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STRUCTURAL CHARACTERISTICS OF NARROW-LEAVED ASH WOOD

Jasmina Popović[1], Milanka Điporović-Momčilović[1], Gordana Radošević[1], Mlađan Popović[1], Dragica Vilotić[1]

[1]University of Belgrade, Belgrade, Serbia
Faculty of Forestry, Department of Wood Science and Technology - Belgrade
e-mail: jasmina.popovic@sfb.bg.ac.rs; milanka.djiporovic@sfb.bg.ac.rs; gordana.radosevic@sfb.bg.ac.rs; mladjan.popovic@sfb.bg.ac.rs; dragica.vilotic@sfb.bg.ac.rs

ABSTRACT

The properties of wood, its behavior during processing, as well as the application and use of wood elements and products, are all consequences of the anatomical structure and chemical composition of wood. The formation of basic anatomical elements of wood (wood cells) proceeds slowly through the metabolic processes. Besides the genetic factors, such processes are influenced by environmental conditions, which cause the dimensional variations of wood structural elements at different positions in the stem. Therefore, the aim of this study was to evaluate the anatomical characteristics and dimensional variations of the most common anatomical elements: wood fibers in the stem of narrow-leaved ash (*Fraxinus angustifolia* Vahl. ssp. *Pannonica* Soo & Simon), represented by the cells that provide the mechanical support and the cells that serve as the transport elements (vessels). Three characteristic cross-sections (transversal, radial, and tangential) of narrow-leaved ash wood were observed and analyzed with the application of scanning electron microscopy (SEM). The integrated system, which includes a *Leica DMLS* light microscope and a *Leica DC 300* camera, coupled with the *Leica IM 1000* software, was used to measure the length, cell-wall thickness, and lumen width of mechanical fibers and vessels.
The results of this study suggest that both the lumen width of vessels and the cell wall thickness of mechanical fibers increase in the radial direction, from the core towards the bark, while the lumen width of fibers decreases in the same direction. In addition, the length of mechanical fibers increases towards the bark, reaching its maximal value at the middle section of the radius. The height position in the tree trunk also shows the influence on the dimensions of the structural elements. Both the length and the cell wall thickness of mechanical fibers decrease towards the top of the trunk, while the lumen width of fibers increases.

Key words: anatomical characteristics, vessel width, fiber length, and fiber cell wall thickness

1. INTRODUCTION

Ash wood occupies 1-2 % of the total forest area in most European countries (Enderle et al., 2019). There are four species of ash (*Fraxinus* spp.) that originate from Europe, with the common ash (*Fraxinus excelsior* L.) being the most widespread and present in almost all European countries, and the other three species limited to southern Europe. A significant and widespread species in the Republic of Serbia is the narrow-leaved ash (*Fraxinus angustifolia* Vahl. Ssp. *Pannonica* Soo & Simon), which can be found in monodominant and mixed forests (Bobinac et al., 2010) of larger river valleys (Danube, Sava, Tisa, West, South, and Grat Morava, Kolubara, Nisava, Timok) (Banković et al., 2008). Most of the mixed forests with narrow-leaved ash include the Common Oak (forest type *Querco-Fraxinetum serbicum* Rud.), but there are also communities with the Willow, Poplar, Alder, and Hornbeam. In the lower reaches of the Sava River, monodominant narrow-leaved ash forests cover 1,401 ha, and the mixed forests of common oak and narrow-leaved ash (*Fraxino angustifoliae-Quercetum roboris* Jovanović et Tomić 1979) and the forests of common oak, narrow-leaved ash, and hornbeam (*Carpino-Fraxino-Quercetum roboris* Miš. et Broz 1962) cover an area of 22.181 ha (Bobinac et al., 2010).

Due to its good physical, mechanical, and aesthetic properties, ash wood is highly valued and has great economic importance in many regions of Europe (Enderle et al., 2019). Ash wood is used for the production of cut and peeled veneer and parquet in construction and shipbuilding, as well as for the production of designed furniture. Due to its elasticity, it is especially suitable for the production of sports equipment (Vilotić, 2000; Šoškić and Popović, 2002; Enderle et al., 2019).

Both the quality and the application value of wood depend on its properties. The anatomical characteristics of wood tissue are the basis for the other properties of wood and are often used to assess its quality (Liu et al., 2020). Due to its biological origin, wood is a heterogeneous material (Bouslimi et al., 2019) whose tissue (xylem) builds a large number of cells that are different in shape, dimensions, orientation (axial and radial), and function in a living tree. The tissue of deciduous species consists of three basic cell types: vessels (conductive elements), libriform or wood fibers, responsible for mechanical strength, and parenchyma, which stores and transports nutrients (Zieminska et al., 2013).

