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Anatomical Characteristics and Assessment of Wood Fiber Quality of Mature Pedunculate Oak (*Quercus robur* L.) Trees Grown in Different Environmental Conditions

Radoslav Lozjanin[1], Dušan Jokanović[2,*], Vesna Nikolić Jokanović[2], Kristina Živanović[3], Ivan Desimirović[4], Marko Marinković[1]

(1) PE "Vojvodina šume", Preradovićeva 2, RS-21132 Petrovaradin, Serbia; **(2)** University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, RS-11030 Belgrade, Serbia; **(3)** University of Belgrade, Institute for Multidisciplinary Research, Department of Plant-Soil and Nano Systems, Kneza Višeslava 1, RS-11030 Belgrade, Serbia; **(4)** PE "Srbijašume", FE "Severni Kučaj", RS-12240 Kučevo, Serbia

*** Correspondence:** e-mail: dusan.jokanovic@sfb.bg.ac.rs

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ABSTRACT

From productive and ecological point of view, *Quercus robur* L. is an extremely important species and the investigation of its anatomical properties is very significant in terms of its technical and technological utilization. The paper deals with the analysis of the wood fiber characteristics (fiber length, fiber lumen width, double-cell wall thickness, Runkel ratio) of mature pedunculate oak trees in the non-flooded (Management Unit "Vinična-Žeravinac-Puk") and flooded area (Management Unit "GVO 83 b"). One of the aims of the study is to determine the wood fiber quality and the possibility of use in paper and pulp production. When the mean values of the analysed anatomical characteristics are compared, the results show slightly higher values in the non-flooded area, except for the Runkel ratio. The radial pattern of the investigated characteristics depending on the age shows growth in the non-flooded area, while the same tendency in the flooded area was found for the fiber length only. It was recorded that fiber lumen width, double-cell wall thickness and Runkel ratio decreased in the final annual rings. The wood fiber quality for use in pulp and paper production is the greatest in the juvenile zone, unlike sapwood zone, regardless of the site conditions.

Keywords: pedunculate oak (*Quercus robur* L.); ecological conditions; variability of wood fiber characteristics; wood fiber quality

INTRODUCTION

Quercus robur L. is one of the most important woody species in Serbia from an economic and ecological point of view (Nikolić 2016). The two most studied oaks in Serbia in terms of ecology and productivity are *Q. robur* and *Q. petraea* Liebl. (Letić et al. 2017, Nikolić Jokanović et al. 2019, Nikolić Jokanović et al. 2020, Radaković and Stajić 2020). Some authors (Feuillat et al. 1997) state that pedunculate oak and sessile oak are the two most important species of oak in European forests (they occupy 27% of the hardwood forests in Europe) and if it is about their physical-mechanical and anatomical properties, wood density is smaller in pedunculate oak (Deret-Varcin 1983, Nepveu 1984, Levy et al. 1992). Also, heartwood it is formed faster in pedunculate oak, which leads to narrower sapwood (Deret-Varcin 1983, Levy et al. 1992, Vilotić 2000). On the other hand, the dimensions of the vessels in oak trees have been analysed in detail (Levanič et al. 2011, Tumajer and Treml 2016). Tsoumis (1991) has found that the radial variation of the anatomical characteristics in the direction from the pith to the bark confirms a significant influence of cambial age on the wood structure. Vilotić (1992) investigated the anatomical characteristics of the *Quercus virgiliana* Ten. trees grown in different ecological conditions and deduced that the obtained differences were due to many factors such as: genotype, soil, climate, terrain topography and forest type.

