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IMPACT OF THE NORWAY SPRUCE SAPWOOD TREATMENT WITH THE STAINING FUNGUS *SYDOWIA POLYSPORA* ON ITS PERMEABILITY AND DYNAMIC MODULUS OF ELASTICITY

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ABSTRACT

The impregnability of the Norway spruce wood is very difficult due to irreversible closing of pit-pairs in the cell walls of tracheids during drying. Therefore, for specific spruce products which have to be better impregnated: (1) with biocides, e.g. poles exposed in humid environment suitable for action of decaying fungi and insects; (2) with polymers or other conservation and modifying substances, e.g. musical instruments at wood-acetylation with anhydride of acetic acid, their good permeability for liquids is very important. Permeability of refractory wood species can be increased by several methods – mechanical, physical, chemical and biological. This study presents a 24-week bio-treatment of spruce sapwood with the wood-staining fungus *Sydowia polyspora* and its effect on permeability as well as on density and dynamic modulus of elasticity of Norway spruce wood. *S. polyspora* was chosen because it does not attack structural polymers of wood but it is fed only by substances stored in lumens of cells and in pit-pairs. The coefficient of axial permeability K of the bio-treated and subsequently dried spruce sapwood increased approximately 5-times, from (0.362×10^{-12} to 1.861×10^{-12}) m^2. On the other hand, the changes in the density ρ and the dynamic modulus of elasticity along the spruce sapwood grain E_L were statistically insignificant, which is an important fact from the point of view of using this bio-treatment method in practice.

Key words: spruce wood, staining fungi, permeability, dynamic modulus of elasticity.

INTRODUCTION

Spruce wood is used in constructions, telecommunications, transport or furniture industry, but also for musical instruments and other special products. In some situations, such as telecommunication pillars, palisades, or string and other musical instruments, it is desirable for the spruce wood to be well permeable for appropriate types of chemical substances. Improved permeability is related specifically to various spruce elements proposed for exterior before their impregnation with biocides, as well as for the defined parts of artistic products and musical instruments with acrylic, epoxy and other synthetic polymers, or with shellac, damara and other natural resins (REINPRECHT 2016).

The researchers NAGYVARY (1988), NAGYVARY *et al.* (2009) and TAI *et al.* (2017) detected unusual minerals in wood of Cremona instruments. The results of the above mentioned researchers indicate the great differences between Cremona instruments which were made from impregnated wood and the modern instruments made from untreated wood.

It turns out that unusual impregnation of wood with chemicals, including borax, might cause superior sound of a musical instrument.

The problem of poor impregnability of spruce wood is caused by its insufficient permeability for liquids. The reason for this is the irreversible attachment of the torus to the porus in the pit-pairs of the cell-walls of tracheids, which in spruce wood occurs at its drying to the moisture content ≤ 30% (KURJATKO and REINPRECHT 1993, DURMAZ *et al*. 2015). For this reason, the spruce wood belongs to the most difficult species to impregnate – refractory species (EN 350 2016). Improved permeability and impregnability of spruce wood can be achieved in a number of ways - mechanical, physical, chemical and biological (REINPRECHT 2016). For example, in the biological ones there at a moisture content of wood above 30% the torus in the still opened pit-pairs is released due to the disturbance of the membranes in the margins at action of convenient species of bacteria, wood-staining fungi or microscopic-fungi. On the other hand, a very important request is made too, that the cell walls of spruce wood should not be disturbed thereby preventing the decrease of its original mechanical properties. From the point of this study, suitable bio-treatments of spruce and other refractory wood species can be made with specific species of bacteria and fungi, which have already been studied in several research studies, for example LIESE and GREAVES 1975, MESSNER *et al*. 2003, LEHRINGER *et al*. 2009, YILDIZ *et al.* 2012.

