Variation in the Microfibril Angles in Resonance and Non-Resonance Spruce Wood (*Picea abies* [L.] Karst.)

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Variation in microfibril angle (MFA) in the S2 of the tangent cell walls of resonance and non-resonance spruce wood (*Picea abies* [L.] Karst.) used in the manufacture of musical instruments was studied. MFA was measured directly after preliminary visualisation of microfibrils in the cell walls. In the tested samples the position of an annual ring in the samples had no significant influence on the MFA. In the resonance wood, MFA values were between two and three times smaller than in the non-resonance wood. In the resonance wood, the differences in the MFA between earlywood and latewood were smaller, and the MFA fluctuations were also smaller.

Keywords: Microfibril angle; Resonance and non-resonance wood; Spruce wood

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INTRODUCTION

Professionals who make or repair string instruments (luthiers) usually visually classify resonance wood first by its macrostructural features, such as the width of annual rings, the percentage of latewood in the annual rings, and the linearity of fibres. The classification based on these features is often misleading. These features do not fully reflect the actual quality of wood desired for making instruments with respect to specific acoustic constant, acoustic resistance, speed of sound propagation, and damping by sound radiation. Practically all these parameters depend on the density and modulus of elasticity of wood (Ono and Norimoto 1984). The best resonance wood shows high elasticity modulus and low density (Abe and Funada 2005; Bucur 2006; Buksnowitz *et al*. 2007; Schwarze *et al.* 2008). These requirements are contradictory, as the elasticity modulus increases with increasing wood density (Kollmann and Côté 1984). Among coniferous species, spruce wood (*Picea abies*) is characterised by low density and high elasticity (Moliński *et al.* 2013), so it is commonly used for the top plate of violin bodies (Veitl 1987).

The density and modulus of elasticity of wood are not the only parameters determining its mechanical quality. The mechanical and, hence, acoustical properties of wood also depend on the distribution of chemical compounds in cell walls; cellulose, in particular, makes structural elements called microfibrils (Cave 1968; Via *et al*. 2009). The highest amount of cellulose occurs in the S2 layer of the secondary cell wall, which is also the thickest. The arrangement of microfibrils in this layer has a significant influence on wood properties (Alteyrac *et al*. 2006; Xu *et al*. 2011). The angle of cellulose microfibrils in the S2 layer is a genetic feature that can be modified by external factors related to the habitat and conditions of tree growth (Lindström *et al.* 1998; Abe and Funada 2005; Tanabe *et al.* 2015). The microfibril angle (MFA) can vary also within individual annual rings. MFA decreases with the passing of the vegetation season and is smallest in the walls of latewood tracheids (Anagnost *et al.* 2002). The arrangement of microfibrils also changes across the tree stem cross-section; in the annual rings near the pith, they make greater angles with the longitudinal axis of the cell. As wood tissue matures, the angle decreases and stabilises in the zone of mature wood (Gorišek and Torelli 1999; Lichtenegger *et al*. 1999; Sarén *et al.* 2004; Fabisiak *et al.* 2006; Jordan *et al*. 2007; Mansfield *et al.* 2009; Watt *et al.* 2010).

The wood suitable for musical instruments should have an arrangement of cellulose microfibrils in the S2 layer of the cell wall that make very small angles with the longitudinal axes of the cells (Mania *et al*. 2015). Only mature wood meets the requirements of resonance wood. In other types of wood, the range of MFA is much greater (Fabisiak *et al*. 2006). The arrangement of microfibrils in cell walls has a notable effect on the acoustic properties of wood. With decreasing MFA, the velocity of acoustic waves in wood increases (Yano 1994; Hori *et al*. 2002; Fabisiak *et al*. 2010; Brémaud 2012).