Numerous studies dealing with the analysis of the wood fiber quality concluded that the best wood fiber quality for pulp and paper production was found in juvenile wood (Tsoumis 1991, Kennedy 1995, Isebrands and Richardson 2014). Juvenile wood, in contrast to mature wood, has a higher twist angle of microfibriles, lower density, thinner cell walls, greater tension in the longitudinal direction and lower strength (Ištok et al. 2017). Although the wood fiber characteristics are, above all, the subject of studies related to the anatomical structures of soft deciduous trees (willow, poplar, etc.), since in these species wood fibers have the largest volume (Tsoumis 1991), there are some studies that dealt with this issue in hard deciduous trees as well (Wani and Khan 2013, Jokanović et al. 2022a, b). Wood fiber dimensions affect the properties of wood for papermaking and are one of the main characteristics that recommend a species for use in the pulp and paper industry. The characteristics of wood fibers on which paper quality depends are: wood fiber length and width, fiber lumen width, thickness of fiber walls, Runkel ratio, coefficient of elasticity, etc. (Zobel and van Buijtenen 1989). The most suitable wood fibers for paper production should be long, thin-walled and elastic (Lundqvist and Gardiner 2011). The strength of paper directly depends on the wood fiber quality, and some legislative documents related to the evaluation of wood raw materials are generally derived from the characteristics of wood fibers (Clark 1985).

The aims of this study were: (i) to determine wood fiber length, double-cell wall thickness and fiber lumen width and to establish the Runkel ratio in mature pedunculate oak wood grown in different site conditions, as well as the variability of these characteristics going from the pith to the bark; (ii)to compare the obtained values of the analysed elements with reference data for other hardwood species; and (iii)to assess wood fiber quality suitable for use in the paper industry.

MATERIALS AND METHODS

For the purpose of the study, a total of six trees were selected and harvested. Three individuals originated from the non-flooded area (Management Unit (MU) "Vinična-Žeravinac-Puk"), while the other three were located at the flooded site (MU "GVO 83b"). The average age of the studied population in the non-flooded area was 122, while in the flooded area, the trees were slightly older and the average age was 132.

Study Area

The studied site in the non-flooded part of the Srem forests, in the Gornji Srem area, includes MU "Vinična-Žeravinac-Puk". The forest is located on flat terrain, the dead cover is poorly represented, and the humification process is very favourable. Ground vegetation is weakly present, as well as shrubs, because there is no flooding influence. From the floristic point of view, it is *Carpino-Fraxino-Quercetum roboris caricetosum remotae* forest on meadow sedges in a non-flooded area. The mentioned forest represents a single-age, mature stand of mixed type and incomplete composition (0.5-0.6). The main purpose of the stand is the production of technical wood, and from silvicultural point of view, restoration is necessary.

The second locality was studied in the flooded part of Srem forests (Donji Srem) and included MU "GVO 83b". The forest is located on flat terrain, the dead cover is poorly represented, and the humification process is favourable. Occasional flooding is present. There is no ground vegetation, while the shrubs are dense. It is a type of *Fraxineto-Quercetum roboris subinundatum* forest on semiglay soils. It is a mixed stand of incomplete composition (0.6). The main purpose of the stand is that it is a special nature reserve of the second category and is intended for transitional management.

Laboratory Work

Discs, thick approximately 5 cm, were taken at the breast height (1.3 m). Radial segments were taken in the north-south direction along the whole diameter going from the pith to the bark. In order to analyse anatomical characteristics (fiber length, fiber lumen width, double-cell wall thickness, Runkel ratio), one half of the radius length was used, including the whole distance from the pith to the bark. The maceration procedure was applied in order to conduct all microscopic analyses and determine wood fiber dimensions.

Maceration is a process of chemically decomposing wood at high temperatures to disintegrate it into pulp. According to Franklin's methodology, the intercellular material was decomposed and single cells suitable for measurements were isolated. Macerate consists of 30% hydrogen peroxide and glacial acetic acid in a volume ratio of 1:1. The wood samples were first thinned to the size of a stick, then placed in a test tube and treated with the mentioned macerate. 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. Wood fiber length was measured as the distance between the initial and the final point. It should be noted that only undamaged and non-deformed samples were taken into consideration. Fiber lumen width was also measured on exactly the same samples with regular shape. Double-cell wall thickness was calculated as the difference between the fiber width and the fiber lumen width. All investigated characteristics were measured using "Boeco" microscope, connected with specialised software with appropriate calibration. Fiber length was measured at 40x magnification, while fiber lumen diameter and double-cell wall thickness were measured at 400x magnification. Four zones within each tree were selected – close to the pith, in the juvenile wood, in the central part of the tree and in the sapwood. The pith included 20 annual rings, while the juvenile zone comprised about 40-45 annual rings. As for the central part of the wood, it consisted of 45-50 annual rings, and, finally, the sapwood includes 10-20 annual rings. It should be noted that 15 fibers in the early and 15 fibers in the late wood were measured in each of the four mentioned zones. Thirty undamaged wood fibers were sampled in each zone, i.e. 120 per tree, or 720 fibers within the entire study area.