Wood cells have different structural characteristics (Zieminska et al., 2013), which depend on a number of factors that have an influence on tree growth, such as genetic origin, cambial age, and growth conditions (Liu et al., 2020). The structural properties of the elements of the xylem anatomical structure are mainly fixed during formation and largely define their functionality, including the transport and storage of water and nutrients and the provision of mechanical support (Crivellaro et al., 2016). For example, the conductive elements (vessels in deciduous species) play a crucial role in transporting water from root to leaf. The speed of water conduction along the tree depends on many factors, but it is traditionally believed that the width of the vessels largely determines the efficiency of water transport and thus the rate of water usage in wood (Gleason et al., 2012). Besides, the conductive elements (vessels) show great anatomical diversity in regard to their size, shape, arrangement, and grouping (Scholz et al., 2013).

One of the most studied properties of wood is density, which is considered a key characteristic that affects the mechanical and physiological performance of wood (Zieminska et al., 2013) and can be used to predict the mechanical properties of wood as well as the cellulose yield and paper quality (Bouslimi et al., 2019). The mechanical properties of wood are considered to be closely related to its density, with wood of higher density tending to be harder and more resistant to failure under stress (Chave et al., 2009). Density, as an important indicator of wood quality, is mostly influenced by anatomical and chemical properties (Bouslimi et al., 2019; De Mil et al., 2018). As a measure of the ratio of cell wall tissue in wood, the density depends on the relation between the cell wall thickness and the cell width, i.e., the volume of cell cavities (lumens) (Lundgren, 2004), where the cell wall density of about 1.53 g/cm^3 is considered constant (Leal et al., 2011). Besides this ratio of cell wall thickness and lumen volume, the density of deciduous species, which are characterized by a complex structure, also depends on anatomical characteristics such as the differences in cell types (fibers, vessels, rays, and parenchyma cells), as well as their share and distribution in wood tissue (Leal et al., 2011, Bouslimi et al., 2019, Zieminska et al., 2013). The conductive elements of deciduous species have very large lumens compared to the other wood cells. Therefore, their size and number have a large influence on wood density and tend to be inversely proportional to this property (Leal et al., 2011). However, the fiber wall fraction is thought to have the greatest impact on wood density, while the vessels and parenchyma affect wood density locally and in other ways (De Mil et al., 2018).

The dimensions of the fibers also affect other properties of wood besides density. Fiber length is one of the most important and thoroughly researched properties. Fiber density and length are positively correlated with shear strength (Lundgren, 2004). On the other hand, the wall thickness and the fiber width affect the bending of fibers and their properties, which are important in papermaking. During paper formation, the thin-walled fibers more easily collapse and bond well with each other, which has a positive effect on the tensile strength, light scattering, and smoothness of the paper surface (Lundgren, 2004).

Besides the above-mentioned, the heterogeneity of wood originates from the large differences in the cell structure, not only between different species but also within the same species and even within the same tree (Bouslimi et al., 2019). Cell parameters such as length, wall thickness, and width vary from early to late wood and from pith to cambium within the tree (Bouslimi et al., 2019). Namely, in addition to genetic origin, the cambial age of trees has a significant impact on the microstructure of trees. The tree growth in the radial direction, due to cambium activity, can be divided into juvenile and mature periods (Liu et al., 2020). The tissue of the wood formed during the juvenile growth period is located in the zone around the pith. The share of juvenile wood depends on the time period when the tree reaches maturity, which varies with regard to the species and the growing conditions (Liu et al., 2020), as well as the age of the tree itself. Wood cells formed during the juvenile period of tree growth are shorter in length and thinner in walls, and they also differ in chemical composition (Bernabei et al., 2000). The frequency and width of the vessels, the length and width of the fibers, and the thickness of the cell wall change with age (Liu et al., 2020). In the radial direction, from the pith towards the bark, the dimensions increase, and the frequency of the vessels and the total width of the growth ring decrease (Salvo et al., 2013). It has also been found that the frequency and surface area of the vessels increase from the base to the top of the tree (Salvo et al., 2013), while the cell wall thickness increases and the fiber width decreases with height (Salvo et al., 2013).

