

Nanohardness and elasticity of cell walls of Scots pine (*Pinus sylvestris* L.) juvenile and mature wood

P. MANIA^{1*} and M. NOWICKI²

¹Department of Wood Science and Thermal Technics, Faculty of Forestry and Wood Technology, Poznań University of Life Sciences, Wojska Polskiego 38/42, 60-627 Poznań, Poland

²Center for Advanced Technology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 10, 61-614 Poznań, Poland

Abstract. The aim of this study was to determine the hardness and reduced modulus of elasticity of juvenile wood of Scots pine (*Pinus sylvestris* L.) using the nanoindentation method, and then to compare the results obtained with those of mature wood. The hardness of juvenile pine wood determined by means of the nanoindentation method was 0.444 GPa while for mature wood it was 0.474 GPa. Statistically significant differences between the values were found. The reduced modulus of elasticity in juvenile wood was 14.0 GPa and 16.4 GPa in mature wood. Thus, the hardness values obtained were about 7% higher, while the modulus of elasticity was 17% higher in mature wood. All determinations were made in the S2-layer of the secondary cell wall.

Key words: nanohardness, cell wall, juvenile wood, mature wood, modulus of elasticity.

1. Introduction

The nanoindentation technique has been gaining popularity in recent years in testing the mechanical properties of various materials [1–3]. In the research of wood, nanohardness allows to determine the heterogeneity of mechanical parameters of the cell wall thickness, related to the arrangement of individual structural compounds. Thus, this method is much more effective than the previously conducted tests of the mechanical properties of cell walls in the tensile test of microtome wood samples, which are time consuming and do not reflect their variability in cell wall thickness. The nanoindentation method allows to determine the variability of mechanical properties of cell walls, which is conditioned e.g. genetically (juvenile and mature wood) and dependent on external factors influencing the quality of the wood created during tree growth. Recently, the nanoindentation method has been used extensively in cellulose fiber studies, which focus on measuring nanohardness and reduced Young's modulus. The earliest literature reports on wood research using this technique date back to 1997, when Wimmer [4] determined the longitudinal hardness and Young's modulus in the S2-layer of the secondary cell wall of spruce wood tracheids, stating that both the hardness and Young's modulus of late wood tracheids were higher than those of early wood tracheids. This was the first time when the nanoindentation technique had been used to study single cell walls. Subsequent experiments were carried out by Gindl in 2004 [5], also on cell wall structures of tracheids of mature spruce wood, stating that only the modulus of elasticity is significantly dependent on the microfibril angle (MFA), while hardness is

dependent on lignin content. This was confirmed by subsequent studies in which cell walls of ten different species of wood were tested with this method and which did not show any significant effect of the species on the hardness of cell walls, but significant differences in the values of modulus of elasticity were found [6]. Yu et al. [7] carried out nanoindentation on bamboo grain in both longitudinal and transverse directions, and the results showed different mechanisms of deformation between directions.

Since the first attempts to determine the nanohardness of wood had been made, many items on this subject have appeared in the literature. Most authors focused on the determination of hardness and reduced modulus of elasticity in mature spruce wood [4, 8] or pine wood [9, 10]. However, there are no reports on the mechanical parameters of juvenile wood or comparison of properties with mature wood.

2. Material and methods

The experimental material was juvenile and mature Scots pine wood (*Pinus sylvestris* L.), obtained from the Murowana Gosolina Experimental Forest Station (geographical coordinates: N 52°32'40.797"; S 17°4'5.132"). The age of the stand was 85 years. Two annual rings, 7 and 74, had been selected for the designations. They can therefore be classified as juvenile wood (ring 7) and mature wood (ring 74).

Two samples with the dimensions of 1.5 (R) × 1 (T) × 5 (L) mm were prepared and used for determination by the nanoindentation method. Each sample contained one analyzed annual ring. The widths of annual rings and individual zones were measured by means of a computer image analyzer equipped with a stereoscopic microscope and a video camera.