Statistical analyses were performed using the STATISTICA software, version 13 (TIBCO Software Inc., 2017). Factorial analysis of variance (ANOVA) followed by Tukey's post hoc test were used to test significance of differences in the selected fiber characteristics between sites and zones, at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

The results showed that site characteristics notable influenced only fiber lumen width (D) and double-cell wall thickness (d) in pedunculate oak trees, whereas no significant differences were observed in terms of fiber length (FL) and Runkel ratio (R) (Table 1). In contrast to the site effect, the ANOVA evidenced that distance from the pith (i.e. zone) and the "site*zone" interaction affected significantly the studied fiber traits.

Based on the obtained results of descriptive statistics (Table 2), it can be concluded that the mean values of all anatomical parameters such as fiber length (FL), fiber lumen width (D) and double-cell wall thickness (d) are slightly higher in the non-flooded area (MU "Vinična-Žeravinac-Puk") compared to the flooded area (MU "GVO 83b"). On the other hand, Runkel ratio is slightly lower in the non-flooded area. Regarding the coefficient of variation (Table 2), the deviation of fiber length (FL) and Runkel ratio (R) is significantly greater in the non-flooded area. The variation of the double-cell wall thickness (d) is approximately the same, while only the fiber lumen width (D) is slightly more variable in the flooded area.

Based on the obtained results (Figure 1), we can deduce that the mean values of fiber length (FL) show an increasing trend from the pith to the bark on both sites. Fiber lumen width (D) in the non-flooded area shows a large increase starting from the pith to the zone of juvenile wood, where the maximum values are reached, followed by a slight decrease in the central part of the tree and then an increase again in the sapwood. On the other hand, in the flooded area, fiber lumen width (D) increases in a radial direction from the pith to the central part of the tree and then its value reduces in the final annual rings. Double-cell wall thickness (d) constantly increases from the pith to the sapwood in the non-flooded area, and a similar pattern was observed on the flooded site with the difference that the value of the mentioned anatomical characteristics records a slight decrease in the sapwood zone.

Runkel ratio (R) shows a trend of constant growth with age in the non-flooded area, while in the flooded habitat conditions it increases significantly up to the zone of juvenile wood, and then records a gradual reduced value. When it comes to more statistically significant differences between the investigated anatomical characteristics (Figure 1), they were recorded only for: fiber lumen width (D) in the juvenile zone, double-cell wall thickness (d) in the sapwood zone and Runkel ratio (R) in the juvenile zone.

The slightly higher mean values of the studied anatomical characteristics of pedunculate oak trees obtained in the non-flooded area can be partly explained by prevailing ecological conditions at the studied sites. Within the non-flooded area, a bit deeper humus-accumulative horizon was established (up to 80 cm) compared to the flooded area (up to 60 cm), which affected the content of nutrients, first of all, nitrogen, which was much more present on fluvisol in the non-flooded area compared to humogley in the flooded area (Nikolić 2016, Letić et al. 2017). As for the total soil porosity, this relation was also more favourable in the non-flooded area on fluvisol compared to the flooded area on humogley (Nikolić 2016, 2019). The same pattern was observed in the ratio of the sand fraction to the clay

Table 1. Results of ANOVA test for fiber characteristics of pedunculate oak wood from flooded and non-flooded areas divided into 4 zones, observed in the direction from pith to bark.

