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VARIABILITY OF WOOD FIBRES OF MATURE PEDUNCULATE OAK IN FLOODED AND NON-FLOODED AREA

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ABSTRACT

The paper deals with anatomical characteristics of wood fibres of *Quercus robur* L. Fiber length, double-cell wall thickness and fiber lumen diameter were analysed from samples in flooded and non-flooded area along Sava river in Serbia. All anatomical elements were measured from pith to bark in order to establish variation with cambial age, not only between researched sites, but also between individuals within each locality. In this study, there was no statistically significant difference only for mean values of fiber length between individual trees at non-flooded area, and in fiber lumen diameter at flooded area. Increasing of mean values of observed anatomical parameters with cambial age is valid for flooded area (MU „Grabovako-vitojevačko ostrvo 20e“), while for another locality (MU “Blata-Malovanci”) it is the case just for double-cell wall thickness. Mean values of all analysed anatomical elements are bigger in flooded area and this could be a consequence of more suitable ecological conditions for *Quercus robur* L. development present in this locality. Obtained variations show nonhomogeneous wood fibre features at both localities.

KEYWORDS: Wood fibres, variability, pedunculate oak, flooded area, non-flooded area.

INTRODUCTION

From ecological and economical point of view, pedunculate oak forests are among the most significant plant associations in Serbia (Nikolić 2016). As for wood fibers, they are mainly used in the pulp and paper industry, while their dimensions should be connected with growth rings width that directly depends on suitability of site conditions (Vilotić et al. 2015). Regarding the mechanical elements that participate in the structure of pedunculate oak wood, apart from wood fibres (libriforms), that are the most deserved for tree stability, fibrous tracheids are also present (Vilotić 2000). Since the pedunculate oak belongs to the ring-porous species, there is a clear difference in the width of the vessels lumen between the early zone, which is lighter colored, and the late zone, which is darker and therefore the growth rings and the boundary between them are clear (Vilotić 2000). It should be noted that in oaks the width of the lumen of the vessels of the early zone is generally about 0.5 mm and they are visible to the naked eye in cross section. Some papers (Gajić and Matijević 1991) examined that anatomical structure of pedunculate oak wood from Ravni Srem is characterized by almost uniform width of annual rings, so relation between early- and latewood is 1:1, and as a result, the transition from early to latewood is abrupt.

In Serbia, anatomical wood structure of *Quercus robur* L. have not been investigated so far. Vilotić (1992) established some differences related to anatomical structure inside the genus *Quercus* and found that these differences are quantitative caused by genotype, pedological, climate and geographical factors, and by plant associations features, as well. Tumajer and Treml (2016) established strong dependence between flooding and growth rings width and as a result it affects variability of dimensions of vessels by pedunculate oak. These authors also concluded that tree-ring width of lowland reference trees is limited by drought stress, and that earlywood conduit size increases with recently increasing temperature. Drobyshev et al. (2008) found that during non-extreme years from climate point of view, pedunculate oak growth depends the most on summer precipitation, while negative effects on its growth are mostly caused by temperature extremes. Levanič et al. (2011) connected growth and anatomical properties of pedunculate oak with its mortality rate and found that variability of anatomical properties is linked a lot with transpiration potential and the other physiological parametres of this species. There are four varieties of *Q. robur* L. in Serbia: praecox, typica, tardissima and tardiflora.

The scope of the paper was to establish variability of wood fibres dimensions (length of fibres, fiber lumen diameter and double cell-wall thickness) depending on cambial age and site conditions and to examine if more suitable ecological conditions (availability of water, deeper humus-accumulative horizon and bigger soil porosity) for *Q. robur* L. growing affect bigger dimensions of wood fibres.

MATERIAL AND METHODS

Study area

As for Ravni Srem area, it consists of two units: Gornji Srem, where a defensive embankment was built in the 1930s and thus excluding the impact of flooding, and Donji Srem which is not defended and where, in addition to atmospheric precipitation and groundwater, a great influence on development characteristics of pedunculate oak has also additional watering through flooding (Nikolić 2016).

