Miha Humar, Viljem Vek, Bojan Bučar[1]

Properties of blue-stained wood

Svojstva drva zaraženoga gljivama plavila

Preliminary paper · Prethodno priopćenje
Received – prispjelo: 17. 1. 2008.
Accepted – prihvaćeno: 5. 6. 2008.
*UDK: 630*844.1*

ABSTRACT • *Discoloration of wood is frequently caused by blue-stain fungi. Among them Aureobasidium pullulans and Sclerophoma pithyophila are reported as the most important staining organism. In previous researches, it was generally considered that blue-stain fungi do not influence mechanical properties. However, there were some opposite results published as well. In order to elucidate this issue, specimens made of Scots pine (Pinus sylvestris) sapwood were exposed to two blue stain fungi A. pullulans and S. pithyophila for periods between two and eight weeks. FTIR, weight, colour and non-destructive modulus of elasticity measurements were performed before and after exposure. The results showed that blue stain fungi, besides considerable discoloration, do not cause any significant damage to wood. Surprisingly the non-destructive MoE analysis showed that modulus of elasticity even slightly increase after fungal exposure.*

Keywords: *Aureobasidium pullulans, colour, FTIR, modulus of elasticity, Pinus sylvestris, sap-stain, Sclerophoma pithyophila*

SAŽETAK • *Promjeni boje drva često uzrokuju gljive plavila. Među tim gljivama najpoznatije su Aureobasidium pullulans i Sclerophome pithyophila. U dosadašnjim radovima prevladavalo je mišljenje da gljive plavila ne mijenjaju mehanička svojstva zaraženog drva, iako su se pojavili i neki kontradiktorni rezultati. Radi razjašnjenja tih suprotnosti, 70 uzoraka bjeljike bijeloga bora (Pinus sylvestris) (veličine 0,5 × 1,0 × 20,0 cm^3) bilo je izloženo djelovanju gljiva Aureobasidium pullulans i Sclerophoma pithyophila u trajanju od dva do osam tjedana, a prema europskoj normi EN 152-1 (1990). Prije i nakon izlaganja gljivama, na istim je uzorcima obavljeno mjerenje mase, boje, FTIR te nedestruktivno mjerenje modula elastičnosti (MoE). Rezultati su pokazali da, osim značajne promjene boje, gljive plavila ne uzrokuju znatnije razaranje drvne tvari. Nedestruktivna metoda mjerenja modula elastičnosti pokazala je slabo povećanje MoE uzoraka nakon izlaganja gljivama.*

Ključne riječi: *Aureobasidium pullulans, boja, FTIR, modul elastičnosti, bijeli bor (Pinus sylvestris), gljive plavila, Sclerophoma pithyophila*

1 INTRODUCTION
1. UVOD

Discoloration of wood is long known phenomenon, which is based on different biotic and abiotic causes. The most important reasons for discoloration are bacteria and fungi, as a result of micro-organism-own pigments (e.g., melanin of blue stain fungi) (Zink and Fengel, 1989). Blue stain is a blue, grey or black striped wood discoloration on sapwood. Fungi causing blue-stain are called blue-stain or sap-stain fungi. Conifers and hardwood, round wood, lumber, finished wood and wood products can be colonized by these organisms (Schmid, 2006). They live on nutrients in the parenchyma cells of sapwood. However, in certain cases, mannanase, pectinase and amylase have been detected

[1] Authors are assistant professor, assistant and associate professor at Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana, Slovenia.
[1] Autori su docent, asistent i izvanredni professor na Odsjeku za znanost o drvu i drvnu tehnologiju Biotehničkog fakulteta, Sveučilište u Ljubljani, Slovenija.

(Schirp *et al.,* 2003). Blue staining of wood is caused by about 100 to 250 fungi belonging to *ascomycetes* and *deuteromycetes* (Kaarik, 1980). *Aureobasidium pullulans* is the main organism causing disfigurement of wood coatings and surface of exposed timber (Sharpe and Dickinson, 1992). This disfigurement of timber in-service is referred to as "bluestain in-service". *A. pullulans* is also associated with the sap-staining of dead wood in the forest and in-service (Ray *et al.,* 2004). Another important blue stain fungus discolouring wood in service is *Sclerophoma pithyophila.* Both species are used in combination in the European standard laboratory test method for determining the effectiveness of preservatives against blue stain fungi (European Standard EN 152-1 & 2, 1990).