Source of variation	FL		D		d		R	
	F	p	F	p	F	p	F	p
Site (S)	0.03	0.873	10.74	<0.001	8.75	0.003	0.57	0.452
Zone (Z)	139.04	<0.001	14.50	<0.001	71.00	<0.001	50.19	<0.001
Interaction SxZ	3.01	0.029	4.76	0.002	3.23	0.022	8.40	<0.001

FL – fiber length (μm); D – fiber lumen diameter (μm); d – double-cell wall thickness (μm); R – Runkel ratio

Table 2. Results of descriptive statistics for the investigated anatomical traits of wood fibers.

Locality	Stat.	FL	D	d	Runkel
Non-flooded	Mean	1377.30	20.21	12.12	0.60
	Max	2128.05	35.28	19.65	0.98
	Min	696.82	12.11	5.98	0.35
	SD	282.74	3.58	2.58	0.10
	CV	20.53	17.74	21.32	16.99
Flooded	Mean	1373.24	19.20	11.63	0.61
	Max	1891.84	30.44	14.60	0.85
	Min	851.28	8.49	2.38	0.34
	SD	223.61	3.60	2.47	0.08
	CV	16.28	18.76	21.25	13.93

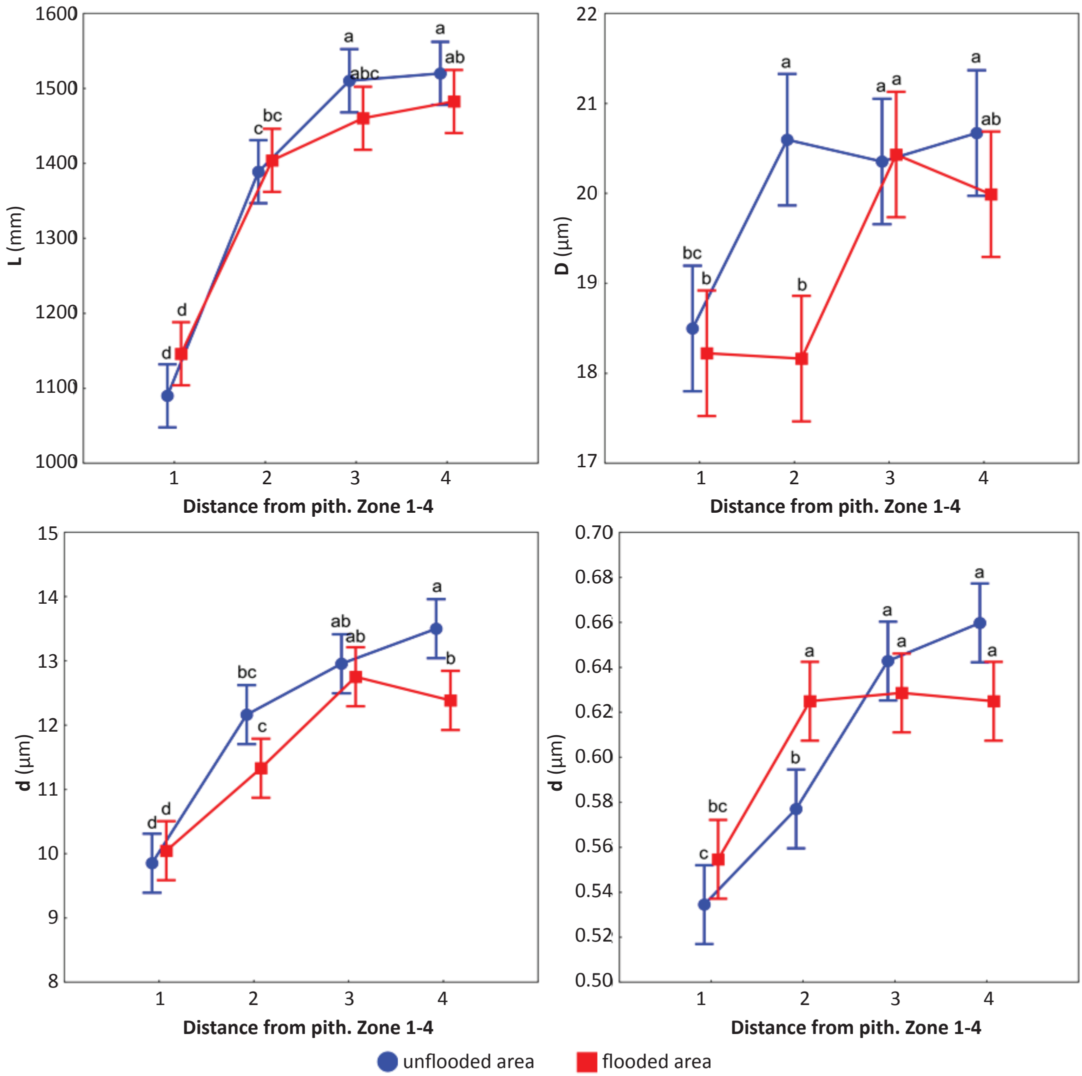

Figure 1. Fiber characteristics of pedunculate oak wood observed in two areas – flooded (red square) and non-flooded (blue circle). Vertical bars denote 0.95 confidence intervals. Results marked with different letter differ significantly at p<0.05 (Tukey's HSD test).

and dust fraction (1:2 at the non-flooded site, i.e. 1:3.5 at the flooded site), which means that the water-air properties and the soil capacity to absorb water are significantly more favourable in the area of Gornji Srem (Nikolić 2016, 2020). The aforementioned affects the slightly higher mean values of the studied anatomical properties of wood fibers within MU "Vinična-Žeravinac-Puk" (non-flooded site conditions), where pedunculate oak satisfies its water needs exclusively through precipitation and groundwater.

The obtained mean values of fiber length at both investigated sites are similar to Mladenova et al. (2017), which are between 1.02 mm for Hungarian oak and 1.46 mm for sessile oak from different sites in Bulgaria. On the other hand, Nazari et al. (2020) established fibers shorter than 1 mm in Persian oak wood (mean value was 0.87 mm). All of these differences were caused by genetic and ecological impacts. Keles and Savaci (2021) found that cambial age significantly affects fiber length in pedunculate oak, gradually increasing from the pith to the bark, which coincides with our results. Sousa et al. (2009) studied wood fibers in cork oak and recorded much higher mean values both for fiber lumen width (23.5 µm), and double-cell wall thickness (18 µm) compared to our results. Some studies (Arend and Fromm 2007, De Micco et al. 2016) concluded that fiber dimensions are sensitive to environmental fluctuations and that they