For the purpose of the research, a total of six trees were selected and harvested. Three trees are located in the area of Gornji Srem (non-flooded area), within the MU „Blata-Malovanci“, while the other three trees are located in the area of Donji Srem (flooded area), within the MU

„Grabovačko-vitojevačko ostrvo 20e". An average age for stems at the first locality (non-flooded area, MU „Blata-Malovanci") is 135 years, while in another, flooded locality (MU „Grabovačko-vitojevačko ostrvo 20e") it is 98 years.

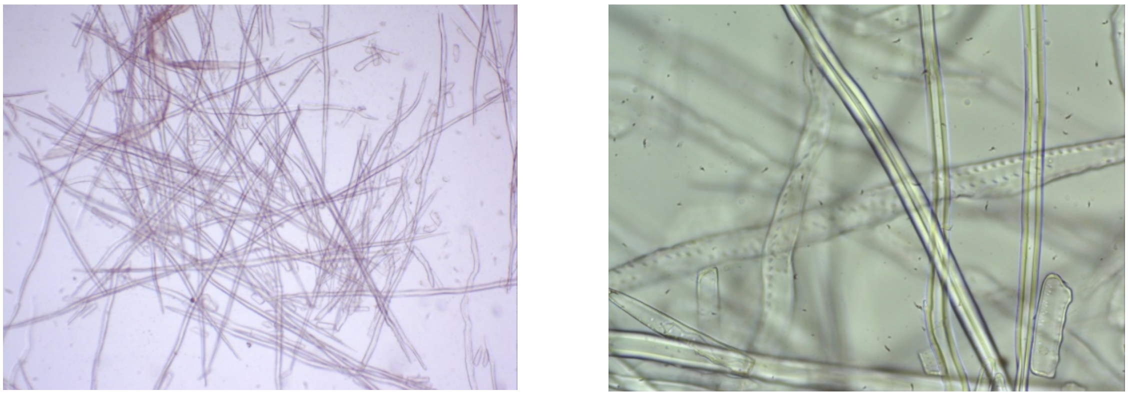
The first locality situated in non-flooded area occupies flat terrain. Dead cover poorly represented, humification process very favourable. There is no flooding in this area, while ground vegetation is sparsely represented. Shrubs are also rarely present, and the forest type is pedunculate oak, hornbeam and ash on meadow blackberries in a non-flooded area. It is high forest of pedunculate oak, hornbeam and ash, mature stand. The health condition of the association is medium, and it is a valuable stand in terms of quality. The main purpose of the stand is the production of technical wood (Nikolić, 2016).

The second site is situated in flooded area. Forest is located on flat terrain. Dead cover is poorly represented with favourable humification process. Flooding is rare and there is no ground vegetation. Shrubs are densely represented. As for the forest type, it belongs to the *Fraxineto-Quecetum roboris subinundatum* association on semiglacial soils (meadow blackberries and alluvial pararendzines) in the occasionally flooded part of Donji Srem (Nikolić, 2016). It is artificially planted even-aged or middle-aged stand of pedunculate oak. Preserved and pure stand with complete canopy (0.7). Health condition of the stand is generally satisfying. As for the quality, it belongs to stand with medium value (21-40% from total volume is technical timber). Medium endangerment from pests and low endangerment from wind. Total area of this department is over 31 ha.

Laboratory work

Discs, approximately 5 cm thick, were cut at breast height, 1.3 m. Radial segments were taken from the south-north direction along the entire length starting from the pith to the bark. For the analysis of the investigated anatomical properties: length of wood fibers – FL (mm), double cell-wall thickness – DCWT (µm) and fiber lumen diameter – FLD (µm), half the length of the test tube was used which includes the segment from the pith to the bark.

In order to perform the necessary microscopic analyses and determine the dimensions of wood fibers, the maceration procedure was applied. Using Franklin's reagent (1945), the intercellular substance decomposed and individual cells suitable for measurements were isolated. The maceration solution consisted of 30% hydrogen peroxide and glacial acetic acid in a volume ratio of 1:1. Samples of wood chopped to the size of a stick were placed in a test tube and treated with the prepared reagent, and then the tubes were closed with corks. 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. The macerates were made in the Laboratory for Tree Anatomy at the Faculty of Forestry in Belgrade. Within each tree, 4 zones were selected. The first near the pith, the second in the juvenile part of the tree, the third which includes the central part of the xylem and the fourth located in the sapwood zone. In each zone, 30 undamaged wood fibers were sampled, which means 120 per tree, or a total of 720 fibers within the entire study area. It should be noted that the juvenile zone comprises about 40 -50 annual rings by all individuals. All investigated dimensions were measured on the same microscope Boeco connected with specialised software with appropriate calibration, and the length of the fibers was measured at x40 magnification, while for double cell-wall thickness and fiber lumen diameter it was conducted at x400 magnification (Fig. 1).



a *b*

Fig. 1: Fiber length: (a) and cell-wall thickness, (b) of pedunculate oak wood.

