Trichoderma viride for Improving Spruce Wood Impregnability

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Impregnability of poles and other products made from spruce or fir wood decreases after the closing of toruses in the pits of tracheids, which usually occurs after their drying up to the fiber saturation point. With the aim of reopening access to the pits in the tracheids, the microscopic fungus *Trichoderma viride* was used for the enzymatic degradation of the pits in Norway spruce. During the summer, freshly cut and debarked spruce bolts were exposed in an inoculation mycelium of *T. viride* for 1, 3, 6, and 9 weeks under exterior conditions. Very good permeability and impregnability of spruce sapwood was observed after 1 or 3 weeks with no apparent change in its mechanical properties (Modulus of Rupture, Impact Bending Strength). On the other hand, previously closed pits of spruce heartwood remained unchanged in all experiments. Generally, coming out from achieved results and knowledge of other researches, bio-treatments of conifers are suitable for improving the impregnability of poles and other rounded timber products with the sapwood zone intact. However, bio-treatments for squared timbers with visible heartwood are usually a less appropriate.

Keywords: Spruce; Impregnability; Biological treatment; Trichoderma viride

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INTRODUCTION

Impregnability of wood depends on its geometric, morphological, and anatomical structure. The opened or closed state of the pits in the tracheids (Fig. 1) has a dominant effect on the permeability of conifers (Siau 1984, 1995). In spruce wood, the pits in the tracheids are already mostly aspirated during the tree growth (in heartwood zone) or during drying of the wood after cutting (sapwood zone). Nearly all the pits of spruce wood are closed when the moisture falls to approximately 30%, which is near the fiber saturation point. Therefore, dried products from spruce sapwood and heartwood are practically non-permeable to liquids and their chemical protectants, which makes its pretreatment to prevent degradation difficult (Kurjatko and Reinprecht 1993; Hansmann *et al*. 2002).

Various methods have been proposed for increasing wood impregnability (Morrell and Morris 2002; Lehringer *et al.* 2009; Pánek and Reinprecht 2011). Well-known are mechanical pretreatments (cutting and incising methods), physical pretreatments (*e.g.*, special ways of seasoning, using of ultrasound waves, laser incising), chemical pretreatments (*e.g.*, using of oxidizing chemicals, and the autocatalytic effect of acetic acid created during steaming of wood), and biological pretreatments.

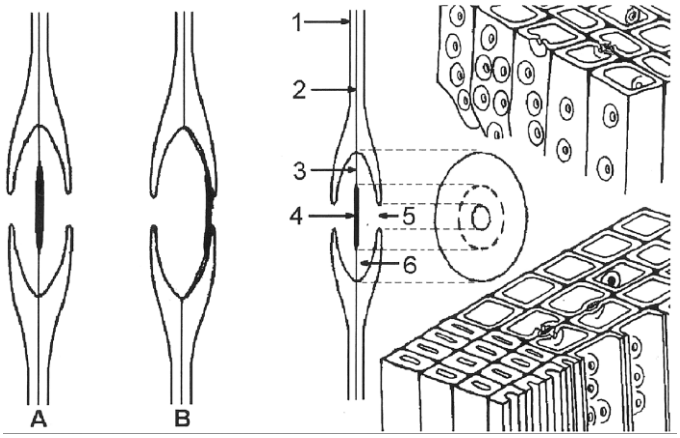

Fig. 1. A – pit in opened state; **B** – closing of the pit during drying: Structure of the typical pit in early wood: 1 cell wall (secondary), 2 middle lamella (and primary wall), 3 margo, 4 torus, 5 porus, 6 inside area of the pit (after Petty 1970).

The biological pretreatment (*i.e.* bio-treatments) of refractory wood species has been studied numerous times using various kinds of bacteria (Liese and Greaves 1975; Burnes *et al.* 2000; Pánek and Reinprecht 2008, 2011; Yildiz *et al.* 2012), pure enzymes (Adolf *et al.* 1972), microscopic fungi (Freitas and Erikson 1969; Rosner *et al.* 1998; Reinprecht and Pánek 2008), wood-staining fungi (Fojutowski 2004, 2005), and also some species of white-rot fungi (Rosner *et al.* 1998). Promising experiments with the white-rot fungus *Physisporinus vitreus* under highly controlled conditions for destruction of only the toruses of the heartwood pits have been done recently (Schwarze *et al.* 2006; Schwarze 2008; Lehringer *et al.* 2009, 2010, 2011a, 2011b). Optimum times of these pre-treatments without the significant effect on mechanical properties and chemical changes have been determined by means of very detailed experiments (Lehringer *et al*. 2011a, 2011b). The experiments have shown that *P. vitreus* destroyed not only toruses of the pits but also caused selective delignification and simultaneous degradation of lignin and polysaccharides in call wall components by highly diffusible ecto-enzymes. Mass losses were relatively low after a longer period of incubation (9 weeks) (Lehringer *et al.* 2010), and the Brinell hardness decreased more significantly only after 7 weeks of the biodegradation process (Lehringer *et al.* 2011a). The mechanism of the degradation process was confirmed by other experiments focused on chemical analyses and the method of microtensile testing (Lehringer *et al.* 2011b). Final results of these experiments showed that optimum incubation period for bio-incising is less than 4 weeks and it is necessary to achieve homogenous wood colonization.

Some kinds of bacteria, fungi, or pure enzymes are useful, since they can degrade margins or toruses in the pits, but they can cause minimal damage to the cell walls of wood (Reinprecht 1996). The non-destroyed cell wall S2 layer is the most important factor for saving the wood's mechanical properties (Adusumalli *et al*. 2010; Eder *et al.* 2013). That is the reason why common brown and white rot fungi are not suitable for pre-treatments. They produce a wide range of enzymes that also destroy cellulose (also in S2 layer) and lignin. Bacteria of the genus *Bacillus* and *Pseudomonas*, wood-staining fungi of the genus *Ceratocystis*, and microscopic fungi of the genus *Trichoderma* can be used for these purposes. *T. viride* also produces mainly hydrolase enzymes that preferentially degrade hemicelluloses; the organism lacks the enzymatic apparatus to induce delignification. So lignin, which can be regarded as a matrix material within the cell wall

structure as a lignocellulosic composite, has a hydrophobic effect and restricted fungus activity. *T. viride* cannot destroy cell walls that contain lignin and also toruses in heartwood tracheid pits after their lignification.