are reduced according to lower water availability. This is closely related to bioecological properties of pedunculate oak whose radial and height growth depend the most on hydrological conditions which significantly affect some anatomical characteristics. Our results completely confirm the aforementioned because more suitable hydrological conditions for pedunculate oak are present in the non-flooded area (water-air properties and more favourable water capacity). As a result, a bit higher mean values of all investigated anatomical parameters were recorded in Gornji Srem area. As for wood fibers in Persian oak (Nazari et al. 2020), highly significant differences were observed at two sites differentiated by altitude and slope. Panshin and de Zeeuw (1980) studied variability of wood fiber dimensions with cambial age in different hardwoods and found reduced values in the sapwood region, which completely coincides with our results. On the other hand, the aforementioned authors found that the dimensions of the double-cell wall thickness increase in the final annual rings, which is compatible with our results in the non-flooded area, while in flooded conditions there is a slight reduction in the value of this element in the sapwood zone.

Helinska-Raczkowska and Fabisiak (1991) concluded that the length of mature anatomical traits, including wood fibers, is from 10% to 20% greater than the length of juvenile wood anatomical elements of oak, and this coincides with our results. Many papers dealt with the variability of wood fibers in different hardwood species such as poplar (Ištok et al. 2017), birch (Luostarinen and Möttönnen 2010) and eucalyptus (Carrillo et al. 2015), showing similar radial patterns. Jokanović et al. (2022a) investigated anatomical properties of pedunculate oak wood fibers in the non-flooded (MU "Raškovica-Smogvica") and flooded (MU "Kupinske grede") area, recording a bit lower mean values of fiber length (1.35 mm, and 1.33 mm, respectively), a bit higher mean values of double-cell wall thickness (13.16 µm, and 12.66 µm, respectively) and almost the same mean values of fiber lumen width as in our paper. On the other hand, the obtained results (Jokanović et al. 2022b) for fiber length of pedunculate oak at the non-flooded and flooded site showed a bit lower mean values in the non-flooded area (1.34 mm) and a bit higher mean values in the flooded (1.41 mm) area compared to our results, while mean values of the double-cell wall thickness and fiber lumen width were very similar.