As in the majority of wood tests carried out with nanoindentation methods, samples were previously embedded in epoxy

*e-mail: przemyslaw.mania@up.poznan.pl

resin [4–6, 11, 12] in order to prevent delamination of the cell wall. The samples were seasoned to a moisture content of about 8%. Then each sample was embedded in a low viscosity epoxy resin, according to the method proposed by Spurr [11]. Using the vacuum method, air bubbles were removed from the intracellular space. A microtome (Leica Ultracut, Leica Microsystems, Wetzlar, Germany), equipped first with a glass knife and then with a diamond knife, was used to smoothly cut the plane surface. The plane used to perform mechanical parameters determination was perpendicular to the cell axis.

Nanoindentation was performed in the UAM Centre for Advanced Technologies on a nanoindenter (AGILENT G200) equipped with a Berkovich type pyramidal tip indenter, using a DCMII measuring head. In the first phase, the indenter approached the sample surface with a surface approach rate of 10 nm/s. After touching the sample, the apparatus was corrected by means of thermal drift. Once the indenter contacted the surface, it was loaded at constant rate of 1 μN/s to an indentation depth of 250 nm. The maximum load was then maintained for about 30 seconds. From the curve recorded during the experiment (Fig. 1), the relation between the depth of the cavity and the force with which we act on it, the key parameters were determined: maximum force (F_{max}), depth under maximum load (h) and the initial slope of the load curve. The analysis of the discussed curve allowed to determine reduced elastic modulus (MOE_r), as follows:

$$MOE_r = \frac{\sqrt{\pi} \times S}{2 \times \sqrt{A}} \text{ (MPa)}, \quad (1)$$

where:

- S – initial slope of the unloading curve,
- A – contact area at F_{max} .

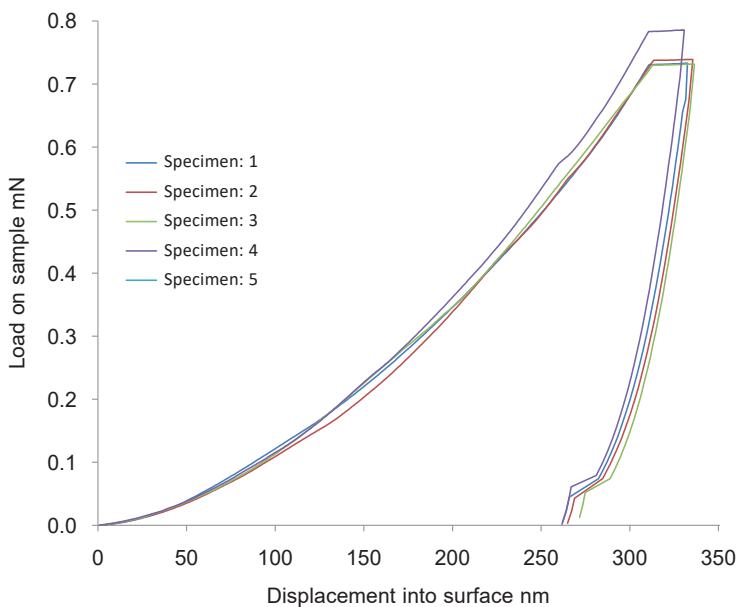


Fig. 1. Load displacement curve of nanoindentation test in a wood-cell wall

Hardness (H), i.e. the mean pressure the wood material will support under load (F_{max}), is defined as:

$$H = \frac{F_{max}}{A} \text{ (MPa)}. \quad (2)$$

Hardness and MOE were measured on every second tracheid of the analyzed ring in the longitudinal direction of the tracheid, from the first cell of early wood to the last cell of late wood. The measurements were taken on the tangential walls of the tracheids in the S2-layer of the secondary cell wall. At least 5 locations were measured on each tracheid. Positioning and marking out of the nanoindentation sites were facilitated by an optical microscope with magnification of 800× coupled with a nanoindenter.

