the 95% confidence level. [2] Boldfaced numbers denote $p < 0.05$.

As for the morphological traits, the mean values of height (H) and root collar diameter (RCD) were the greatest for *Quercus robur* and the lowest for *Q. petraea*. According to the values of the coefficient of variation (CV, %), H had a low degree of variability (0–10%) between the species, whereas the values of this coefficient for RCD were moderate (10–20%) (Table 2).

Regarding the anatomical traits of the studied oak species, *Q. robur* had the lowest mean values of the proportion of latewood, bark and pith, and the highest of the proportion of wood and earlywood. On the other hand, the lowest mean value of vessels width (VW) was determined for *Q. petraea* and the highest for *Q. frainetto*. Considering the obtained values of F-ratio, the variables that contributed the most to the differentiation of species were the proportion of wood and vessel width (Table 3; Fig. 2). Most of the analyzed anatomical traits exhibited a moderate degree of variability (CV = 10–20%) (Table 3). Significantly smaller average dimensions of morphological traits in *Q. petraea* (Table 2) can be associated with the biological properties of the seed, as well as the degree of germination.

Orešković et al. (2006) conclude that *Q. robur* and *Q. petraea* develop a simple root system in a container, which is characterized by the presence of one or more strong taproots that dominate their thickness, and several thin and branched lateral roots. The same authors state that in the Bosnaplast 18 container, which was also used in our paper, the lowest degree of air drying of the roots was recorded in the lower opening of the container, where the largest number of veins is located. Otherwise, air drying of the taproot and branched adventitious roots has a positive effect on its development, i.e. it does not cause deformations of the roots, which are reflected in the rotating and

backward growth of the roots in height (Orešković et al. 2006). In the aforementioned paper, the development of *Q. robur* and *Q. petraea* seedlings in five different types of containers was monitored over a period f four years, and the values obtained for one-year-old *Q. petraea* seedlings in the Bosnaplast container are: H =11 cm, RCD = 3mm, while in the same type of container for one-year-old *Q. robur* seedlings were obtained significantly higher values for both morphological traits: H = 26.3 cm, RCD = 4 mm. The same tendency is observed in our paper when it comes to the development characteristics of the seedlings of these two species – both morphological traits are significantly larger in *Q. robur* compared to *Q. petraea*. If a comparison is made with the aforementioned values (Orešković et al. 2006), we can conclude that our results show higher mean values of the height in *Q. petraea* and root collar diameter in *Q. robur*, but also a significantly lower height in *Q. robur* seedlings, while the same values were obtained for the root collar diameter in *Q. petraea* seedlings.

