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Mold inhibition on unseasoned southern pine

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Abstract

Concerns about indoor air quality due to mold growth have increased dramatically in the United States. In the absence of moisture management, fungicides need to be developed for indoor use to control mold establishment. An ideal fungicide for prevention of indoor mold growth on wood-based materials needs to specifically prevent spore germination and provide long-term protection under conditions of high humidity. Fungicides intended for indoor use must exhibit no mammalian toxicity, be odorless and emit no VOCs. Classes of compounds meeting one or more of these criteria include acids, phenolic compounds (antioxidants), pharmaceuticals, commercial and experimental wood preservatives, food preservatives, and plant essential oils. Wood preservatives and food preservatives were initially screened at various concentrations for inhibitory properties to mold fungi on 2% malt agar (MA). Many compounds that inhibited the test fungi on MA failed to substantially inhibit mold growth on unseasoned southern pine at higher concentrations than the minimal inhibitory concentration determined on MA. Subsequently, compounds were screened on unseasoned southern pine stakes. Pine stakes were dipped for 15 seconds in varying concentrations of the test chemicals according to the ASTM standard test method D4445 for controlling mold on unseasoned lumber. Stakes were challenged with *Penicillium chrysogenum* PH02, *Aspergillus niger* 2.242, and *Trichoderma viride* ATCC 20476 spore preparations. Following a 4-wk incubation, stakes were rated from 0-5 with 5 representing heavy mold growth. An inihibition rating of 0 to 1 is indicative of successful mold inhibition. The best overall average ratings for wood preservatives were seen in stakes treated with 5% Bor-A-plus or Cu+, which were highly inhibitory to all test fungi. High concentrations of ethanolamine (10%) and thujaplicin (10%) were inhibitory to all test fungi. Pine resin (50%) solely inhibited *P. chrysogenum.* Of the food preservatives tested, five percent sodium benzoate and potassium sorbate inhibited all test fungi, while calcium propionate selectively inhibited *A. niger*. Pharmaceutical antifungals such as voriconazole (2.5mg/mL), thiabendazole (25mg/mL), and miconazole (20mg/mL) completely inhibited all test fungi on unseasoned pine, while other azole-derivatives failed to inhibit mold growth. Nystatin (10,000 units/mL) inhibited only *A. niger*. A combination of effective chemicals should be considered as one strategy to provide long-term protection of wood-based building materials from mold establishment.

Key words: Mold fungi, fungicide, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride*

Introduction

Concerns about indoor air quality (IAQ) issues have been elevated by steady media coverage of mold infestations in residential and commercial structures. Besides increasing public awareness, a number of factors indicate a genuine increase in the incidence of mold infestations in new and existing structures containing cellulose-based materials. Any number of reasons may be responsible for mold infestations in existing structures, such as original design, poor construction practices, poor site drainage, leaky roofs or plumbing, inadequate insulation, improper ventilation, etc (Clausen 2002).

Scheduling changes during new construction can result in delayed installation of materials and extended exposure of an open structure and building components to the elements. The incidence of mold on new building materials is common, but the consumer's reaction has changed. New products, such as trusses and dimension lumber, are being rejected at an alarming rate by consumers due to the presence of mold. Short-term strategies for inhibiting mold on such products, such as dipping in a fungicide, are cost-prohibitive. One company has resorted to washing trusses, while on a tractor-trailer, in a car wash to remove any surface mold just prior to delivery. Ideally, determining and controlling the sources of moisture, as well as rapidly drying building components will prevent further mold growth. In the absence of moisture management, economical, non-toxic, VOC-free fungicides (mildewcides) that are suitable for indoor use are needed to protect wood in-service from repeated mold infestations.

