Curbing Indoor Mold Growth with Mold Inhibitors

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Abstract

Environmentally acceptable mold inhibitors are needed to curb the growth of mold fungi in woodframe housing when moisture management measures fail. Excess indoor moisture can lead to rapid mold establishment which, in turn, can have deleterious affects on indoor air quality. Compounds with known mold inhibitory properties and low mammalian toxicity, such as food preservatives, pharmaceuticals, agricultural fungicides, wood preservatives, or plant extractives were evaluated individually and in combination for their ability to inhibit three common mold fungi on wood-based building materials. Dip-treatment of southern pine wood and southern pine oriented strandboard with azole-derivatives and plant extractives combined with borate-based compounds effectively prevented mold growth.

Introduction

Mold spores are inherently resistant to chemicals, UV, and even most wood preservatives. Mold growth can occur on wood in 24 to 48 hours if temperature and humidity conditions are optimal. Moisture is the key ingredient and any number of problems may attribute to excess moisture in existing structures, such as flawed design, poor construction practices or maintenance, poor site drainage, leaky roofs or plumbing, inadequate insulation, improper ventilation, etc. (2). A nutrient is also necessary, although accumulated detritus on virtually any material will support growth of the vegetative state of the fungus. Simple sugars from wood provide ample nutrition for growth of mold fungi, leaving wood-based building materials particularly vunerable to mold growth under conditions of chronic moisture exposure. Since even the best moisture management practices cannot prevent eventual moisture intrusion, economical mold inhibitors that are suitable for interior use are needed. In addition to being effective against mold fungi, they must be nontoxic, nonvolatile, environmentally acceptable, safe to handle, and possess low solubility (8). Surface treatment of dimension lumber or engineered products with mold inhibitors would add an additional layer of protection for in-service wood products and lessen the impact of current indoor air quality issues. This strategy is being used to some degree in the manufacture of gypsum board (5, 6) and oriented strandboard (OSB). The goal of this research was to evaluate compounds, such as food preservatives, wood preservatives, agricultural fungicides, pharmaceuticals, or natural plant extractives, with known fungicidal properties for their ability to inhibit growth of mold fungi on southern pine and OSB. Further, combinations of the compounds were evaluated for synergistic efficacy against mold fungi.

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Materials and Methods

Test Chemicals

***Experimental and Commercial Wood Preservatives and Additives.* —** Disodium octaborate tetrahydrate (5% wt/v) (Pole Maintenance Co, Columbus, NE);

CuBor (5% v/v); Cu+ (a zinc, boron combination) (5% v/v); and ethanolamine (25% v/v) (Sigma Chemical, St. Louis, MO) were diluted from the concentrations shown in parentheses until they demonstrated no mold inhibition. Additionally, a solution containing 5 percent boric acid (National Boraxx, Cleveland, OH), 25 percent propionic acid (JT Baker, Phillipsburg, NJ), 55 percent dimethylcocoamine (Lonza Inc., Fair Lawn, NJ), and 15 percent polyethylene glycol (Sigma, St. Louis, MO) was diluted from 2 percent v/v of the concentrate until no mold inhibition occurred.

Food Preservatives. **—** Sodium acetate, sodium benzoate, calcium propionate, potassium sorbate, sodium formate, and sodium nitrite (Sigma Chemical) were each diluted from 5 percent wt/v to the point of no mold inhibition.

Plant Extractives. **—** Thujaplicin (isopropyltropolone) (Cedarome Canada, Inc., Montreal, Quebec), pine resin, and soybean ester (The Heavens Group, LLC, Rolla, MO) were each diluted from 100 percent (neat) v/v in 70 percent ethanol to the point of no mold inhibition.

Pharmaceuticals and Agricultural Fungicides. **—** Triazole (5% wt/v), sodium triazole (5% wt/v), difluconazole (2% wt/v), thiabendazole (5% wt/v) (Sigma Chemical, St. Louis, MO), and voriconazole (1% wt/v) (Pfizer Inc., NY, NY) were tested at concentrations shown in parentheses and diluted to the point of no mold inhibition. A single concentration of miconazole (Pharmacia Upjohn, Kalamazoo, MI) (2% wt/v) and clotrimazole (1% v/v suspension in polyethylene glycol) (TARO Pharmaceuticals, Bramalea, Ontario) was tested, due to product availability.