The production of high-quality wood products requires a thorough knowledge of wood properties, especially its anatomical characteristics, which can have a great impact on the manufacturing process and product performance (Leal et al., 2007). The existing wide variability of anatomical characteristics complicates the accurate assessment of wood performances. Hence, a better knowledge and understanding of the variability within wood species would be useful for wood quality research and wood application (Bouslimi et al., 2019). Narrow-leaved ash belongs to the fast-growing species that has an industrial value, and therefore, there are growing interests for this species in Europe (Guler et al., 2015). Therefore, this paper presents the investigation of the anatomical properties of narrow-leaved ash wood at different positions in the radial direction and along the height of the tree, sampled from the area of Morović (Republic of Serbia), as an insufficiently studied species.

2. MATERIAL AND METHODS

The samples of narrow-leaved ash (*Fraxinus angustifolia* Vahl. ssp. *Pannonica* Soó & Simon) were taken from the forest governed by the *Morović* Forest Administration of the *Vojvodinašume* State Enterprise, Serbia. The three sampled trees were selected by the mean-stand tree method (Mirković, 1972). The sample discs of 5 cm in thickness were cut from each trunk at three different height positions (1.3 m, ½, and 3/4 of the trunk height).

Table 1: *Dendrometric characteristics of sampled trees*

Trunk	Age (years)	Height - h (m)	Diameter (cm)		
			h=1.3 m	½ h	¾ h
1	73	29.20	26.50	19.35	15.90
2	73	31.10	30.75	20.50	18.80
3	70	28.80	24.25	19.20	15.75

The radial pieces of a 1x1 cm cross-section were cut out of each sampled disc, from the pith to the bark. These pieces were used to analyze parts close to the pith **(A)**, from the middle **(B),** and near the bark **(C)**, as shown in Fig. 1.

Preparation of samples for scanning electron microscopy (SEM) imaging

Samples for SEM microscopy were cut from the middle of the radial test piece (**B**) taken from the sampled disc (Figure 1) at a height of 1.3 m from the tree. A microtome knife (*Leitz Wetzler, Germany*) was used to cut transversal, radial, and tangential samples of 60–80 µm in thickness and 1 x 1 cm in format. After being cleaned of dust particles, grease, and other impurities, the evaporator (*model Leica EM SCD005*) was used to cover the samples with a 10-15 nm thick layer of gold.

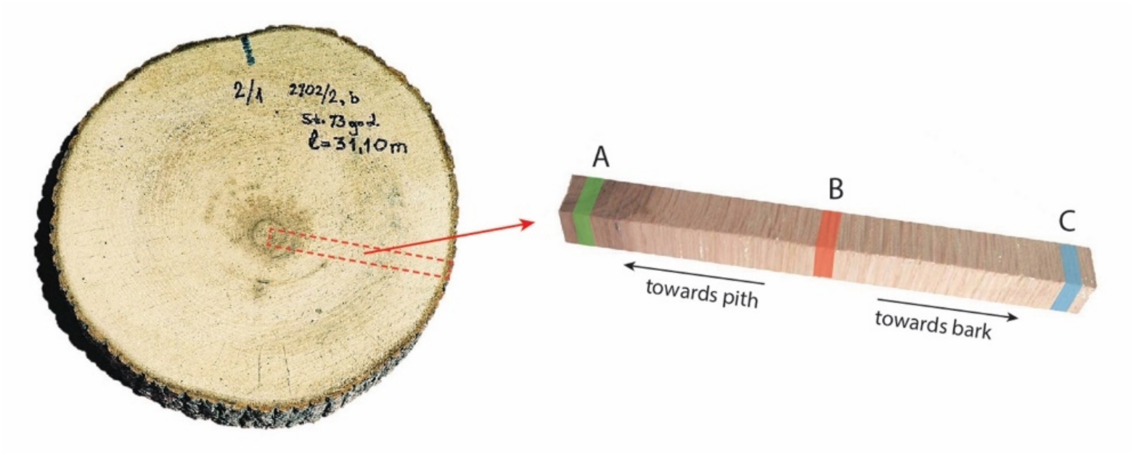

Figure 1:*Radial piece used in anatomical characterization*

Scanning electron microscopy (SEM) imaging

The prepared samples were scanned using the SEM microscope *JEOL JSM-6610LV* (*Tokyo, Japan*) at an operating voltage of 20 kV and a vacuum of 20 µPa. The distance between the samples and the electron source during the scan was about 10 mm, and the time of data acquisition was 30 s per image. The Wolfram fiber was used as the electron source (cathode) for the electron cannon of the microscope.

Preparation of samples for measuring vessel width

A microtome knife (*Leitz Wetzler, Germany*) was used to cut the 20 µm thick samples in a transversal direction out of each section of the radial test piece (**A**, **B,** and **C**, Figure 1) taken from the breast height (1.3 m) of the tree.

