WOOD RESEARCH
53 (3): 2008
1-8

BIO-TREATMENT OF SPRUCE WOOD FOR IMPROVING OF ITS PERMEABILITY AND SOAKING PART 2: DIRECT TREATMENT WITH THE FUNGUS *TRICHODERMA VIRIDE*

Ladislav Reinprecht, Miloš Pánek
Faculty of Wood Sciences and Technology, Technical University in Zvolen, Slovak Republic

ABSTRACT

Poor impregnability of spruce wood can be improved by selected microscopic fungi which destroy the toruses in pits. In this experiment, from one healthy green spruce log were immediately prepared sap- and heartwood specimens which were treated in Kolle´s flasks with the microscopic fungus *Trichoderma viride* at a temperature of 20 °C, during 1, 3, 6 and 9 weeks. After bio-treatment an increase of the axial permeability and kinetics of water soaking of spruce sap zones was observed, however, the impregnability of its heart-zones remained unchanged.

KEY WORDS: spruce, *Trichoderma viride*, torus opening, permeability, soaking

INTRODUCTION

The depth of impregnation of dry spruce wood is only small because the system of pits in its tracheids is closed. One way of how to keep the pits permanently in an open state is to bio-degrade the toruses using selected species of fungi or bacteria. Suitable are species which produce only hydrolase type of enzymes (such as pectinases), and do not destroy the structure of cell walls (Liese et al. 1995). In the part 1 of this work we opened the system of pits in spruce wood with the bacterium *Bacillus subtilis*, or by means of an undefined microorganism in non-sterile water (Pánek and Reinprecht 2007). Biological pretreatments of wood were examined in many works which proved the suitability of these processes for improving the impregnability of less permeable wood species.

One of the most suitable microorganism for coniferous bio-treatment is a microscopic fungus *Trichoderma viride*. It has also antagonist effect against growth of wood destroying fungi (Score et al. 1998, Tichý 1975). Lindgren (1952) researched colonisation of pine wood by microscopic fungi *Trichoderma* sp. during few months. He observed better impregnability of sapwood zone but changes in heartwood zone were only minimal. Freitas and Erickson

(1969) analysed influence of some species of microscopic fungi for increasing the permeability of alder wood for creosote oil. They used these four species of fungi isolated from the surface of wooden boles: *Trichoderma* sp., *Gliocladium roseum, Fusarium* sp. and *Chaetomium conchlioides.* Microscopic fungi were sprayed to circumference zone of boles at which after four weeks of bio-treatment the hyphae were found out in the depth of 20 mm. Impregnability of boles increased at cold soaking and mainly at vacuum impregnation. The best results were achieved with using of the microscopic fungi *Trichoderma* sp. and *Gliocladium roseum*. The influence of bio-treatment of spruce wood by various fungi species was researched by Rosner et al. (1998). The work was focused on the improving of spruce impregnability caused by the following fungi species: *Phanerochaete chrysosporium, Dichomitus squalens, Trichoderma aureoviride* and *Trichoderma viride.* The green logs were pretreated during the period of 1 – 4 weeks and subsequently impregnated with the creosote oil. Colonisation of samples by fungi was observed by light microscope and electron microscope, at which the quantification was done by ergosterol method. After about one week the entire sapwood zone was colonised. *Dichomitus squalens* colonised also the heartwood. The depth of penetration of creosote oil corresponded to the depth of colonisation of samples by fungi. The observation by electron microscope has shown that the increase of permeability was caused more by enzymatic opening of pits then by physical penetration of hyphae through the membranes. Fojutowski (2005) observed the influence of fungi causing blue-stain on absorptiveness of Scots pine wood. The wet sapwood samples of freshly cut pine were infected with pure culture of fungi: *Ceratocystis penicillata, Cladosporium herbarum, Discula pinicola,* or with their mixture. Action of the fungi lasted 1, 3 or 12 months and the ability of the blue-stained wood to impregnation increased particularly after 3 and 12 months. More researches demonstrated that selected fungi are able to increase the permeability of spruce wood mainly in its sapwood zone and to cause only minimum decrease of its mechanical properties.

MATERIAL AND METHODS

In the previous experiment "combined ponding – bio-treatment with fungus" the green spruce logs were primarily ponded in non-sterile water during 16 weeks, and then the individual sapwood and heartwood specimens prepared from the ponded logs were subsequently exposed to the fungus *Trichoderma viride* (Pánek et al. 2005).