Test Fungi

Aspergillus niger 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 were maintained on 2 percent malt agar (Difco, Detroit, MI). Individual spore preparations were prepared by washing the surface of a 2-week-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.

Mold Test

Specimens (7 by 20 mm 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 specimens was 48 percent by weight (n = 3). Southern pine OSB specimens (11 by 20 mm cross section by 7 cm long) were cut from a full sheet of OSB and conditioned to 70 percent relative humidity (RH). Seven random replicate stakes were dip-treated for ~ 15 seconds invarious concentrations of individual or combination biocides and held in a covered container overnight according to the ASTM standard test method D4445-91 (1). Specimens were arranged over four layers of blotting paper that had been saturated with 30 mL DI water and a polyethylene mesh spacer in sterile disposable Petri dishes (150 by 25 mm) (B-D Falcon, Los Angeles, CA). Untreated stakes dipped in DI water served as a control for water-based test chemicals. Stakes dipped in 70 percent ethanol served as a control for test chemicals of low aqueous solubility. Stakes were sprayed with 1 ml of mold spore inoculum, sealed in polyethylene bags to prevent drying and incubated at 27°C and 70 percent RH for 4 weeks. Following incubation, stakes were individually rated for mold growth on a scale of 0 to 5 with 0 representing clean specimens and 5 representing heavy mold growth on all surfaces.

Statistical Analysis

For compounds showing mold inhibition, the minimum concentration of fungicide that results in effective resistance to mold growth (0 rating on specimen) was statistically estimated as the minimal fungicidal concentration (MFC90) that will give at least a 90 percent probability of a 0 rating. Using SAS V8.2 (7), ratings were modeled as ordinal responses in cumulative complementary log-log models, which modeled the probability of ratings as functions of the logarithm of fungicidal concentrations.

Results and Discussion

Biocide efficacy is routinely tested on unseasoned pine for several reasons. First, building with unseasoned lumber is an accepted practice in some regions of the United States. Second, it reflects reality, because wood and wood-based products rarely remain dry throughout their entire service life. Third, if a biocide can protect unseasoned wood from mold growth, then it should have equal or greater success at protecting dry wood and wood-based products from mold establishment (4).

Average mold ratings for seven replicate specimens and corresponding chemical concentrations are shown in **Table 1.** Five percent was selected as the maximum concentration tested for mold inhibition. In most instances, except where noted, dip-treated solid pine specimens were challenged with individual test fungi. Sodium benzoate and potassium sorbate inhibited all test fungi at 2.5 percent. Calcium propionate was effective only against *A. niger* **(Table 1).** Five percent sodium acetate actually accelerated mold growth. Food preservatives are generally intended for short-term preserva-

Table 1. — ***Mold resistance of unseasoned pine dip-treated with various chemicals.***