Preparation of samples for measuring fiber dimensions

The parts of the radial test pieces (**A**, **B,** and **C**, Figure 1), taken from a tree height of 1.3 m and which remained after preparation of samples for measuring vessel width, were further cut into smaller pieces with dimensions of about 2 x 2 x 20 mm using a microtome knife (*Leitz Wetzler, Germany*). The same procedure was also applied for the middle part of the radial test pieces (**B**), sampled at 1/2 and 3/4 of the tree height. The comminuted material was then macerated in order to measure the fiber dimensions. The maceration was performed according to the Schulz method by treatment with potassium chlorate ($KClO_3$) and concentrated nitric acid (68% HNO_3) (Chamberlain, 1932).

Measuring the dimensions of anatomical elements

The acquisition of digital photographs of prepared samples and then the precise electronic measurements of anatomical elements are all enabled by using the system consisting of the *Leica DMLS* light microscope and the camera, the *Leica DC 300* (*Switzerland*), with the software support of the *Leica IM 1000*.

The samples prepared for the measurements of vessel width were monitored and photographed using a microscope at 5x magnification (Figure 2 a). The width of 30 vessels was measured at each sampled position. The width of the vessels in the tangential direction (tangential width) was measured due to its smaller variability in relation to the radial one (Vasiljević, 1967).

Macerated wood fragments were transferred to a glass holder with an anatomical needle, observed under a microscope, and photographed. The fiber length was determined using digital photographs of samples (macerates) taken at 5x magnification (Figure 2 b), while the magnification of 20x was used to measure the thickness of the cell wall and lumen width. Each prepared sample was used to measure the length of 100 fibers, while the thickness of the cell wall and the lumen width were measured on 50 fibers.

The mean value of the length, the cell wall thickness, and the lumen width of narrow-leaved ash wood fibers for each position in the radial direction (**A**, **B,** and **C**) at 1.3 m height and at 1.3 m 1/2 and

3/4 of the tree height (for position **B**) was calculated as the arithmetic mean of the measured values for all three sampled trees.

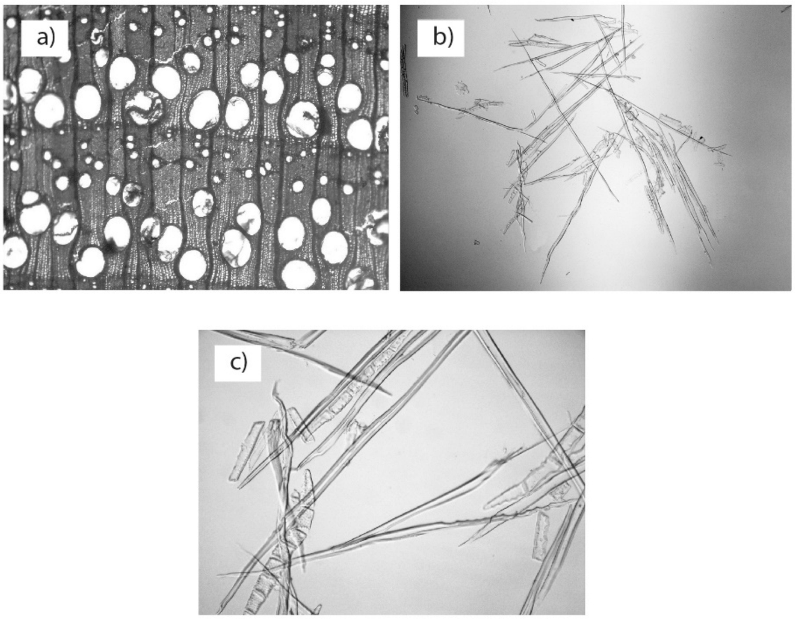

Figure 2: *The photographs of the samples for measuring the dimensions of anatomical elements of narrow-leaved ash wood: a) width of vessels (magnification 5x), b) fiber length (magnification 5x), and c) cell wall thickness and fiber lumen width (magnification 20x)*

Statistical calculations

The single-factor ANOVA, at a confidence level of 95%, was used for the comparison and evaluation of differences between the mean values of the results obtained from different sample groups.

3. RESULTS

Microscopic structure of narrow-leaved ash wood

The observation and analysis of three characteristic cross-sections of wood using SEM microscopy (Figure 3) have provided data concerning the type, arrangement, and interrelationship of anatomical elements in the narrow-leaved ash wood structure.