Freitas and Erikson (1969) increased the impregnability of alder poles to creosote oil by using a four week bio-treatment process with the microscopic fungi *Trichoderma* sp., *Gliocladium roseum*, *Fusarium* sp., and *Chaetomium conchlioides*. Rosner *et al.* (1998) applied the microscopic fungi *Trichoderma viride* and *T. aureoviride*, and white-rot fungi *Phanerochaete chrysosporium* and *Dichomitus squalens* to improve the impregnability of Norway spruce wood to creosote oil. Various analyses (*e.g.* the light and electron microscopy, as well as the ergosterol method that quantifies fungi biomass in wood), have shown colonization of all parts of spruce sapwood already during one week of its bio-treatments and then significant improvement of its impregnability. *Trichoderma* species are also used as antagonistic organisms against wood-destroying fungi or bacteria (Bruce and Highley 1991; Ejechi 1997; Score *et al.* 1998), and commercially for enzyme production (Verma *et al.* 2007).

Suitable species of fungi (*e.g., Trichoderma* sp. or *Physisporinus vitreus*) are well known to improve the permeability of selected zones of conifers (*e.g.*, spruce, fir, and pine). However, the main problem is to achieve homogenous growth and homogenous enzymatic effect of the applied fungus throughout the volume of the sapwood or even heartwood. In previous works, authors have analyzed the effects of the microscopic fungus *Trichoderma viride* Pers. 1402 for improving the permeability and impregnability of Norway spruce wood by two methods carried out in Kolle's flasks. The first involves bio-treatments of small sapwood and heartwood samples from short logs (35 cm long) previously ponded for 4 months in unsterile water (Pánek *et al.* 2005). The second involves bio-treatments of small samples from freshly cut logs (Reinprecht and Pánek 2008).

The aim of this work was to examine the effects of the fungus *Trichoderma viride* on freshly cut and debarked Norway spruce logs – through the study of microscopic structure, permeability, and selected mechanical properties of the sapwood and heartwood zones.

MATERIAL AND METHODS

Spruce Wood

Norway spruce (*Picea abies* L. Karst) green debarked bolts, *i.e.* short logs, prepared from 35- to 37-year-old trees, with a medium diameter from 225 to 260 mm and with a constant length of 600 mm were used in these experiments. Sapwood zone in the logs had thickness dimensions from 3 to 4 cm. The sapwood zone was distinguished by the high moisture content difference between the sapwood and heartwood zones in freshly cut logs from trees in the growing season, as determined visually and by moisture content measurements. At a moisture of 0%, the medium density of the sapwood was 398.3 kg/m^3 and of the heartwood 339.5 kg/m^3, respectively. Surfaces of all bolts (4 for experiment, 1 control) were initially sterilized by 800 W of UV-radiation for 30 minutes, and then their frontal surfaces were painted with PVAc latex.

Bio-treatment of Spruce Bolts by the Microscopic Fungus *T. viride*

All rounded surfaces of spruce bolts were inoculated with a *Trichoderma viride* suspension in distilled water. One spruce bolt was inoculated with *T. viride* suspension prepared from spores and 8-week-old mycelium growth on the surface of Czapek-Dox agar in 10 Petri dishes with a diameter of 120 mm. Inoculated spruce bolts were then stored under wet conditions in plastic vessels 300 x 450 x 700 mm without direct contact with distilled water. Water was only in the bottom of plastic vessels, while bolts were standing on pads, and the top of each plastic vessel was gently covered with PVC film. Bio-treatment was carried out for 1, 3, 6, and 9 weeks (T1, T3, T6, and T9) under outdoor conditions during the summer from the beginning of June to the beginning of August with temperatures ranging from 5 to 35 °C.

Permeability, Impregnability, Strength, and Microscopic Analyses of Spruce Bolts Bio-treated by the Microscopic Fungus *T. viride*

Spruce samples were prepared immediately after each bio-treatment process, especially from the sapwood (depth from the surfaces in radial direction was 1 to 3 cm) and heartwood (depth from the surfaces in radial direction was 6 to 8 cm) zone of bolts (Fig. 2).

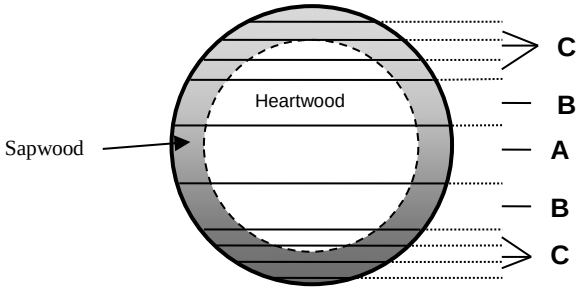

Fig. 2. Scheme of testing samples preparation from one bolt with the length of 600 mm. **A** zone – samples for the coefficient of permeability (n=15); **B** zone – samples for the water uptake (n=5) and for the impregnability (n=5); **C** zone – samples for the modulus of rupture (n=10) and for the impact bending strength (n=10)

Treated samples were sterilized for 5 h at 90 °C, conditioned at two moisture content levels of 0 and 8% ±1%, and finally analyzed in accordance with the work of Pánek and Reinprecht (2011):

1) Coefficient of axial permeability (K).

The value of *K* was determined on the basis of the Darcy´s law.

$$K = (V \,.\, \eta \,.\, L) : (A \,.\, \tau \,.\, \Delta p) \qquad [m^2] \qquad (1)$$

Experimental conditions were as follows: steady flow of 20 °C distilled water in the longitudinal direction of samples; L is the length of samples in the longitudinal "axial" direction (0.015 m); A is the inputting transversal area of samples for flow (1.10^{-4} m^2); Δp is the pressure difference (1.10^5 Pa); V is the transported volume of distilled water (5.10^{-6} m^3); η is the dynamic viscosity of distilled water at 20 °C (1.10^{-3}Pa.s); τ is the time of flow (s); initial moisture of samples w = 8±1 %; and the number of samples in each series is n = 15.