In a study of wood from eight species of spruce, Shen *et al.* (2002) established that the optimum MFA, with respect to susceptibility to vibration (damping of sound radiation), is between 9° and 13°. Earlier, Liu *et al*. (2001) analysed spruce wood properties by the vibration method and showed that they are strongly correlated with the crystallinity index of cellulose. Others (Äkerholm and Salmén 2003) have noted the effect of the degree of lignification of cell walls. These reports demonstrate that high quality resonance wood is characterised by the smallest cyclic inhomogeneity, within both the individual annual rings and in the whole board of the resonance box (Spycher *et al*. 2008; Dinulică *et al*. 2015). The index of cyclic inhomogeneity is defined as a ratio of a given feature value in late- and earlywood in individual annual rings (Moliński *et al.* 2013). In individual wood species, the variation in properties depends not only on the cambial age of annual rings but also on the conditions of tree growth (Lindström *et al.* 1998).

The wood visually classified as resonance can differ in quality. To the best of our knowledge, literature in the field provides scarce information on determination of differences/similarities in variation of ultrastructure of resonance wood of different quality. As the inhomogeneity in wood ultrastructure is known to have significant effects on its acoustic parameters, this study established the differences in MFA in the S2 layer of secondary cell walls within individual annual rings of resonance spruce wood, which was classified as very good or good by violin-makers. The results were compared with the corresponding features of non-resonance spruce wood.

EXPERIMENTAL

Materials

The study was performed on resonance spruce wood from trees growing in Poland, Slovakia, and Bosnia-Herzegovina and on non-resonance spruce wood from Poland. The resonance wood from Bosnia-Herzegovina and Poland was purchased from an Italian importer of spruce and sycamore wood (Toone Wood International, Cremona, Italy). The wedges were cut out from trees more than 100 years old, from the near circumference zone, so they contained only mature wood tissue. The wedges were of the same size: 480 mm in length (L), 130 mm in width in the radial direction (R), 50 mm in width in the tangential direction (T) at the thicker end, and 27 mm at the thinner end. The first wedge, from a tree growing in the mountains of east Bosnia-Herzegovina (near Mazoče; 43°24′02″N, 18°48′08″E), was classified as very high quality resonance wood. The second wedge, from a tree in Poland near Istebna in the Beskid Śląski Mountains (49°33′50″N, 18°53′38″E), was classified as good resonance wood. According to luthiers, spruce trees growing in this area of Poland provide the country's best wood for musical instrument making. The third sample was obtained from the Katedra Náuky o Dreve at the University of Technology in Zvolen (Slovakia) in the form of a small plank (500 mm [L], 150 mm [R], 30 mm [T]), which was cut from a wedge (also from mature wood) qualified for making a violin resonance box. The wedge came from a tree growing in the mountains near Zvolen (48°34′14″N, 19°07′03″E). The small plank showed a linear arrangement of fibres and annual rings of uniform width. The Zvolen sample was classified as good resonance wood. For comparison, a non-resonance sample of spruce wood (showing no features of resonance wood) was selected from a 100-year-old tree growing in the Olsztyn Forest Division. This plank was cut from the mature wood zone, was approximately 500 mm (L) × 160 mm (R) × 30 mm (T), and had varied annual ring widths and a high percentage of latewood. The wood was classified visually on the basis of macrostructural features (the width of annual rings, contribution of latewood and linearity of fibres arrangement) by qualified violin-maker.

Samples of 480 mm (L) × 130 mm (R) × 24 mm (T) were cut from the wedges (Fig. 1a). A small piece of approximately 20 mm in thickness along the grains was cut from the front of the plank, and the macrostructural parameters were determined in this small piece (Fig. 1b). The remaining body of the sample was cut into two parts and subjected to machining to a thickness of 9 mm. From one of the slabs, a sample of 8 mm in thickness was cut to determine the MFA (Fig. 1c). Samples of the same dimensions were cut from the Zvolen slab and the non-resonance slab from the Olsztyn.