This paper deals with a bio-treatment of the Norway spruce wood with the wood-staining fungus *Sydowia polyspora*. The objectives are based on some similar research studies carried out in laboratories of the Faculty of Wood Sciences and Technology in Zvolen (e.g. REINPRECHT and PÁNEK 2008, PÁNEK and REINPRECHT 2011, PÁNEK *et al.* 2013), when the microscopic fungus *Trichoderma viride* and the bacterium *Bacillus subtilis* were applied in a bio-treatment and permeability improving of spruce logs.

MATERIALS AND METHODS

Wood material

The samples from the Norway spruce (*Picea abies* (L.) Karst.) sapwood with the dimension of 30 mm × 30 mm × 15 mm (R × T × L) were used for the permeability analyses, and with the dimension of 10 mm × 10 mm × 400 mm (R × T × L) were used for the density and the dynamic modulus of elasticity analyses. The samples, prepared from the freshly cut tree, had not knots or other growth inhomogeneities, and their moisture content was approximately of 144% (Fig. 1).

Fig. 1 Sapwood zones in the cross sections of the Norway spruce logs marked by blue colour.

The permeability test was performed with 216 samples. The samples were divided into three sets, when each set consisted of 72 samples. The samples of the first set (subsequently subjected to the bio-treatment) and the second set (native without bio-treatment) were immediately immersed into the distilled water with addition of xylene to prevent growth of microorganisms and to ensure their high moisture content, i.e. ≥ 100% (permanently wet state). The samples of the third set were dried and conditioned at temperature of 25 °C and relative humidity of air 43% to reach their 8 ± 1% moisture content (dried state).

The density and the dynamic modulus of elasticity analyses were performed with 32 samples (16 native + 16 bio-treated with staining fungus), which prior to testing were firstly dried and conditioned, in order to achieve 8 ± 1% moisture content.

Staining fungus

In the experiment the wood-staining fungus *Sydowia polyspora* (Bref. & Tavel) E. Mủll., syn. *Hormonena dematioides* Lagerb. & Melin was used, which frequently occurs in moist coniferous wood species. It does not attack structural polymers of wood, feeds only on reserve substances stored in the lumens of cells and pits of cell walls, and causes grey-green colouration of wood (REINPRECHT 2016).

Biological treatment of wood

The fresh spruce sapwood samples were sterilised for 35 minutes in autoclave (PS 121, Chirana Brno, Czechoslovakia) at the temperature of 121 °C and pressure of 125 kPa. Subsequently, they were transferred into inoculation box (Merci Ferrera, Italy) where they were again sterilised by a germicidal lamp with the power of 0.74 kW, and finally inoculated by immersion for 2–3 seconds into a nutrient solution containing the conidia of the wood-staining fungus *Sydowia polyspora*. The inoculated samples were stored in sterilised glass containers on stainless steel pads placed on a layer of Czapek-Dox medium with 10 mm gaps between them. The actual incubation of samples in the fungus *S. polyspora* lasted 24 weeks.

Permeability of wood

The spruce sapwood samples, before their permeability determination, were immersed into distilled water for 24 hours. The permeability measurements were performed in a laboratory device proposed by REGINÁČ *et al.* (1977). The coefficient of axial permeability K (m^2) of the Norway spruce sapwood samples was calculated by the Darcy low valid for the stationary transport of liquids (SIAU 1984), using Eq. 1:

$$K = \frac{V.\eta.\ell}{A.\tau.\Delta p} \tag{1}$$

where: V (5×10^{-6} m^3) is the measured volume of distilled water flowed through the sample; η (1×10^{-3} Pa.s^{-1}) is the dynamic viscosity of distilled water at 20 °C; ℓ (0.015 m) is the length of sample in longitudinal direction; A (1×10^{-4} m^2) is the entry area through which the distilled water enters the sample; τ (60 s) is the flow time of the distilled water; and Δp (1×10^5 Pa) is a pressure gradient.