2) Water uptake - kinetics of water soaking (S).

Experimental conditions were as follows: dimension of samples 20 x 20 x 30 mm (radial x tangential x longitudinal); soaking in distilled water at 20 °C valued after 15, 30, 45 min, 1, 2, 4, 8 h, and 1, 2, 3, 6, 8, 10, 16, 21 days,

$$S = /(m_w - m_0) : m_0/ . 100 \quad [\%] \qquad (2)$$

where m_0 is the weight of sample in the oven dry state (dried at 103±2 °C); m_w is the weight of sample in a defined stage of soaking. In each series 5 samples were tested.

3) Impregnability (R, I_S).

The impregnability was determined on the basis of the retention of distilled water in samples (R in kg•m^{-3}), or on the basis of the degree of sample saturation (I_S in % by Equation 3) with water,

$$I_S = (R / R_{max}) . 100 \quad [\%] \qquad (3)$$

where R is the achieved retention of water in the sample, and R_{max} is the theoretically maximum retention of water in the sample with a defined density (porosity) and moisture. The following experimental conditions were used: dimension of samples 20 x 20 x 100 mm (radial x tangential x longitudinal); impregnation by modified Lowry pressure technique at p = 0.8 MPa during 5, 15, and 150 minutes; initial moisture of samples 8%; frontal surfaces of samples were or were not painted before impregnation by paraffin; 5 samples in each series.

4) Modulus of rupture (MOR) and impact bending strength (IB)

The *MOR* was determined in accordance with the ASTM D143-94 standard (2000) using modified dimensions of samples. Experimental conditions were as follows: dimension of samples 10 x 10 x 120 mm (radial x tangential x longitudinal); load acting on samples in their tangential direction; moisture of samples 8%; 10 samples in each series.

5) Microscopic analyses

Samples from sapwood and heartwood were gold coated, and then analyses were carried out with a Scanning Electron microscope RAM-TESCAN-VEGA TS 5130. Sapwood samples were prepared from a zone 1 to 3 cm deep, and heartwood samples 6 to 8 cm deep from the surface of a bolt in the radial direction.

Statistical Evaluation

All of the results obtained from the bio-treated spruce sapwood and heartwood samples were compared with the results obtained from the biologically untreated ones. The results were statistically evaluated by mean values, graphs with confidence intervals, standard deviations, and Duncan´s test.

RESULTS AND DISCUSSION

Coefficient of Permeability and Water Soaking

The coefficient of axial permeability (*K*) in the case of spruce sapwood pretreated with *Trichoderma viride* increased significantly (Fig. 3). It could not be determined for the control and the bio-treated spruce heartwood samples due to a large number of closed pits (Photo 6). For bio-treated sapwood, this coefficient increased approximately 11-times after 1 week of *T. viride* action in comparison to untreated sapwood. Values changed from $K = 0.12 \times 10^{-12}$ m^2 to 1.47×10^{-12} m^2; after 9 weeks, this coefficient increased approximately 18-times to $K = 2.13 \times 10^{-12}$ m^2 (Fig. 3). Mean values of *K* did not increase gradually with the time of bio-treatment. This can be explained by the non-homogenous nature of biological processes and also by a non-homogenous amount of early and late wood in tested samples with different portion of the pits in tracheids. Another factor could be the homogeneity of colonization of each bio-treated log.

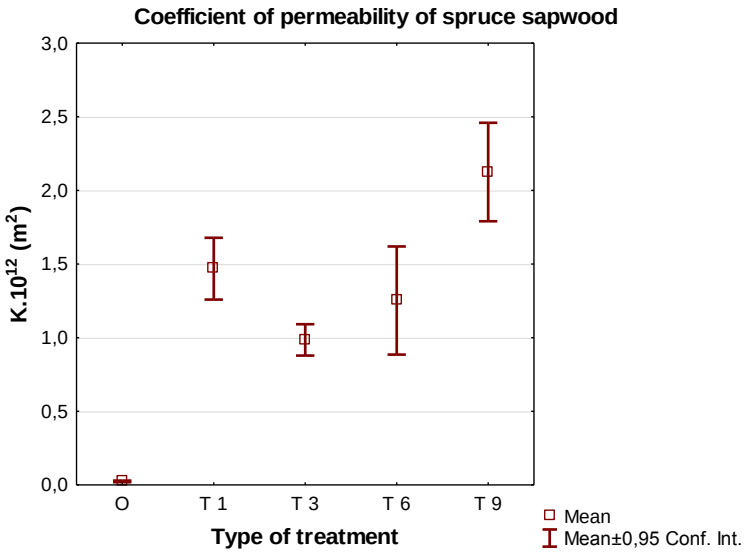

Fig. 3. Coefficient of axial permeability (*K*) of spruce sapwood from bolts bio-treated with the microscopic fungus *Trichoderma viride* (Control – O; Treated for 1, 3, 6 and 9 weeks – T1, T3, T6 and T9).

The water uptake (*S*), measured from 15 min to 21 days, increased only for the bio-treated spruce sapwood. This increase was significant in the initial stages of the soaking test from 0 min to 4 h but particularly in the first 15 min (Fig. 4). After 1 week of bio-treatment, the soaking S_{15min} increased by 67% (1 week - T1). The effect of the fungus *Trichoderma viride* was more pronounced at longer times of its action. For instance, after 3 weeks of bio-treatment, the soaking S_{15min} had already increased by 115% (3 weeks - T3). However, the kinetics of water soaking remained stable for 3, 6, and 9 weeks of bio-treatments.

The water uptake (*S*) of the bio-treated spruce heartwood remained practically unchanged (Fig. 5). Some small changes were observed (mainly after longer period of soaking when the factor of porosity of wood is dominant and opening system of the pits is less important) mainly due to different densities of the samples in tested series (each series was prepared from a different spruce bolt with a different medium density of wood).