According to Wheeler et al. (1989), fibers are classified into three groups: 1 – short fibers with a length less than 0.90 mm; 2 – fibers of medium length between 0.90 and 1.60 mm, and 3 – fibers longer than 1.60 mm. Obtained results for an average fiber length of pedunculate oak wood (Tab. 1) are 1.34 mm and 1.41 mm, respectively, so these fibres belong to group 2.

Statistical analysis

All the above-mentioned 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 fiber characteristics between sites and in direction from pith to bark. The one-way analysis of variance (ANOVA) and Post hoc Tukey`s test was used to test significance of differences between trees within each site.

RESULTS AND DISCUSSION

Results of statistical analysis are presented in Tab. 1.

Tab. 1: Statistical values of fiber dimensions of pedunculate oak wood from two sites.

Site	Value	FL (mm)	DCWT (µm)	FLD (µm)
MU „Blata-Malovanci“ (non-flooded area)	N_{mean}	1.34	11.83	18.89
	N_{min}	0.71	2.00	8.28
	N_{max}	1.96	21.62	29.18
	S_d	0.22	2.70	3.53
	C_v	16.42	22.82	18.69
MU „Grabovačko-vitojevačko ostrvo 20e“ (flooded-area)	N_{mean}	1.41	12.00	19.60
	N_{min}	0.77	5.28	10.02
	N_{max}	1.91	19.33	30.36
	S_d	0.21	2.54	3.29
	C_v	14.89	21.17	16.79

N_{mean} – mean value, N_{min} – minimum, N_{max} – maximum, S_d – standard deviation; C_v – coefficient of variation.

Comparing the researched sites, we can conclude that average values of all of three mesured parameters are greater in locality MU „Grabovačko-vitojevačko ostrvo 20e“ than in MU „Blata-Malovanci“ (Tab. 1). Based on the Tab. 1, comparing the researched sites, we can conclude that an average fibres length is greater in MU „Grabovačko-vitojevačko ostrvo 20e“ (1.41 mm) than in MU „Blata-Malovanci“ (1.34 mm). The average values of double cell-wall thickness are 12.00 µm and 11.83 µm, respectively, while the mean values of fiber lumen diameter are also a bit greater in MU „Grabovačko-vitojevačko ostrvo 20e“ (19.60 µm) than in MU „Blata-Malovanci“ (18.89 µm). Based on obtained coefficient of variation (Tab. 1) we can deduce that all three measured anatomical elements are more variable at non-flooded than at flooded-area.

Tab. 2: Results of analysis of variance for fiber characteristics of pedunculate oak wood from two sites and in direction from pith to bark, divided into four zones.

Source of variation	**Fiber length L (mm)**		**Double cell wall thickness d (µm)**		**Fiber lumen diameter D (µm)**	
	F	**p**	**F**	**p**	**F**	**p**
Site	18,78	**0,000017**	0,96	0,328751	8,68	**0,003330**
Zones of distance from pith	20,47	**0,000000**	61,60	**0,000000**	25,24	**0,000000**
Site * Zones of distance from pith	51,14	**0,000000**	2,96	**0,031477**	0,43	0,734301

Bold values show statistically significant differences depending on source of variation.

Significant differences in fiber characteristics are detected, and they depend in distance from pith and sites. Significant differences in fiber length and fiber lumen diameter were detected between sites (Tab. 2). Due to flooding effect, flooded area is considered to be more favorable for longer fibers. Difference was non-significant in double cell wall thickness. According to the results, the change in fiber diameter would be mainly the result change of inner fiber lumen diameter, without significant changes of cell walls.