In this experiment the green spruce specimens were directly exposed to the microscopic fungus *Trichoderma viride* (Pers.) 1402. Specimens (a/ 30x30x15 mm for the coefficient of axial permeability testing (n = 90), b/ 20x20x30 mm for the kinetics of water soaking testing (n = 50)) were prepared from one healthy spruce log (*Picea abies* Karst. L.) and then immediately exposed to the bio-treatment process (Fig. 1). Medium density of the sapwood specimens was 421.2 kg/m^3, and that of the heartwood ones was 359.8 kg/m^3.

Attack of the sapwood and heartwood specimens by the fungus *T. viride* was accomplished in ½ liter Kolle's flasks. All surfaces of these specimens were first sterilized with UV radiation and then contacted with the fungus mycelium growth already 4 weeks on the Czapek-Dox agar medium in flasks. The contact of specimens with *T. viride* mycelia lasted 1, 3, 6 or 9 weeks at a temperature of 20 ± 2 °C. After the bio-attack all specimens were sterilized for 5 hours at 90 °C.

Specimens for the determination of the coefficient of permeability were then conditioned to a moisture content of 12 %, and those for the determination of the kinetics of water soaking were dried to a moisture content of 0 %. Microscopic analyses were carried out at selected specimens with the aim to study changes in the structure of pit membranes and in cell walls.

The coefficient of permeability in axial direction was determined on the basis of the Darcy low:

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

where: V – transported volume of distilled water (m^3), η – dynamic viscosity of distilled water at 20 °C (Pa.s), L – specimen length in axial direction = 0.015 m, A – axial surface allocated for the flow transport (m^2), τ - time of flow (s), Δp – pressure difference (Pa).

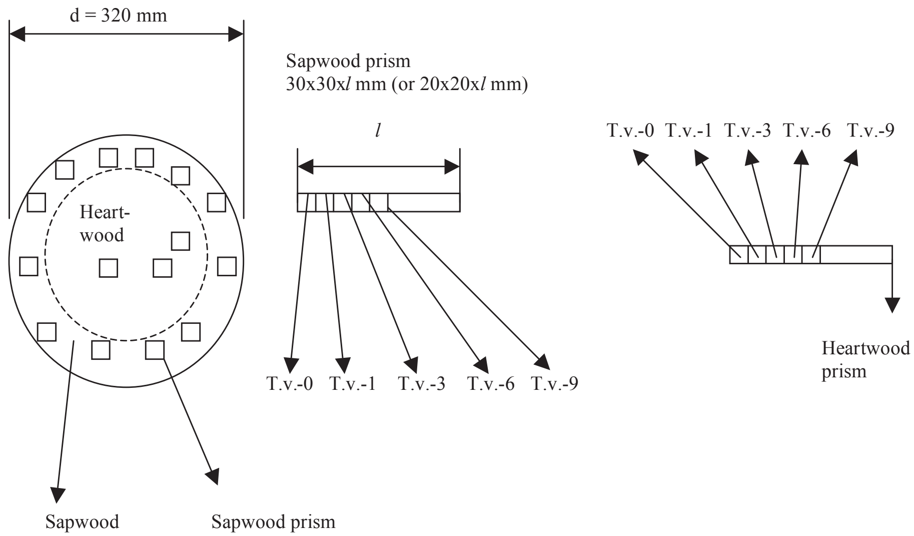

Fig. 1: Scheme of samples preparation from the green spruce log
(Note: T.v.-1 = Sample exposed to the fungus T. viride during 1 week)

The kinetics of soaking in distilled water was determined in the interval from 15 minutes to 21 days in these times: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, and 21 days.

RESULTS AND DISCUSSION

Results of this work are presented in the Tab. 1, Fig. 2-5.

Bio-treatment of spruce sapwood with the microscopic fungus *Trichoderma viride* had a positive effect on its impregnability. The coefficients of permeability of dry (w~12%)sapwood specimens increased after 1 week of exposure to *T. viride* by 1.8 times, after 3 weeks by 5.5 times, after 6 weeks by 7.1 times, and after 9 weeks by 8.7 times (Tab. 1). The permeability of 6 or 9-week bio-treated and then dried sap zones ($K = 3.20.10^{-12}$ or $3.92.10^{-12}$ m^2) was even comparable with the permeability of green sap zones of spruce having originally opened pits ($K = 3.85.10^{-12}$ m^2). Permanent increase of the permeability of sapwood specimens treated with *T. viride* was caused by destroying the pits between tracheids (Fig. 5B and 5C).

