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Wood Fibre Characteristics of Pedunculate Oak (*Quercus R obur* L.) Growing in Different Ecological Conditions

Svojstva drvnih vlakanaca hrasta lužnjaka sa staništa različitih ekoloških uvjeta

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ABSTRACT • *Anatomical properties of pedunculate oak (Quercus robur L.) wood have not been previously investigated in Serbia, so bearing in mind economic-productive and ecological significance of this species, it was one of the main reasons why this research was conducted. In this paper, fibre length (FL), double cell-wall thickness (DCWT) and fibre lumen diameter (FLD) were measured in pedunculate oak wood from two different sites. The study was conducted at two sites (Donji Srem – MU „Raškovica-Smogvica" and Gornji Srem – MU „Kupinske grede"), situated along the Sava River and characterised by different ecological conditions, including flooding regimes. Measurements were conducted in radial direction, from pith to bark, in order to assess variation of investigated characteristics with cambial age, between two sites and between individual trees within each site. All measured characteristics from both sites increase going from pith to bark apart from FL in the area of Donji Srem – MU „Kupinske grede" that reaches maximum value in the central part of xylem and then decreases. Hydrological site conditions affect the dimensions of pedunculate oak wood fibres and these values are a bit higher in Gornji Srem – MU „Raškovica-Smogvica", due to a greater quantity of available water.*

KEYWORDS: *pedunculate oak (Quercus robur L.); fibre characteristics; Gornji Srem; Donji Srem; wood variation*

SAŽETAK • *Anatomska svojstva drva hrasta lužnjaka (Quercus robur L.) do sada nisu istraživana na području Srbije, a imajući na umu golemo značenje te vrste u ekonomsko-proizvodnome i ekološkom smislu, to je bio jedan od glavnih razloga za provedbu ovog istraživanja. U radu je izmjerena duljina vlakanaca (DV), dvostruka debljina stijenki (DDS) i promjer lumena vlakanaca (PLV) drva hrasta lužnjaka s dva različita staništa. Istraživanje je provedeno na staništima Gornji Srem – GJ „Raškovica-Smogvica" i Donji Srem – GJ „Kupinske grede", koja se prostiru duž rijeke Save, a karakteriziraju ih različiti ekološki uvjeti, uključujući i režim plavljenja. Mjerenja su provedena u radijalnom smjeru, od srčike prema kori, kako bi se utvrdile varijacije istraživanih svojstava ovisno*

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o starosti kambija, o različitosti dvaju lokaliteta te o razlikama među stablima unutar istog lokaliteta. Utvrđeno je da se sve izmjerene vrijednosti drva s oba lokaliteta povećavaju u smjeru od srčike prema kori, osim DV-a na području Donjeg Srema – GJ „Kupinske grede", koji maksimalnu vrijednost dosežu u središnjem dijelu ksilema i potom se smanjuju. Hidrološki uvjeti staništa uvelike utječu na dimenzije drvnih vlakanaca hrasta lužnjaka i te su vrijednosti zbog veće količine raspoložive vode nešto veće u Gornjem Sremu, u GJ „Raškovica-Smogvica".

KLJUČNE RIJEČI: *hrast lužnjak (Quercus robur L.); svojstva drvnih vlakanaca; Gornji Srem, Donji Srem; varijabilnost drva*

1 INTRODUCTION
1. UVOD

Pedunculate oak (*Quercus robur* L.) forests in the area of Ravni Srem (Serbia) are of exceptional importance from ecology and economic point of view (Nikolić, 2016). Its wood is considered to be of high quality and is used for different purposes, mainly veneer, floor-boards, furniture and construction wood. The entire complex of hygrophilous forests in this area is divided into two spatial-geographical units: Gornji Srem, where a defensive embankment was built in the 1930s so that the impact of flooding is excluded, and Donji Srem, which is not defended and where, in addition to atmospheric precipitation and groundwater, additional watering through flooding also has a significant influence on development characteristics of pedunculate oak. In Serbia, both pedunculate (*Q. robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.) have been investigated in terms of growth and development (Letić *et al*., 2017; Nikolić Jokanović *et al*., 2019; Nikolić Jokanović *et al*., 2020; Radaković and Stajić, 2021). From these studies, it was concluded that water is the main ecological factor related to pedunculate and sessile oak growth and development. Its shortage can affect the decline of the species. Feuillat *et al.* (1997) emphasised that pedunculate and sessile are two major oak species in European forests (27 % of the area of European broad-leaved forests). They are seldom distinguished during management operations and never after felling.