As for the variability of dimensions of observed elements with cambial age, we can conclude that in MU „Grabovačko-Vitojevačko ostrvo 20e“ values of all three measured parameters increase going from pith to bark (Figs. 2, 3, and 4). On the other side, in MU „Blata-Malovanci“ the same tendency is valid only for double-cell wall thickness (Fig. 3). As for changing of fiber length with cambial age in MU „Blata-Malovanci“, these values gradually increase and reach culmination in central wood zone, while in the last zone close to bark there is no significant fluctuations (Fig. 2). Fiber lumen diameter in MU „Blata-Malovanci“ increase from pith going through the juvenile and central zone, while in the sapwood zone is obvious degressive trend (Fig. 4).

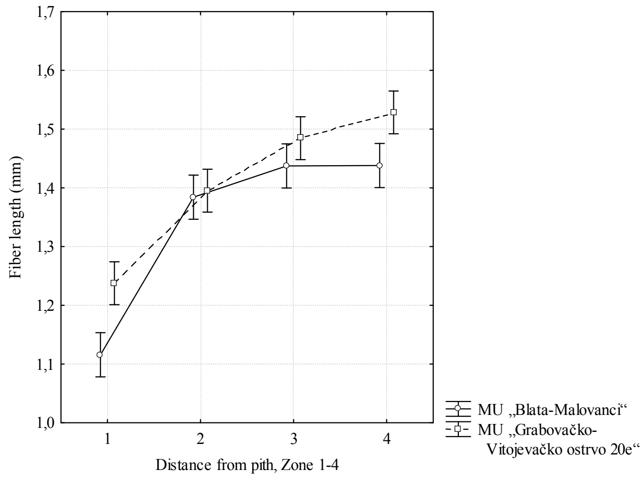


Fig. 2: Radial distribution of fiber length of pedunculate oak wood from two sites. (Bars on graph represent standard error).

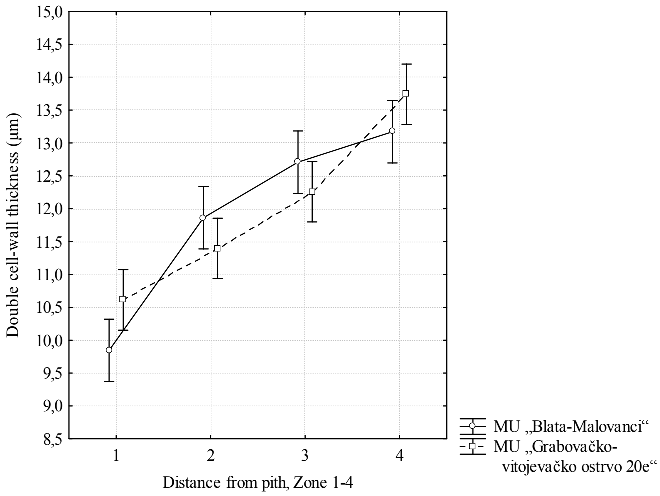


Fig. 3: Radial distribution of double cell-wall thickness of pedunculate oak wood from two sites. (Bars on graph represent standard error).

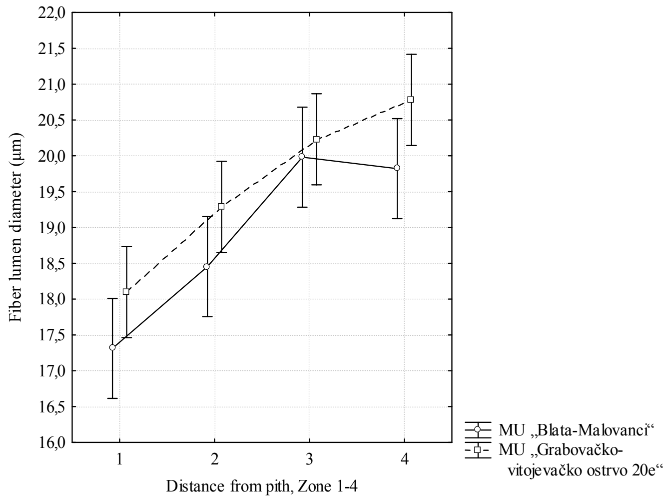


Fig. 4: Radial distribution of fiber lumen diameter of pedunculate oak wood from two sites. (Bars on graph represent standard error).