Dynamic modulus of elasticity of wood

For obtaining the dynamic modulus of elasticity along the wood grain E_L (Pa) the resonant dynamic method was used – applying the measuring device MEARFA (Fig. 2).

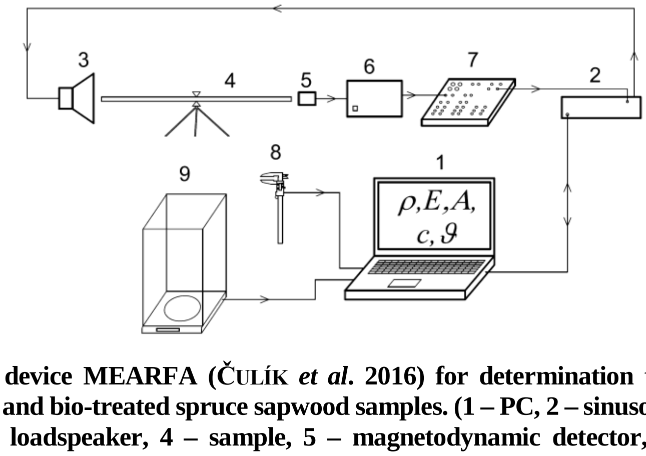

Fig. 2 The measuring device MEARFA (ČULÍK *et al.* 2016) for determination the dynamic modulus of elasticity of the natural and bio-treated spruce sapwood samples. (1 – PC, 2 – sinusoidal signal generator and detector response, 3 – loadspeaker, 4 – sample, 5 – magnetodynamic detector, 6 – low-pass filter, 7 – preamplifier, 8 – digital gauge, 9 – scales).

The principle of this method searches for the resonant frequency f_r of a vibrating body of the bar shape. The dynamic modulus of elasticity E_L was calculated according to Eq. 2:

$$E_{\mathrm{L}} = 4.\ell.f_{\mathrm{r}}^2.\rho \quad (2)$$

where: ℓ (0.4 m) is the length of spruce sample; ρ (kg.m^{-3}) is the density of spruce sample – see Table 1; and f_r (Hz) is the fundamental resonant frequency.

RESULTS AND DISCUSSION

The bio-treated Norway spruce sapwood samples, i.e. after treatment with the wood-staining fungus *Sydowia polyspora* and after subsequent drying, had the coefficient of axial permeability $K = 1.861 \times 10^{-12}$ m^2, i.e. 5-times higher comparing to the untreated native dried samples (0.362×10^{-12} m^2), (Tables 1, 2 and 3). The permeability of the bio-treated samples was even more than 2-times higher in relation to the fresh permanently wet native samples with still naturally open pit-pairs (0.904×10^{-12} m^2), (Table 1).

PÁNEK *et al*. (2013) in their study of the Norway spruce sapwood inoculated with the microscopic fungus *Trichoderma viride* found out that its coefficient of axial permeability increased approximately 11-times after 1-week incubation in this fungus in comparison to dried untreated sapwood, from $K = 0.12 \times 10^{-12}$ m^2 to 1.47×10^{-12} m^2, while after 9 weeks this coefficient increased approximately 18-times to $K = 2.13 \times 10^{-12}$ m^2.

According to PÁNEK and REINPRECHT (2011) the maximum improvement, a 59-fold increase of the coefficient of permeability of spruce sapwood (on the value of $K = 7.24 \times 10^{-12}$ m^2), was observed after 9 weeks of the *Bacillus subtilis* action in the laboratory at 30 °C – when the bacteria suspension was added to spruce logs each week.

Tab. 1 Influence of the bio-treatment of the Norway spruce sapwood with the staining fungus *Sydowia polyspora* on its permeability, density and dynamic modulus of elasticity.