In this paper, a measurement of microfibril angle was made using light microscopy and a computer image analyzer (direct method). The digital microscopy software (Motic Images Plus 3.0) applied allows for measurements with an accuracy of 0.01°.

Microtome slices of about 20 μm in thickness were cut out from wooden blocks to prepare microscope slides. The number of slices obtained from each sample was dependent on the width of annual rings. The slices were then immersed in a 20% $Cu(NO_3)_2$ solution and heated for 3 to 5 h at 80°C. The procedure briefly described above is a modification of the method proposed by Wang et al. [13] and then described by Fabisiak and Mania [14]. 20 to 30 MFAs measurements were made from each slice, but no more than two MFAs from a single tracheid.

3. Results and discussion

The width of annual rings in the wood sample analyzed is shown in Fig. 2. This figure is based on data obtained from direct measurements. Data were approximated by a linear function.

Average ring width for the entire board studied was 1.51 mm and the range between the minimum and maximum values was close to 3.93 mm. Analyzing the general trend of changes in the width of annual rings, it can be stated that this parameter decreases significant to about 18–20 growth ring. Based on the analysis of the width of annual rings, 7th and 74th

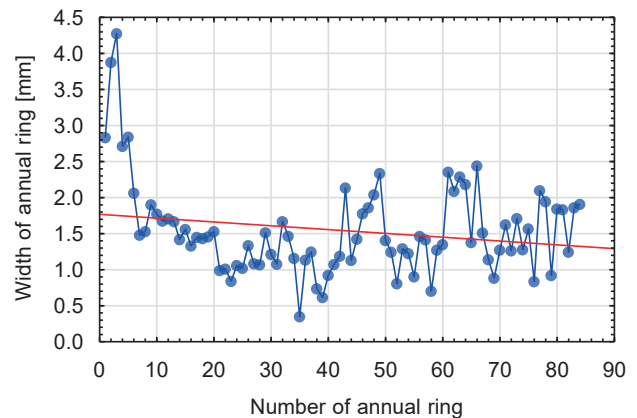


Fig. 2. Radial variations of ring width in pine wood

ring were selected for further analysis. The widths of rings 7 and 74 correspond to the median of this value for juvenile and mature wood.

The widths of the early- (ew), transition (tw) and latewood (lw) zones for the two annual rings analyzed are shown in Table 1. Early-, transition- and latewood zones were determined on the basis of microstructure, macrostructure and wood density data in individual zones. An additional feature was MFA and its distribution within individual annual rings.

Table 1
Width of individual pine wood zones

Number of annual rings	Wood zone	Value [mm]
7	ew	1.14
	tw	0.13
	lw	0.21
74	ew	0.61
	tw	0.28
	lw	0.37

The table shows that the average width of ring 7 was 1.48 and 74 1.26 mm. There were also differences in the widths of individual zones. The share of late wood in juvenile wood was 14.2%, while in mature wood it was 29.4%. This development of both the width of annual rings and the proportion of late wood is characteristic for coniferous species, where wider annual rings with a low proportion of late wood occur at the pith. As we move away from the pith, the width of annual growths decreases and the share of late wood increases [15]. The number of tracheids in individual annual growths was also different. In the 7th growth, in juvenile wood, 46 tracheids were recorded, while in the 74th growth, there were 54 tracheids.

Table 2 presents the results of the reduced modulus of elasticity for the two annual growths analyzed, separately for early- (ew), transition (tw) and latewood (lw), together with basic statistical parameters.