Tab. 3: Results of analysis of variance for mean values of fiber characteristics of pedunculate oak wood within population from each site.

Source of variation	Site	Fiber length L (mm)		Double cell wall thickness d (µm)		Fiber lumen diameter D (µm)	
		F	p	F	p	F	p
Trees	MU „Blata-Malovanci“	1,56	0,213400	12,37	**0,000006**	14,46	**0,000001**
	MU „Grabovačko-vitojevačko ostrvo 20e“	4,07	**0,017822**	28,96	**0,000000**	2,40	0,092515

Bold values show statistically significant differences depending on source of variation.

Tab. 4: Fiber characteristics of pedunculate oak wood within population from each site depending on distance from pith (Post hoc Tukey's HSD test).

Site	Distance from pith, Zone 1-4	Fiber length L (mm)	Double cell wall thickness d (µm)	Fiber lumen diameter D (µm)
MU „Blata-Malovanci“ (non-flooded area)	1	1,12a*	9,84c	17,31a
	2	1,38b	11,86a	18,45a
	3	1,44b	12,71ab	19,98b
	4	1,44b	13,17b	19,82b
MU „Grabovačko-vitojevačko ostrvo 20e“ (flooded area)	1	1,24b	10,61a	18,10c
	2	1,40c	11,40a	19,29a
	3	1,48a	12,26b	20,23ab
	4	1,53a	13,74c	20,78b

*Average values in the same column with different letter (a, b, c) are statistically different for $p < 0.05$ (Post hoc Tukey's HSD test).

Comparing mean values inside researched localities between individual trees, there was significant difference in this values of double cell-wall thickness and fiber lumen diameter among trees in MU „Blata-Malovanci“ (non-flooded area). At locality MU „Grabovačko-vitojevačko ostrvo 20e“ (flooded-area) there was significant difference in mean values of fiber length and double cell-wall thickness among trees (Tab. 3). In this study, difference was non-significant only in mean values of fiber length between individual trees at non-flooded area, and in fiber lumen diameter at flooded area. This may be explained by differences in its microenvironment and response of each tree to those differences (Zobel and van Buijtenen 1989, Tsoumis 1991, Ištok et al. 2017).

There are statistically significant differences in values of measured elements by fibres inside the site depending on distance from the pith (Tab. 4). It should be noted that less significant variation exists by anatomical elements for trees at locality MU „Grabovačko-vitojevačko ostrvo 20e“ (flooded-area) and all measured medium values are bigger at this site than in MU „Blata-Malovanci“ (non-flooded area). These differences could be explained in the light of present ecological conditions at non-flooded and flooded area. MU "Blata-Malovanci", which belongs to non-flooded area, is located on drier site conditions which confirms the presence of *Carpinus betulus* that is one of the main wood species in this locality. The non-flooded area is also located on automorphic soil which its needs for water satisfies from rainfalls. On the other side, flooded-area belongs to hygrophilous forests and the soil type is hydromorphic, so these associations use water both from rainfalls and groundwater (Nikolić 2016). The surface soil horizon is deeper and richer with nutrients at flooded area (Nikolić 2016) which affects more suitable site conditions for *Quercus robur* L. growth and development and it is connected with its bigger wood fibres dimensions at flooded area. Soil porosity is also a bit more suitable and the groundwater level is much closer to the surface at flooded area which contributes to better watering regime of *Quercus robur* L. in this locality (Nikolić 2016).

Fiber morphology are the key elements responsible for the wood strength and play an important role in determining the qualitative and quantitative wood properties and specific usage of lignocellulosic materials (Gryc and Vavrčik 2005, Nazari et al. 2021). Gričar et al. (2013) found connection between anatomical and hydrological elements of pedunculate oak in floodplain forests in the valley of the river Krka in Slovenia. Cochard and Tyree (1990) examined vessels length and width by oaks at different sites and deduced that vessels in shoots formed in current year are much shorter than at the other places by *Quercus rubra* and *Quercus alba*. Villar-Salvador et al. (1997) found that water deficit is the main factor that affects variability of wood anatomy, while low winter temperatures are less significant by some hardwoods such as *Quercus coccifera* and *Quercus ilex*. By diffuse-porous species was found that low quantity of precipitation is linked with narrow growth rings, greater participation of earlywood vessels, lower participation of latewood vessels, lower vessels diameter and shorter wood fibers (Leal et al. 2004). Garcia-Gonzales and Eckstein (2003) established by pedunculate oak a strong dependence between vessels width and precipitation quantity during late winter – early spring and that affects quantity of available water during ontogenetic development which is related to vessels forming.