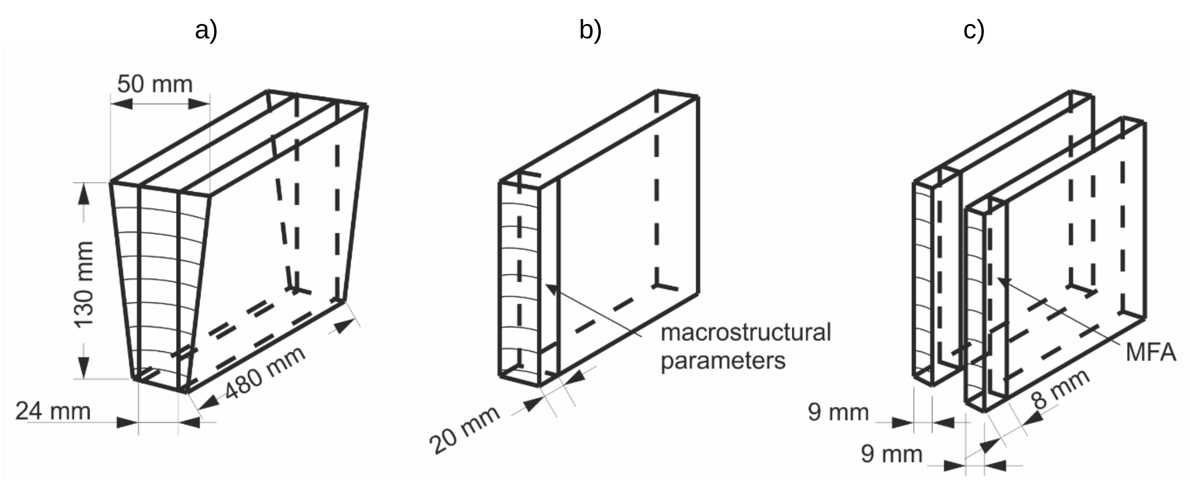

Fig. 1. Diagram of sample cutting

Methods

Macrostructure measurements (ring width, latewood width) were performed with a BIOTRONIK electronic growth ring device (BEPD-5, Warsaw, Poland). The widths of annual rings and latewood zones were measured along the entire width of the plank (in the cross section, Fig. 1b), to an accuracy of 0.01 mm, at 7% moisture content. Measurements of the ultrastructure were performed with a polarised light microscope coupled with a computer image analyser using the Motic Images Plus 2.0 program (Motic Incorporation Ltd, Hong Kong, China). The MFA values in the S2 layer of secondary cell wall were measured by the direct method after earlier visualisation of the microfibrils, as previously described (Fabisiak *et al*. 2006; Wang *et al*. 2001). To visualise the fibril arrangement the samples were heated in a 20% cobalt chloride solution at 80 °C for 3 to 5 h. MFA in selected annual rings in each sample were measured to an accuracy of 0.1° (Fig. 2).

Tangentially oriented slices 20 µm in thickness were cut from the small slab using a sledge microtome, and microscopic preparations were made from these slices. In selected annual rings, MFA measurements were made in slices cut off at certain defined distances from the border of the preceding annual ring. Care was taken to cut off the slices from the start of the annual ring until its end so the distance between the positions of the neighbouring slices was about 0.2 mm. Depending on the width of the annual rings and the percentage of latewood, the number of preparations selected for MFA measurements differed (47 preparations in the Mazoče sample, 68 in the Istebna sample, 64 in the Zvolen sample, and 56 in the non-resonance sample). In the earlywood section of each preparation, at least 30 MFA were measured. In the latewood sections of the preparations, at least 20 angles were measured, but no more than 2 angles were in the same tracheid. Statistical analysis was performed using Statistica 10.0 software (Dell, Round Rock, TX, USA). The descriptive statistics and single factor analysis of variance (ANOVA) were applied. All tests were carried out for the significance level of $p<0.05$.

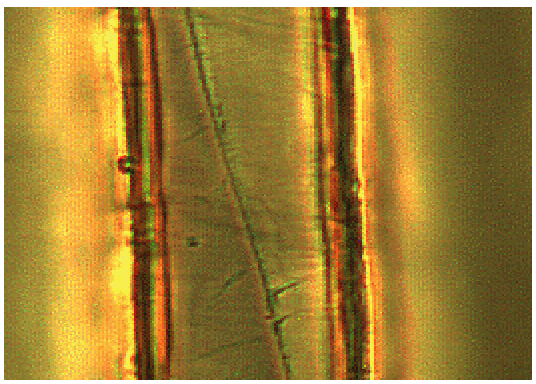

Fig. 2. Tangential cross-section of a spruce wood sample subjected to $CoCl_2$, showing a crack along the microfibrils