Table 2
Reduced modulus of elasticity in juvenile and mature pine wood

	MOE [MPa]			
	min	mean	max	±SD
7 ew	12932	13318	13867	263.4
7 tw	14221	14645	14925	305.1
7 lw	15785	17525	18614	943.8
74 ew	12464	13326	14231	461.6
74 tw	17662	18412	19065	423.5
74 lw	19728	20462	21161	544.4

Juvenile wood (ring 7) is characterized by lower modulus of elasticity than mature wood. The exception is early wood, where the results are very close to each other in both rings and

are about 13.3 GPa. The ANOVA analysis showed no significant differences between these cases (Tukey's test $p = 0.9949$). The difference between the average values for transition wood was about 4 GPa and for latewood about 3 GPa. In both cases the differences are statistically significant at $\alpha = 0.05$. These results do not differ significantly from the literature results, where the modulus of elasticity ranges from 13.5 to 21.5 GPa [4, 5, 9, 16].

Figure 3 shows the average MOE values for individual tracheids in the annual rings analyzed. Each point is an average of 5 indentations made in the S2-layer on tracheids in one row. The Figure shows that the two dependents overlap only in the initial phase, i.e. within the earlywood, where, as mentioned above, the differences were insignificant. In the middle of the growth ring, the highest differences between the two increments can be observed in the transition wood. There were more late tracheids in mature wood, which is due to the higher proportion of latewood in ring 74 (Table 1).

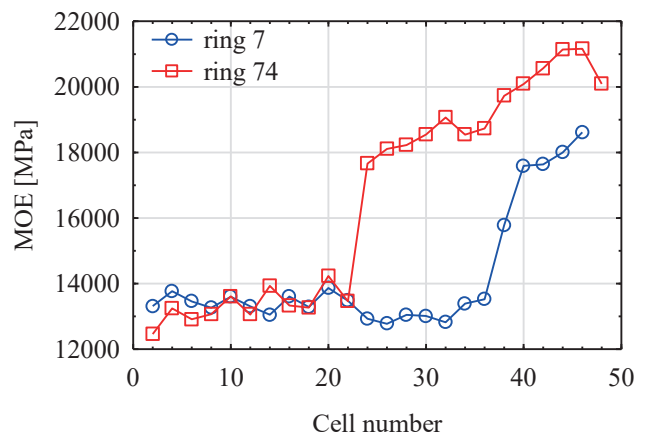


Fig. 3. Average values of reduced modulus of elasticity in individual tracheids

One of the reasons for the differentiation of the modulus of elasticity is the orientation of the microfibril in the S2-layer of the secondary cell wall. The angle at which the microfibril is oriented has a significant impact on the mechanical parameters of wood. The strength of wood and the modulus of elasticity are higher the lower the inclination angle of the microfibril (MFA) [13, 15–19]. The development of MFA within individual annual growths is shown in Fig. 4. Each point is an average of 30 angle measurements on a microscope preparation.

Analysis of the data contained in Fig. 4 shows that the microfibril angle decreases with the maturation of wood tissue. Thus, in juvenile wood, the microfibril runs at a greater angle to the longitudinal axis of the cells than in mature wood. Such shaping of MFA in individual tissues has been described by many authors [20–26]. The largest difference in MFA values was observed in latewood, while the smallest difference was observed in earlywood, as it was 8.4° and 3.0°, respectively, and is statistically significant. The large variation in the average microfibril angle of starting in the transition wood is the reason

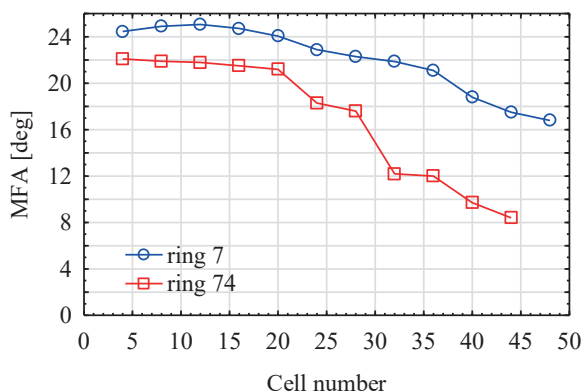


Fig. 4. Average values of the microfibril angle in the annual rings analyzed

for the variation in the modulus of elasticity in both tissues. Very similar MFA values in early wood can result in similar MOE values. Almost twice the angle of inclination of microfibril in juvenile wood is probably the reason for the smaller MOE in this tissue.

