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Long-term efficacy of wood dip-treated with multicomponent biocides

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ABSTRACT

Biocides designed for prevention of indoor mold growth on wood-based materials need to provide long-term protection under conditions of high humidity. Specimens of kiln-dried southern pine and unseasoned southern pine, aspen, and Douglas-fir were dip-treated with borate-dimethylcocoamine (DMCA) supplemented with voriconazole, thiabendazole, or thujaplicin and evaluated at 4-week intervals for inhibition of three mold fungi, *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma viride*, using the ASTM D4445 mold test. After 8 weeks, all treatments of aspen and Douglas-fir inhibited growth of test fungi, while kiln-dried pine only inhibited *A. niger* and unseasoned pine only inhibited *T. viride* despite average chemical retentions in pine up to four-fold greater than those in aspen and Douglas-fir. After 12 weeks, aspen and Douglas-fir treated with the thiabendazole-containing biocide continued to provide protection against growth of the test fungi. The thujaplicin-containing biocide provided partial protection of aspen and Douglas-fir for 12 weeks but failed to protect pine samples beyond 4 weeks. A comparison of tebuconazole, propiconazole, voriconazole, and thiabendazole in the borate-DMCA biocide system showed that thiabendazole and voriconazole provided superior protection against the test fungi, particularly *T. viride*. We conclude that multicomponent biocides with thiabendazole protect aspen and Douglas-fir against growth of test fungi for at least 12 weeks under the conditions of the ASTM 4445 mold test.

Key words: Mold fungi, biocide, borate, azole, thujaplicin

1. INTRODUCTION

In order to address increased public concern about indoor air quality (IAQ) issues, wood protection systems need to be developed that go beyond the scope of traditional wood preservation, which primarily focuses on protecting wood used in outdoor applications. The strategy employed for developing indoor wood protection systems is quite different than that of wood preservation formulations intended for outdoor applications.

Ideally, building components should not bear the responsibility for prevention of mold growth in indoor climates. But in reality, predisposing conditions often exist, such as

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architectural design or improper construction practices that cannot or are not being controlled during construction. In addition, a number of factors may result in excess moisture in existing structures, such as poor site drainage, leaky roofs or plumbing, inadequate insulation, or improper ventilation to name a few (Clausen 2002). Improvements to building component durability are one reactionary measure until proactive approaches are developed. No matter how meticulous the maintenance on a building, eventually every structure will encounter a moisture event that may be as obvious as flooding or as subtle as a chronic leak inside a wall that only becomes obvious in advance stages of biological activity (Clausen and Yang 2004). Since even the best moisture management practices cannot prevent eventual moisture intrusion, economical, nontoxic, stable, nonvolatile biocides that are suitable for interior use are needed. In addition, they should be environmentally acceptable, safe to handle, and possess low solubility (Zabel and Morrell 1992). Surface treatment of dimension lumber or incorporating a biocide into engineered products would add an additional layer of protection for in-service wood products. Clearly, treating construction materials with an effective biocide would lessen the impact of current IAQ issues. This strategy is already being employed in the manufacture of some building materials, such as gypsum (Fogel and Lloyd 2000; 2002) and oriented strandboard. In addition to being effective against mold fungi, biocides must be nonvolatile, environmentally acceptable, safe to handle, and possess low solubility (Zabel and Morrell 1992). In this study, we evaluate the long-term efficacy of multicomponent biocides on several species of wood and compare the efficacy of several azoles in the multicomponent system.

2. EXPERIMENTAL METHODS

2.1 Test Organisms

Mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476, were grown on 2% malt agar, and a mixed spore preparation was prepared by washing the surface of a 2-wk-old culture of each fungus with 10 mL of sterile deionized (DI) water according to the American Society for Testing and Materials (ASTM) standard D4445-91 (ASTM 1998). Spore suspensions were transferred to a spray bottle, diluted to 100 mL with DI water, and adjusted to deliver 1 mL inoculum per spray that contained approximately 10^7 spores per mL.

2.2 Test Chemicals

The borate-DMCA base was comprised of 5% boric acid (National Boraxx, Cleveland, OH), 25% propionic acid (JT Baker, Phillipsburg, NJ), 55% dimethylcocoamine (Lonza Inc., Fair Lawn, NJ), and 15% polyethylene glycol (Sigma, St. Louis, MO). Two percent borate-DMCA base was supplemented with 0.1% voriconazole (Pfizer Inc., New York, NY), 0.1% thiabendazole (Sigma-Aldrich Chemical, St. Louis, MO) in 70% ethanol, 0.1% tebuconazole (Bayer Corporation, Pittsburgh, PA), 0.1% propiconaole (Janssen Pharmaceutica, Titusville, NJ), or 0.5% thujaplicin (isopropyltropolone, Cedarome Canada, Inc., Brossard, Quebec) in 70% ethanol.