Wood density and shrinkage are lower in pedunculate oak (Deret-Varcin, 1983; Nepveu, 1984; Levy *et al.,* 1992), heartwood formation is quicker, thus leading to a thinner sapwood zone (Deret-Varcin, 1983; Levy *et al.,* 1992), while wood colour is slightly different (Klumpers *et al*., 1993). On the other hand, vessel dimensions in oak wood structure have been studied in more detail (Tumajer and Tremsl, 2016; Levanič *et al*., 2011). Levanič *et al.* (2011) linked growth and anatomical properties of pedunculate oak to its mortality rate and claimed that variability in anatomical properties affects transpiration potential and other physiological parameters of this species. Xylem anatomy has direct implications on tree ecophysiology. The hydraulic functioning of trees largely depends on anatomical features (Zimmermann, 1983). Jacobsen *et al.* (2005) examined the connection between xylem fibres and vessels cavitation resistance by different hardwoods. At the cellular level, they found that increased cavitation resistance and stem mechanical strength were associated with increased thickness of fibre cell walls. Increased vessel wall thickness was not correlated to xylem density, while xylem conductive efficiency was correlated with increased hydraulic vessel lumen diameter and decreased fibre wall area. Hacke *et al.* (2001) established that fibre and vessel properties are indeed correlated and found a possible role for fibres in increased vessel implosion resistance.

Wood anatomical characteristics greatly influence its properties and quality. Regarding mechanical elements in the structure of pedunculate oak wood, in addition to wood fibres (libriforms), fibrous tracheids are also present (Vilotić, 2000). Fibres have the greatest area in the structure of pedunculate oak wood (40-60 %). In line with that, the vessel area in the earlywood is 40 % and in the latewood 8 %, while the ray area is 15-30 % (Wagrenführ and Scheiber, 2006).

Radial variation of anatomical characteristics from pith to bark shows a big influence of cambial age on wood structure (Tsoumis, 1991). This could point to transition from juvenile to mature wood on the cross-section of wood. In a certain site, variation is very high, generally higher than between sites, and for Serbian Pedunculate oak populations this assumption was confirmed by DNA markers (Kesić *et al*., 2021).

Some papers (Mladenova *et al*., 2017; Nazari *et al*., 2020; Keleş and Savaci, 2021) investigated fibre characteristics, mainly fibre length in wood of different oak species. Obtained results showed a big dependence of its dimensions on site conditions. Vilotić (1992), established some differences related to the anatomical structure inside the genus *Quercus* and claimed that these differences are quantitatively caused by genotype, pedology, climate, topography, and features of plant associations. However, little attention has been given to determining different patterns of fibre characteristics variation.

The aim of this research is to provide preliminary results on the intra- and inter-population variability of wood fibre characteristics (fibre length – FL, double cell wall thickness – DCWT and fibre lumen diameter

– FLD) of pedunculate oak on the territory of Serbia, as well as to determine how these traits change with age, going from pith to bark of the tree.

2 MATERIALS AND METHODS
2. MATERIJALI I METODE

2.1 Study area
2.1. Istraživana staništa

For the purpose of this research, a total of six trees were selected and harvested. Three trees were located in the area of Gornji Srem, within the Management Unit (MU) „Raškovica-Smogvica", while the other three were located in the area of Donji Srem, within the Management Unit (MU) „Kupinske grede". It should be emphasised that all stems were planted from the seed.