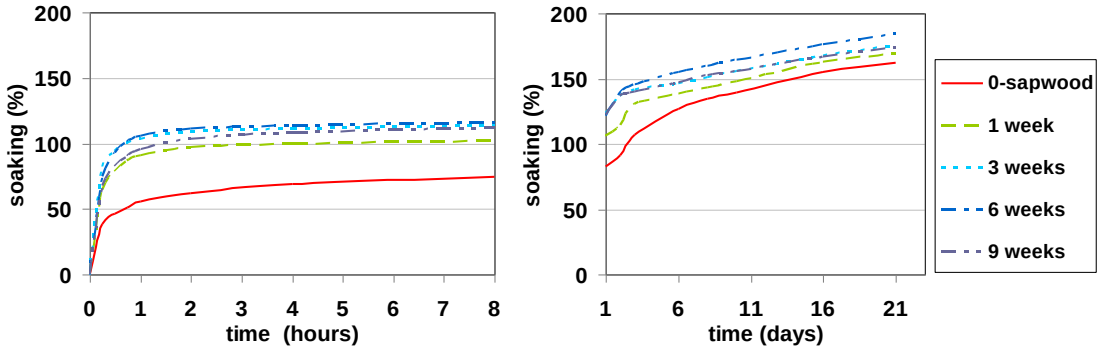

Fig. 4. Water soaking (*S*) of spruce sapwood bio-treated with the microscopic fungus *T. viride* for 0 (control), 1, 3, 6, and 9 weeks

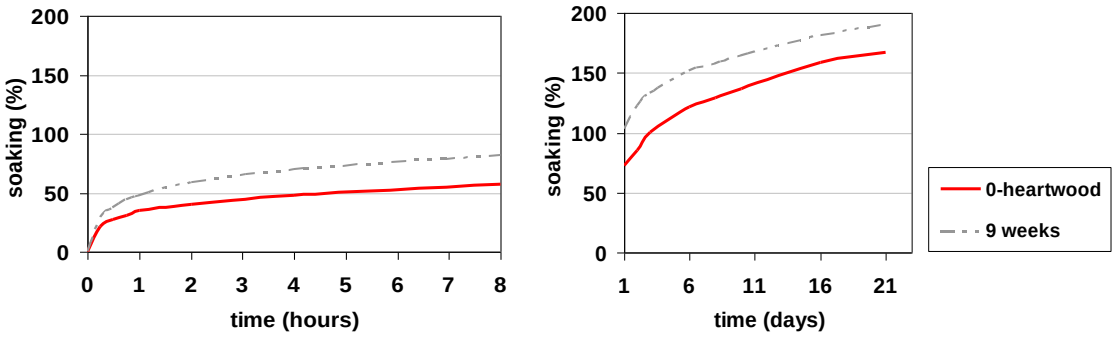

Fig. 5. Water soaking (*S*) of spruce heartwood bio-treated with the microscopic fungus *T. viride* for 0 (control) and 9 weeks

Impregnability

The retention (*R*) and the degree of saturation (I_S) of the bio-treated spruce sapwood samples in comparison with untreated ones increased very similarly (Table 1; Fig 6). The *R* values for the bio-treated spruce sapwood impregnated for 5 min with the paraffined ends increased 3-times after 1 week of treatment with *T. viride*, or 3.5-times after 9 weeks bio-treatment. Differences between the retentions (*R*) of the control and bio-treated sapwood samples were smaller after 15 min of impregnation, and also for samples with the non-paraffined ends after 150 min (Table 1).

The I_S values of the bio-treated sapwood samples, both with paraffined frontal surfaces (Fig. 6A) and non-paraffined ones (Fig. 6B), increased significantly at shorter impregnation processes lasting 5 and 15 minutes. Differences in I_S values between the untreated (*i.e.* control) and the bio-treated samples were higher and more significant with the paraffin sealed ends (Fig. 6A). For sapwood treated 1 week with *T. viride* the $I_{S\text{-}5min}$ value increased by 210%, and after 6 or 9 weeks by 222% or 258%, respectively. A high increase of the *I*s values due to *T. viride* was also measured after 15 min of pressure impregnation; however, after 150 min, only small or no differences were observed between treated and control specimens with non-paraffined ends (Fig. 6B).

Table 1. Retentions (*R*) of Bio-Treated (T1, T3, T6, T9) and Control (O) Spruce Sapwood and Heartwood Samples with Paraffined and Non-Paraffined Frontal Surfaces at the Pressure Impregnation Process

Bio-treatment with *Trichoderma viride*		Retention – *R* (kg/m^3)					
		R - paraffined			*R* – non-paraffined		
		5 min.	15 min.	150 min.	5 min.	15 min.	150 min.
Sapwood	(weeks)						
O-control	0	116.1 (25.2)	187.4 (41.0)	431.5 (77.8)	245.1 (78.3)	335.9 (91.1)	572.5 (71.4)
T1	1	354.5 (8.1)	474.9 (12.9)	500.2 (10.8)	381.9 (7.8)	474.8 (16.4)	503.2 (10.4)
T3	3	339.3 (29.3)	447.8 (14.1)	530.2 (18.7)	424.8 (22.8)	474.4 (12.3)	530.6 (20.7)
T6	6	368.6 (65.8)	446.9 (54.0)	567.7 (22.7)	430.4 (36.1)	507.4 (21.9)	565.8 (19.2)
T9	9	405.1 (46.1)	461.6 (21.5)	518.3 (31.5)	456.9 (36.9)	502.4 (22.9)	547.1 (12.4)
Heartwood	(weeks)						
O-control	0	79.8 (23.9)	120.8 (35.5)	269.5 (59.4)	125.0 (6.1)	184.2 (7.6)	388.5 (20.7)
T9	9	78.7 (13.5)	123.8 (23.2)	288.9 (56.7)	166.6 (23.1)	248.1 (34.2)	508.1 (51.9)

Note: Arithmetic means in each series are from five samples (n = 5). Numbers in the parentheses are the standard deviations.