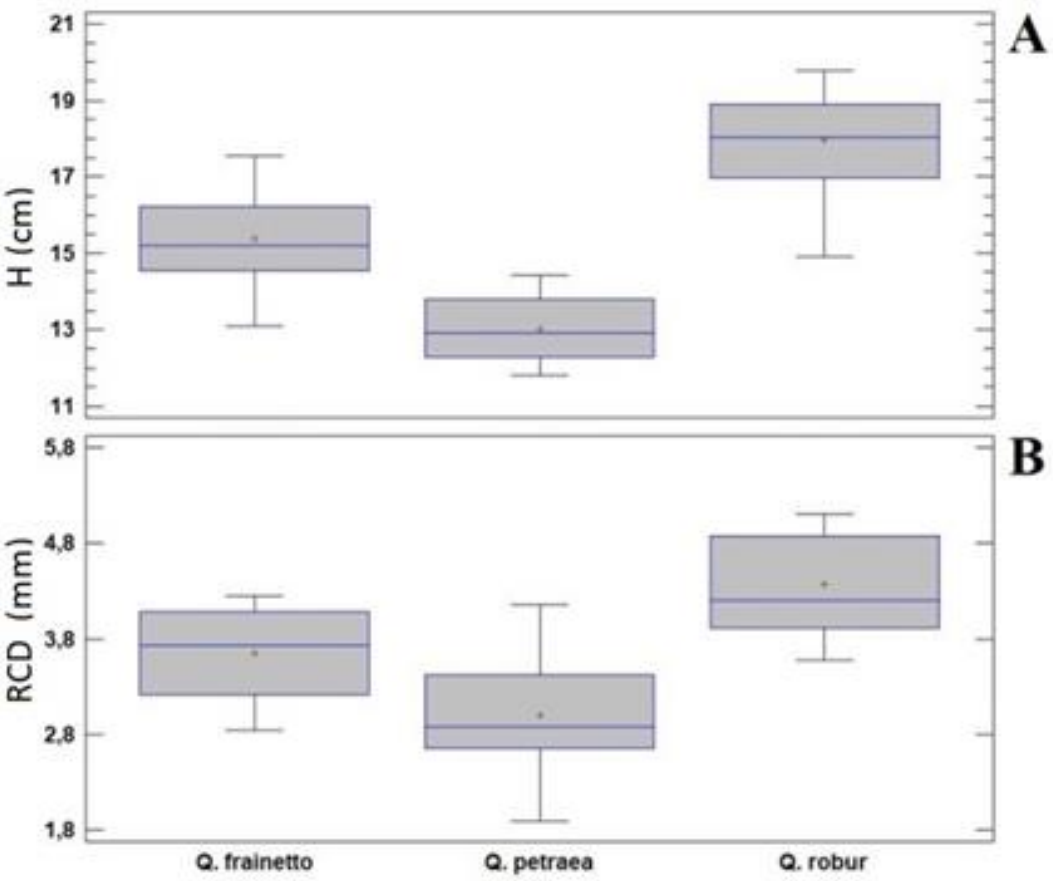

Figure 1. Box-and-whiskers plots of basic statistical parameters for seedling morphological traits of oak species: (A) H - height (cm); (B) RCD – root collar diameter (mm). Legend: middle sign – mean, middle line – median, box – lower and upper quartile, whiskers – range.

