International Biodeterioration & Biodegradation 107 (2016) 154–157

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Short communication

Fungal pigments from spalting fungi attenuating blue stain in *Pinus* spp.

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
6 November 2015
Accepted 23 November 2015
Available online 10 December 2015

Keywords:
Blue sap-stain attenuation
Fungal pigments
Spalting fungi
Pinus spp.
Scytalidium spp.
Chlorociboria spp.

ABSTRACT

Fungal pigments from spalting fungi *Scytalidium cuboideum* (red), *Scytalidium ganodermophthorum* (yellow) and *Chlorociboria aeruginosa* (green), were used to attenuate the presence of blue sap-stain in *Pinus* spp. samples. Pigments, filtered from liquid cultures of spalting fungi, were vacuum impregnated into pine samples with three different levels of blue staining: 1 [0–50%], 2 [50–90%] and 3 [100%]. The CIE L*a*b* color difference at wood surfaces, before vs. after treatment, was quantified with a chroma meter and the internal color coverage in the core of the samples with digital images. Light microscopy was used to observe the penetration of pigment in the microstructure of blue stained pine samples. Results show that fungal pigments are suitable candidates to attenuate the appearance of blue stain on the wood surface. Pigments produced by *S. cuboideum* showed a higher color difference regardless of the blue stain level, but the highest color difference was obtained in samples with staining level 1. The percentage of internal color coverage of pigments was significantly higher for green and red pigments in staining levels 1 and 2. The proposed treatment may be used to improve the value of blue stained wood allowing its commercialization into the niche of environmentally friendly products.

1. Introduction

Fungal pigments found in spalted wood (i.e. fungal colored wood), have been used for centuries by woodworkers, carvers and turners to increase the aesthetic value of their products (Blanchette et al., 1992). By processing spalted wood these artists can create decorative items, such as fine art mosaics, bowls, and furniture, which hold an interesting niche market in North America and Europe (Donovan and Nicholls, 2003; Robinson, 2012).

After years of research, scientists have developed procedures for producing spalting in controlled conditions. Zone lines, bleaching and wood coloring spalting-types can now be produced while avoiding excessive mass and strength losses and decreasing incubation time, therefore increasing the efficiency of the treatment (Gignac and Dian-Qing, 2012; Robinson, 2012; Robinson et al., 2007, 2013). In addition, methods to produce and isolate fungal pigments for wood coloring are being developed (Maeda et al., 2003; Robinson et al., 2012a, 2014a). As a result, the fungal incubation stage can now be skipped and the pigments applied as any other finishing treatment if required (Robinson et al., 2011, 2012b; Weber et al., 2014).

Although blue sap-stain technically is a form of spalting, it has not generated the same interest among artists and consumers as have zone lines and other pigments (Robinson et al., 2013). Blue stain is produced by ophiostomatoid fungi as well as some other fungi, that can metabolize substances accumulated inside the sapwood's resin canals, parenchyma cells and tracheid lumina (Robinson, 1962). For industrial wood processing, the presence of blue stain is considered a defect rather than an attribute, precisely because of the changes that the melanized hyphae produce on the natural color of the wood (Behrendt et al., 1995). In countries like Chile, New Zealand and Canada, blue stain can be a major issue as the dominant pine species are highly susceptible.

In order to reverse the negative impact of blue stain, some companies have tried to capitalize on blue stained wood with products like Denim Pine™. Unfortunately, these initiatives have seen little commercial success. An alternative approach, consisting of repurposing blue stained wood into decorative uses, was proposed by Robinson and collaborators (Robinson et al., 2013). The authors reasoned that the addition of further colorization would

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http://dx.doi.org/10.1016/j.ibiod.2015.11.020
0964-8305/

make blue stained wood more attractive for decorative purposes. Thus, they inoculated mountain pine beetle blue stained wood with the spalting fungi *Scytalidium cuboideum* and *Scytalidium ganodermophthorum* to add reddish and yellowish color to the samples. Acceptable results were achieved after 12 weeks of incubation, with colorization that persisted after planing of the wood surface. Although the recoloring of the blue stained wood with other spalting fungi was successful, it still required live inoculation and a substantial incubation time. Use of extracted fungal pigments from spalting fungi could improve the original treatment by decreasing the incubation time, to hours instead of weeks, and by promoting an even colorization of wood fibers that could eventually attenuate the presence of blue stain.

The purpose of this work was to test pigments extracted from established spalting fungi *S. cuboideum* (red), *S. ganodermophthorum* (yellow) and *Chlorociboria aeruginosa* (green) as color modifiers of blue stain in *Pinus* spp. samples. Successful use of the pigments in this type of application could revolutionize the spalting process and help industry commercialize a process that has long been relegated to small business owners and crafters.