Mold resistance of wood-based building materials can affect the extent of indoor mold growth and therefore indoor air quality (IAQ). Laks *et al.* (2002) evaluated four common commercial untreated sheathing panel types, i.e. southern pine and Douglas-fir plywood, oriented strand board, and gypsum board for mold resistance. Their results showed that all of these products were prone to mold growth within one week of inoculation with mold spores and incubation at 27^{o}C and 100% relative humidity (RH). Moreover, all panels had been pre-conditioned for one week at 27^{o}C and 100% RH and showed signs of mold establishment prior to inoculation with the test fungi! Clearly, treating these products with a mildewcide would lessen the impact on IAQ issues.

Nontoxic mildewcide would be considered an oxymoron by many scientists in this field. Mildewcides generally need to be quite toxic to prevent spore germination, which would preclude their use in inhabited environments. Spores are quite resistant to heat, drying, and UV. Most studies on fungicides in construction products have concentrated on borates. Borates would be a desirable addition to a multi-component mildewcide because they exhibit low toxicity and are widely used for insect control and as a fire retardant. Fogel and Lloyd (2002) showed that commercial levels of borate significantly reduced the levels of mold growth on solid wood, wood composites and gypsum wallboard.

Examples of highly effective mildewcides for indoor use are those added to paint formulations. In addition to being effective against mold fungi, they must be nonvolatile, environmentally acceptable, safe to handle, and possess low solubility (Zabel and Morrell 1992). Categories of potential mildewcides include some commercial and experimental

wood preservatives, food preservatives, plant extractives, or pharmaceuticals. Low mammalian toxicity is desirable for experimental wood preservatives intended to inhibit decay fungi. But mildewcidal properties are equally important, since mold fungi readily grow on the surface of wood treated with many current wood preservatives, which can otherwise adequately inhibit decay fungi. Food preservatives may be ingested, yet are specifically designed to inhibit mold during the relatively short shelf life of food products. Some plant extractives have been shown to have antifungal and antibacterial properties. Pharmaceutical antifungals, such as the azole derivatives, are specific inhibitors of fungal ergosterol biosynthesis and do not display mammalian toxicity.

The objective of this study was to evaluate potential mildewcides for their ability to inhibit mold fungi in agar and on unseasoned southern pine.

Materials and Methods:

Test fungi
Aspergillus niger 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 were maintained on 2% malt agar (Difco, Detroit, MI, USA). Individual spore preparations were prepared by washing the surface of a 2-wk old culture of each fungus with 10 mL deionized (DI) water and transferring the liquid spore suspension to a spray bottle. Each spore suspension was diluted to 100 mL with DI water. The spray bottle was adjusted to deliver 1 mL inoculum/spray.

Test chemicals
Chemicals in the following categories were evaluated for mold inhibitory properties in agar or on wood at concentrations shown in Tables 1 and 2a-2d.

Experimental and commercial wood preservatives or additives; Disodium octaborate tetrahydrate (DOT) (Pole Maintenance Co, Columbus, NE); N-hydroxynaphthalimide (NHA) (Aldrich Chemical Co., Milwaukee, WI); Bor-A-Plus (a boron, propionate combination), CuBor (a copper, boron combination), and Cu+ (a zinc, boron combination) (provided by Michael West); and ethanolamine (Sigma Chemical, St. Louis, MO).

Food preservatives; Sodium acetate, sodium benzoate, calcium propionate, potassium sorbate, sodium formate, and sodium nitrite (Sigma Chemical).

Plant extractives; Thujaplicin (isopropyltropolone) (provided by Cedarome Northwest), pine resin, and soybean ester (provided by The Heavens Group, LLC, Rolla, MO).

Pharmaceuticals; Miconazole (Pharmacia Upjohn, Kalamazoo, MI); ibuprofen (IB), triazole, sodium triazole, difluconazole, amphotericin B, nystatin, and thiabendazole (Sigma Chemical, St. Louis, MO); voriconazole (Pfizer Inc., NY, NY), itraconazole (Janssen Pharmaceutica Products, Titusville, NJ), clotrimazole (suspension in polyethylene glycol) (TARO Pharmaceuticals, Bramalea, Ontario).