Modulus of elasticity of cell walls also increases with decreasing MFA. The relation between these two parameters, shown in Fig. 5, can be approximated with a linear function, for the range of MFA values measured. According to the determination coefficient of this relation, the modulus of elasticity of cell walls in the axial direction is 82% in ring 7 and 74% in ring 74, dependent on MFA.

The higher modulus of elasticity of latewood, apart from the MFA variation, is also due to other differences in annual growth. Latewood has a higher degree of cellulose crystallinity [27] than early wood.

Table 3 presents the results of nanohardness (H) measurements for the two annual growths analyzed, separately for early- (ew), transition (tw) and latewood (lw), together with basic statistical parameters.

Table 3 shows that the variability of this property is lower than for the MOE. Average nanohardness values are higher for mature wood. The differences between the hardness of early-

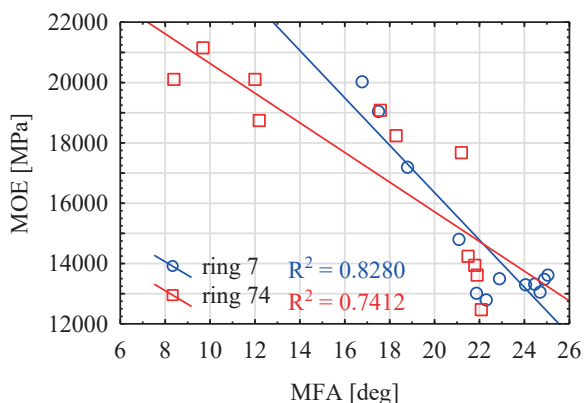


Fig. 5. Relationship between microfibril angle of the material studied and their reduced modulus of elasticity

Table 3
Hardness in juvenile and mature pine wood

	H [MPa]			
	min	mean	max	±SD
7 ew	433	439	458	3.51
7 tw	462	451	467	5.10
7 lw	463	466	469	2.49
74 ew	462	469	475	4.52
74 tw	477	485	488	3.37
74 lw	483	489	495	4.68

and latewood, within a single annual ring, are small. In juvenile wood, latewood has a higher hardness of approx. 6%, while in mature wood of only approx. 4%, and these differences are statistically significant, at a significance level of $\alpha = 0.05$. Similar results of nanohardness were also obtained in single tracheids by Wimmer et al. [4] and Vincent et al. [10]. To check how the hardness changed on the width of the annual increment, Fig. 6 was prepared.

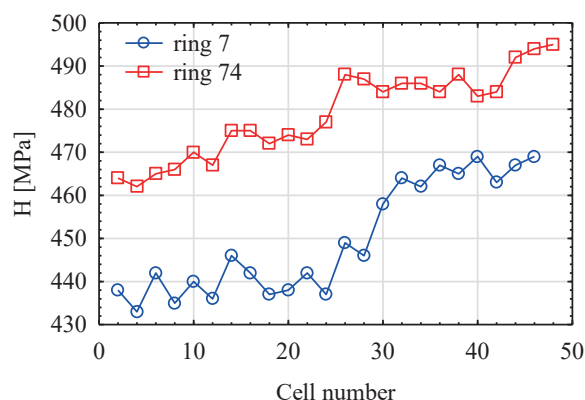


Fig. 6. Average hardness values in individual tracheids

Both curves show a similar course, but they are spread out in relation to each other. The greatest differences can be observed in the earlywood and transition wood zone, where the differences in individual tracheids exceed 30 MPa. In the late wood zone, the differences for both tissues are lower and amount to about 23 MPa. Within a single annual increment in mature wood only, statistically insignificant differences between transition and latewood were shown. In other cases, the differences are significant. A slight differentiation of hardness values within single annual rings is less dependent on MFA and more on the share of lignin in the cell wall [25]. It is the matrix that encrusts the cell wall and is mainly responsible for the hardness of the wood. Early wood has a higher lignin content. However, the differences are not massive, as they stand at about 2% [28, 29]. A similar relationship can be found with juvenile and mature wood. Mature wood is characterized by a lower share of lignin by even about 10% [29].