RESULTS AND DISCUSSION

The variation in macrostructural features of the resonance and non-resonance wood was evaluated in the samples of the same size, cut in the radial direction. As all samples studied were of the same size, the number of annual rings differed depending on the width of annual rings. The sample of resonance wood from the vicinity of Mazoče (Bośnia-Herzegovina) comprised 120 annual rings, from Istebna (Poland) 86, from Zvolen (Slovakia) 69 and non-resonance sample from Olsztyn (Poland) comprised 77 annual rings. The width of annual rings and the contribution of latewood in them and the variation in these parameters along the width of the rings determine the wood homogeneity and significantly determine its resonance properties. The average width of annual rings in these samples was 1.30 mm, 1.51 mm, 1.46 mm, and 1.58 mm, respectively, although the sample from Olsztyn showed high variation. The variation coefficient of the non-resonance sample reached almost 40%, over twice that of the resonance wood. The wood for violin making should be characterised by low density so a small contribution of latewood. The mean percentage of latewood in the resonance wood did not exceed 20%; it was 13.4%, 19.5%, and 17.3% in the Mazoče, Istebna, and Zvolen samples, respectively. In the non-resonance sample, this value was much higher (approximately 33%).

The radial variations in the average MFA of early- and latewood, in selected annual rings of the samples, are illustrated in Fig. 3. The x-axis shows the numbers of