The narrow-leaved ash belongs to the large ring-porous species, with clearly visible growth rings, which can be seen in the transversal cross section (Figure 3 a). In addition to the noticeable vessels of early wood, the transversal cross-section (Figure 3 a) also shows vessels of late wood, with narrower lumens, rays (radial parenchyma), and wood fibers. At higher magnification, the parenchyma cells surrounding the vessels are clearly visible (Fig. 3 b). The vessels of the early wood are arranged individually, or in groups of two (Fig. 3 b), very close to each other. These vessels have wide lumens and are visible to the naked eye. According to the literature data, the width of the lumen of the early wood vessels is 150 - 350 µm, while the width of the late wood vessels is in the range of 15 - 80 µm (Vilotić, 2000).

The longitudinally cut conductive elements (vessels), mechanical fibers, and cells of rays are visible on the radial cross-section of the narrow-leaved ash wood (Figure 3 c). The tangential cross-section (Figure 3 d) shows rays (radial parenchyma), walls and lumens of mechanical fibers, and the segments of vessels. The rays are homocellular in structure, about 10 cells high, and are mostly biseriate ones (Figure 3 d). According to the literature data, rays can be uniseriate, and their height is up to 0.5 mm (Vilotić, 2000), accounting for about 15% of the wood structure (Sisojević, 1982). The share of mechanical fibers, which make up the basic mass of narrow-leaved ash wood tissue, ranges from 50 to 72% (Šoškić and Popović, 2002). The fibers are 0.15 to 1.6 mm in length (Šoškić and Popović, 2002), with very pointed ends, wide lumens, and without living content. The pits are simple, cracked, and angled.

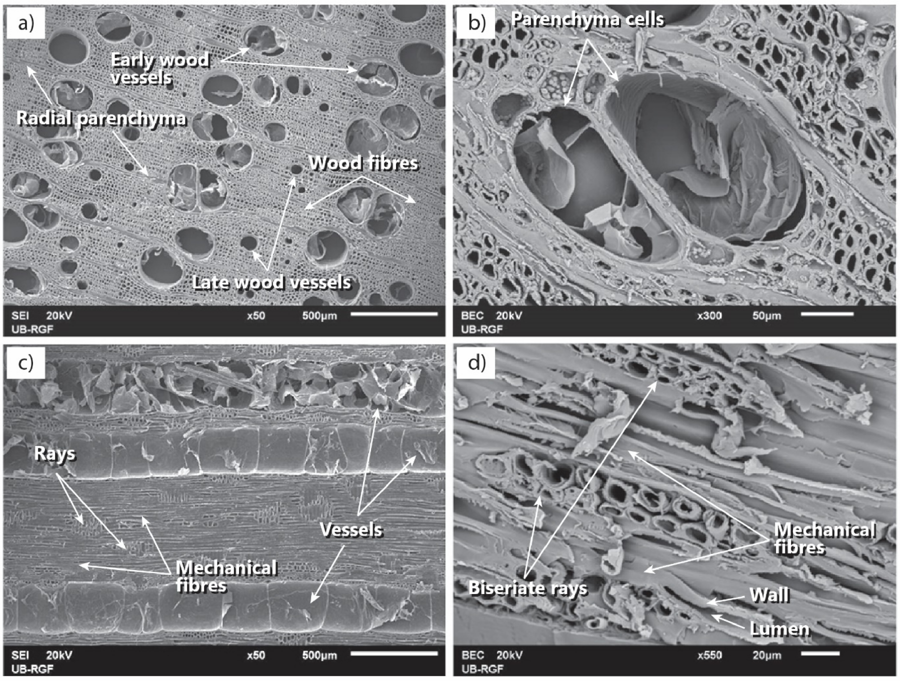

Figure 3: *SEM microphotographs of the transversal (a and b), radial (c), and tangential (d) cross-section of narrow-leaved ash wood*

Dimensions of anatomical elements of narrow-leaved ash wood

The graphs in Figures 4 through 7 show the dimensions of the anatomical elements of narrow-leaved ash wood. Figure 4 shows the mean values for the tangential width of the lumen in vessels at different positions in the radial direction and at the tree height of 1.3 m.