The first site, in the area of Forestry Holding (FH) „Morović", within the MU „Raškovica-Smogvica" is situated on flat terrain, the dead cover is poorly represented, the humification process is very favourable and there is no flooding. This is a type of ash and pedunculate oak forest with maple and hornbeam and a rich floor of shrubs in the non-flooded part of Gornji Srem on the driest options of marsh blackberries and meadow blackberries with signs of leaching. The mechanical texture of the soil is lighter (clay + powder are represented with about 75 %), which affects the content of air in the soil increases, and the amount of total water decreases.

The second site is situated in the lowland belt area of Forestry Holding (FH) „Kupinovo", within the MU „Kupinske grede". The dead cover is moderately represented, while the humification process is favourable. The site is characterised by regular flooding, the shrubs are medium dense present, with some weeds cover. As for the forest type, it is ash and pedunculate oak association on moderately moist marsh blackberries. There is also a high forest of ash and poplar, a two-story, ripening stand. The stand is medium endangered by wind, and its quality is very high. It is primarily intended for the production of technical wood.

2.2 Laboratory work
2.2. Laboratorijsko istraživanje

Discs, approximately 5 cm thick, were cut at breast height (1.3 m). Radial segments were taken from the north-south section along the entire radius, starting from pith to bark. To analyse the wood anatomical characteristics (FL (mm), DCWT (μm) and FLD (μm)), half the length of the test tube (one radius) was used which includes the segment from pith to bark (Figure 1).

In order to perform the necessary microscopic analyses and determine the dimensions of wood fibres, a maceration procedure was applied. It is a process of chemical decomposition of wood mass in order to dis-

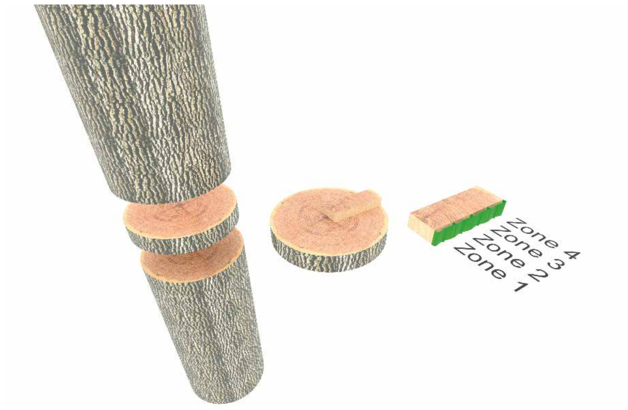

Figure 1 Scheme of disc sampling and test tube preparation
Slika 1. Shema uzimanja ispitnih kolutova i izrade ispitnih uzoraka

integrate intercellular spaces and perform the necessary measurements. Using Franklin's reagent (1945), the intercellular substance was decomposed, and individual cells suitable for measurements were isolated. The maceration solution consists of 30% hydrogen peroxide and glacial acetic acid in a volume ratio of 1:1. Samples of wood primarily chopped to the size of a stick were placed in a test tube and treated with the prepared reagent. The material thus prepared was thermally treated in an oven at 65°C for 24 hours until it turned into pulp. After rinsing with distilled water, measurable single xylem cells were obtained.

Within each tree, four zones were selected – first near the pith, second in the juvenile part of the tree, third including the central part of the xylem and fourth located in the sapwood zone. Thirty undamaged wood fibres were sampled in each zone, meaning 120 per tree, or 720 fibres within the entire study area. Trees from the first site were 122, 121 and 119 years old, while trees from the second site were 120, 180 and 175 years old. In this research, the juvenile wood includes about 40 annual rings apart from two trunks from MU „Kupinske grede" (Donji Srem) that are 180 years old, so by these trees juvenile wood occupies about 60-70 growth rings. All investigated characteristics were measured using „Boeco" microscope, connected with specialised software with appropriate calibration. FL was measured at 40x magnification, while DCWT and FLD were measured at 400x magnification.