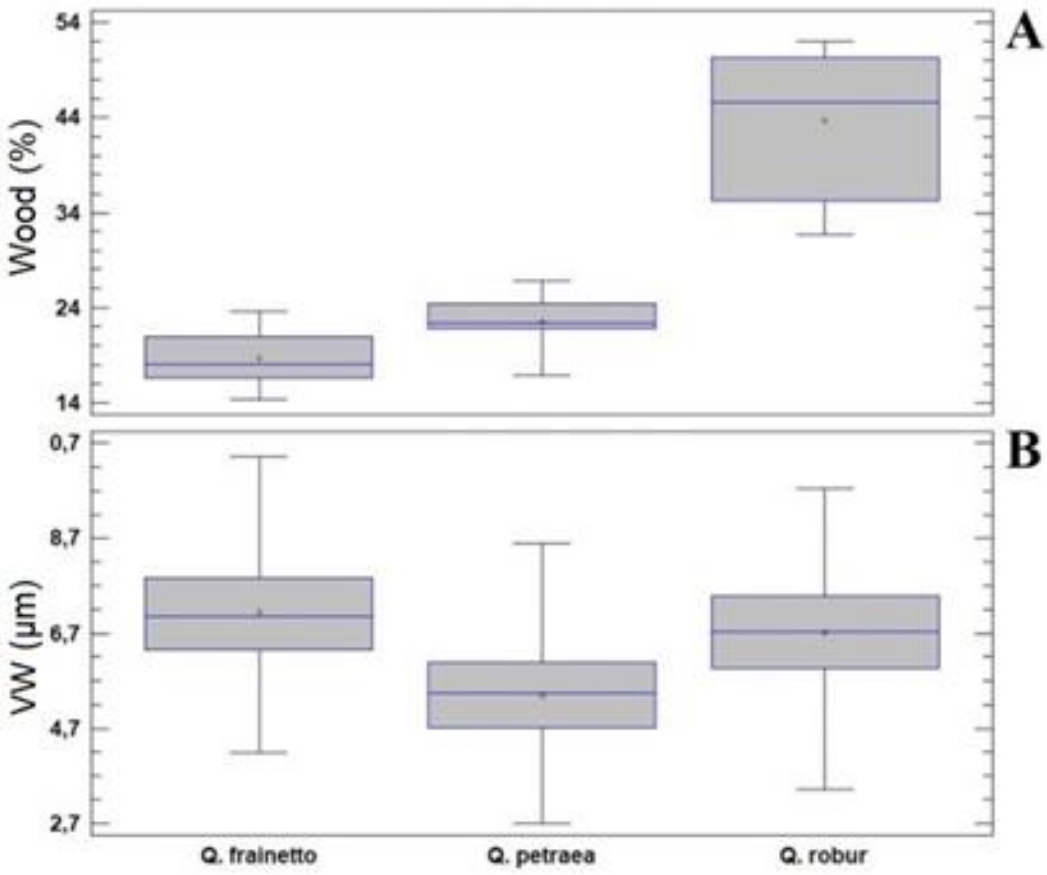

Figure 2. Box-and-whiskers plots of basic statistical parameters for oak seedling anatomical traits with the highest values of F-ratio: (A) Wood (%); (B) VW – vessels width (µm). Legend: middle sign – mean, middle line – median, box – lower and upper quartile, whiskers – range.

Popović et al. (2014) analyzed the influence of different container types on the dimensions of the morphological traits of one-year-old *Q. robur* seedlings and obtained the following results: in the Bosnaplast 12 container, the mean height was 21 cm, while the mean root collar diameter was 3.22 mm, while in the Bosnaplast 18 container the following values were obtained: the mean height was 22.1 cm, and the mean root collar diameter was 3.96 mm, respectively. The mean heights obtained for the *Q. robur* seedlings in our paper are significantly lower, while the average values of the root collar diameter, which is more important for assessing the quality of the seedlings, are significantly higher.

It should be noted there are no many papers that deal with anatomical traits of juvenile seedlings – what's more, dimensions of mechanical and conductive elements linked to morphological parameters may be closely related to seedlings quality. Jokanović et al. (2024) analyzed the influence of three container types (one biodegradable and two made of plastic) on the morphological and anatomical dimensions of one-year-old black pine and Scots pine seedlings. In plastic containers, the aforementioned authors obtained mean height values for Scots pine of 8 cm and for black pine of 10.5 cm, while the mean values of the root collar diameter were 1.3 mm for Scots pine, and 1.5 mm for black pine, respectively. By comparing with the results obtained in our paper, it can be stated that significantly higher mean values of both morphological traits were recorded in all three oak species compared to Scots and black

pine. This is also related to the biology of the species, as well as the differences in the dynamics of growth and development in the juvenile stage between oaks and pines. Regarding the proportion of pith, wood and bark in plastic containers (Jokanović et al. 2024) for both pines, approximately the same ratios were obtained – wood is represented over 60 %, while pith and bark participate with about 20 % each. This significantly deviates from the results obtained in our paper, where wood proportion is 43.63 % by *Q. robur*, then 22.46 % by *Q. petraea*, and finally 18.70 % by *Q. frainetto*, which means that a significantly larger number of cambial cells differentiate into xylem in pines compared to oaks. On the other hand, the pith proportion in all three oaks is significantly higher compared to pines (about 35 % in *Q. robur*, and over 55 % in the other two species), which means that forming of elements who are in charge of storing nutrients is much more intense in oaks. The intensive formation of pith causes earlier heartwood and mechanical elements forming, which are related to the strength and density of the tree. The participation of the bark is similar to values obtained for pines, while its proportion is slightly higher in *Q. frainetto*, which means that the seedlings of this species in the juvenile stage are equipped with a bit stronger physical barrier for the pests and pathogens attack. The aforementioned authors (Jokanović et al. 2024) analyzed also the dimensions of tracheids width for one-year-old seedlings of Scots pine and black pine and for plastic containers they found the following average values: about 2 microns in Scots pine, and about 3 microns in black pine seedlings, respectively. In our paper the mean values of the vessels width were significantly larger, particularly in *Q. frainetto* and *Q. robur* seedlings.

Mijatović et al. (2022) investigated the effect of three container types (two made of plastic and one biodegradable) on the development and growth of one-year-old *Q. robur* seedlings and for plastic containers the following values were recorded: for Bosnaplast 18, mean height was 17.10 cm, and mean root collar diameter was 4.85 mm, while for Hiko, mean height was 17.24 cm, and mean root collar diameter was 5.35 mm, respectively. Our results are very similar to these for both studied morphological traits. The same authors found that root intensity was highest for seedlings produced in biodegradable container, because they have the largest volume. However, some other papers (Kolevska et al. 2020) for conifers reported higher root intensity values for container with smaller volume, which can be explained that oak species need much more root growing space than pines.