Norway spruce sapwood		$K \times 10^{12}$ (m^2)	ρ (kg.m^{-3})	E_L (GPa)
Bio-treated by *S. polyspora* and dried	MV	1.861	378	10.03
	SD	0.456	13.5	2.58
Native dried	MV	0.362	374	9.98
	SD	0.124	20.4	2.41
Native permanently wet	MV	0.904	-	-
	SD	0.607	-	-

MV – mean value, SD – standard deviation.

Tab. 2 One-way ANOVA for the changes of permeability, density and dynamic modulus of elasticity of the Norway spruce sapwood pre-treated with the staining fungus *Sydowia polyspora*.

Properties	Sum of Squares	df	Variance	F-test	p-value
K 10^{12} (m^2)	37.4796	2	18.73981	39.9393	0.000000
ρ (kg.m^{-3})	2661.3	2	1330.635	0.736551	0.479995
E_L (GPa)	18.26	2	9.13	0.89	0.41

Tab. 3 Duncan´s test for the changes of permeability of the Norway spruce sapwood pre-treated with the staining fungus *Sydowia polyspora*.

State of spruce sapwood		Native permanently wet	Bio-treated by *S. polyspora* and dried	Native dried
$K \times 10^{12}$ (m^2)		**0.904**	**1.861**	**0.362**
State of spruce sapwood	Native permanently wet	-	0.000011	0.028680
	Bio-treated by *S. polyspora* and dried	0.000011	-	0.000011
	Native dried	0.028680	0.00011	-

Comparing the wood-permeability-improving effect of the staining-fungus *Sydowia polyspora* with similar effects of some other specific microscopic fungi and bacteria (e.g. more research works in this field were cited by PÁNEK and REINPRECHT 2011 and PÁNEK *et al.* 2013) it was proved, that *S. polyspora* does not belong to the best improvers of wood impregnability.

Indirectly, on the basis of the stable "statistically unaffected" densities and moduli of elasticity of the native and bio-treated spruce sapwood samples in the dried state – after drying and conditioning on approximately of 8% moisture content (Tables 1, 2 and 3), it was confirmed that the staining fungus *S. polyspora* is able to damage unclosed pits-pairs in tracheids (present in fresh wet sapwood), however, without degradation of tracheid's cell walls.

The modulus of rupture *MOR*, the modulus of elasticity *MOE* and mainly the impact bending strength *IB* are sensitive mechanical properties used for indication of all types of deterioration in the wood structure (Wilcox 1978). They are often used to detect various initial changes in the molecular and anatomical structure of wood damaged by wood-decaying fungi, however, also for a potential change of cell walls structure due to bacteria, microscopic fungi – moulds, and wood-staining fungi. PÁNEK and REINPRECHT (2011) and PÁNEK *et al.* (2013) did not observe any negative effect of the bacterium *Bacillus subtilis* and the microscopic fungus *Trichoderma viride* acting in the spruce sapwood on these above mentioned sensitive mechanical properties. Similar results with bacteria were obtained in studies of KNUTH (1964) and UNLIGIL (1972), who observed only a minimum decrease of these mechanical wood properties, however, EFRANSJAH *et al.* (1989) observed a 17% decrease of the modulus of rupture in bending after 5 months ponding of spruce logs in presence of the bacterium *Bacillus subtilis*.

CONCLUSIONS

Biological treatment of the Norway spruce sapwood with the wood-staining fungus *Sydowia polyspora* for a time-period of 24 weeks significantly increased its permeability in dried state – about more than 100 % in relation to permanently wet samples, and about 400 % in relation to dried samples having prominently closed pit-pairs in tracheids. However, cell walls of tracheids and other cells in spruce sapwood stayed undamaged, as it was indirectly confirmed by determination of unchanged values of the density and the dynamic modulus of elasticity for the bio-treated samples.

REFERENCES

ČULÍK M., DANIHELOVÁ A., DANIHELOVÁ Z. 2016. Wood for musical instruments. In Akustika, 25: 66–72.