Statistical analyses were performed using the statistical program STATISTICA 7.0 (StatSoft Inc. 2004). Factorial analysis of variance (ANOVA) was used to test significance of differences in selected fibre characteristics between sites and in pith to bark direction. The one-way analysis of variance (ANOVA) was used to test significance of differences between tress within each site and Tukey's post hoc test for comparison between four zones within populations.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

Statistical values of fibre characteristics of pedunculate oak wood from both sites are given in Table 1.

In MU „Raškovica-Smogvica" within Gornji Srem, the groundwater level is quite deep, usually 2-2.5 m and 3-3.5 m (Nikolić, 2016). However, nearby is the River Bosut, which presumably contributes to additional wetting of this habitat, provides favourable water-air properties of humogley soil (Nikolić, 2016). The humus-accumulative horizon goes to a depth of 55 cm, and the ratio of the total sand fraction to the total clay and powder fraction is 1: 2.5 (Nikolić, 2016).

On the other hand, MU „Kupinske grede" is characterized by fluvisol-type soil, whose humus-accumulative horizon (0-35 cm) is shallower than humogley, which means that this soil is poorer in nutrients than the previous one (Nikolić, 2016). Also, MU „Kupinske grede" is in a depression where, due to the granulometric composition of fluvisol and low permeability, water stagnates (anaerobic conditions) and its uptake by pedunculate oak is difficult. The ratio of the total sand fraction to the total clay and powder fraction is 1:19, which means that the water-air properties of this soil and water capacity are much worse than those of the humogley present in MU „Raškovica-Smogvica" (Nikolić, 2016). Based on the mentioned habitat conditions prevailing in the two investigated sites, it can be concluded that ecological, primarily hydrological conditions in the area of MU „Raškovica-Smogvica" are more favourable for pedunculate oak development than site conditions in the area of MU „Kupinske grede". As a result, greater dimensions of wood fibres were determined in Gornji Srem – MU „Raškovica-Smogvica".

Table 1 Statistical values of fibre characteristics of pedunculate oak wood from studied sites
Tablica 1. Statističke vrijednosti svojstava vlakanaca drva hrasta lužnjaka s oba staništa

Site *Stanište*	**Value** *Vrijednost*	**FL** / *DV*, mm	**DCWT** / *DDS*, mm	**FLD** / *PLV*, mm
Gornji Srem	N_{mean}	1.35	13.16	20.14
	N_{min}	0.71	6.98	12.08
	N_{max}	1.97	22.19	28.93
	S_d	0.25	2.57	3.09
	Cv	18.52	19.53	15.34
Donji Srem	N_{mean}	1.33	12.66	19.76
	N_{min}	0.65	5.62	9.90
	N_{max}	1.96	19.90	31.12
	S_d	0.22	2.52	3.90
	Cv	16.54	19.91	19.74

N_{mean} – mean value / *aritmetička sredina*, N_{min} – minimum value / *minimum*, N_{max} – maximum value / *maksimum, Sd* – standard deviation / *standardna devijacija*, *Cv* – coefficient of variation / *koeficijent varijacije*, FL – fibre length / *DV – duljina vlakanaca*, DCWT – double cell wall thickness / *DDS – dvostruka debljina stijenki*, FLD – fibre lumen diameter / *PLV – promjer lumena vlakanaca*

Based on the results shown in Table 1, we can conclude that mean values of all investigated characteristics were greater in the area of Gornji Srem, MU „Raškovica-Smogvica". From the calculated coefficient of variation (Table 1), we can deduce that FL is more variable in MU „Raškovica-Smogvica", while other two characteristics are more variable in the area of MU „Kupinske grede".

The mean values of FL are in the range reported by Mladenova *et al*. (2017), between 1.02 mm for Hungarian oak and 1.46 mm for sessile oak from different sites in Bulgaria. However, Nazari *et al*. (2020) reported fibres shorter than 1 mm in Persian oak wood (mean value was 0.87 mm). All of these differences could be explained by individual genetic and silvicultural impacts. Keleş and Savaci (2021) determined that seasonality considerably influenced fibre length in pedunculate oak wood. Longer fibres in the first growing period (1.22 mm) are closer to the results obtained from this research, although the sampling approach was different. Sousa *et al*. (2009) examined the wood anatomy of the cork oak. They established greater mean values of fibre width and double wall-thickness than in this research (approximately 23.5 μm, and 18 μm, respectively).