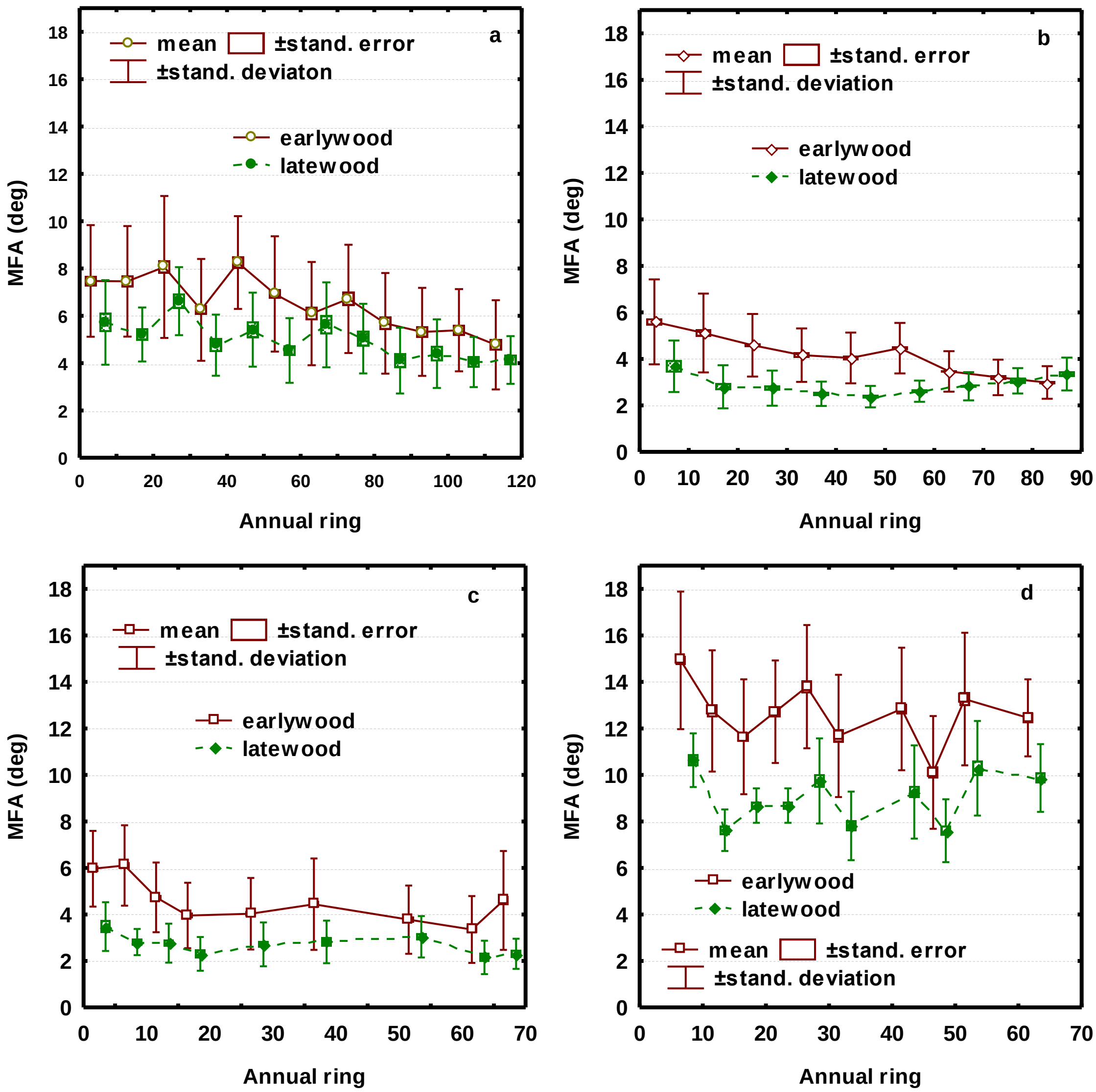

Fig. 3. Mean MFA in early- and latewood tracheids in the resonance samples from a) Mazoče, b) Istebna, c) Zvolen, and non-resonance samples d) from Olsztyn. (The OX axis gives subsequent number of annual ring in a given sample)

annual rings and not their cambial age. Each plot point is a mean value from approximately 90 to 180 measurements in the earlywood zone and 30 to 60 measurements in the latewood zone, on a single annual ring. To characterise the scatter of the results also the range of variation MFA, standard error, and standard deviation are marked.

In all samples, the MFA in the tracheids formed in earlywood were higher than those in latewood. Within individual annual rings, the differences between the MFA from these two zones was much smaller in the resonance wood than in the non-resonance wood. In resonance wood, the smallest differences between the MFA from these two zones were found in the Istebna sample. In two of the annual rings analysed (69 and 75), the MFA values from the early and latewood were very similar, and, in ring 81, the MFA in tracheids from latewood was 0.4° greater than that in earlywood. The Istebna sample showed the greatest difference in MFA between early and latewood of the same annual ring (2.3°) at annual ring 14. These results were consistent with those reported in Sahlberg *et al.* (1997) and Reiterer *et al*. (1998).

Very small differences between the MFA in tracheids in early- and latewood were found in the Mazoče and Zvolen samples. Within the same annual ring, the differences varied from 0.5° (annual ring 66) to 3.8° (annual ring 51) in the Mazoče sample and from 0.74° (annual ring 54) to 3.3° (annual ring 10) in the Zvolen sample. The greatest differences in MFA between early- and latewood were noted in the non-resonance sample, in which the differences varied from 2.5° (annual ring 49) to 5.2° (annual ring 11) within the same annual ring. However, it should be emphasised that the differences in spruce wood, both resonance and non-resonance, were smaller than in other species of wood (Yano 1994; Donaldson 2008; Keunecke *et al.* 2009).

Analysis of the average MFA in the tracheids of early- and latewood showed that the smallest fluctuations in this parameter occurred in the Istebna sample. In the earlywood, the highest MFA was 5.6° and the lowest was 3°, so that the variation in MFA over the sample of 86 annual rings was 2.6°. The MFA fluctuations in latewood were much smaller, such that the maximum MFA was 3.6° and the minimum was 2.4°.

The range of variation in MFA in the Zvolen sample was similar to that of the Istebna sample. In the Zvolen sample, MFA values varied from 6.1° to 3.4° in earlywood and from 3.5° to 2.2° in latewood. Therefore, the average MFA in earlywood was 4.2° in the Istebna sample and 4.5° in the Zvolen sample (Fig. 4). In the tracheids from latewood zones, the corresponding values were 2.9° (Istebna) and 2.7° (Zvolen). Higher MFA values and their greater fluctuations were noted in the Mazoče sample. Over the range of 120 annual rings, the MFA values in earlywood varied from 8.3° to 4.8° and from 6.6° to 4.1° in latewood (Fig. 3). For all zones, the average MFA calculated was 6.6° in earlywood and 5.6° in latewood (Fig. 4).