Although, blue stain fungi belongs to the same group of fungi as soft rot fungi (Troya *et al.,* 1990), it is generally assumed that blue stain fungi do not cause any or only little cell wall attack, and therefore strength properties are hardly affected (Schmid 2006). Thus the damage of wood is mainly cosmetic. It is supposed, that fungal hyphae are growing on the internal face of the cell walls without any enzymatical alteration on the surface. On the other hand, some hyphen of blue stain fungi have been seen in inside the parenchyma cell walls (Liese, 1964), or even between cell walls in middle lamella region (Rose *et al.,* 1999), which can affect mechanical properties. Furthermore, it is known for some time that blue stain fungi produce intra and extra-cellular enzymes, some of which can degrade polysaccharides and pectins. The, presence of lignin splitting enzymes was also reported (Troya *et al.,* 1990; Sharpe and Dickinson, 1992). The aim of our research was to evaluate whether blue stain fungi could anyhow influence mechanical properties of blue-stained wood. Such researches are nowadays much easier, as non-destructive techniques for determination of mechanical properties are widely available. These methods enable us to compare the modulus of elasticity before and after fungal exposure at the same specimen, avoiding wood heterogeneity.

2 MATERIAL AND METHODS
2. MATERIJAL I METODE

2.1 Sample preparation
2.1. Priprema uzoraka

Samples ($0.5 \times 1.0 \times 20.0$ cm^3) were made of Scots pine sapwood (*Pinus sylvestris*). Orientation and quality of wood meet the requirements of the standard EN 113 (1996). Afterwards, the samples were exposed to blue stain fungi for the period ranging between 2 and 8 weeks, according to the standard EN 152-1 (1990). *Aureobasidium pullulans* (de Barry) Arnaud (ZIM L060) and *Sclerophoma pithyophila* (Corda) Hohn (ZIM L070) (Raspor *et al.,* 1995) were used in this experiment. In total, 70 specimens were exposed to blue stain fungi.

2.2 Evaluation of modulus of elasticity
2.2. Određivanje modula elastičnosti

Modulus of elasticity (MoE) was determined before and after fungal exposure. Specimens were oven dried prior to MoE measurements. Because of difficulties encountered in measuring the axial vibrations, flexural vibration modes were used to characterize elastic parameters. Considering the hypothesis of the homogeneity of geometrical and mechanical properties along the sample, basic dynamics theorems can be applied to obtain the motion equation of first vibrations. Analysis was performed on specimen with clamped–free end conditions. During the test, the lateral displacement was measured of vibrating sample in damped vibration with known vibration mode. As an inductive proximity sensor was used, a small piece of metal foil of neglecting mass was glued on the surface of each sample. The damped frequency was obtained by FFT analysis of the exponentially decayed displacement signals detected in time domain. For determination of Young modulus of samples, we used the frequency equation deducted from Bernoulli model, which was assumed as acceptable because of the relatively high length-to-depth sample ratio, (E - Young modulus, N/m^2, ν - natural frequency, s^{-1}, $C = 3.51563$ – constant derived from Bernoulli equation, ρ – density, kg m^{-3}, l – free sample length, m, h – sample height, m) (Timoshenko *et al.,* 1974). Measurements were performed on seven replicates.

$$E = \frac{48 \cdot \pi^2 \cdot l^2 \cdot \rho \cdot v^2}{C^2 \cdot h^2} \qquad (1)$$

2.3 Chemical analysis (CNS) of wood
2.3. Kemijska (CNS) analiza drva

Prior to nitrogen and carbon analysis, wood blocks that were used for MoE measurements, were milled into particles (MESH 80) and homogenized. Approximately, 0.2 g of an oven dry sample was combusted in the oxygen atmosphere at 1350°C in LECO 2000-CNS analyzer to determine carbon and nitrogen content.