Runkel ratio, as one of the parameters of the wood fibers for use in the pulp and paper industry, was determined in *Populus bolleana* (Xu et al. 2006), in the plantations of *Leucaena leucocephala* (Oluwadare et al. 2007and in *Juglans regia* (Wani and Khan 2013). Runkel ratio was also investigated in some oak species in order to determine their suitability for papermaking (Bakhshi et al. 2012, Elmas and Öztürk 2022). Wood fibers with a high Runkel ratio produce coarser paper with less cross-linking surface compared to fibers with a low Runkel ratio (Ashori and Nourbakhsh 2009). A lower value of Runkel ratio also indicates the probability of fibers to overlap, creating strong interconnections, which has a positive effect on the strength of the paper (Istikowati et al. 2016). Based on this, it can be concluded that the closer the value of the Runkel ratio is to zero, the higher the strength and qualitative value of the produced paper. Tension wood occurs in poplars, but also in some hard deciduous species, especially in very old trees, and has a negative impact on the physical-mechanical properties of the wood, and thus on the paper quality as a final product (Fang et al. 2008). The same authors found that the paper quality obtained from the gelatinous fibers of tension wood is poor. Antolković (2018) found that the mean value of the Runkel ratio in juvenile white poplar wood was 0.28, while for *Populus bolleana* (Xu et al. 2006) it was 0.66, which is more than double higher.

Therefore, the Runkel ratio obtained in our paper is significantly higher compared to its value for domestic poplars. However, it can also be deduced that the wood fibers of pedunculate oak are somewhat more suitable for the production of paper and pulp compared to allochthonous poplars. Antolković (2018) established that poplar wood fibers from natural populations have significantly better performance for paper production than fast-growing clones planted in plantations. As the best time for maximum use of fibers for this purpose, it is recommended to cut the tree during its transition from juvenile to mature growth phase. Qualitative elements of wood fibers for paper production were also investigated in eucalyptus (Ohshima et al. 2005, Pirralho et al. 2014) and the range of variation of the Runkel ratio was in the interval 0.54-0.67, which is close to results obtained in our study. Wani and Khan (2013) analyzed the Runkel ratio in *Juglans regia* and found that the values ranged between 0.6 and 1, which means that in some cases they reached the limit value, which is why they are not recommended for paper and pulp production.

CONCLUSIONS

In the paper were studied the anatomical characteristics (fiber length, fiber width, double-cell wall thickness, Runkel ratio) of wood fibers in pedunculate oak (*Q. robur* L.) from different ecological conditions. One of the key reasons for conducting such analysis is to determine the wood fiber quality and the possibility of using *Q. robur* for the purpose of papermaking. Based on the radial pattern of the Runkel ratio, it can be concluded that the most suitable wood fibers for pulp and paper production are located in the pith and juvenile zone of the tree, regardless of the site conditions. The results obtained in the paper show that the wood fibers of *Q. robur* grown at non-flooded site are longer and with lower Runkel ratio compared to flooded habitat. It can be deduced that wood fibers from non-flooded area are more suitable for papermaking than those from the other site. Fast-growing species, such as willow, poplar, etc., whose wood fibers are longer, more elastic and thinner-walled, are more suitable for paper and pulp industry compared to *Q. robur*. However, taking into consideration that Runkel classification results in our study are between 0 and 1, *Q. robur* may also be recommended for use in papermaking.

Author Contributions

RL, DJ and VNJ conceived and designed the research; KŽ, ID and MM carried out the field measurements; DJ and KŽ performed laboratory analysis; RL, VNJ and ID processed the data and performed the statistical analysis; MM supervised the research and helped to draft the manuscript; RL and DJ wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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