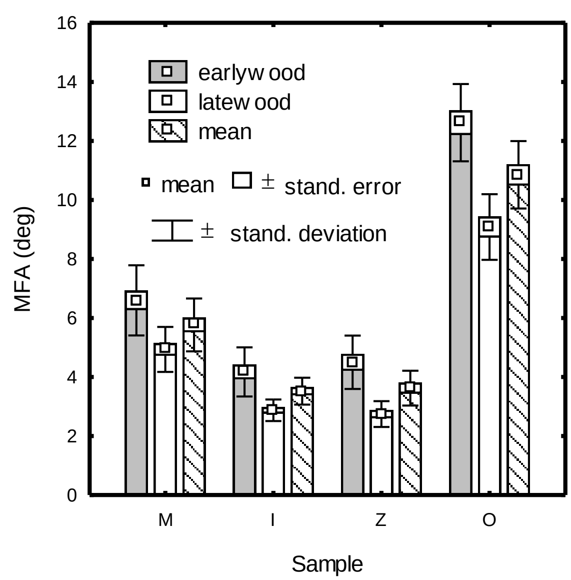

Fig. 4. Average MFA for earlywood, latewood, and for entire samples: M - Mazoče; I - Istebna; Z - Zvolen; O – Olsztyn

The MFA values for entire annual rings and for entire samples (Fig. 4 and Fig. 5) were calculated as weighted means (MFA_w) of those determined for early wood and latewood according to Eq. 1, otherwise known as the rule of mixtures (Gibson and Ashby 1997),

$$MFA_w = MFA_{ew} \times u_{ew} + MFA_{lw} \times u_{lw} \quad (1)$$

where MFA_{ew} and MFA_{lw} stand for the MFA of earlywood and latewood, respectively, and u_{ew} and u_{lw} stand for the earlywood and latewood content, respectively, in annual rings.

Paakkari and Serimaa (1984) have reported small differences in the average MFA values in early- and latewood, not exceeding 1° in spruce wood. Similarly small differences in the MFA values between early- and latewood spruce were found by Gorisek and Torelli (1999) and confirmed by Hori *et al.* (2002), who obtained MFA values of 6° in earlywood and 5° in latewood. Sahlberg *et al*. (1997) reported slightly larger MFA values of 8.1° in latewood tracheids and 9.0° in earlywood. Very small variation MFA in the tracheid walls of earlywood and latewood, sometimes not exceeding 3^{o}, in spruce has been also reported by Brändström (2001) and Eder *et al*. (2009).

In the non-resonance wood, the MFA values were greater than in the resonance samples, and the fluctuations in MFA were also greater. In the earlywood tracheids, the MFA varied from 14.9° to 10.1°, and in latewood from 10.6° to 7.6° (Fig. 3). These values were approximately three times higher than the corresponding ones in the Zvolen and Istebna samples, and twice greater than those from the Mazoče sample (Fig. 4). These results were similar to those obtained previously for non-resonance spruce wood (Sarén *et al*. 2004; Keunecke *et al.* 2009). According to the single factor analysis of variance (ANOVA), the differences in the mean MFA values for the samples compared are statistically significant (Table 1). Prior to this analysis it was checked whether the conditions for its application are satisfied (normal distribution and uniformity of conditional variances).

Table 1. Weighted Mean and Index of Cyclic Inhomogeneity of the MFA in Resonance and Non-Resonance Spruce Wood

Source of Variation	SSB	df	MSB	SSE	df	MSE	F	p
Weighted Mean MFA	359.2937	3	119.7646	26.5762	40	0.6644	180.2579	0.0000^{s}
Index of Cyclic Inhomogeneity MFA	0.0955	3	0.0318	0.4837	40	0.0120	2.6340	0.0630^{ns}

SSB – sum squares between groups; MSB – mean squares between groups; SSE – sum squares within groups; MSE – mean squares within groups; df – degrees of freedom; F – value of test function; p – level of significance; ^{s}significant differences; ^{ns}not significant differences