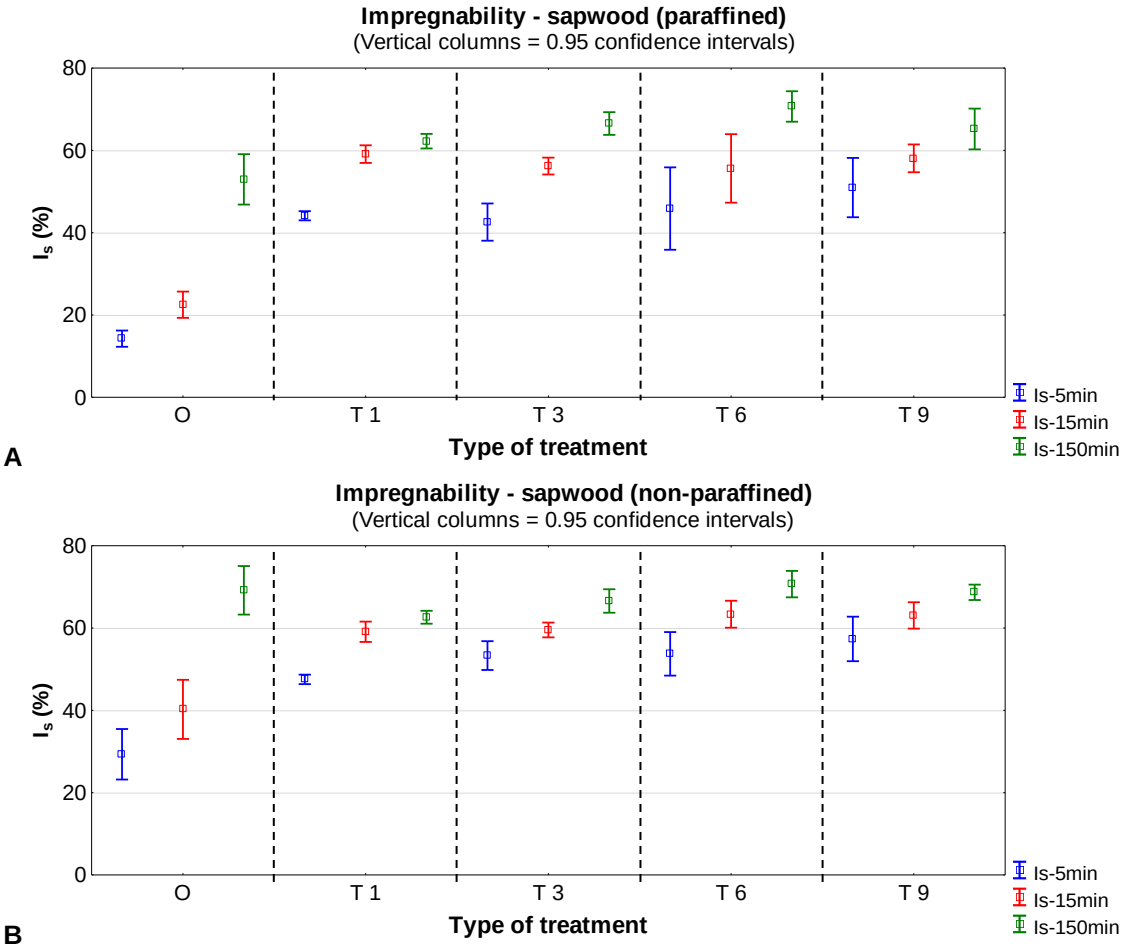

Fig. 6. Degree of saturation (I_S) of bio-treated (T1, T3, T6, T9) and control (O) spruce sapwood samples with paraffined (A) and non-paraffined (B) frontal surfaces with the pressure impregnation process

Finally, it can be said that pretreatments of spruce logs with the fungus *T. viride* should be important for shortening the time of sapwood zone impregnation when predetermined values of impregnability (I_S or R), and also the depth of penetration have to be achieved. Of course, the maximum values of impregnability (R_{max}) cannot be increased by this method because they depend only on the porosity and the density of wood.

The fungus *T. viride* did not have any significant influence on the impregnability (R, I_S) of spruce heartwood (Table 1, Figs. 7A and 7B). Smaller changes in the impregnability of non-paraffined heartwood were probably caused by different densities of samples in individual bio-treated series, when each series was prepared from one log (Fig. 7B).

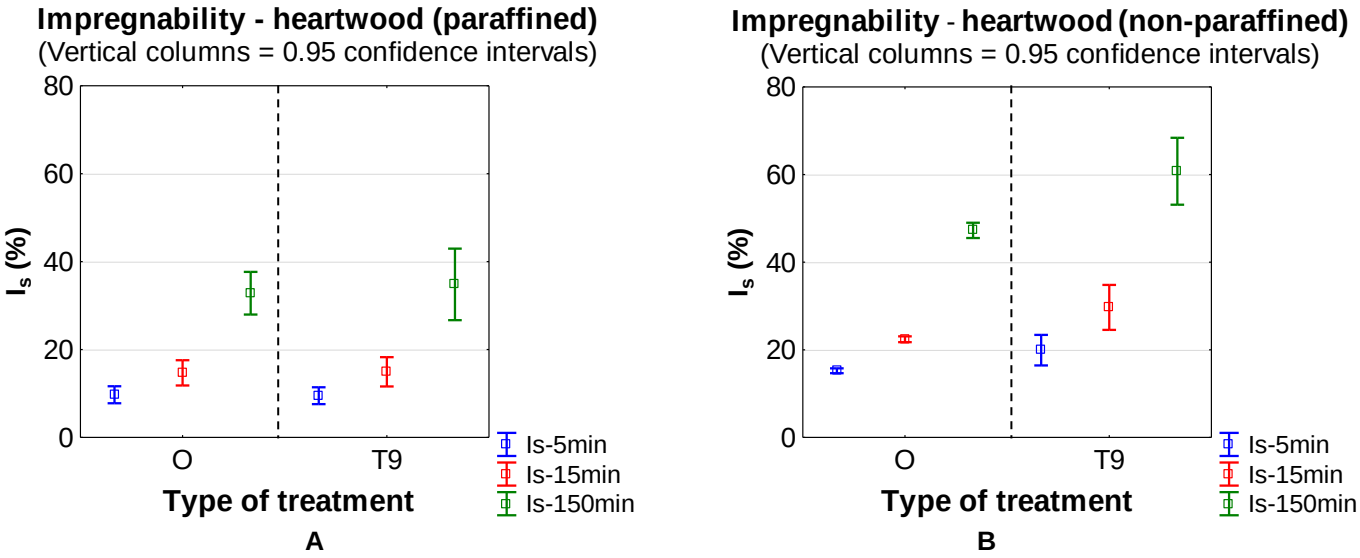

Fig. 7. Degree of saturation (I_S) of bio-treated (T9) and control (O) spruce heartwood samples with paraffined (A) and non-paraffined (B) frontal surfaces at pressure impregnation process