2.4 FTIR and colour measurements
2.4. FTIR i mjerenje boje drva

FTIR spectra were recorded with the Perkin Elmer FTIR Spectrum One Spectrometer, using Abrasive Pad 600 Grit-Coated, PK/100 (Perkin Elmer) paper. DRIFT spectra of wood samples were recorded between 4000 cm^{-1} and 450 cm^{-1}. Colour of the specimens was recorded with HP Scanjet 4800 scanner. Scanner was chosen, as specimens were to narrow for measurements with colorimeter. Colour obtained with scanner and colorimeter gives comparable results (Noč, 2006). The reported values are the average value of seven replicate measurements. The colour was expressed in Cie L*a*b* format.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

As expected, the exposure of Scots pine wood specimens to blue stain fungi resulted in considerable

Table 1 Colour changes of Scots pine sapwood exposed to blue stain fungi
Tablica 1. Promjena boje bjeljike bijelog bora nakon izlaganja gljivama plavila

Incubation time (weeks) *Vrijeme izlaganja gljivama (tjedni)*	*Sclerophoma pithyophila*				*Aureobasidium pullulans*			
	*L**	*a**	*b**	Δ*E*	*L**	*a**	*b**	Δ*E*
0	85.1	3.8	13.7	0.0	85.1	3.8	13.7	0.0
2	66.7	3.1	10.7	18.7	82.6	3.8	14.6	2.6
4	63.8	2.6	9.0	21.8	79.7	2.8	12.8	5.6
6	57.5	2.5	7.5	28.3	71.9	3.1	11.3	13.4
8	58.4	2.5	7.7	27.4	70.1	3.0	10.1	15.4

Table 2 Modulus of Elasticity, mass changes, carbon and nitrogen content of Scots pine sapwood after exposure to blue stain fungi
Tablica 2. Modul elastičnosti, promjene mase, sadržaj ugljika i dušika bjeljike bijelog bora nakon izlaganja gljivama plavila

Incubation time (weeks) *Vrijeme izlaganja gljivama (tjedni)*	*Sclerophoma pithyophila*				*Aureobasidium pullulans*			
	Δ *m* %	Δ *MoE* %	*C* %	*N* %	Δ *m* %	Δ *MoE* %	*C* %	*N* %
0	0.8	1.2	47.63	0.0352	0.8	1.2	47.63	0.0352
2	0.6	2.8	46.33	0.0205	0.2	1.7	47.00	0.0160
4	0.3	3.6	46.55	0.0181	0.0	1.5	47.02	0.0163
6	0.2	2.5	46.48	0.0175	0.1	2.9	45.60	0.0164
8	0.8	2.7	46.27	0.0181	0.8	1.7	45.97	0.0162

colour changes. The first signs were visible after the second week of exposure. However, colour changes of specimens exposed to *S. pithyophila* (Δ*E* = 18.7) for two weeks, were more prominent than the ones exposed to *A. pullulans* (Δ*E* = 2.6). *S. pithyophila* remained more active all the time of exposure. Within the exposure time, the specimens became darker, less reddish and yellowish and more bluish and greenish (Table 1). Maximum colour change was observed after the sixth week at *S. pithyophila* and after eight week at *A. pullulans.* The most important reason for observed changes is melanin excreted by those two staining fungi (Schmid, 2006).

Mass changes of wood specimens exposed to blue stain fungi were insignificant. No mass losses were observed in any of the cases. Quite the contrary, all specimens gain some weight; firstly because specimens were immersed to malt agar suspension prior to fungal exposure, as proposed by EN 152-1 standard (1990), and secondly, as fungi contributes to the mass of the specimens with their biomass and excreted compounds like melamine. However, changes of mass are relatively small and are always smaller than 1 % (Table 2). These measurements are in line with the data presented by Higley (1999).