DURMAZ S., YILDIZ U.C., YILDIZ S. 2015. Alkaline enzyme treatment of spruce wood to increase permeability. In BioResources, 10(3): 4403–4410.

EFRANSJAH F., KILBERTUS G., BUCUR V. 1989. Impact of water storage on mechanical properties of spruce as detected by ultrasonics. In Wood and Science Technology, 23(1): 35–42.

EN 350 2016. Durability of wood and wood-based products. Testing and classification of the durability to biological agents of wood and wood-based materials. Brussels.

KNUTH D.T. 1964. Bacteria associated with wood products and their effects on certain chemical and physical properties of wood. PhD Thesis, Univ. Wisconsin, Madison, USA, 186 p.

KURJATKO S., REINPRECHT L. 1993. Transport látok v dreve. (Transport of substances in wood – English Abstract). Monograph, Zvolen : Technical University in Zvolen, Slovakia, 110 p.

LEHRINGER C.H., RICHTER K., SCHWARZE F.W.M.R., MILITZ H. 2009. A review on promising approaches for liquid permeability improvement in softwoods. In Wood and Fiber Science, 41(4): 373–385.
LIESE W., GREAVES H. 1975. Micromorphology of bacterial attack. In Biological Transformation of Wood by Microorganisms, Berlin : Springer-Verlag, pp. 74–88.
MESSNER K., BRUCE A., BONGERS H.P.M. 2003. Treatability of refractory wood species after fungal pre-treatment. In Processing of the Second European Conference on Wood Modification, Göttingen, Germany, pp. 389–401.
NAGYVARY J. 1988. The chemistry of a Stradivarius. In Chemical & Engineering News, 66(21): 24–31.
NAGYVARY J, GUILLEMETTE, R.N, SPIEGELMAN C.H. 2009. Mineral preservatives in the wood of Stradivari and Guarneri. In PLOS ONE, 4(1): e4245.
PÁNEK M., REINPRECHT L. 2011. *Bacillus subtilis* for improving spruce wood impregnability. In BioResources, 6(3): 2912–2931.
PÁNEK M., REINPRECHT, L. MAMOŇOVÁ, M. 2013. *Trichoderma viride* for improving spruce wood impregnability. In BioResources, 8(2): 1731–1746.
REGINÁČ L., ČOP D., ŠTEFKA V. 1977. Zariadenie na skúšanie priepustnosti pórovitých materiálov najmä dreva pre kvapaliny a plyny. (A device for testing the permeability of porous materials, preferentially from wood).Patent ČSSR 5308-77.
REINPRECHT L., PÁNEK M. 2008. Bio-treatment of spruce wood for improving of its permeability and soaking - Part 2: Direct treatment with the fungus *Trichoderma viride*. In Wood Research, 53(3): 1–8.
REINPRECHT L. 2016. Wood deterioration, protection and maintenance. Chichester : John Wiley and Sons, Ltd, UK, 357 p.
SIAU J. F. 1984. Transport processes in wood. Berlin – Heidelberg – New York – Tokyo : Springer-Verlag, 245 p.
TAI H. CH., LI G. CH., HUANG S. J., JHU CH. R., CHUNG J. H., WANG BO. Y., HSU CH. S., BRANDMAIR B., CHUNG D. T., CHEN H. M., CHAN J. CH. CH. 2017. Unique chemical properties of Stradivari's wood. In Proceedings of the National Academy of Sciences, 114 (1): 27–32.
UNLIGIL H.H. 1972. Penetrability and strength of white spruce after ponding. In Forest Products Journal 22(9): 92–100.
WILCOX W.W. 1978. Review of literature on the effects of early stages of decay on wood strength. In Wood and Fiber, 9: 252-257.
YILDIZ S., CANAKCI S., YILDIZ U. C., OZGENC O., TOMAK E. D. 2012. Improving of the impregnability of refractory spruce wood by *Bacillus lichteniformis* pretreatment. In BioResources, 7(1): 565–577.

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