Test chemical	(%)	Test organisms		
		A. niger	*T. viride*	*P. chrysogenum*
Food preservative				
Na acetate	5.0	5.0	5.0	5.0
Na benzoate	1.0	4.6	4.4	4.9
	2.0	2.9	3.1	2.4
	2.5	1.7	1.0	0.0
	3.0	0.4	0.4	0.0
	5.0	0.0	0.7	0.0
Ca propionate	1.0	5.0	4.6	4.9
	2.0	5.0	4.7	5.0
	5.0	0.9	3.3	2.0
K sorbate	1.0	5.0	4.9	5.0
	2.0	2.6	2.9	2.6
	2.5	0.0	0.7	0.0
	3.0	0.0	1.0	0.0
	5.0	0.0	0.0	1.7
Na formate	1.0	5.0	4.3	4.6
	2.0	5.0	4.1	4.7
	5.0	4.7	4.0	3.3
Na nitrite	5.0	5.0	5.0	4.9
Wood preservative				
DOT	2.0	5.0	4.7	4.0
	5.0	3.7	0.7	4.9
CuBor	2.0	4.9	3.3	4.7
	5.0	5.0	2.1	5.0
Borate-quat	0.5	5.0	5.0	5.0
	1.0	4.1	5.0	3.3
	1.5	4.3	2.1	3.1
	2.0	4.4	0.6	0.4
	2.5	4.3	1.4	0.4
	5.0	0.0	0.0	0.9
Cu-borate-zinc	1.0	4.7	4.7	3.3
	1.5	4.3	2.7	2.3
	2.0	2.6	0.6	0.0
	3.0	0.0	2.0	0.3
	5.0	0.0	0.0	0.0
Additive				
Ethanolamine	5.0	3.1	1.9	1.4
	10.0	0.9	2.4	1.6
	15.0	0.3	0.0	0.6
	25.0	0.0	0.0	0.0
Plant extractive				
Thujaplicin	0.4	5.0	1.0	0.0
	0.8	0.0	0.0	0.0
	1.5	0.0	0.0	0.0
Pine resin	100.0	5.0	3.9	4.1
Soybean ester	100.0	5.0	5.0	5.0
Pharmaceuticals				
Miconazole	2.0	0.0	0.0	0.0
Triazole	3.0	5.0	5.0	5.0
	5.0	4.6	4.9	3.6
Sodium triazole	3.0	5.0	5.0	5.0
	5.0	4.0	2.1	2.4
Difluconazole	2.0	5.0	5.0	4.7
Clotrimazole	1.0	5.0	3.7	3.4
Voriconazole	0.016	0.0	2.4	0.0
	0.031	0.0	1.3	0.0
	0.063	0.1	0.4	0.0
	0.125	0.0	0.1	0.0
Agricultural fungicide				
Thiabendazole	0.0098	0.0	2.5	0.0
	0.019	0.0	0.0	0.0
	0.039	0.0	0.0	0.0
Multi-component systems				
Borate-quat + azole[a]	2.0 + 0.1[b]	0.0	5.0	5.0
Borate-quat + thuja[a]	2.0 + 0.0[b]	0.0	5.0	5.0
Untreated control	0.0	5.0	5.0	5.0
Ethanol control	70.0	5.0	5.0	5.0

[a] Tested on solid pine and pine OSB.
[b] 2.0% borate-quat concentrate + 0.1% azole or thuja component.

tion of foods. However, their low mammalian toxicity makes them safe for use as an indoor mold inhibitor (3).

Disodium octaborate tetrahydrate and copper borate are safe to handle and economical. Neither were effective mold inhibitors at 5 percent, although both demonstrated partial inhibition of *T. viride*. 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).

Eighth percent (0.8%) thujaplicin, diluted in 70 percent ethanol was inhibitory to all test fungi. Thujaplicin, an extractive from western redcedar, has a pleasant odor and is not water-soluble. To rule out effects of the ethanol diluent, control stakes were dip-treated in 70 percent ethanol and challenged with test fungi. Ethanol did not inhibit growth of test fungi. Pine resin (100%) did not significantly inhibit test fungi. Concentrated soybean ester greatly accelerated mold growth.

Azoles gave mixed results for mold inhibition in the stake test. Triazole, difluconazole, and clotrimazole failed to inhibit test fungi at the concentrations tested. In some instances, chemical concentrations were limited by product availability. Voriconazole,

Table 2. ~ *Minimal fungicidal concentration (MFC_{90}) that will give at least a 90 percent probability of a 0 mold growth rating.*

Test chemical	MFC_{90}
Sodium benzoate	4.39
Calcium propionate	33.14
Potassium sorbate	3.69
Ethanolamine	24.16
voriconazole	0.04
Thiabendazole	0.02
Thujaplicin	0.78
Borate/quat combo	7.56
Cu-borate/zinc combo	4.08

miconazole, and thiabendazole completely inhibited growth of the test fungi at 2.5, 20, and 25 mg/mL, respectively. 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, resulting in extremely low mammalian toxicity, but cost remains a disadvantage.

Only test chemicals that substantially inhibited molds were analyzed for MFC90 **(Table 2).** Thiabendazole, voriconazole, and thujaplicin had a calculated MFC90 of 0.02, 0.04, and 0.78 percent, respectively and were further tested in combination with the borate/quat test solution. The combination of borate/quat solution combined with either voriconazole, thiabendazole, or thujaplicin displayed synergism against a mixed mold inoculum on solid pine and pine oriented strandboard when combined at a lower concentration than the MFC90 of the borate/quat component **(Table 2).** Testing of the individual azole and quat component, in the form of dimethylcocoamine, revealed that both components were responsible for control of mold fungi (4).

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