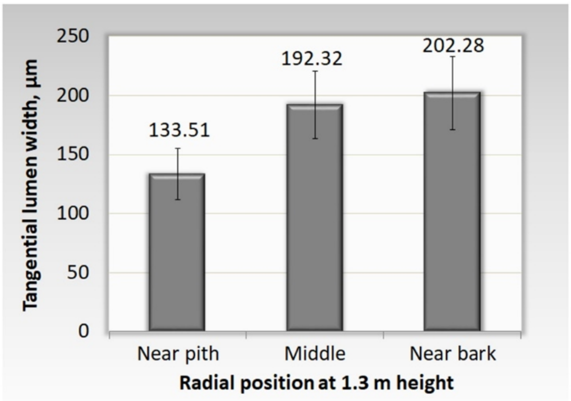

Figure 4: *The lumen tangential width of the vessels in narrow-leaved ash wood (height 1.3 m)*

The mean values of the lumen tangential widths of the vessels, measured in the narrow-leaved ash samples taken from 1.3 m height, range from 133.51 μm in the section near the pith to 202.28 μm, as measured in the outer growth rings. The differences in the mean values of the tangential width of vessels for all three radial positions are found to be statistically significant (Table 2). The minimum value of the vessel's tangential width of 93.66 μm was determined at the pit section, while the maximum value of 293.14 μm was recorded at the position near the bark. Slightly higher values of the tangential width of early wood vessels, ranging from 192.20 to 230.34 μm, were found in the plantation-grown samples of *Fraxinus angustifoliae* Vahl., aged 25 to 38 years, in Turkey (Güler et al., 2009). Differences in the vessel width of the samples examined in this work and the samples of narrow-leaved ash originating from Turkey may be a consequence of different growth conditions.

The mean values of the mechanical fiber length at different positions along the diameter of the trunk at 1.3 m height, as well as different positions along the height of the tree, are shown in Figure 5.

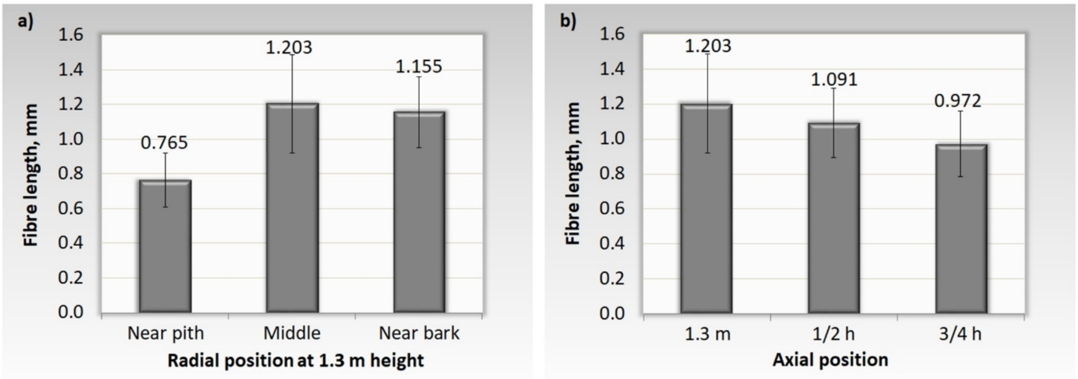

Figure 5: *The length of mechanical fibers of narrow-leaved ash wood in regard to: a) different radial positions measured at the 1.3 m height of the trunk; and b) different height positions along the tree trunk measured at the middle radial section*

The length of mechanical fibers in the samples taken at a tree height of 1.3 m ranges from 0.765 mm (near the pith) to 1.203 mm (in the middle section). However, the maximum fiber length at 1.3 m height of 1.794 mm was measured in the section near the bark, while the lowest value of 0.448 mm was recorded in the section near the pith. At the same time, there was no statistical difference in the fiber length, at 1.3 m height, between the middle section and the section near the bark (Table 2).

In addition, it can be noticed that the fiber length tends to decrease with higher vertical position along the tree trunk, i.e., from 1.203 mm (at 1.3 m) to 0.972 mm, as measured in the middle section at 3/4 of the tree height. At this height, the smallest fiber length for all tree samples was 0.500 mm, measured at the middle section. The maximum value of 1.724 mm was found at a height of 1.3 m, also measured in the middle section. Differences in the fiber length values of narrow-leaved ash wood that exist between samples from different tree heights are statistically significant (Table 2). The fiber length values, determined in this work, are in accordance with the values ranging from 0.15 to 1.6 mm given by Šoškić and Popović (2002) for *Fraxinus excelsior* L., as well as with the values of fiber length of narrow-leaved ash grown in Turkey ranging from 1.15 to 1.33 mm (Güler et al., 2009).

The mean values for the cell wall thickness of mechanical fibers of the mature narrow-leaved ash wood with regard to the radial position are shown in Figure 6 a), while Figure 6 b) shows the cell wall thickness along the height of the tree, measured at the middle radial section.

