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The effect of natural product treatment of southern yellow pine on fungi causing blue stain and mold

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ABSTRACT

Blue stain and mold growth on wood can be aesthetically unappealing, but mold growth can also potentially trigger health related issues. In this study, a screening of the effect of selected natural products and derivatives of natural products including essential oils, plant based monomers, and shellfish exoskeleton compounds on the inhibition of blue stain fungi and mold fungi in southern yellow pine veneers and cellulose filter paper. The treatment of the substrates, the weight percent gain of the natural products, and the inhibition effect is presented. The natural products have been investigated previously, but most have not been applied to wood or investigated with regards to blue stain or mold growth. The specimens were treated by dipping, wrapping in foil, and then heating at 105 °C for 24 h in order to encourage reaction or grafting of the natural products to the wood. A selection of seven additives each exhibited significant protection against blue stain colonization in Petri dish tests and these were studied more in-depth. These included tea tree oil, propyl gallate, hydrogenated gum oil, salicylic acid, cinnamon bark oil, butylene oxide, and furfural. The salicylic acid, tea tree oil, and cinnamon bark oil had the least amount of mold growth after four weeks in the mold chamber test, and have been previously reported to have a mechanism of antifungal activity resulting from their ability to disrupt the fungal cell wall. Propyl gallate veneer was the only specimen that had a lower pH and that would be considered unfavorable to mold growth. While furfural, salicylic acid, and cinnamon bark oil treated veneers all had more hydrophobic surfaces when compared to untreated wood.

1. Introduction

Colonization of wood by mold and blue stain fungi can occur. There are several types of mold that usually appear in both interior and exterior surfaces due to the presence of nutrients and sufficient moisture. Mold growth may produce odor, discolored patches and the fungal spores can be detrimental to the health of those with allergies (Clausen, 2000).

Blue stain fungi causes dislocation in wood because the fungal hyphae have brown melanin pigment and the blue black color on wood results from the refraction of light in combination with the substrate (Zink and Fengel, 1990; Bardage, 1997). Blue stain is mainly a surface phenomenon that decreases the aesthetic value of wood, however it can penetrate into the wood structure via the medullary rays, colonizing the tracheids and fibers. Blue stain fungi live on low molecular weight substances present in wood without degrading the wood cell wall (Schmidt, 2006).

In an effort to prevent fungal growth on wood, chemical biocides are commonly used in wood treatments. These can include triazoles, barium metaborates and zinc oxides, which if added in sufficient amounts will result in insignificant growth of fungi. In order to minimize the use of conventional biocides, a plethora of studies involving natural compounds that will prevent fungal growth and yet not detrimentally affect the environment, have been investigated. A selection of the natural compounds and those that can be derived from natural compounds has been compiled from the literature. Many screening studies have been done regarding essential oils, with tea tree oil and cinnamon bark oil most often reported as showing the greatest range of inhibition against bacteria, yeast, molds, and bacteriophages (Maruzzella et al. 1960; Chao et al., 2000; Yang and Clausen, 2007). Tea trea oil (*Melaleuca alternifolia*) has been shown to alter fungal membrane properties and compromise membrane-associated functions (Hammer et al. 2004). Cinnamon bark oil, mostly composed of (E)-

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cinnamaldehyde has been reported to have anti-fungal activity which has also been linked to membrane disruption and the lipophilicity of the hydrocarbon chain (Shreaz et al. 2011). Alkylene oxides, such as butylene oxide which can be derived from ethanol, have been studied and found to provide biological resistance to brown and white rot fungi and termites, by lowering the moisture content and changing the character of the cell wall (Sondheimer and Woodward, 1953; Rowell and Gutzmer, 1975; Rowell and Ellis, 1984; Rowell, 2006; Pandey et al. 2010). Chitosan, a biopolymer derived from chitin, has also been widely shown to protect wood from fungi in the past 20 years (Lee et al., 1992; Larnøy et al. 2006). The nitrogen containing polysaccharide chitosan, derived from shellfish exoskeletons, has been reported to disrupt the cell-wall membrane of fungi (Alfredsen et al., 2004). Anti-oxidants such as propyl gallate, a derivative of gallic acid from tannins, have been reported to be effective in antifungal activity over a broad spectrum of fungi due to their radical scavenging activity (Aruoma et al. 1993; Hsu et al. 2009). Stilbenes, found in wood with a diphenyl structure, have long been demonstrated to provide anti-fungal activity by binding to cell walls (Kingsbury et al. 2012) and most optical whiteners used in the paper industry are based on stillbene chemistry (Hart, 1981). In this study, the paper whitener, Leucophor S, containing a stillbene within the chemical structure is investigated. Additionally, rosin, consisting mainly of a abietic acid, a natural diterpene resin acid, has shown to be anti-microbial, partially due to the ability to act as a chemical modulator that can even open large conductance calcium activated K^+ channels (BK channels) González et al. (2010) have demonstrated to protect wood against fungi, both in the dehydrogenated form (Resigral) and in the hydrogenated form (Hydrogral) (Eslyn and Wolter, 1981; Liang et al. 2013). Benzoic derivatives such as salicylic acid, naturally derived from the Willow tree, have been shown to be anti-fungal agents due to their ability to enter the fungal cell wall and lower the internal cell pH (Amborabé et al. 2002).