Mechanical Properties

The modulus of rupture (*MOR*) and the impact bending strength (*IB*) are sensitive mechanical properties of wood. Changes in these properties can be used as a means to detect various initial changes in its molecular and anatomical structure. However, no negative effects of bio-treatments by the fungus *Trichoderma viride* on the mechanical properties of spruce were observed (Table 2). Some differences of mean values of *MOR* and *IB* were in good correspondence with different densities of tested series (*e.g.*, from $\rho_{O\text{-control}}$ = 390 kg/m^3 to ρ_{T3} = 417 kg/m^3 for *MOR*; from $\rho_{T\text{-}6}$ = 400 kg/m^3 to ρ_{T9} = 426 kg/m^3 for *IB*).

Mechanical properties of spruce pretreated with *T. viride* corresponded well with microscopic analyses, which showed enzymatic degradation only at the pits (outage of toruses) in the sapwood zone without the destruction of the cell walls of tracheids for both the sapwood and the heartwood (Photos 1 to 10). Results reported by Fojutowski (2004) with blue-stain fungi, which mainly destroy pits, were very similar, *i.e.* they have negligible negative effect on the mechanical properties of wood.

Table 2. Modulus of Rupture (*MOR*) and Impact Bending Strength (*IB*) of Spruce Sapwood and Heartwood without Treatment (O-control) and After Bio-treatments with the Fungus *T. viride*

Bio-treatment of spruce with *Trichoderma viride*		Modulus of Rupture		Impact Bending Strength	
		MOR (MPa)	Density (kg/m^3)	IB (J/cm^2)	Density (kg/m^3)
Sapwood	(weeks)				
O-control	0	66.4 (9.4)	390 (32.6)	4.01 (0.79)	404 (31.7)
T1	1	67.0 (3.2)	409 (16.1)	4.30 (0.60)	411 (9.1)
T3	3	69.0 (4.8)*	417 (22.2)*	4.91 (0.68)***	420 (11.2)
T6	6	71.6 (8.0)**	407 (22.1)	3.70 (1.03)	400 (43.3)
T9	9	66.2 (4.6)	405 (23.8)	4.90 (1.15)***	426 (44.8)
Heartwood	(weeks)				
O-control	0	54.6 (8.9)	342 (17.6)	2.61 (0.60)	340 (28.8)
T9	9	54.8 (8.1)	333 (13.0)	2.23 (1.00)	332 (25.0)

Note: Arithmetic means in each series are from ten samples (n = 10). Numbers in the parentheses are the standard deviations.
Duncan´s test: Statistical differences in mechanical properties evaluated in relation to the O-control untreated samples at the 99.9% significance level (***); at the 99% significance level (**) and at the 95% significance level (*).

Microscopic Analyses

Results from the permeability, water uptake, impregnability, and strength tests were compared with results of microscopic analyses. Pits of tracheids in the bio-treated spruce sapwood were usually intensively damaged, while pits in the bio-treated spruce heartwood remained undamaged (Photos 1 to 10). No more expressive intensity of toruses degradation in sapwood was observed by SEM after prolongation of pre-treatments for 3, 6, and 9 weeks of (Photos 4, 5, 7, 8). The typical falling out of toruses was often observed; however, cell walls of tracheids were not damaged in either the sapwood or heartwood.

Comparison of Biological Pre-Treatments of Spruce Wood by the Bacterium *B. subtilis* and Microscopic Fungus *T. viride*

The optimum fungal pretreatment of debarked spruce logs with the microscopic fungus *Trichoderma viride*, taking into account technology process efficacy, should take place within 1 to 3 weeks (See values of *K, S, R, Is* in Fig. 3, 4, 6 and Tab. 1).

Further prolongation of bio-treatments on 6 and 9 weeks showed only small increases of impregnability. This can be explained by lower activity of fungus *T. viride* on bolts due to the consumption of most of the useful nourishment from their surface layers (SEM showed no hyphal growth deeper in samples and no more expressive degradation of toruses after 6 or 9 weeks of bio-treatments – Photos 4, 5, 7, and 8).
T. viride grows only on the surfaces of bolts, and toruses of pits are destroyed by enzymes diffusion into the wood. For practice, the use of green wet logs with free water in lumens is important. High moisture content is necessary for the opened state of pits, and free water in wood cells is a necessary condition for enzymes diffusion transport using water as the medium.