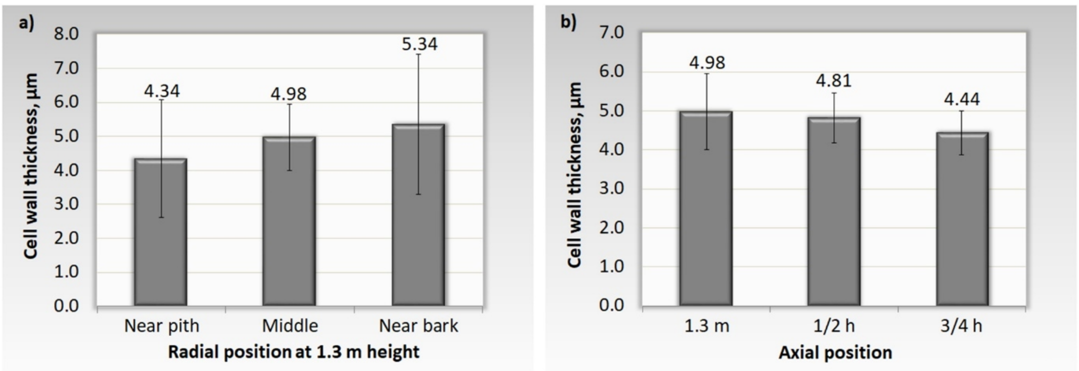

Figure 6: *Cell wall thickness of mechanical fibers of narrow-leaved ash wood with regard to: a) different radial positions measured at the 1.3 m height of the trunk; and b) different height positions along the tree trunk measured at the middle radial section*

There is a trend of increase in cell wall thickness in the radial direction, from the pith towards the bark, of narrow-leaved ash wood fibers, measured at 1.3 m tree height. The cell wall thickness of the mechanical fibers of the samples from this height ranges from 4.34 µm (near the pith) to 5.34 µm, as measured in the section near the bark. Statistical analysis showed that the values of the cell wall thickness of fibers near the pith differ significantly compared to the samples from the other two positions (Table 2). When moving from the base to the top of the tree, the cell wall thickness of the mechanical fibers decreases from 4.98 µm at 1.3 m height to 4.44 µm, as recorded at 3/4 of the height of the narrow-leaved ash tree. The cell wall thickness of the fibers at 3/4 of the tree height shows statistically significant differences compared to the samples in the lower parts of the tree. The obtained results are in accordance with the values ranging from 3.57 to 5.47 µm for the cell wall thickness of ash fibers given by Güler et al. (2009).

Figure 7 a) shows the mean values of the lumen width of the fibers with regard to the radial position, while Figure 7 b) shows the lumen width with regard to the position along the height of the tree.

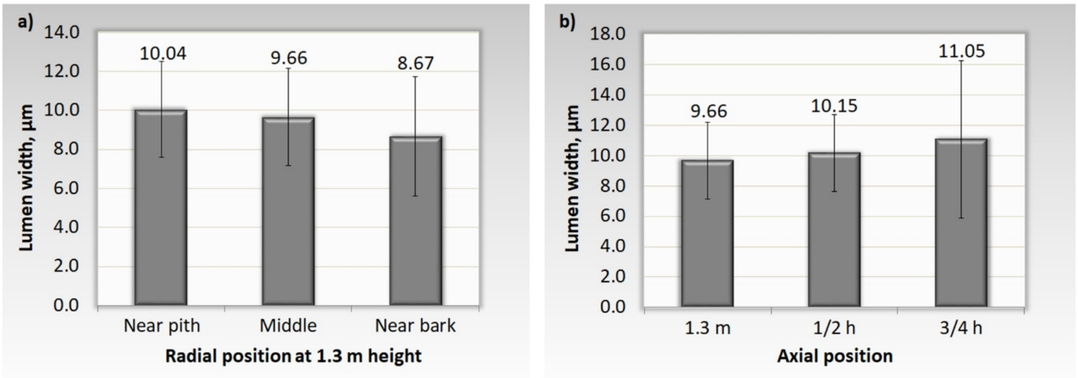

Figure 7: *Mechanical fibers lumen width of narrow-leaved ash wood in regard to: a) different radial positions measured at the 1.3 m height of the trunk; and b) different height positions along the tree trunk measured at the middle radial section*

The lumen width of mechanical fibers decreases in the radial direction, from the pith to the bark, and increases from the base to the top of the tree. At 1.3 m height, the smallest lumen width of 8.67 µm was measured in fiber cells from near the bark. This value has a significant difference in comparison to the other samples taken from the same height (1.3 m). The values of lumen widths of fibers sampled from 3/4 of the tree height also differ significantly compared to the samples from 1.3 m and 1/4 of the tree height (Table 2). The measured values are slightly lower compared to the values of 14.85 - 9.71 µm given by Güler et al. (2009) for the lumen width of narrow-leaved ash.