Agar test method
Wood preservatives and food preservatives were added either alone or in combination at various concentrations to a sterile 2% solution of malt agar, which had been cooled to 45°C (Table 1). Solidified agar was inoculated with 1 mL of a mixed fungal spore preparation and incubated at 27°C and 70% relative humidity (RH) for 3 weeks. 3-Iodo-2-propynyl butyl carbamate (IPBC) served as a positive control for our test system. The negative control consisted of malt agar inoculated with the mixed spore preparation. Plates were rated for growth according to the percent coverage of the plate surface.

Stake test method
Stakes (7 x 20mm cross section by 7 cm long) were cut from unseasoned southern pine mill ends from a Mississippi sawmill and stored at 0°C. Average moisture content of the stakes was 48 % by weight (n=3). Seven random replicate stakes were dip-treated for ~15 seconds in varying concentrations of the test chemicals and held in a covered container overnight according to the ASTM standard test method D4445-91 (Table 2). Stakes were arranged over 7-10 layers of 125 mm filter paper that was saturated with 30 mL DI water and a polyethylene mesh spacer in sterile disposable Petri dishes (150 x 25 mm) (B-D Falcon, Los Angeles, CA, USA). Each dish contained stakes treated with a single concentration of test solution per test fungus. Untreated stakes served as a control for water based test chemicals. Stakes dipped in 95% ethanol served as a control for test chemicals of low solubility. Stakes were sprayed with 1 ml of fungal spore inoculum, sealed in zip lock polyethylene bags to prevent drying and incubated at 27°C and 70% RH for 4 weeks. Following incubation, stakes were individually rated for mold growth on a scale of 0-5 with 5 representing heavy mold growth.

Results and Discussion:

Agar test results
Preliminary screening of potential mildewcides on agar was a rapid means of eliminating chemicals that cannot possibly meet the rigorous criteria of the stake test method on unseasoned wood. Table 1 shows results of the agar inhibition test on individual chemicals and combinations of wood preservatives and food preservatives. Individually, 1.0% disodium octaborate tetrahydrate (DOT), 1.0% N-hydroxynaphthalimide (NHA), 0.1% Bor-A-Plus, and 1.0% CuBor were inhibitory to all test fungi. There appeared to be a synergistic effect from the combination of DOT plus NHA (0.5% + 0.1%), and to a lesser degree CuBor plus NHA (0.5% + 0.1%) and CuBor plus ibuprofen (IB) (0.5% + 0.05%). Ibuprofen, a phenyl propionic acid derivative, has previously been shown to be fungicidal to 15 brown-rot basidiomycetes in malt agar at a concentration of 100ug/mL (Clausen, 1996). In these combinations, a lower concentration of each component inhibited test fungi more effectively than higher concentrations of the individual components. The triple combination of low concentrations of sodium benzoate, sodium nitrite, and potassium sorbate completely inhibited test fungi in agar.

Table 1. Inhibition of a mixed spore suspension on malt agar containing various concentrations and combinations of test chemicals.

Test chemical(s)	Concentration (%)	Surface coverage (%)
DOT	0.1	100
"	0.5	100
"	1.0	1
DOT + NHA	0.1+0.1	100
"	0.5+0.1	0
"	1.0+0.1	0
DOT + IB	0.5+0.05	90
"	1.0+0.05	25
"	1.5+0.05	2
NHA	0.1	100
"	0.5	20
"	1.0	0
NHA + IB	0.1+0.05	100
"	0.1+0.1	90
"	0.5+0.1	50
IB	0.05	80
"	0.1	50
"	0.5	80
Bor-A-Plus	0.1	0
"	0.5	0
"	1.0	0
Bor-A-Plus + NHA	0.1+0.05	0
"	0.1+0.1	0
"	0.1+0.5	0
Bor-A-Plus + IB	0.5+0.01	0
"	0.5+0.1	1
"	0.1+0.05	5
CuBor	0.1	100
"	0.5	100
"	1.0	0
CuBor + NHA	0.1+0.1	100
"	0.1+0.5	100
"	0.5+0.1	25
CuBor + IB	0.5+0.01	25
"	0.5+0.05	5
"	0.5+0.1	1
"	0.1+0.05	2
Na benzoate+Na nitrite+K sorbate	0.3+0.3+0.3	0
IPBC	0.01	0
"	0.05	0
"	0.1	0
None	0	100