2.3 Mold Test

Specimens (7 by 20 mm in cross section by 7 cm long) cut from unseasoned southern pine mill ends from a Mississippi sawmill, unseasoned Douglas-fir (provided by Weyerhaeuser Company, Federal Way, WA), and unseasoned aspen were stored at 0°C. Kiln-dried southern yellow pine specimens were cut to the same dimensions and stored at 27°C and 70% relative humidity (RH). Five random replicate specimens of each wood type were dip-treated for ~15 seconds in 2% borate-DMCA base containing thiabendazole, voriconazole, or thujaplicin and

held in a covered container overnight according to the ASTM standard test method D4445-91 (ASTM 1998). For a comparison of azoles, five specimens of unseasoned southern pine were dip-treated with 2% borate-DMCA base containing propiconazole or tebuconazole. Specimens were arranged over four layers of blotting paper that was 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, USA). Untreated stakes dipped in DI water served as a control for water-based test chemicals. Stakes dipped in 70% ethanol served as a control for test chemicals of low solubility. Stakes were sprayed with 1 mL of the mold spore inoculum, sealed in polyethylene bags to prevent drying and incubated at 27°C and 70% relative humidity (RH) for 4 weeks. Following incubation, stakes were individually rated for mold growth on a scale of 0 to 5 with 5 representing heavy mold growth. Following the 4-week ratings, all specimens except those treated with propiconazole and tebuconazole were reinoculated and reevaluated for mold inhibition at 4-week intervals. Moisture in the test apparatus was adjusted to provide 100% RH, and specimens were incubated at 27°C and 70% RH.

2.4 Chemical Retention

Retention of test chemical was determined by weighing conditioned specimens pre- and post-dipping and calculating the average amount of chemical retained during the 15-second dip based on the average volume of the test specimen. Retentions were expressed as kg/m^3.

3. RESULTS AND DISCUSSION

At moisture contents greater than 20%, mold establishment can occur on unseasoned wood in 24 to 48 hours if temperatures permit and rapid drying of the wood does not occur. The high moisture content of the test method used in this study provides a particularly rigorous evaluation of potential biocides. If a biocide is efficacious on unseasoned wood, it is more likely to provide long-term protection in high moisture situations. To test the long-term efficacy of the multicomponent formulations, treated specimens were rated for mold growth and reinoculated with test fungi at 4-week intervals. Moisture content of the test apparatus was maintained at 100% RH throughout the test.

Average retention rates for multicomponent biocides are shown in Table 1. Kiln-dried pine had the highest average retention for all three moldicides. Average retentions did not vary greatly between the water-based voriconazole preparation and the ethanol-based thiabendazole and thujaplicin-containing preparations. The greatest variability in retention between the preparations was seen in unseasoned pine, which had an average moisture content of 48% by weight (n = 3). In comparison, unseasoned Douglas-fir and aspen had an average moisture content of 26% and 54% by weight, respectively (n = 3). Overall, average retentions for unseasoned aspen and Douglas-fir were ~0.50 kg/m^3.

Table 1: Average retention rate of chemical treatments on different wood species and conditions

Treatment[a]	Retention [kg/m^3]			
	Pine kiln-dried	Pine unseasoned	Aspen unseasoned	Doug.-fir unseasoned
0.1% voriconazole	2.34	2.19	0.52	0.46
0.1% thiabendazole	1.89	0.83	0.56	0.54
0.5% thujaplicin	1.76	1.28	0.49	0.56

[a]2% borate-DMCA base plus supplemental component.

Previous studies showed that multicomponent biocide systems combining a borate-DMCA base supplemented with 0.1% voriconazole, 0.1% thiabendazole, or 0.5% thujaplicin inhibited *A. niger*, *P. chrysogenum*, and *T. viride* for 4 weeks (Clausen and Yang 2003, 2004). After 8 weeks, all treatments inhibited growth of the test fungi on aspen and Douglas-fir. The azole-containing biocides protected kiln-dried pine from *A. niger* and unseasoned pine from *T. viride*, even though pine chemical retentions were up to four-fold greater than those of aspen and Douglas-fir. The thujaplicin-containing biocide offered no protection of pine, either kiln-dried or unseasoned, beyond 4 weeks. After 12 weeks, only aspen treated with the three experimental formulations continued to inhibit test fungi. Despite retentions up to four-fold lower in aspen and Douglas-fir than in pine, treatments of these two wood species provided protection for 4 to 8 weeks longer in the ASTM mold test.