These studies have allowed for much greater insight into different classes of materials for further investigation as substitutes to harmful biocides that are of interest to replace. However, little has been reported on their application of these compounds to untreated wood for protection against blue stain and mold fungi.

The objective of this study is to take the most promising compounds in the literature and perform blue stain and mold tests on Southern Yellow Pine (SYP) veneers. Since these are surface effects, dipping of the natural additives is applied to the samples. Further, modification of the wood pH, change in color and surface hydrophobicity are also presented and correlated to the fungal growth results.

2. Materials and methods

2.1. Materials

The stilbene based fluorescent dye, Leucophor S, was received from Clariant, gum rosin, Resigral (dehydrogenated rosin), and Hydrogral (hydrogenated rosin) were received from DRT (France). Butyl oxide, tea tree oil, cinnamon oil, salicylic acid, propiconazole (reference fungicide), furfural (reference natural pesticide), propyl gallate, and all other chemicals were used as received from Sigma–Aldrich.

2.2. Test specimens

Carolina Southern yellow pine (SYP) veneers (*Pinus palustris*) that were cut by the half-round slicing method were supplied by Capital Crispin Veneer (London). After conditioning at 65% RH, the veneers had approximately 14 ± 1% moisture content (MC) and a density of 530 ± 20 kg m^{-3}. The SYP specimens of 0.6 mm thickness were cut to 50 × 50 mm^2 for the Petri dish tests and 50 × 100 mm^2 for the mold chamber test.

2.3. Dip and heat treatment

The SYP veneers were dipped in the additive mixtures for 5 s, with 4 samples of each additive, immediately wrapped in foil, and placed in an oven at 105 °C for 24 h. The samples were then removed and tested.

2.4. Measurement of weight percent gain (WPG) and leaching of additives

The uptake of the additive was calculated by correcting for the 14% MC of the originally 65% RH conditioned wood samples and then gravimetrically calculating the weight gained in the 4 wood samples of each additive, removed from the oven after dipping and heat treatment. The leaching of additives was conducted by placing them in water that was changed 10 times over 14 days according to the standard EN-84:1997.[1]

2.5. pH measurements

The pH of wood was measured by adding 0.25 g of ground-up veneer to 5 ml of de-ionized water and agitating for 24 h. The pH of the water solution containing the wood particles was then measured using a Mettler Toledo MP 225 pH-meter. *2.6. Contact angle measurements*.

Static contact angle measurements were performed on the wood to air substrate using a contact angle meter, CAM 200 (KSV). Measurements were done on earlywood annual rings only and averages of 7 measurements, taken after 1s after the drop was placed are reported.

2.6. Blue stain test

Malt agar plates (2.5 w/w% malt extract, 1.5 w/w% agar) were used in which a cellulose filter paper (Whatman 1) cut to dimensions of 50 × 50 mm^2 were placed beside a SYP veneer specimen of 50 × 50 mm^2. This was done in duplicate for every sample. The unleached heat treated samples (described in 2.3), were placed in malt agar plates prior to inoculation with a mixed spore suspension containing 10^5 spores ml^{-1} of Aureobasidium pullulans (de Bary) Arnaud, strain P 268, source Hann. Münden and Sydowia polyspora (Bref. & Tavel) E. Müller (syn. Sclerophoma pithyophila (Corda) v. Höhnel) strain S231, source Hann. Münden. The spore suspension was applied by spraying approximately 1 ml. After inoculation, the malt agar plates were sealed with Parafilm® and incubated at 28 °C. The samples were continuously evaluated for blue stain growth and results from 3, to 32 days are presented.

2.7. Environmental test chamber (mold chamber)

A self-contained plexiglass environmental chamber was used containing water, a soil bed, and sample hangers to allow suspension of the samples 5 cm above the inoculated soil. Duplicates of the heat treated unleached samples (section 2.3) were made, sterilized by γ radiation, and placed in different locations in the chamber. The chamber was covered with a pitched cover to prevent condensation falling on the samples. The tank was sealed and the humidity monitored to be maintained at 95% RH. The previously sterilized soil was inoculated with three test fungi, two mold fungi