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Evaluation of White-Rot Fungal Growth on Southern Yellow Pine Wood Chips Pretreated with Blue-Stain Fungi

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White-rotting basidiomycetes do not colonize on southern yellow pine. This study seeks to reduce the resinous extractive content of southern yellow pine by treating it with blue stain fungi. The mycelial growth of wood-inhabiting ligninolytic white-rot fungi can be achieved on pretreated southern yellow pine wood. *Aureobasidium, Ceratocystis,* and *Ophiostoma* spp. removed 70% to 100% of the extractives from the southern yellow pine wood within a period of 3 to 6 days. *Griofora fondosa, Hericium erinaceus, and Pleurotus ostreatus* colonized readily after the treatment. As a result, ligninolytic white-rot fungi can be easily colonized on southern yellow pines pretreated with blue stain fungi.

Keywords: Abnormal fruiting bodies, aerial mycelia, blue stain fungi, white-rot, white-rot fungi, wood decay, southern yellow pine.

INTRODUCTION

There are eleven species of southern yellow pine: longleaf pine (*Pinus palustris)*; short leaf pine (*P. echinata*); slash pine (*P. elliottii*); southern Forida slash pine; loblolly pine (*P. taeda*); pitch pine (*P. rigida*); spruce pine (*P. grabra*); Virginia pine (*P. virginiana*); sand pine (*P. clausa*); pond pine (*P. serotina*); table mountain pine (*P. pungens*). Four species of southern yellow pine (loblolly, short leaf, long leaf, and slash pines) account for about 93% of all standing timber. These trees generally grow in the southeast and south central states of the United States (Hoadley, 1990). The southern yellow pine usually consists of more than 90% hemicelluloses, cellulose, and lignin. Lignocellulosic materials are made up of polymeric cellulose, amorphous hemicelluloses, and complex lignin. Wood extractives range from 3% to 9% (Koch, 1972) and 1% to 5% (Zabel and Morrell, 1992) of the total dry weight of wood. In exceptional cases, the extractives may represent 10% to 40% (Zabel and Morrell, 1992). These extractives are more soluble in neutral, nonpolar, organic solvents and cold water. Softwood such as southern yellow pine inhibits mycelial growth by ligninolytic white-rot basidiomycetes, but hardwood does not. Wood extractives may be responsible for the mycelia growth of white-rotting fungi.

The *Aureobasidium*, *Ceratocystis*, and *Ophiostoma* spp., which are of the class of Ascomycetes, are initial wood colonizers. The most commonly isolated blue stain fungus

is *Aureobasidium* pullulans (Eaton and Hale, 1993). These blue stain fungi attack wood chips, logs, sawn timber, veneer, sawdust, and wood products; they penetrate rapidly and deeply into the sapwood of conifers, resin canals, and epithelial cells and invade ray parenchyma cells. At the same time, these fungi grow mainly in ray parenchyma cells where they assimilate available nutrients, mainly the nonstructural wood components. The extractives are simple sugars (such as starch, glucose, fructose, and sucrose), phenolic compounds (such as stilbenes, tannins, phlobaphenes, flavanoids, and lignans), lipids (triglycerides, fatty acids, resin acids, sterols, and steryl esters), and waxes, alkaloids, and tropolones found in parachyma cells and in the lumen of other cells (Gao and Breuil, 1995; Zabel and Morrell, 1992). As they grow on wood and penetrate into it, the pigmented hyphae discolor it. Although the fungi do not degrade the major components of wood cell walls, lignin, and cellulose, the metabolic action substantially reduces wood extractives (Blanchette et al., 1992). The primary objective of this study was to select the isolate best capable of removing the wood extractives in the southern yellow pine wood waste. A second objective was to facilitate the growth of the white-rot fungi on pretreated southern yellow pine chips.