Significant differences in FL and DCWT were detected between two sites (Table 2). Studies by Arend and Fromm (2007) and De Micco *et al.* (2016) reported that fibre dimensions are sensitive to environmental fluctuations, indicating that their dimensions decreased with low water availability. Our results coincide with these because longer wood fibres of pedunculated oak were determined in the area with better water-air properties and more favourable water capacity (MU „Raškovica-Smogvica"), compared to another site (MU „Kupinske grede"). Those site conditions, due to water stagnation, shallower humus-accumulative horizon and less suitable ratio of the total sand fraction to the total clay and powder fraction, are less desirable for pedunculated oak development. Likewise, highly significant differences in Persian oak fibre length were observed at two sites differentiated by altitude and slope (Nazari *et al*. 2020).

As for changing of observed characteristics with cambial age (distance from pith divided into four zones) (Table 2, Figure 2, 3 and 4), we can conclude that all characteristics increased with cambial age from pith to bark in the area of MU „Raškovica-Smogvica" (Gornji Srem). On the other side, in the area of MU „Kupinske grede" (Donji Srem), there is a linear increase of an average DCWT and FLD with cambial age (Figure 3 and 4), while FL gradually increases starting

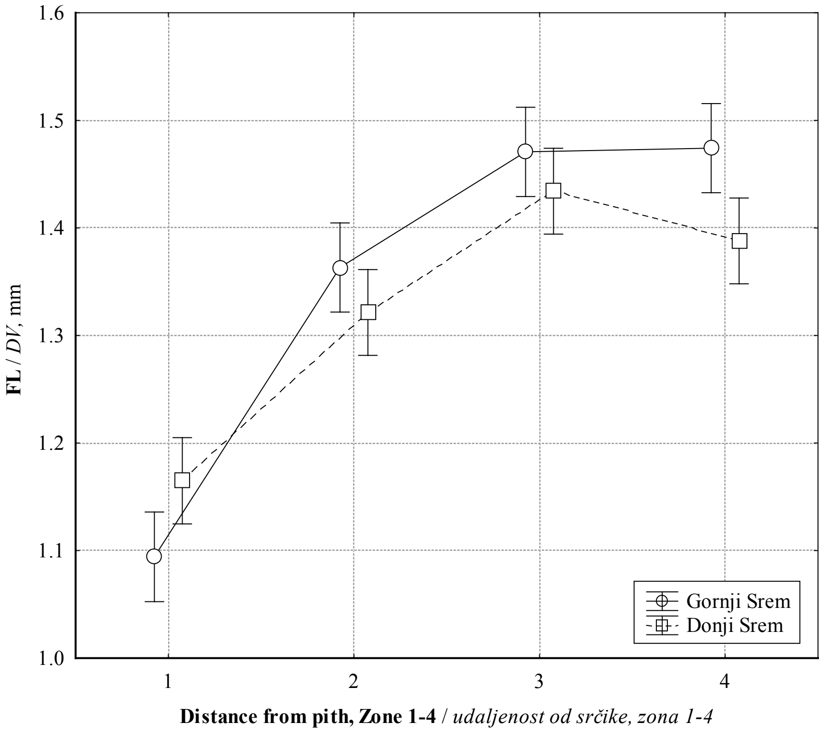

Figure 2 Radial distribution of fibre length (FL) of pedunculate oak wood from two sites
Slika 2. Raspored duljine vlakanaca (DV) drva hrasta lužnjaka s dva staništa u radijalnom smjeru