Tab. 1: The coefficients of the axial permeability (K) of spruce sapwood in the natural state (green wet state w >> 30 %), in untreated state – control (dried at w = 12 %), and after bio-treatment with the fungus Trichoderma viride during 1, 3, 6 or 9 weeks (dried at w = 12 %)

COEFFICIENT OF PERMEABILITY OF SPRUCE SAPWOOD								
Kind of bio-treatment	Time of bio-treatment with the fungus *Trichoderma viride*							
	1 week		3 weeks		6 weeks		9 weeks	
	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)
T. viride	0.82	28.4	2.49	57.2	3.20	51.7	3.92	42.7
Control - untreated	$K = 0.45.10^{-12}$ m^2 (v = 34.2 %)							
Green wet state	$K = 3.85.10^{-12}$ m^2 (v = 21.7 %)							

Note: v – coefficient of variability, n – number of specimens at each group = 15

When measuring the kinetics of water soaking of spruce sapwood, there an increase of water uptake by about 82.3 % (1 week of bio-treatment with *T. viride*) to by about 145.8 % (6 weeks of bio-treatment with *T. viride*) was observed during the first 15 minutes. Further prolongation of the bio-treatment process to 9 weeks did not have any greater effect on the speed of soaking (increase by about 144.7 % in comparison with soaking of untreated spruce sapwood) (Fig. 2 and 3).

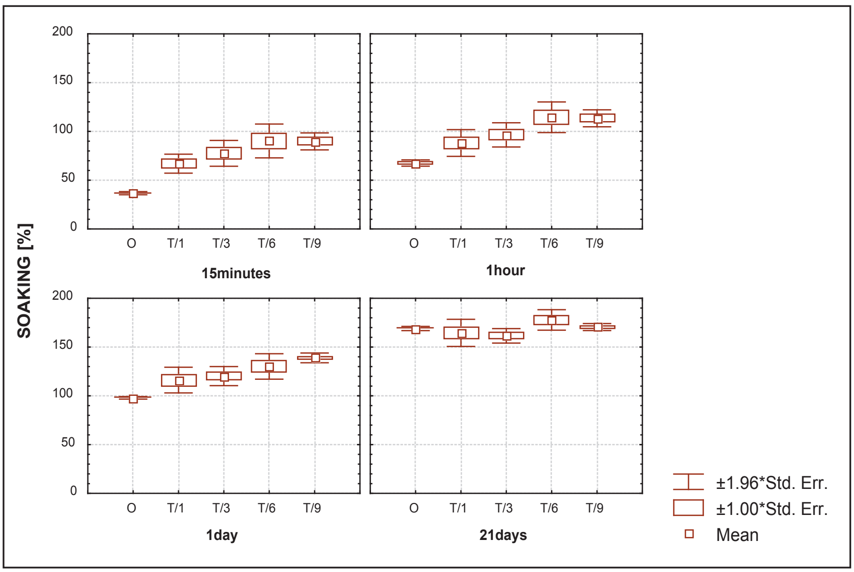

Fig. 2: Two side confidence intervals of water soaking of untreated spruce sapwood (O) and spruce sapwood bio-treated for 1, 3, 6 and 9 weeks with the microscopic fungus Trichoderma viride (T/1, T/3, T/6 and T/9) – soaking in distilled water measured after 15 minutes, 1 hour, 1 day and 21 days
Note: n – number of specimens at each group = 5

Differences in the water uptake of both the bio-treaded and untreated specimens were successively smaller during a longer time of soaking. After 1 hour of soaking the water uptake of bio-treated sap zones was higher from 30.3 % to 69.4 %, after 1 day of soaking from 18.6 % to 41.9 %, at which after 21 days of soaking there were not observed any relevant changes (Fig. 2 and 3).

For the bio-treated spruce heartwood there was not determined a more significant increase of water uptake in the initial periods of soaking (Fig. 4). This result can be explained by different anatomical and chemical structure of pit membranes in sapwood and heartwood (Côte 1963), e.g. *T. viride* does not create suitable enzymes for heartwood pits decomposition (Fig. 5D). Lesser changes of water uptake after longer periods of soaking were caused only by the density variation between sets of specimens (Fig. 4).