Montagnoli et al. (2021) consider that the amount of vascular cambium cells that divide depends on the dimensions of the container, which may be related to an earlier onset of the cambial growth in larger containers or may be related to an overall higher cambial activity. However, in the same paper was concluded that the thickness of the xylem layer, as well as the direction of the wood rays, do not depend on the container type. Jelić et al. (2014) investigated the influence of containers on the dimensions of the morphological traits of one-year-old *Pinus pinea* seedlings and deduced that seedlings grown in containers of larger volume have higher mean values of the height and root collar diameter, so they can be used for afforestation of more difficult sites, because the quality of the seedlings depends on, first of all, its thickness. However, the aforementioned authors note that the development of the root system and potential deformations must be taken into consideration when selecting seedlings that should be used for afforestation.

Growth characteristics of one-year-old *Q. frainetto* seedlings in full light conditions were investigated by Šušić et al. (2019) and significantly smaller average

values of height (11.3 cm vs 15.40 cm) and almost the same values of the mean root collar diameter (3.80 mm vs 3.65 mm) were obtained compared to our paper. The same authors note that *Q. frainetto* is a meso-xerothermic oak species that should have greater management importance in the Republic of Serbia and southeastern Europe, in general, having in mind the climate changes. Kanjevac (2020) dealt with the biology and possibilities of natural restoration of sessile oak forests situated in the area of northeastern Serbia. The aforementioned author noted the dominance of trees with low vitality, thinning crowns, in a certain stage of decline. The occurrence of these trees decline correlates with the age, but also with the trend of global warming – it is about stands over 160 years old that are very thinned and with the occurrence of an understory of species with high biological strength that threaten to eliminate sessile oak. Popović et al. (2021) found that natural regeneration of *Q. frainetto* populations is very difficult and therefore is mainly restored in vegetative way which additionally affects reducing of genetic diversity. Multi-flush growth is typical of *Q. frainetto* and may be related to the optimal growing conditions (Le Hir et al. 2005). The long-term drought stress affect the multi-flash growth in *Q. robur* plants, while the onset of cambium forming can be prolonged for the period after the drought stress (Spiess et al. 2012). Bobinac et al. (2012) establish that multi-flush growth of the young *Q. robur* plants is very important for the success of the natural regeneration of pedunculate oak forests.

Arenas-Navarro et al. (2020) found negative relationships between vessel diameter and frequency and concluded, for oak species, that wide vessels in lower frequency lead to lower wood density. Due to the fact that wider vessels are more vulnerable because of the biophysical constraints on water transport to canopies, trees tend to produce conduits no wider than those permitted by embolism risk given conditions such as water availability, temperature, air humidity, soil type, rooting depth, etc. (Olson et al. 2018). Some papers (Sousa et al. 2009; Gupta and Gupta 2020; Percolla et al. 2021) found that the occurrence of small vessels (less than 15 µm) or vasicentric tracheids (transitive conductive elements by angiosperms) appears to be an anatomical structure to survive for oaks during drought conditions in different environments around the world.

4 Conclusions

The results obtained in our paper should contribute to better understanding of the development characteristics of the investigated oaks in the analyzed container type. *Quercus robur* (L.) seedlings have significantly better morphological characteristics compared to the other oak species. As for anatomical traits, the greatest ratio of total wood and early wood was also recorded in *Q. robur*, while the highest values of bark, pith and vessels width were detected in *Quercus frainetto* (Ten). Bearing in mind that trees with wider conduits are at higher risk of embolism, we can conclude, based on the obtained results, that *Quercus petraea* (Matt.) Liebl. with the lowest values of vessels width, might be the most suitable for arid habitats reforestation. In order to obtain reliable indicators related to the quality of the planting material, it is necessary to conduct multi-year investigation, which would enable us to get feedback from the field regarding the degree of reception and survival of the seedlings, as well as their morphological (radial and height increment) and anatomical (participation of wood, bark, and pith, width of conductive and mechanical elements) traits.

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