Nitrogen content in uninfected pine sapwood was 0.0352%. Immediately after exposure, nitrogen content dropped significantly, to 0.0205% at *S. pithyophila* and to 0.0160% at *A. pullulans*, and remained almost constant within time of exposure (Table 2). This indicates that blue stain fungi rapidly consumed nitrogen within the first two weeks of exposure. The remained nitrogen is either in biologically unavailable form, or it was used for chitin and protein synthesis and is present in wood as a constituent of fungal hyphae. Similar as nitrogen content, carbon content also decreased from initial 47.63%, to final 46.27 at *S. pithyophila* and to 45.97% at *A. pullulans.*

Unexpectedly, modulus of elasticity (MoE) of blue stained specimens did not decrease but even slightly increased (Table 2). The first increase of MoE was observed immediately after sterilization, even with specimens that were not exposed to wood decay fungi at all. Similar phenomenon is reported for heat treated wood, where MoE firstly increases within temperature of treatment and when temperatures overreach 150°C, MoE starts decreasing (Finnish Thermowood Association, 2003). This increase of MoE is assigned to the fact that after water evaporation, cellulose hydroxyl groups form hydrogen bonds between neighbouring micro-fibrils, which results in improved MoE of steam-sterilised wood. Another possible explanation for increased MoE values of blue-stained wood is the presumption that melanin could interact with wood functional groups, and additionally crosslink wood components. However, this is only a presumption, which is very difficult to be proved.

Infra red spectra confirm the above results. There were no differences in IR peaks between control unexposed pine wood and blue-stained wood even after eight weeks of exposure (Figure 1). This confirms that blue stain fungi did not change lignin, cellulose and hemicelluloses structure. Additionally, it seems that the amount of melanin in wood is too low to be observed from FTIR spectra. Secondly, as functional groups of

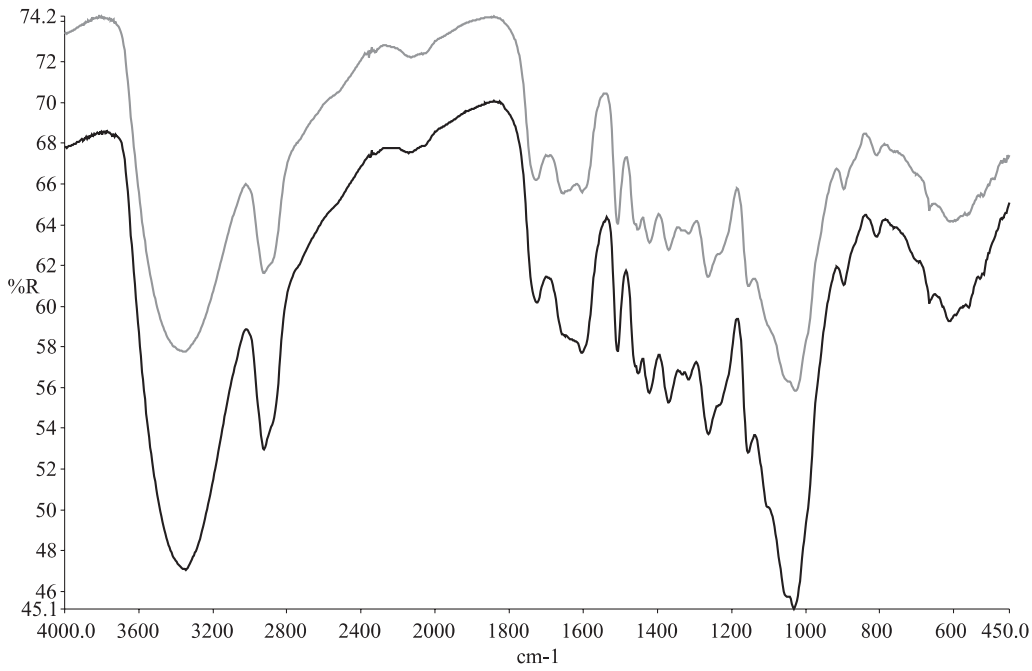

Figure 1 FTIR spectra of *Pinus* sylvestris sapwood before (lower line) and after eight-week exposure to *Sclerophoma pithyophila* (upper line)
Slika 1. FTIR spektri bjeljike bijelog bora prije (donja linija) i nakon osam tjedana izlaganja gljivi *Sclerophoma pithyophila* (gornja linija)

melanin are relatively similar to lignin functional groups, melanin cannot be resolved from FTIR spectra (Butler and Day, 1998).

This data are important from application point of view. Blue stained wood can, therefore, be used for various construction applications. And secondly if albino blue stain fungi are utilised for biocontrol applications (Farrell *et al.,* 1993), it could be presumed that those fungi do not significantly influence mechanical properties of colonised wood.