4. Conclusions

The aim of this study was to determine the hardness and reduced modulus of elasticity of juvenile and mature Scots pine wood using the nanoindentation method. This method has become a powerful tool in determining the properties of wood on a nano scale. The results obtained allow us to conclude that the hardness and modulus of elasticity, determined in the S2-layer of the secondary cell wall, are lower in juvenile wood than in mature wood. The exception is MOE of earlywood, which is almost identical for both types of tissue. There were no statistically significant differences between these cases. However, this property is highly dependent on MFA, and MFA values for both tissue types were very similar. Much smaller variation of values both within individual annual increments and when comparing the annual increments analyzed points to nanohardness. In juvenile wood the difference between the hardness of late and early wood is 30 MPa, and in mature wood it amounts only to 20 MPa. However, these differences are statistically significant.

Acknowledgements. The paper was financed within the framework of the Ministry of Science and Higher Education program entitled ‘Regional Initiative of Excellence’ in the years 2019–2022, Project No. 005/RID/2018/19.

Conflicts of interest: The authors declare no conflict of interest.

REFERENCES

- [1] K.P. Mróz, S. Kucharski, K. Doliński, A. Bigos, G. Mikułowski, E. Beltowska-Lehman, and P. Nolbrzak, “Failure modes of coatings on steel substrate”, *Bull. Pol. Ac.: Tech.* 64(1), 249–256 (2016).
- [2] R. Major, P. Lacki, J.M. Lackner, and B. Major, “Modelling of nanoindentation to simulate thin layer behaviour”, *Bull. Pol. Ac.: Tech.* 54(2), 189–198 (2006).
- [3] W. Orłowicz, M. Mróz, M. Tupaj, A. Trytek, M. Jacek, and M. Radoń, “Morphology and Material Properties of Carbides in High (24%) Chromium Cast Iron”, *Arch. Foundry Eng.* 18(1), 77–80 (2018).
- [4] R. Wimmer, B.N. Lucas, W.C. Oliver, and T.Y. Tsui, “Longitudinal hardness and Young’s modulus of spruce tracheid secondary walls using nanoindentation technique”, *Wood Sci Technol* 31, 131–141 (1997).
- [5] W. Gindl, H.S. Gupta, T. Schoberl, H.C. Lichtenegger, and P. Fratzl, “Mechanical properties of spruce wood cell walls by nanoindentation”, *Appl. Phys. A* 79, 2069–2073 (2004).
- [6] Y. Wu, S. Wang, D. Zhou, C. Xing, Y. Zhang, and Z. Cai, “Evaluation of elastic modulus and hardness of crop stalks cell walls by nano-indentation”, *Bioresour. Technol.* 101, 2867–2871 (2010).
- [7] Y. Yu, B. Fei, B. Zhang, and X. Yu, “Cell-wall mechanical properties of bamboo investigated by in-situ imaging nanoindentation”, *Wood Fiber Sci* 39, 527–535 (2007).
- [8] W. Gindl and H.S. Gupta, “Cell-wall hardness and Young’s modulus of melamine-modified spruce wood by nano-indentation”, *Compos Part A-Appl S* 33, 1141–1145 (2002).
- [9] Y. Huang, B. Fei, Y. Yu, S. Wang, Z. Shi, and R. Zhao, “Modulus of elasticity and hardness of compression and opposite wood cell walls of masson pine”, *BioResources* 7(3) 3028–3037 (2012).
- [10] B. Vincent, Q. Tong, N. Terziev, G. Daniel, C. Bustos, W.E. Escobar, and I. Duchesne, “A comparison of nanoindentation cell wall hardness and Brinell wood harness in jack pine (*Pinus banksiana* Lamb.)”, *Wood Sci Technol.* 48(1), 7–22 (2014).