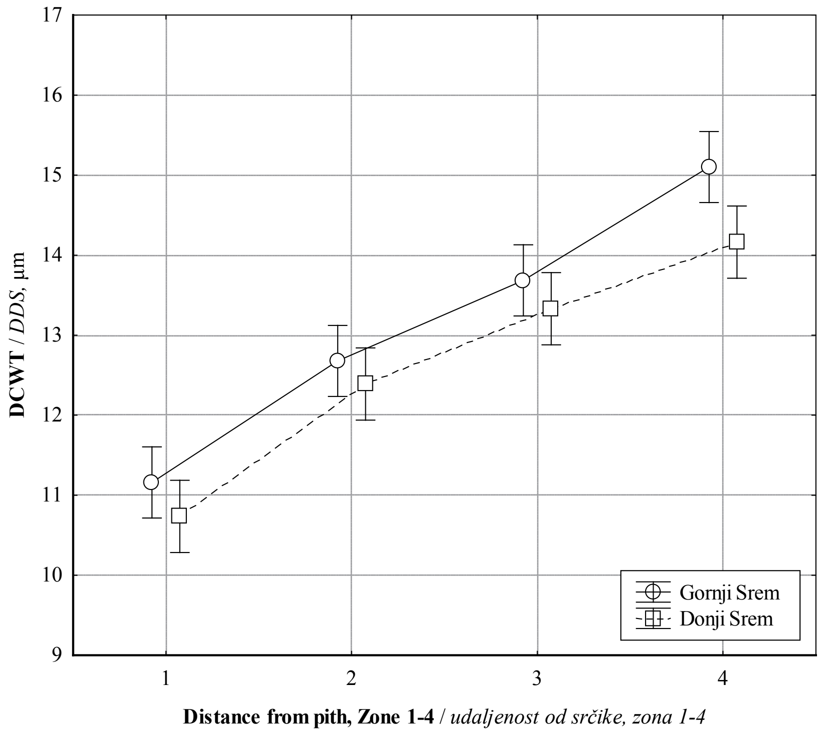

Figure 3 Radial distribution of double cell-wall thickness (DCWT) of pedunculate oak wood from two sites
Slika 3. Raspored dvostruke debljine stijenki (DDS) drva hrasta lužnjaka s dva staništa u radijalnom smjeru

from the pith, and then along juvenile and central xylem zone. In the final zone (sapwood zone), a gradual reduction of FL can be noticed, bearing in mind that they previously reached their culmination (Figure 2). Panshin and de Zeeuw's (1980) early findings report that hardwood fibres exhibit appreciable postcambial elongation, considering juvenile and mature phases. The juvenile phase reflects a rapid increase in cell length, while the functioning of the mature cambium is stabilised in the mature phase. Old age in trees brings a reduction in fibre length. The same authors find a similar pattern of radial increase in cell-wall thickness.