Stake test results

Testing potential mildewcides on unseasoned wood simulated a realistic, yet rigorous test. Green (unseasoned) pine typically contains in excess of 100% moisture by weight (Simpson and TenWolde 1999). At moisture contents greater than 20%, mold establishment can occur in 24-48 hrs if temperatures permit and rapid drying of the wood does not occur. Tables 2a-d show results of the stake test on individual chemicals and combinations of chemicals tested in this study. Results are expressed as the average rating of seven replicate stakes on a scale of 0-5 with 0 indicating complete inhibition and 5 indicating heavy mold growth. Five percent Bor-A-Plus, a combination of boron and propionate, and Cu+, a zinc and copper combination, were highly effective against all test fungi (Table 2a). Neither DOT nor CuBor were effective at 5% against the test fungi.

Table 2a. Mold inhibition by wood preservatives on unseasoned southern pine.

Wood preservative	Concentration (%)	Average rating		
		Penicillium chrysogenum	*Aspergillus niger*	*Trichoderma viride*
IPBC	0.02	2.6	3.1	3.9
	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
Cu +	1.0	3.3	4.7	4.7
	1.5	4.6	5.0	4.7
	2.0	5.0	5.0	5.0
	5.0	0.0	0.0	0.0
DOT	1.0	4.1	4.9	4.3
	1.5	4.4	4.7	4.7
	2.0	4.0	5.0	4.7
	5.0	4.9	3.7	0.7
NHA	1.0	3.4	4.7	4.0
	1.5	3.7	4.6	3.6
	2.0	3.4	5.0	2.4
	5.0	3.6	3.7	3.4
CuBor	1.0	3.1	4.7	4.4
	1.5	4.3	5.0	5.0
	2.0	4.7	4.9	3.3
	5.0	5.0	5.0	2.1
Bor-A-Plus	0.5	5.0	4.7	5.0
	1.0	3.6	4.1	5.0
	1.5	5.0	3.9	4.4
	5.0	1.9	0.0	0.0
Ethanolamine	5.0	1.4	3.1	1.9
	10.0	1.6	0.9	2.4
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.0

Ten percent ethanolamine, a common additive in wood preservatives, inhibited *A. niger* completely and was moderately inhibitory to *P. chrysogenum* (1.6) and *T. viride* (2.4). Results on the synergistic effects of wood preservative combinations showed that Cu +/DOT (1.5%), NHA/DOT (2%), CuBor/NHA (2%), and CuBor/IB (2%) were unable to inhibit test fungi on unseasoned pine (data not shown).

Sodium benzoate and potassium sorbate inhibited all test fungi, while calcium propionate was effective only against *A. niger* (Table 2b). Five percent sodium acetate actually accelerated mold growth. Food preservatives are generally intended for short-term preservation of foods. However, their low mammalian toxicity makes them ideal candidates for an indoor wood mildewcide.

Table 2b. Mold inhibition by food preservatives on unseasoned southern pine.

Food preservative	Concentration (%)	Average rating		
		Penicillium chrysogenum	*Aspergillus niger*	*Trichoderma viride*
Na Acetate	0.5	5.0	5.0	5.0
	5.0	5.0	5.0	5.0
Na benzoate	1.0	4.6	4.4	4.9
	2.0	4.9	5.0	4.9
	5.0	0.0	0.0	0.7
Ca propionate	1.0	4.9	5.0	4.6
	2.0	5.0	5.0	4.7
	5.0	3.0	0.9	3.6
K sorbate	1.0	5.0	5.0	4.9
	2.0	4.7	5.0	4.9
	5.0	0.0	0.0	1.6
Na formate	1.0	4.6	5.0	4.3
	2.0	4.7	5.0	4.1
	5.0	3.3	4.7	4.0
Na nitrite	1.0	5.0	5.0	4.1
	2.0	4.7	5.0	3.7
	5.0	4.9	5.0	5.0