Table 2. *Statistical comparison of anatomical element dimensions of narrow-leaved ash wood depending on the radial position and the position along the tree height*

Radial direction (at 1.3 m)			***Position along the tree height***		
	P	***F/Fcrit***		***P***	***F/Fcrit***
		Width of vessels (1.3 m)			
near pith / near bark	9.92E-82	159.99*			
middle / near bark	0.001142	2.78*			
near pith / middle	4.03E-70	127.91*			
		Fiber length			
near pith / near bark	5.75E-29	48.02*	***1.3 m/ ½ h***	0.001664	2.61*
middle / near bark	0.321295	0.25	***½h / ¾ h***	6.06E-08	7.95*
near pith / middle	1.33E-25	40.60*	***1.3m /¾ h***	5.15E-10	11.03*
		Cell wall thickness of fiber			
near pith / near bark	3.32E-05	4.60*	***1.3 m/ ½ h***	0.13339	0.58
middle / near bark	0.088735	0.75	***½h / ¾ h***	4.15E-06	5.74*
near pith / middle	0.000764	3.00*	***1.3m /¾ h***	8.56E-07	6.61*
		Lumen width of fiber			
near pith / near bark	0.000195	3.69*	***1.3 m/ ½ h***	0.095318	0.72
middle / near bark	0.0122	1.65*	***½h / ¾ h***	0.022678	1.35*
near pith / middle	0.23019	0.37	***1.3m /¾ h***	0.009305	1.77*

h – tree height; **denotes a statistically significant difference at the confidence level of 95 %*

4. DISCUSSION

Through the analysis of the results obtained in this work, it was found that there is variability in the dimensions of the anatomical elements of the narrow-leaved ash wood, both in the radial direction and in the height of the tree. The fiber length is an important anatomical property of wood that affects its mechanical characteristics and behavior during processing (Lundgren, 2004), while the fiber wall thickness has a crucial influence on the density of wood. Having in mind that the dimensions of anatomical elements influence the properties of wood, it could be expected that there will be variability in the properties and quality of the wood of narrow-leaved ash, both in the radial direction and along the height of the tree. In addition, it was found that the differences in the dimensions of the anatomical elements are particularly pronounced in the section near the pith and in the upper parts of the tree (3/4 of the tree height). Lower values of cell wall thickness and fibber length in the section near the pith are in accordance with the characteristics of fibers that are formed in the juvenile period of tree growth. At the same time, the lower cell wall thickness and the higher values for the lumen width of the fibers in the section near the pith indicate a lower density of this part of the tissue and thus poorer mechanical properties of these parts of the xylem. However, the fibers of the thinner cell walls present in the section near the pith and at 3/4 of the tree height, according to the literature, are suitable for paper manufacturing (Lundgren, 2004).

In addition to its key role in transporting water in a living tree (Gleason et al., 2012), the vessel width in the same way affects the efficiency of chemical transport during chemical wood processing. Based on that, the obtained results indicate a better conductive function of the central and ash tree tissue to the bark. On the other side, although based on the fiber wall thickness in the middle section and in the section near the bark, a higher density and thus better wood quality can be expected, the higher values for the tangential width of vessels in these parts of the narrow-leaved ash wood can result in a negative effect on density, especially in early wood zones where the vessels are arranged very close to each other.

5. CONCLUSIONS

The results presented in this paper confirm the variability of all examined dimensions of structural anatomical elements of narrow-leaved ash wood with regard to its position on the tree. Simultaneously, considering the evident influence of anatomical properties on other properties of wood, the presented results indicate possible variability of properties (density and mechanical properties), i.e., the quality of narrow-leaved ash wood, both in the radial and axial directions. The variability of the dimensions of the anatomical elements of narrow-leaved ash wood is especially pronounced in the section near the pith and in the upper parts of the tree. In the radial direction, from the pith towards the bark, the cell wall thickness shows a decreasing trend, while the lumen width of fiber increases. The same trend was observed in the axial direction, from the base to the top of the tree. Accordingly, the lower density and poorer mechanical properties of wood in the section near the pith and in the upper parts of the tree can be expected. Therefore, the application of these parts of the tree trunk for paper manufacturing can be considered.

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