4 CONCLUSIONS
4. ZAKLJUČCI

Aureobasidium pullulans and *Sclerophoma pithyophila* affect significantly blue-stained pine wood specimens. However, the results of the experiments showed that this change is only aesthetic and does not influence weight or mechanical properties of blue-stained wood.

Acknowledgments
Zahvala
Authors would like to acknowledge Slovenian Research Agency for financial support in the framework of the programs L4-0820-0481 and L4-7163-0481.

5 REFERENCES
5. LITERATURA

1. Butler, M.J.; Day, A.W. 1998: Fungal melanins: a review. Journal of Microbiology 44: 1115-1136.
2. Cristina, R.M.; Reyes, L.; Alvarez, J.; Andaur, A.; Beltrán, C.; Rivera, D. 1999: Decrease in the mechanical resistance of blue stained and "orange stained" *Pinus radiata* wood. The international research group on wood protection, Stockholm, IRG/WP 99-10313, 6.
3. European Committee for standardization. 1989: Wood preservatives; Determination of the toxic values against wood destroying basidiomycetes cultured an agar medium. EN 113. Brussels, 14.
4. European Committee for standardization. 1996: Test methods for determining the protective effectiveness of a preservative treatment against blue stain in service – Part 1: Brushing procedure. EN 152-1. Brussels, 16.
5. Farrell, R.L., Blanchette R.A., Brush T.S., Hadar Y., Iverson S., Krisa K., Wendler P.A., Zimmerman W. 1993: Cartapip™: a biopulping product for control of pitch and resin acid problems in pulp mills. Journal of Biotechnology 30: 115-122.
6. Finnish Thermowood Association. 2003: Thermowood Handbook. Helsinki: Finnish Thermowood Association.
7. Higley, T.L. 1999: Biodeterioration of Wood. In: Wood Handbook., Madison: Forest Products Laboratory, 17.
8. Kaarik, A. 1980: Fungi causing sap stain in wood. Swedish University of Agriculture Science. Department of Forest Products. 114.
9. Liese, W. 1964: Über den Abbau verholzter Zellwände durch Moderfäulepilze. Holz als Roh- und Werkstoff 22: 289–295.
10. Noč, L. 2006: Comparison of colour measurements with a colorimeter and an optical reader. Graduation thesis, University of Ljubljana, Biotechnical faculty, Ljubljana, 56.
11. Raspor, P.; Smole-Možina, S.; Podjavoršek, J.; Pohleven, F.; Gogala, N.; Nekrep, F.V.; Rogelj, I.; Hacin, J. 1995: ZIM: Collection of industrial microorganisms. Ljubljana: Biotechnical faculty.
12. Ray, M.J.; Dickinson, D. J.; Buck, M. 2004: *Aureobasidium* or *Hormonema*? A Genetic Approach. The international research group on wood protection, Stockholm, IRG/WP 04-10529, 13.

13. Schirp, A.; Farrell, R.L.; Kreber, B.; Singh, A.P. 2003: Advances in understanding the ability of sapstaining fungi to produce cell wall-degrading enzymes. Wood and Fiber Science 35: 434–444.
14. Schmidt, O. 2006: Wood and Tree Fungi. Berlin: Springer.
15. Sharpe, P.R.; Dickinson, D.J. 1992: Blue stain in service on wood surface coatings Part 1. The nutritional requirements of *Aureobasidium pullulans*. The international research group on wood protection, Stockholm, IRG/WP 92-1556, 14.
16. Timoshenko, S.; Young, D.H.; Weaver, W. 1974: Vibration problems in engineering. Fourth edition. John Wiley & Sons, New York.
17. Troya De, M.T.; Navarrete, A.M.; Relano, E. 1990: Analysis of the degradation of carbohydrates by blue-stain fungi. The international research group on wood protection, Stockholm, IRG/WP 90-1457, 13.
18. Zink, P.; Fengel, D. 1989: Studies on the coloring matter of blue-stain fungi. Holzforschung 43: 371–374.

Corresponding address:

Assistant Professor MIHA HUMAR, PhD

Department of Wood Science and Technology
Biotechnical Faculty, University of Ljubljana
Jamnikarjeva 101
1000 Ljubljana
Slovenia
E-mail: miha.humar@bf.uni-lj.si