2. Materials and methods

Five blue stained pine boards, kiln-dried to 12% moisture content (KD-12), were purchased in a local lumber store in Corvallis, Oregon, and processed to obtain 14 × 14 × 14 mm wood blocks with clean radial, tangential and transverse faces. Since most pine species are highly susceptible to blue stain, identification to genus level was considered sufficient and realistic for this experiment. Identification by light microscopy was performed on samples obtained from each board to confirm the presence of windows-like cross-fields, distinctive of pine species. After processing, the blocks were allowed to equilibrate to ambient temperature for 7 days to 10–12% moisture content. Blocks were then classified according to the severity of blue stain. Classification included three levels, according to the volumetric presence of blue stain in the blocks: 1 [0–50%], 2 [50–90%] and 3 [100%]. Nine blocks from each staining level were selected for the experiment. Digital images (TIFF, 1200 dpi resolution) of selected tangential, radial and transverse faces were acquired from each selected block in an Epson Perfection V370 Photo Scanner. The blue stained area, in selected sections, was quantified by processing digital images in the software Image J (ImageJ 1.48v, Wayne Rasband, National Institutes of Health, USA). The color of the tangential and radial faces was evaluated in a Konica Minolta CR-5 chroma meter in the CIE L*a*b* color space.

Three fungal species, isolated by researchers from the Applied Mycology Laboratory of The Department of Wood Science and Engineering at Oregon State University and registered in The University of Alberta Culture Collection, were selected for the experiment. Selection criteria included their ability to produce extracellular pigments and their frequent use in spalting. *S. cuboideum* (Sacc. & Ellis) Singler & Kang (strain UAMH 11517) producing red pigments, *S. ganodermophthorum* Kang Sigler (strain UAMH 10320) producing yellow pigments and *Chlorociboria aeruginosa* (Nyl.) Kanouse (strain UAMH 11657) producing green pigments, were cultured in liquid media (2% malt extract) in a glass flask stirred at 110 rpm for 6 weeks. The media were then filtered using Whatman Cat. No. 1002150 filter paper to collect the colored supernatants.

Color of pigmenting solutions was recorded on the chroma meter (as above), colors obtained were: [$L^* = 81.46$, $a^* = 9.35$, $b^* = 10.81$] for *S. cuboideum*; [$L^* = 93.30$, $a^* = -4.07$, $b^* = 20.04$] for *S. ganodermophthorum*; and [$L^* = 86.19$, $a^* = -14.21$, $b^* = 2.93$] for *C. aeruginosa*. Pigmenting solutions were impregnated into wood samples within the following 24 h after color determination.

Wood blocks from each staining level were impregnated (n = 3) with red, yellow and green water-based pigmenting solution, in three different 250 mL glass beakers. Samples were placed in the flask and the pigmenting solutions were added to complete 170 ml. A plastic mesh was used to ensure the total immersion of the sample into the solutions. Afterwards, the beakers were located inside a glass desiccator connected to a vacuum network. Vacuum impregnation was performed at room temperature, 64.3 kPa for 24 h. After treatment, wood blocks were dried overnight at 65 °C for 24 h and color and digital images of tangential and radial faces were measured and acquired, respectively, in the chroma meter and scanner (as described above). Samples were then ripped along the axial axis for acquisition of digital images from the inner sections. Internal color coverage was calculated from digital images by using ImageJ (as above). Color difference in tangential and radial surface, after vs before treatment, were calculated from CIE L*a*b* parameters using the relation (ASTM D2244-15a, 2015): $\Delta E = [(L^*_{final} - L^*_{initial})^2 + (a^*_{final} - a^*_{initial})^2 + (b^*_{final} - b^*_{initial})^2]^{1/2}$. Where:

ΔE: total color difference
L*: lightness (0–100)
a*: greenness-redness (−60 to 60)
b*: blueness-yellowness (−60 to 60)

Pigment penetration in vacuum impregnated samples was examined using light microscopy. Pine blocks were soaked in distilled water for 2 days, and then 20 μm sections were obtained from the blocks using a disposable blade (Type S35, Feather Safety Razor Co., Japan) bolted to a microtome blade-holder. Microtome sections were mounted directly on glass slides and covered with a cover slip. No mounting reagent or contrasting pigments were used to avoid alterations in the visualization of fungal pigments in wood cells. The sections were examined using a light microscope (Carl Zeiss, Germany) at various magnifications. A Canon Power-Shot A640 digital camera attached to the microscope was used to take photographs of wood microstructure.

Analysis of variance (ANOVA) was used to test the significance of blue stain level and pigment color, on color differences and internal color coverage. The least significant difference (L.S.D.) test was run to determine the location of the differences. All statistics were performed on SAS 9.4.

3. Results

The ANOVA showed a highly significant effect (p-value < 0.001) between pigment color (PC) and blue stain level (BSL) in terms of surface color and internal color. No interaction between factors (PC x BSL) was detected.

The significantly greatest color difference was obtained with samples impregnated with pigments from *S. cuboideum* (red) (ΔE = 11.26) (Fig. 1A and C). These samples also displayed one of the higher internal color coverages (25.07%). Conversely, samples impregnated with pigments from *C. aeruginosa* (green) showed the lowest color difference (ΔE = 5.25), although their internal color coverage was not statistically different from that of samples treated with *S. cuboideum* (red). Color difference achieved by samples impregnated with pigments from *S. ganodermophthorum* (yellow) was slightly superior than that of samples treated with pigments from *C. aeruginosa* (green). However, the samples impregnated with yellow pigments achieved the significantly lowest internal color coverage (5.19%).

Wood samples with stain level 1 showed the highest color difference after treatment (ΔE = 9.49) and a high internal color coverage (21.90%) (Fig. 1B and D). Unfortunately, the internal results