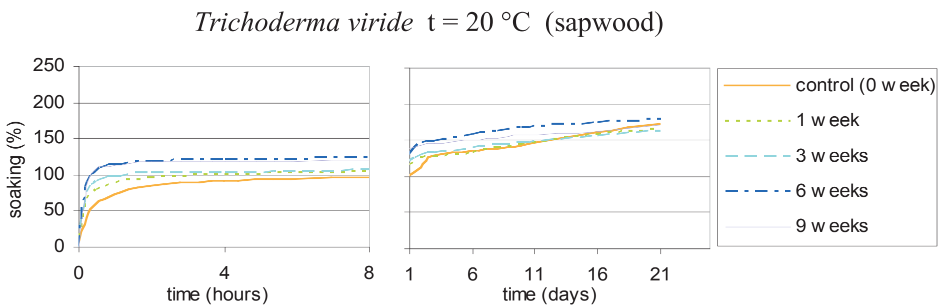

Fig. 3: The kinetics of water soaking of spruce sapwood specimens bio-treated with Trichoderma viride (1, 3, 6, or 9 weeks) in comparison with control sapwood specimens
Note: Soaking was measured in these times: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, 21 days.
n – number of specimens at each group (for each curve line) = 5

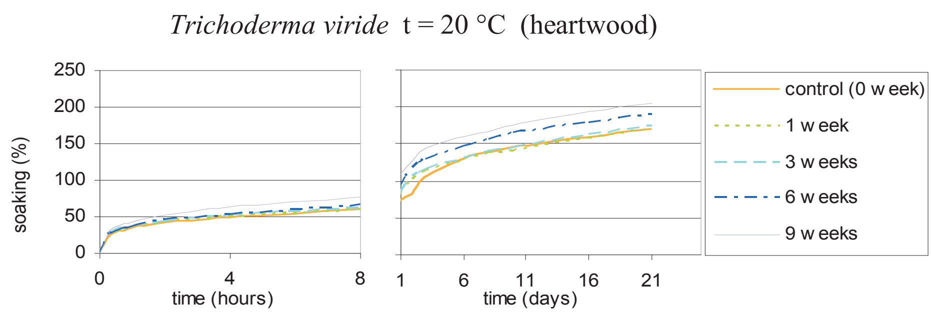

Fig. 4: The kinetics of water soaking of spruce heartwood specimens bio-treated with Trichoderma viride (1, 3, 6, or 9 weeks) in comparison with control heartwood specimens
Note: Soaking was measured in these times: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, 21 days.
n – number of specimens at each group (for each curve line) = 5