Table 2c shows stake results from plant extractives. Ten percent thujaplicin, diluted in 95% ethanol was highly inhibitory to all test fungi. Thujaplicin, an extractive from western red cedar, has a pleasant odor and is not water-soluble. To determine if the ethanol diluent affected the results, control stakes were dip-treated with 95% ethanol. Ethanol did not inhibit growth of *A. niger* (5.0) or *T. viride* (5.0) in the control stakes, but was partially inhibitory to *P. chrysogenum* (0.5). Pine resin (50%) selectively inhibited *P. chrysogenum*. Concentrated soybean ester greatly accelerated mold growth.

Table 2c. Mold inhibition by plant extractives on unseasoned southern pine.

Plant extractives	Concentration	Average rating		
	(%)	*Penicillium chrysogenum*	*Aspergillus niger*	*Trichoderma viride*
Thujaplicin	10.0	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.6
	100.0	0.0	0.0	0.6
Pine resin	100.0	4.1	5.0	3.9
	50.0	1.9	4.4	3.9
Soybean ester	100.0	5.0	5.0	5.0

Test results for pharmaceuticals on unseasoned pine are shown in Table 2d. A number of azoles, nystatin and amphotericin B gave mixed results for mold inhibition in the stake test. Triazole, difluconazole, clotrimazole and amphotericin B failed to inhibit test fungi at the concentrations tested. In some instances, chemical concentrations were limited by product availability. Nystatin selectively and solely inhibited *A. niger*. Voriconazole, miconazole and thiabendazole completely inhibited growth of the test fungi at 2.5, 20 and

Table 2d. Mold inhibition by pharmaceuticals on unseasoned southern pine.

Pharmaceutical	Concentration	Average rating		
	(%)	*Penicillium chrysogenum*	*Aspergillus niger*	*Trichoderma viride*
Miconazole	2.0	0.0	0.0	0.0
1,2,4-Triazole	3.0	5.0	5.0	5.0
	5.0	3.6	4.6	4.9
1,2,4-Na-Triazole	3.0	5.0	5.0	5.0
	5.0	2.4	4.0	2.1
Thiabendazole	2.5	0.0	0.0	0.0
	5.0	0.0	0.0	0.0
Voriconazole	1.0	0.0	0.0	0.0
	0.5	0.0	0.6	0.3
	0.25	0.0	0.0	0.0
Itraconazole	10.0	1.9	0.3	4.1
Clotrimazole	1.0	3.4	5.0	3.7
Difluconazole	2.0	4.7	5.0	5.0
Amphotericin B	0.25	4.9	5.0	4.9
Nystatin	10,000 units/mL	2.9	0.0	5.0

25 mg/mL, respectively. Itraconazole selectively inhibited *A. niger*. Triazole antifungals generally inhibit fungal ergosterol biosynthesis; some azoles, such as voriconazole, inhibit fungal cytochrome P-450 enzymes selectively over other mammalian cytochrome P-450 enzymes.

Conclusions:

A chemical's ability to inhibit mold growth on agar cannot reliably predict the mildewcidal properties of the chemical on unseasoned wood. Minimal inhibitory concentrations of an effective mildewcide need to be higher ($\geq$10 times higher) in wood than in agar. This phenomenon is commonly known for chemicals that inhibit wood decay basidiomycetes (Clausen 1996) and may be more exaggerated for mold inhibitors due to the inherent chemical resistance of mold spores. A mildewcide intended for long-term protection of indoor wood-based materials needs to be non-toxic, non-volatile, odorless, and insoluble. Combinations of chemicals that successfully inhibit a variety of representative mold fungi on unseasoned pine stakes will be evaluated for a multi-component mildewcide.

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