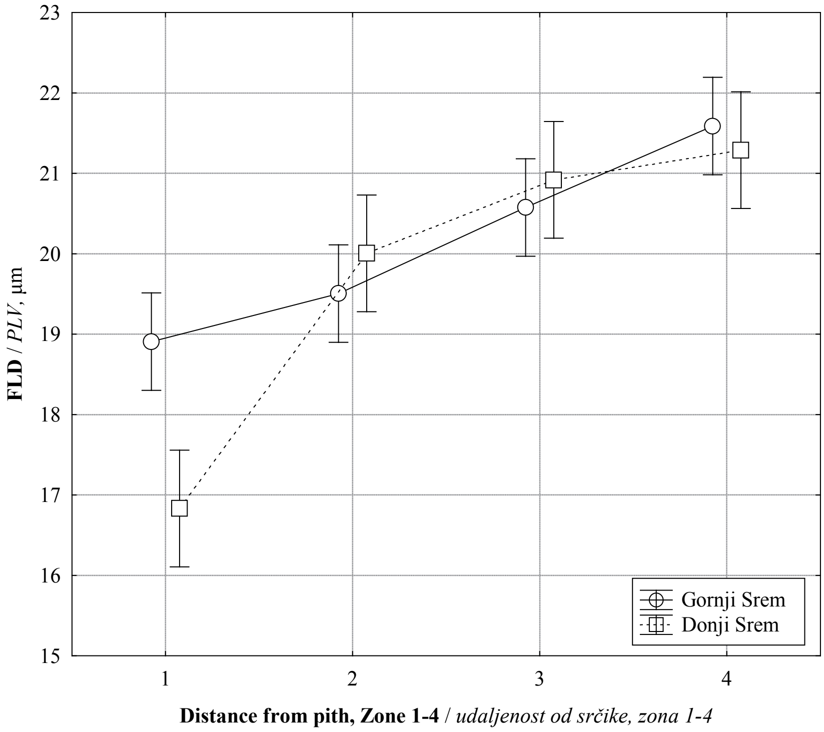

Figure 4 Radial distribution of fibre lumen diameter (FLD) of pedunculate oak wood from two sites
Slika 4. Raspored promjera lumena vlakanaca (PLV) drva hrasta lužnjaka s dva staništa u radijalnom smjeru

Table 2 Results of analysis of variance for fibre characteristics of pedunculate oak wood from two sites and in pith to bark direction, divided into 4 zones
Tablica 2. Rezultati analize varijance svojstava vlakanaca drva hrasta lužnjaka s dva staništa i u smjeru od srčike prema kori; podjela u četiri zone

Source of variation *Izvor varijabilnosti*	**FL** / *DV*, mm		**DCWT** / *DDS*, µm		**FLD** / *PLV*, µm	
	F	*p*	*F*	*p*	*F*	*p*
Site / *stanište*	13.57	**0.000247**	9.68	**0.001932**	2.53	0.111889
Zones of distance from pith *zone udaljenosti od srčike*	28.65	**0.000000**	94.05	**0.000000**	41.65	**0.000000**
Site × Zones of distance from pith *stanište × zone udaljenosti od srčike*	55.67	**0.000000**	0.85	0.468224	6.03	**0.000465**

FL –fibre length / *DV – duljina vlakanaca*, DCWT – double cell wall thickness / *DDS – dvostruka debljina stijenki*, FLD – fibre lumen diameter / *PLV – promjer lumena vlakanaca*

Rao *et al.* (1997) showed that latewood libriform fibre diameter and wall thickness in pedunculate oak increased significantly with ring number from pith. Determined mean values of fibre diameter and double cell wall thickness are a bit lower than this research results. Helinska-Raczkowska and Fabisiak (1991) concluded that fibre length in sessile oak wood increased in the first 30 growth rings. Based on the radial fibre length variation in eight oak species, other authors concluded that the juvenile wood usually comprises 30-40 annual rings (Hamilton, 1961; Farmer, 1969; Taylor, 1979; Petrić and Ščukanec, 1980; Furukawa *et al*., 1983). In this research, the juvenile wood includes about 60 annual rings bearing in mind that trees are older than 120 years apart from two trunks from MU „Kupinske grede" (Donji Srem) that are 180 years old, so in these trees, juvenile wood occupies a bit more than 60 growth rings. According to Eaton *et al*. (2016), the most valuable oak wood is produced in high mixed forests on fertile sites with long economic rotations, about 130 years for pedunculate oak. This implies that some of the used trees are possibly overmature. The same authors (Helinska-Raczkowska and Fabisiak, 1991) deduced that the length of mature anatomical elements, including wood fibres, is from 10 to 20 % greater than the length of juvenile wood anatomical elements of oak, and this coincides with our results. Variations in fibre characteristics were investigated in other hardwood species, such as poplar (Ištok *et al.*, 2017), birch (Luostarinen and Möttönnen, 2010) and eucalyptus (Carrillo *et al.*, 2015), showing similar differences and trends in radial variations.