METHODS AND MATERIALS

Fungi

Dikaryotic isolates of mushroom-producing white-rot basidiomycetes *Grifola frondosa* (Dicks: Fr.) S.F. Gray (M-10), *Hericium erinaceus* (Bulliard: Fries) Persoon (M-13), *Pleurotus ostreatus* (Jacquin: Fries) Kummer (FP–101509), (HHB–9790) (m-21). The blue stain fungus *Aureobasidium pullulans* (deBary) Arnand [MDX 18], *Ceratocystis coerulescens* (Munch) Bakeshe [C-262], and *C. pilifera* (fries) Moreau [RWD 9472B] were all obtained from the Center for Forest Mycology Research at the Forest Products Laboratory (USDA Forest Service, Madison, WI). A colorless isolate, Cartapip 97, *Ophiostoma piliferum*, was obtained from Clariant Co. Charlotte, NC. The fungi were maintained on 1.5% (w/v) malt extract (Bacto, Difco, Detroit, MI) and 2% (w/v) agar (Bacto, Difco).

The mycelial isolates of white-rotting fungi and blue stain fungi were plated on 1.5% (w/v) malt extract agar (MEA). Malt extract agar 90-mm-diameter plates were inoculated with a mycelium/agar plug (6 mm diameter) of a young, actively growing margin of the colony at the center of the plate and incubated at 24°C in the dark for 1 to 2 weeks or until mycelial growth had covered the entire surface of the MEA plates.

Southern yellow pine wood waste—Southern yellow pine wood chips were obtained from Bienville National Forest, Mississippi. One hundred grams (dry weight 49%) of frozen southern yellow pine (*Pinus* spp.) chips of various sizes (0.5 to 3.5 cm by 0.2 to 0.25 cm) and 100 mL distilled water were added to Pyrex^{R} storage dishes (Corning No. 3250). Each dish was autoclaved and inoculated with actively growing mycelia from a 1/2 MEA plate with blue stain fungi. After mixing well, they were incubated at 24°C in the dark for 7 days. For the colorless isolates (lyophilized fungal cells), the storage dish was inoculated with 1×10^9 (1×) and 2×10^9 (2×) spores of Cartapip per dish. They were mixed and incubated for 41/2 days. At the end of the incubation period, all dishes were

autoclaved. They were inoculated with actively growing mycelia of white-rotting fungi from a half MEA plate, mixed well, and incubated at 24°C in the dark.

Resinous extractives determination—After treatment, the southern yellow pine chips were oven dried at 50°C and ground into 30-mesh sawdust with a Wiley mill (Authur H Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). The oven-dried sawdust (1 g dry weight) was extracted in a Soxhlet extractor with diethyl ether overnight (Brush et al., 1994). The data constitute a percentage weight loss on the basis of dry weight in a vacuum oven at 50°C.

RESULTS AND DISCUSSION

The wood extractives are mostly low molecular weight compounds that are easily extracted from wood by solvents such as water, alcohol, ether, or benzene. The percentage of the extractives accounted for 9.02% (with 0.04 standard deviation) of the dry weight of the southern yellow pine wood chips (Table 1). The southern yellow pine chips were treated with four different isolates of *Aureobasidium pullulans*, *Ceratocystis coerulescens*, *C. pilifera*, and Cartapip. They removed 70% to 100% of the extractives (diethyl ether extractible) of dry weight of pine wood chips (Table 1). After treatment with blue stain fungi, *Ceratocystis pilifera* removed 95% and Cartapip removed 100% of the extractives. Seventy percent of the extractives were removed by *Aureobasidium pullulans* and *Ceratocystis coerulescens*.

Fig. 1. Mycelial growth of *Griofola frondosa* on treated southern yellow pine chips.

All the blue stain fungi attacked the southern yellow pine chips; they all colonized the entire surface of the softwood chips within 2 to 3 days. Microscopic examination showed that the mycelia had penetrated into wood chips; they also appeared in the resin canal and the ray parenchyma cells after 2 to 3 days incubation.