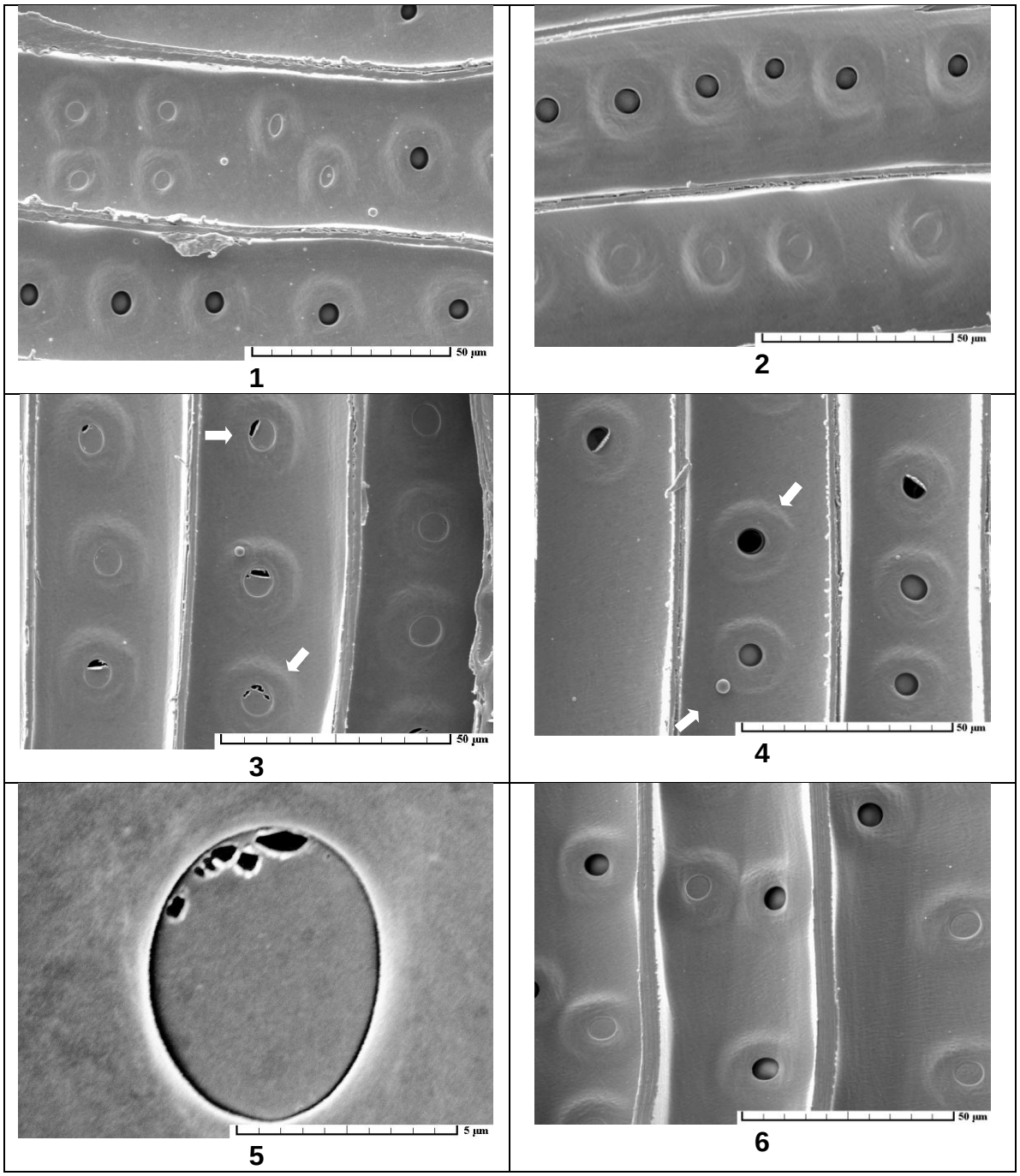

Photo 1: Non-destroyed toruses in pits of untreated spruce sapwood.
Photo 2: Non-destroyed toruses in pits of untreated spruce heartwood.
Photo 3: Partial degradation of toruses in pits (both *arrows*) of spruce sapwood treated with the microscopic fungus *T. viride* after 6 weeks (type T6).
Photo 4: Typical more significant falling out of toruses (down *arrow*) from pit (top *arrow*) of spruce sapwood after *T. viride* degradation (type T9).
Photo 5: Detail of damaged torus in pit of bio-treated spruce sapwood – initial stadium of margo and border of torus degradation by the *T. viride* (type T3).
Photo 6: Toruses in pits of spruce heartwood were not destroyed with *T. viride* even after 9 weeks (type T9).