Comparing mean values between individual trees inside study sites, we can establish significant differences in fibre characteristics among trees within both

Table 3 Results of analysis of variance for fibre characteristics of pedunculate oak wood within-population from each site
Tablica 3. Rezultati analize varijance svojstava vlakanaca drva hrasta lužnjaka unutar populacije svakog staništa

Source of variation *Izvor varijabilnosti*	Site *Stanište*	FL / *DV*, mm		DCWT / *DDS*, µm		FLD / *PLV*, µm	
		F	*p*	*F*	*p*	*F*	*p*
Trees *stabla*	Gornji Srem	6.45	0.001776	3.762	0.024167	8.81	0.000185
	Donji Srem	17.38	0.000000	21.88	0.000000	58.22	0.000000

FL –fibre length / *DV – duljina vlakanaca*, DCWT – double cell wall thickness / *DDS – dvostruka debljina stijenki*, FLD – fibre lumen diameter / *PLV – promjer lumena vlakanaca*

Table 4 Fibre characteristics of pedunculate oak wood within population from each site depending on distance from pith (Post hoc Tukey's HSD test)
Tablica 4. Svojstva vlakanaca drva hrasta lužnjaka unutar populacije svakog staništa ovisno o udaljenosti od srčike (Post hoc Tukeyjev HSD test)

Site *Stanište*	Distance from pith, Zone 1-4 *Udaljenost od srčike, zona 1-4*	FL / *DV*, mm	DCWT / *DDS*, µm	FLD / *PLV*, µm
Gornji Srem	1	1.09b	11.16a	18.91a
	2	1.36c	12.68b	19.50ab
	3	1.47a	13.69c	20.58bc
	4	1.47a	15.10d	21.59c
Donji Srem	1	1.16c	10.69a	16.81b
	2	1.32a	12.39b	20.01a
	3	1.43b	13.33c	20.92a
	4	1.39ab	14.16d	21.29a

FL –fibre length / *DV – duljina vlakanaca*, DCWT – double cell wall thickness / *DDS – dvostruka debljina stijenki*, FLD – fibre lumen diameter / *PLV – promjer lumena vlakanaca*
*Average values in the same column with different letter (a, b, c, d) are statistically different for p <0.05 (Post hoc Tukey's HSD test). / *Prosječne vrijednosti u istom stupcu s različitim slovom (a, b, c, d) statistički su različite za r <0,05 (Post hoc Tukeyjev HSD test).*

pedunculate oak populations, all highly significant in Donji Srem (Table 3). This could be explained by more differences in the microenvironment of each tree, as well as by differences in the genetic constitution of each tree (Tsoumis, 1991), especially in Donji Srem.

Observing FL in different zones from the pith outwards within sites, we can conclude that in the juvenile wood of all trees from both sites this parameter is statistically less significant compared to the other zones (Table 4). Going from pith to bark within the mature wood, FL increases in the area of MU „Raškovica-Smogvica" until the last zone. However, in trees from another site (MU „Kupinske grede"), it decreases in the last zone. DCWT statistically significantly increases from pith to bark. FLD increases significantly with cambial age in the MU „Raškovica-Smogvica", while in another site, trees have significantly narrower fibre lumens in the juvenile wood and its value is somewhat the same in the other three zones going to the bark, so there are no statistically significant differences in these three zones (Table 4).

4 CONCLUSIONS
4. ZAKLJUČAK

In this paper, anatomical characteristics of wood fibres (FL, DCWT and FLD) of pedunculate oak in the area of Ravni Srem were investigated. All measured characteristics increase from pith to bark apart from FL in MU „Kupinske grede" – Donji Srem that reaches maximum values in the central part of the wood and then decreases towards the bark. Mean values of all three measured characteristics are greater in the area of Gornji Srem.

Based on the calculated coefficient of variation, it was concluded that FL is more variable in MU „Raškovica-Smogvica", unlike the other two characteristics, which are more variable in the area of MU „Kupinske grede". Dimensions of all measured wood fibre characteristics have greater values in the area of MU „Raškovica-Smogvica", due to more favourable hydrological conditions for growth and development of pedunculate oak.

Regarding tree age, results obtained in this study indicate the presence of too mature trees of this species, explaining radial trends in FL. More detailed information on wood properties of pedunculate oak from these two sites would better characterise wood quality and possible alternative use of too mature trees. The recommendation is to monitor fluctuations of groundwater level during longer time in order to establish complete relation between hydrological factors and anatomical characteristics of pedunculate oak wood fibres.

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