Figure 5 presents the mean MFA values for the annual rings analysed as a function of the ring position in the sample width. Single factor analysis of variance (ANOVA) was used to check the statistical significance of the differences in the MFA in the resonance samples and in the non-resonance sample (Table 2). For the tested samples of the position of the annual ring did not have a significant impact on the MFA. The Fisher test statistic was smaller than the tabulated value for all samples (for Mazoče $F_{tab.(12;13;0.05)}$=2.6037; for Istebna $F_{tab.(10;11;0.05)}$=2.8536; for Zvolen and Olsztyn $F_{tab.(9;10;0.05)}$=3.0204). This result confirmed the well-known observation that the ultrastructure of tracheid walls is highly uniform in the region of mature wood.

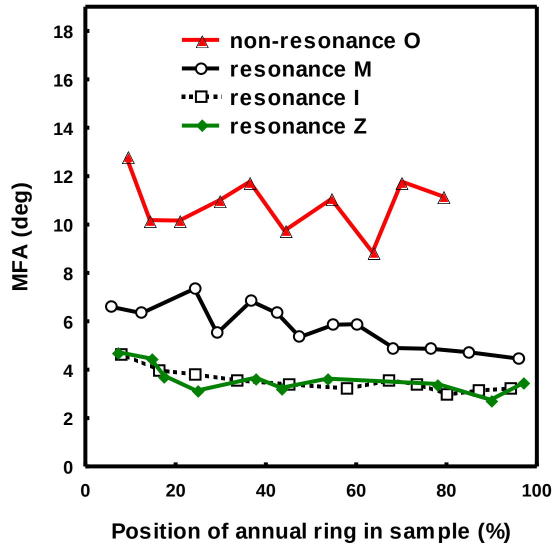

Fig. 5. Average MFA in the annual rings versus the position of the ring in the sample: M - Mazoče; I - Istebna; Z - Zvolen; O – Olsztyn

Table 2. ANOVA of MFA in Annual Rings in Spruce Wood Samples

Source of Variation	Sample		SSB	df	MSB	SSE	df	MSE	F	p
Position of Annual Ring in Sample	Resonance	M	19.2230	12	1.6019	22.6329	13	1.7409	0.9201	0.5542^{ns}
		I	4.1736	10	0.4173	13.3850	11	1.2168	0.3429	0.9485^{ns}
		Z	6.2876	9	0.6986	18.2298	10	1.8229	0.3832	0.9176^{ns}
	Non-resonance	O	23.4688	9	2.6075	65.5726	10	6.5572	0.3976	0.9095^{ns}

SSB – sum squares between groups; MSB – mean squares between groups; SSE – sum squares within groups; MSE – mean squares within groups; df – degrees of freedom; F – value of test function; p – level of significance; ^{ns}not significant differences; M- Mazoče; I-Istebna; Z-Zvolen; O-Olsztyn

Resonance wood is characterised by low cyclic inhomogeneity of properties influencing the wood quality, including the MFA. The index of ultrastructure inhomogeneity, defined as a ratio of the MFA values in late- and earlywood tracheids in given annual ring. In the resonance wood it takes values from 0.62 to 0.76, while for the non-resonance sample the value of this ratio falls at the middle of this range (0.72). Although the mean MFA for the samples studied differed statistically significantly, the differences in the inhomogeneity indices were statistically insignificant (Table 1, Fig. 6), which suggests that it is a feature of the species.

According to Roszyk *et al.* (2013), from mature wood from a 60-year-old pine tree (*Pinus sylvestris* L.), the mean from 30 annual rings was 18.2° for earlywood and 11.1° for latewood. Thus, the values of MFA were higher than in the spruce wood studied, but the MFA inhomogeneity index for the pine wood was 0.61 and was close to the lower value for spruce wood.