- [11] A.R. Spurr, “A low-viscosity epoxy resin embedding medium for electron microscopy”, *J Ultra Res.* 26, 31–43 (1969).
- [12] S.H. Lee, S.Q. Wang, G.M. Pharr, M. Kant, and D. Penumadu, “Mechanical properties and creep behavior of lyocell fibers by nanoindentation and nano-tensile testing”, *Holzforschung* 61, 254–260 (2007).
- [13] H.H. Wang, Drummond J.G., S.M. Reath, K. Hunt, and P.A. Watson, An improved fibril angle measurement method for wood fibres. *Wood Sci. Technol.* 34: 493–503 (2001).
- [14] E. Fabisiak and P. Mania, “Variation in the Microfibril Angles in Resonance and Non-Resonance Spruce Wood (*Picea abies* [L.] Karst.)”, *BioResources* 11(4), 8496–8508 (2016).
- [15] F. Kollmann, W.A. Côté, Principles of wood science and technology, B. Heideberg, 1968.
- [16] A. Jäger, T. Bader, K. Hofstetter, and J. Eberhardsteiner, “The relation between indentation modulus, microfibril angle, and elastic properties of wood cell walls”, *Composites: Part A* 42(6), 677–685 (2011).
- [17] I.D. Cave, “Modeling the structure of the softwood cell wall for computation of mechanical properties”, *Wood Sci. Technol.* 10(1), 19–28 (1976).
- [18] J.M. Dinwoodie, Timber, its nature and behaviour, Van Nostrand Reinhold Co. Ltd, 1981.
- [19] I.D. Cave and J.C.F. Walker, “Stiffness of wood in fast-grown plantation softwoods: the influence of microfibril angle”, *Forest Prod. J.* 44(5), 43–48 (1994).
- [20] A. Reiterer, H. Lichtenegger, S.E. Stanzl-Tschegg, and P. Fratzl, “Experimental evidence for a mechanical function of the cellulose spiral angle in wood cellulose walls”, *Philos. Mag. A* 79, 2173–2186 (1999).
- [21] E. Roszyk, P. Mania, and W. Moliński, “The influence of microfibril angle on creep of wood under tensile stress along the grains”, *Wood Res-Slovakia* 57(3), 347 – 358 (2012).
- [22] L.A. Donaldson, “Within- and between-tree variation in microfibril angle in *Pinus radiata*”, *N. Z. J. Forest. Sci.* 22, 77–86 (1992).
- [23] H. Lichtenegger, A. Reiterer, S. Stanzl-Tschegg, and P. Fratzl, “Variation of cellulose microfibril angles in softwoods and hardwoods – a possible strategy of mechanical optimization”, *J. Struct. Biol.* 128, 257–269 (1999).
- [24] J.R. Barnett and V.A. Bonham, “Cellulose microfibril angle in the cell wall of wood fibres”, *Biological Reviews.* 79(2), 461–472 (2004).
- [25] E. Fabisiak and W. Moliński, “Variation in the microfibril angle within individual annual rings in wood of larch (*Larix decidua* Mill.) from plantation culture”, *Ann. WULS SGGW For. Wood Technol.* 61, 207–213 (2007).
- [26] C.L. Lee, “Crystallinity of wood cellulose fibers”, *For. Prod. J.* 11, 108–112 (1961).
- [27] Y.T. Wu and J.W. Wilson, “Lignification within coniferous growth zones”, *Pulp Pap. Mag. Can.* 68(4), 159–164 (1967).
- [28] W. Gindl, M. Grabner, and R. Wimmer, “The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width”, *Trees* 14(7), 409–414 (2000).
- [29] L.A. Donaldson, “Seasonal changes in lignin distribution during tracheid development in *Pinus radiata* D. Don”, *Wood Sci. Technol.* 25, 15–24 (1991).