Some papers connect growth dynamics and ending of timber forming with climate and physiological factors. Rossi et al. (2006) established that growth rate reaches maximal value during summer maximum of the photoperiod, while Lachaud (1989) found that high content of abscisic acid recorded in the stem after cell division marks the end of cell production. Ištok et al. (2017) researched wood fiber properties in the juvenile wood of white poplar at two different sites and found significant differences, before all between fiber length and double cell-wall thickness. In the same paper was established that fiber length increased in radial direction from pith to bark which coincides with our results. As for the other two dimensions (double cell-wall thickness and fiber lumen diameter), Ištok et al. (2017) also found significant variations with cambial age, but the trend wasn't so consistent like in case of fiber length. Vilotić et al. (2015) researched dimensions of wood fibers by *Paulownia elongata* at two different sites and it should be noted there was performed two fertlizer treatments with different concentrations of nutrients, while one locality at both sites was left as control. Based on obtained results, it was deduced there was no influence of fertilizer on fiber length at one site, while at another were established much greater dimensions compared to control site. The same authors found that there is no significant impact of fertilizer to fiber lumen diameter on both sites.

Rao et al. (1997) established that latewood libriform fibre diameter and wall thickness both increased significantly with cambial age in radial direction. On the other side, the same authors found that the number of wood fibres per mm^2 decreases going from the pith to the bark. The same paper (Rao et al. 1997) obtained similar average values to our paper for fibre lumen diameter and double fibre wall thickness. Dong et al. (2021) investigated fiber characteristics of wood of *Crataegus azarolus* L. and comparing to our results, we can deduce that mean values of fibre length for pedunculate oak are much bigger, while for the other two anatomical elements (fiber lumen diameter and double-cell wall thickness) obtained values are very similar.

Helinska-Raczkowska and Fabisiak (1991) concluded by sessile oak wood that fibre length increases from pith up to 30th ring. Based on radial fibre length variation in eight oak species other authors concluded that the juvenile zone usually comprises 30-40 annual rings (Hamilton 1961, Farmer 1969, Taylor 1979, Petrič and Scukanec 1980, Furukawa et al. 1983) which roughly coincides with our results. The same authors (Helinska-Raczkowska and Fabisiak 1991) deduced that the length of the mature anatomical elements, including wood fibres, is from 10 to 20% greater than the length of the juvenile anatomical elements of oak that is also confirmed by our results. As for average values of fibre lumen diameter and double cell-wall thicknes by cork oak, Sousa et al. (2009) obtained greater dimensions than in our paper, unlike mean values for fibre length (approximately 1.15 mm) which were significantly less than by pedunculate oak in our paper.

CONCLUSIONS

In the paper anatomical properties of wood fibres of pedunculate oak – FL (mm), DCWT (µm) and FLD (µm) were analysed depending on cambial age at two different sites (flooded and non-flooded locality). As for obtained results, we can establish that all three measured anatomical elements increase from pith to bark at flooded area, while in non-flooded locality, only double-cell wall thickness rises in radial direction, while two other elements reach culmination in the central part of the xylem. Mean values are bigger in flooded area for all three anatomical parameters. On the other side, based on obtained coefficient of variation, all three parameters are more variable at non-flooded area. Bigger mean values of all anatomical features of wood fibres at flooded area can be connected with more suitable site (ecological) conditions in this locality. An average groundwater level is much closer to the surface at flooded area which affects better development and growth characteristics of *Quercus robur* L. and this impacts to bigger wood fibre dimensions. It should be noted that surface soil horizon is also deeper at flooded area, and the porosity of this soil is a bit more suitable. The recommendation for the research in the future is to conduct permanent monitoring of groundwater fluctuations and to examine how it affects the rate of radial growth which is closely connected with wood fibre dimensions.

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