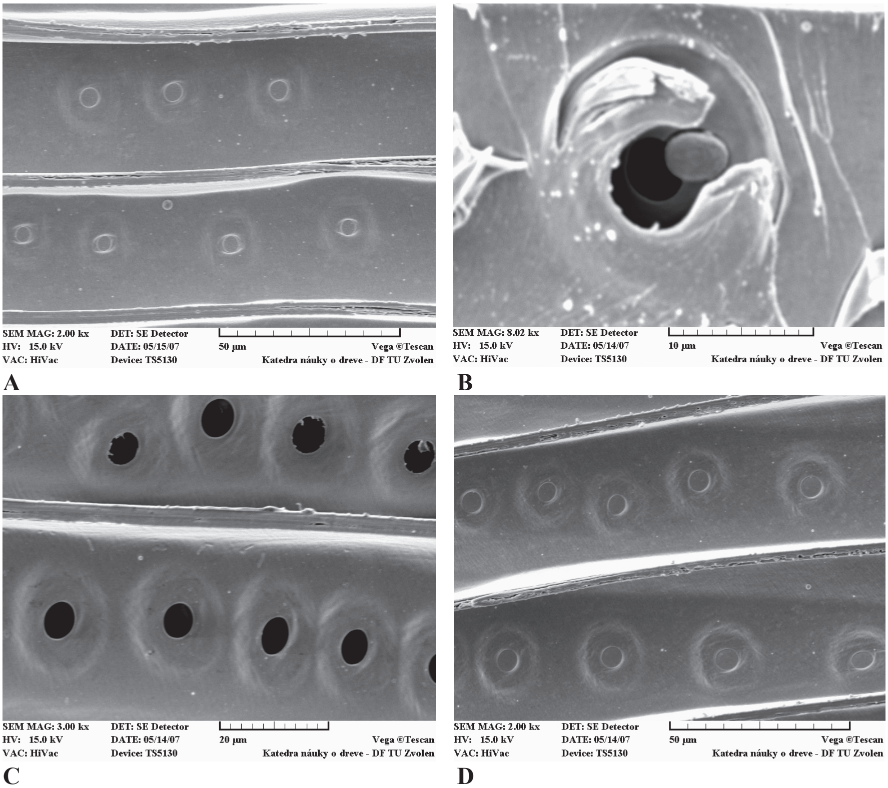

Fig. 5: Changes in the structure of spruce wood after bio-treatment with the fungus Trichoderma viride
A – *closed pits in the tracheids of untreated spruce sapwood – control specimen,*
B - *detail on the liberated torus in spring tracheid of spruce sapwood after 1 week of bio-treatment with T. viride,*
C – *total degradation of toruses of the pits of spruce sapwood tracheids after 3 weeks of bio-treatment with T. viride,*
D – *closed pits in the tracheids of spruce heartwood after 6 weeks of bio-treatment with T. viride.*

CONCLUSIONS

Bio-treatment of spruce wood with the microscopic fungus *Trichoderma viride* has resulted into the following conclusions:

- the bio-attack of pit membranes was observed only in sap zones,
- the permeability of sap zones improved 2-9 times (in dry state at w = 12 %) with the bio-attack prolongation, at which after 6-9 weeks of *T. viride* action it was already similar to the permeability of green sap zones,
- the kinetics of water soaking of bio-treated sap zones evidently increased mainly in the first periods of dipping,

- the pit membranes in spruce heartwood zones were closed also after all bio-attack processes, and as a result the kinetics of heartwood soaking was not improved,
- the bio-treatment of spruce wood with *T. viride*, which coincides well with the opinions of other researchers, could be convenient for improving the impregnability of sap zones of poles and other structural products for their 3rd and 4th hazard classes of exposure (EN 335-1).

ACKNOWLEDGEMENT

The authors wish to express their thanks to the Grant agencies of the Slovak Republic (Grant No. 1/4377/07) for the financial support of this work.

REFERENCES

1. Côte, W. A., 1963: Structural factors affecting the permeability of wood. Journal Polymer Sci., C 2, (5): 231-242
2. EN 335-1: Durability of wood and wood-based products. Definition of hazard classes of biological attack. Part 1: General
3. Fojutowski, A., 2005: The influence of fungi causing blue-stain on absorptiveness of Scots pine wood. IRG/WP/05-10565, 5 pp.
4. Freitas, A.R., Erickson, H.D., 1969: Propagation of molds on Red Alder roundwood – their effect on oil soak treatments. For. Prod. J., 19, (10): 45-52
5. Liese, W., Schmidt, O., Schmidt, U., 1995: On the behavior of hardwood pits towards bacteria during water storage. Holzforschung, 49: 389-393
6. Lindgren, R.M., 1952: Permeability of Southern pine as affected by mold and other fungus infection. Proc. Am., Wood preservation Assoc., 48, Pp. 158-174
7. Pánek, M., Reinprecht, L., Babiak, M., 2005: Improving of spruce wood impregnability with *Bacillus subtilis* and *Trichoderma viride*. In: Workshop COST Action E37, Oslo, Norway, http://bfafh.de/cost37.htm, http://bfafh.de/inst4/45/pdf/4impreg.pdf, 11 pp.
8. Pánek, M., Reinprecht, L., 2008: Bio-treatment of spruce wood for improving of its permeability and soaking. Part 1: Direct treatment with the bacterium *Bacillus subtilis*. Wood Research, 53(2): 1-12
9. Rosner, B., Messner, K., Tucker, E., Bruce, A.. 1998: Improved preservative penetration of spruce after pre-treatment with selected fungi. I. Fungal pretreatment of pole sections. IRG/WP/98-40117, 14 pp.
10. Score, A. J., Bruce, A., King, B., Palfreyman, J. W., 1998: The biological control of *Serpula lacrymans* by *Trichoderma* species. Holzforschung, 52, (2): 124-132
11. Tichý, V., 1975: Importance of synergism and antagonistic effects during the degradation of wood by fungi. Drevársky Výskum - Wood Research, 20, (1): 37-57

Ladislav Reinprecht
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: reinpret@vsld.tuzvo.sk

Miloš Pánek
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: panek@vsld.tuzvo.sk