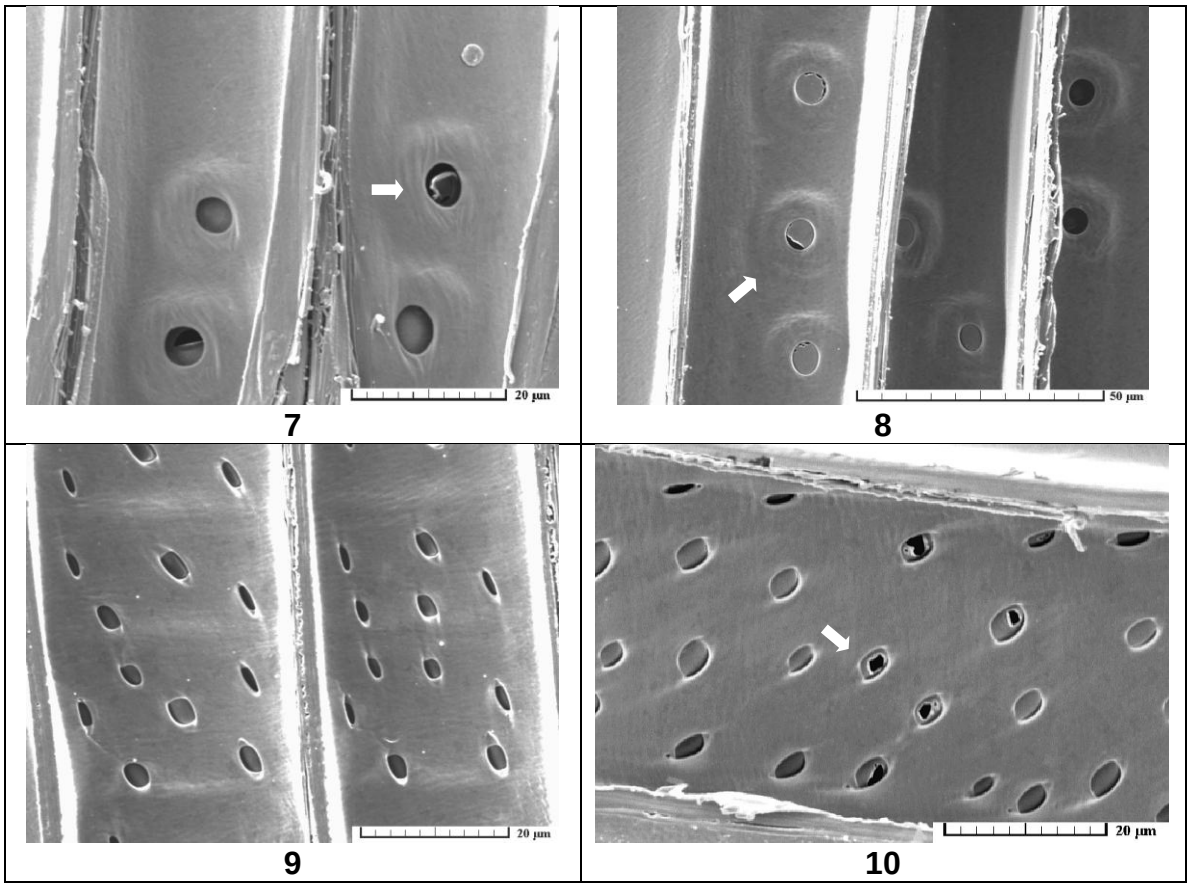

Photo 7: Fallen out torus (*arrow*) in the bio-damaged spruce sapwood pit – situated in the lumen of tracheid (type T9).
Photo 8: Degradation of margo and borders of toruses in pits (*arrow*) of the bio-treated spruce sapwood tracheid (type T1).
Photo 9: Non-destroyed pits in untreated spruce sapwood ray parenchyma cells.
Photo 10: Enzymatic degradation of pits (*arrow*) in ray parenchyma cells after 3 weeks pretreatment with *T. viride* (type T3).

The time of bio-treatment of wet logs is also connected with their biological protection with this fungus. On the other hand, the main disadvantage in comparison with bacterial pretreatment is a more complicated application of fungal spores on all surfaces of wood poles. Another disadvantage of such bio-treatments is the limited time for use of this technology during the year in some territories because the optimum temperature for the activity is about 20 to 30 °C; lower temperatures (below 3 °C) decrease or inhibit their activity.

In comparison with our previous tests with the bacterium *Bacillus subtilis* (Pánek and Reinprecht 2011), the following conclusions can be drawn:

a. *T. viride* acted more quickly, causing a significant increase of the spruce sapwood permeability already after 1 week; *e.g.* it caused 2-times higher increase of the coefficient of axial permeability compared to *B. subtilis*.
b. *B. subtilis* acted more slowly; however, at longer times (from 3 to 9 weeks) it caused more apparent changes in pits of spruce sapwood and more expressive increase of its permeability and impregnability.

c. Microscopic analyses showed different degradation of spruce sapwood pits in tracheids by the bacterium *B. subtilis* and the fungus *T. viride; B. subtilis* degraded toruses of pits in direct contact with them, while *T. viride* destroyed mainly margo of the pits (Photos 4 and 7), which caused the falling out of the toruses into lumens. This could be one of the reasons accounting for a faster effect of the *T. viride* on spruce sapwood relative to increasing the permeability. Destroying of pits by *T. viride* was probably caused by the diffusion of its enzymes from wood surfaces inside the timber because no hyphal growth could be observed in the tracheids with destroyed pits. Similar results were reported by Rosner *et al.* (1998).
d. Both bio-treatments (with *B. subtilis* and *T. viride*) proved that the attack of these organisms during 1 to 9 weeks of exposure did not impact the bending strength and the modulus of rupture of spruce sapwood or heartwood.
e. The greatest problem of bio-treatments of spruce (or of other refractory conifers) with bacteria and microscopic fungi is a minimum increase of the impregnability of their heartwood zones.

The results obtained with the action of *T. viride* on spruce logs corresponded well with works of Lindgren (1952), Freitas and Erikson (1969), Rosner *et al.* (1998), and Messner *et al.* (2003), who have also observed an increase of impregnability of refractory sapwoods but not of refractory heartwoods after treatment with microscopic fungi.

The problem of heartwood treatment is connected with the closed state and different chemical structure of pits in tracheids. Older pits fulfill mainly a mechanical function in living trees, and toruses are closed and lignificated (Côte 1963; Liese and Greaves 1975). Therefore the used technology with *T. viride* is suitable only for the pre-treatment of poles. In squared timber the surface sapwood layer 3 or 5 cm thick is partially or fully removed during saw-milling operations, thus rendering such timber unsuitable for the described treatment.

Today, it is possible to increase the permeability of spruce and other coniferous heartwood through the use of the specific-cultivated species of white-rot fungi with highly controlled processes of bio-treatment (Schwarze *et al.* 2006; Schwarze 2008; Lehringer *et al.* 2009, 2010). However, this research is only in the early stages of investigation. Another possibility involves the incising of wooden cell walls with traditional white-rot (*Trametes versicolor, Irpex lacteus, etc.*) or staining (*Ophiostoma piceae, etc.*) fungi (Yang 2009), followed by strengthening of the damaged wood with suitable modification chemicals (Wan *et al.* 2006).

CONCLUSIONS

On the basis of structural, physical, and mechanical property analyses of spruce wood, which was in the form of short debarked logs, intentionally attacked by the microscopic fungus *Trichoderma viride* for 1 to 9 weeks, it can be concluded that:

1. A significant increase of permeability and impregnability characteristic of the spruce sapwood (*K*, *S*, *R*, and I_S) was obtained already after 1 week of regulated bio-treatment applied to the exterior.

2. Bio-treatments carried out for 3 weeks are better for technologies in practice. Such a time period provides a technological reserve for more homogenous pre-treatment in order to compensate for the variability of conditions (lower temperatures, lower activity of fungus, *etc*.) during not so strictly controlled processes.
3. The mechanical properties (*MOR* and *IB*) of the bio-treated spruce sapwood or also heartwood were not affected by *T. viride* activity. These results corresponded with microscopic analyses, which have shown only the degradation of pits of spruce sapwood tracheids, while the cell walls were not destroyed. Cell walls were not destroyed because they are a natural composite that contains hydrophobic lignin. *T. viride* does not have an enzymatic apparatus for destruction of such complicated composite structures.
4. The spruce heartwood zone remained unchanged, and the pits of tracheids in this zone were not enzymatic degraded, even after 9 weeks of bio-treatment by *T. viride*. This finding is attributed to the above-mentioned conclusion, because toruses in older heartwood tracheids are closed and significantly lignified.
5. On the basis of these results, it is evident that the action of the fungus *T. viride* can be useful only for pretreatment of green poles and similar rounded products from spruce wood with a possibility of achieving quicker and more homogenous preservation of their sapwood zones.

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