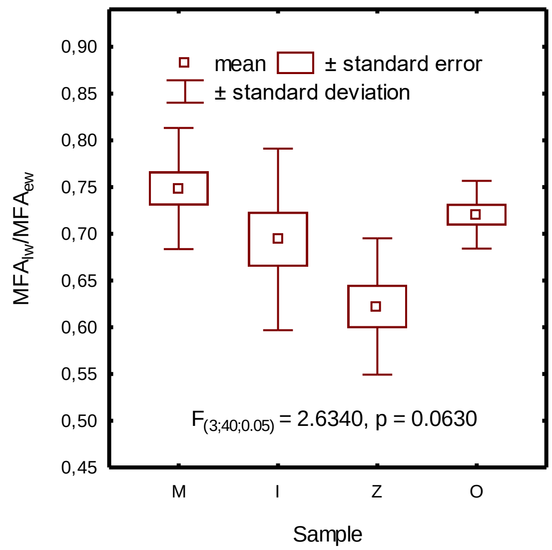

Fig. 6. Index of cyclic inhomogeneity of the MFA in the tested samples: M – Mazoče, I – Istebna, Z – Zvolen, O - Olsztyn

For the mature larch wood (*Larix decidua* Mill.), (comprising 16 annual rings), the mean MFA was 22.5^o for earlywood and 11.5^o for latewood (Fabisiak *et al*. 2006). Also for larch wood, the values of MFA in early- and latewood were higher than in pine and spruce wood and the inhomogeneity index was lower and equal to 0.5. This result confirms the already known fact of a considerable decrease in MFA with increasing distance along the radius in the width of annual rings in mature wood. The result also confirms the report of Abe *et al.* (1992) and Anagnost *et al.* (2002), for example, saying that MFA is greater in the walls of the first cells formed at the beginning of the vegetation season than in the cells formed at the end of the season. Similar results for *Cryptomeria japonica* wood were reported by Moriizumi *et al.* (1973), who also concluded that the differences in the MFA in earlywood and latewood were a feature of the species.

Comparison of the results with literature data shows that the MFA in spruce wood was smaller than in wood from the other coniferous species. Also lower, in comparison to the other coniferous species, was the variation in MFA along the radius in mature of spruce wood. These features of spruce wood tissue classify it as the resonance wood that can be used for construction of musical instruments. As follows from the literature data the relations between MFA in the cell walls and the mechanical parameters of wood under tensile stress along the grains demonstrate that the elasticity modulus of wood and cell walls is the higher for smaller MFA (Mark and Gillis 1973; Cave and Walker 1994; Groom *et al*. 2002). This character of the relations is essential for resonance wood as by definition should show a high elasticity modulus and low density. According to Bendtsen and Senft (1986) as well as Cave and Walker (1994), the main parameter determining the rigidity of wood in longitudinal direction is the microfibril angle in S2 layer of the secondary cell wall. Earlier Cave (1968) has shown that the rigidity of cell wall increases fivefold when the mean MFA decreases from 40 to 10°. Via *et al.* (2009), who used the IR spectroscopy and X-ray diffraction methods to establish the effect of lignin and cellulose content, MFA, density and sample position at the radius of the tree in the wood of Longleaf pine, have reported a fourfold increase in the wood rigidity upon MFA decrease from 40^o to 5^o.

CONCLUSIONS

1. In resonance and non-resonance spruce wood, the MFA values in the S2 layer of the secondary cell wall of tracheids in earlywood were greater than in latewood tracheids.
2. Within individual annual rings, the differences in MFA values between early- and latewood were smaller in the resonance wood samples. In the resonance wood, the smallest differences not exceeding 2.3° were noted in the Istebna sample. In the non-resonance wood, the difference in the MFA between early- and latewood in the same annual ring varied from 2.5° to 5.2°.
3. The average MFA values in early- and latewood zones showed that MFA values and their fluctuations along the stem radius were smaller in resonance than in non-resonance wood. The smallest MFA values were measured in the samples from Istebna and Zvolen. Mean MFA values for all zones of earlywood in these samples were 4.2° and 4.5°, respectively, and 2.9° and 2.7°, respectively, for all zones of latewood. In the non-resonance wood, the corresponding values were about three times greater than those for the Zvolen and Istebna samples, and about twice greater than those for the Mazoče sample.
4. According to the ANOVA results, in the tested wood sample, the position of the annual ring had no significant impact on MFA values.

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