The fungi that cause the decay of wood are brown-rot (red-rot) and white-rot in wood, which are found in forests, in stored logs, standing trees, on stumps, woodland, and elsewhere. Brown-rot basidiomycetes attack mainly softwoods and occasionally also hardwood. White-rot basidiomycetes attack mainly hardwood but only rarely softwood. Our study demonstrated that white rot fungi; *Griofola frondosa*, *Hericium erinaceus*, and *Pleurotus ostreatus* did not grow or else grew poorly on untreated southern yellow pine chips (Table 1). *Griofola frondosa* did not grow on untreated pine chips but dense, filamentous heavy mycelial growth occurred on the entire surface of treated southern yellow pine chips (Fig. 1). Mycelial growth of *Griofola frondosa* covered 80% to 85% of pine chips treated with *Aureobasidium pullulans* and *Ceratocystis coerulescens*. However, white-rot fungi covered the entire surface of pine chips treated with *Ceratocystis pilifera* and colorless isolates Cartapip (Table 1). The mycelial growth of *Hericium erinaceus* and *Pleurotus ostreatus* was filamentous but this growth was not dense or heavy. However, they both produced abnormal fruiting bodies on treated southern yellow pine chips without special treatment e.g., the use of light/dark cycles, added humidity, or decreased temperature (fig. 2 and 3).

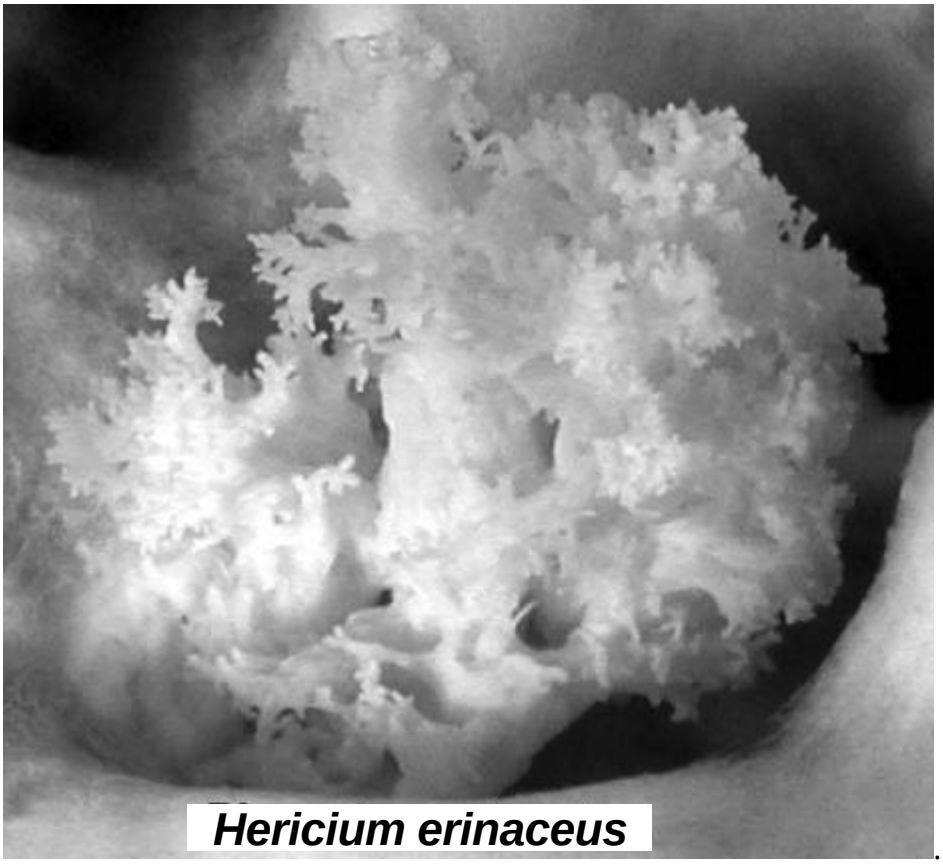

Fig. 2. Abnormal fruiting body formation of *Hericium erinaceus*.

The *Hericium erinaceus* and *Pleurotus ostreatus* grew as aerial mycelia on the surface of southern yellow pine chips. The aerial mycelia of *Hericium erinaceus* were elevated 24mm to 35 mm and elongated 28 mm to 33 mm beyond the surface of pine chips as shown in Fig. 2. However, in comparison, the abnormal fruiting bodies, aerial mycelia, of *Pleurotus ostreatus* remained relatively flat, although they experienced some elongation (up to 20 mm to 30 mm) on the surface of pine chips (Fig. 3). The growth of abnormal fruiting bodies was pale yellowish to whitish in color and as the cultures age, the mycelial became yellowish brown.

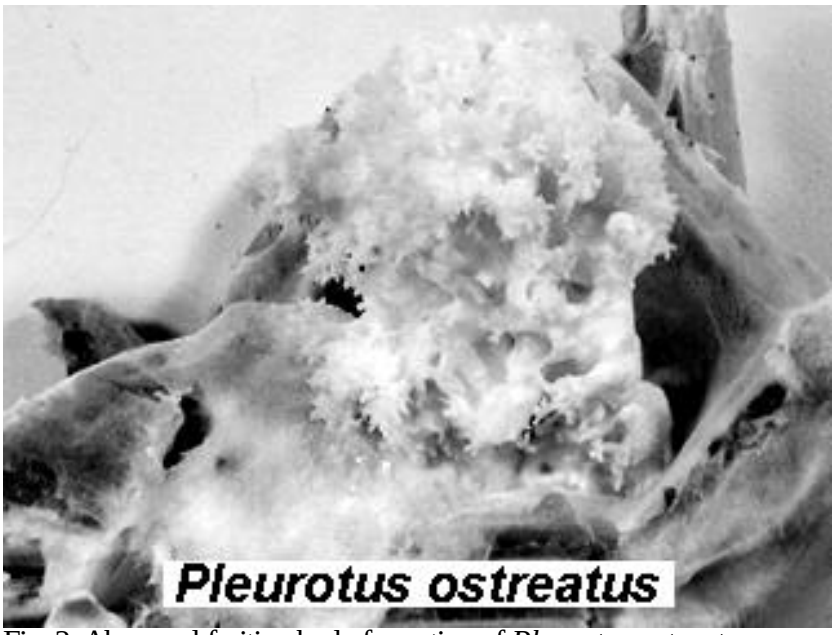

Fig. 2. Abnormal fruiting body formation of *Pleurotus ostreatus*.

In sum, the results of this study demonstrate that blue stain fungi, *Ceratocystis pilifera*, and Cartapip can remove 95% to 100% of the extractives from southern yellow pine. White rot fungi, *Grifola frondosa*, *Hericium erinaceus*, and *Pleurotus ostreatus* can be easily colonized on treated pine chips.

Table 1. Mycelial growth on treated southern yellow pine with blue stain fungi[a].

Extractives concentration SYP			Mycelial growth on treated SYP chips		
	% extractive	% removed	*Grifola frondosa*, M10	*Hericium erinaceus*, M13	*Pleurotus ostreatus*, M21
Control	9.02 +/- 0.04%	0	0-1	1-2	1
MDX-18 Aureobasidium pullulans	2.73	69.67	3-4+++	2+*	2-3++*
C-256 Ceratocystis coerulescens	2.69	70.18	3-4+++	2+*	2-3++*
RWD 9427 C. pilifera	0.05	94.45	5+++	4++	3-4, ++*
Cartapip 1X Ophiostoma piliferum	0.009	99.9	5+++	2-3++*	3-4, ++*
Cartapip 2X Ophiostoma piliferum	0.009	99.9	5+++	5+++	2++*

[a]Legend

5	Covered the entire surface area of chips with sparse mycelial growth
4	Covered the most chips (75% to 90%) but grown sparsely
3	50% to 75%, sparsely
2	25% to 50%, sparsely
1	5% to 25%, sparsely
0	No growth
*	Abnormal fruiting bodies in storage dish without any treatment for fruiting
+	Sparse and transparent growth
++	Filamentous but not heavy mycelial growth
+++	Dense, filamentous heavy mycelial growth

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