Quaternary ammonium compounds are known to inhibit decay fungi (Butcher *et al.* 1977; Preston *et al*. 1987). Dimethylcocoamine (DMCA), a tertiary amine intermediate in the manufacture of quaternary ammonium compounds, is one of the components of the borate-DMCA base. We have previously shown that at the levels used in this study, the DMCA alone is not responsible for inhibition of the mold fungi (Clausen and Yang in press). Borates alone are only marginally effective at controlling mold fungi (Barnes *et al.* 1989). Indeed, 15% DOT was unable to substantially inhibit mold fungi (Clausen and West, in press), and the minimum fungicidal concentration (MFC_{90}) has been estimated as 7.6% for the borate-DMCA base used in this study (3.7 mg/mL boric acid) (Clausen and Yang 2005). The estimated MFC_{90} for voriconazole, thiabendazole, and thujaplicin are 0.043%, 0.016%, and 0.78%, respectively. Components in the combination biocides in this study act synergistically to offer protection against mold and decay fungi as well as *Reticulitermes* termites (Clausen and Yang 2004).

Table 2: Average mold ratings for three multicomponent biocides evaluated at 4-week intervals

Test chemical[a]	Specimen type[b]	Condition	Time [weeks]	Average mold rating[c]		
				T. viride	*P. chrysogenum*	*A. niger*
Thiabendazole	Aspen	unseasoned	4	0	0	0
			8	0	0	0
			12	0.2	0	0.8
	Doug-fir	unseasoned	4	0	0	0
			8	0	0	0
			12	2.4	0.8	0.4
	SYP	unseasoned	4	0.8	0	2.2
			8	1.6	4.2	4.2
			12	1.8	4.4	4.6
	SYP	kiln-dried	4	0	0	0
			8	3.8	4.4	0
			12	4.8	4.6	2.4
Voriconazole	Aspen	unseasoned	4	0	0	0
			8	0	0.4	1
			12	0.4	1.8	3.2
	Doug-fir	unseasoned	4	0	0	0
			8	0.2	0	0
			12	1	5	4
	SYP	unseasoned	4	0.4	0	0
			8	0.4	4	3
			12	0.4	3.8	4
	SYP	kiln-dried	4	0	0	0
			8	3.4	4.8	1
			12	4.4	4.8	4.2
Thujaplicin	Aspen	unseasoned	4	0	0	0
			8	1.8	0.8	0.8
			12	2	1.4	2.6
	Doug-fir	unseasoned	4	0	0	0
			8	0	0	0
			12	2.8	2.8	2.6
	SYP	unseasoned	4	2.6	0.6	1.4
			8	3.4	3.6	4.8
			12	3.6	4.2	4.8
	SYP	kiln-dried	4	0	0	0
			8	4.4	3.6	1.8
			12	4.4	3.4	4.4
Control	Aspen	unseasoned	4	3	4.4	4
	Doug-fir	unseasoned	4	4.4	3.2	2.8
	SYP	unseasoned	4	5	5	5
	SYP	kiln-dried	4	4.7	3.6	4.3

[a]2% borate-DMCA base plus supplemental component.
[b]SYP, southern yellow pine.
[c]Average rating of 0 equals a clean specimen and 5 equals complete coverage of the specimen.

To compare the effectiveness of azoles commonly used by the wood preservation industry, tebuconazole and propiconazole were compared with thiabendazole and voriconazole in a 4-week mold test. Comparative specimen ratings are shown in Table 3. At the same concentration, voriconazole and thiabendazole provided greater protection from the test fungi, particularly *T. viride,* compared with propiconazole and tebuconazole. This is due, in part, to the mode of action for voriconazole. Whereas many azoles rely on inhibition of several membrane-bound enzymes and membrane lipid biosynthesis as a mode of action, voriconazole specifically inhibits cytochrome P-450-dependent 14α-sterol demethylase (P-450_{DM}) as the mode of action. It has been demonstrated to be about 250 times more active against fungal P-450_{DM} than mammalian P-450_{DM} (Hitchcock *et al.* 1995). Not only is voriconazole less toxic to humans, but it has a stronger avidity for 14α-lanosterol demethylation found in mold fungi (Clausen and Yang 2005).

Table 3: Comparative mold resistance of southern pine dip-treated with four azole compounds

Test chemical[a]	Average mold rating[b]		
	T. viride	*P. chrysogenum*	*A. niger*
Tebuconazole	4.2	1.8	0.6
Propiconazole	3.2	0.2	0.6
Voriconazole	0.4	0	0
Thiabendazole	0.8	0	2.2
Control	5	5	5

[a] 2% borate-DMCA base plus 0.1% of the respective azole compound.
[b] 4-week rating: 0 equals a clean specimen and 5 equals complete coverage of the specimen.

4. CONCLUSIONS

Treatment of aspen, Douglas-fir, and pine with multicomponent biocides containing a borate-DMCA base supplemented with an azole or thujaplicin revealed variability in long-term efficacy of the biocides against three test fungi. The best results were observed for thiabendazole-treated aspen and Douglas-fir. For the voriconazole-treated specimens, aspen inhibited *T. viride* and *P. chrysogenum* for 12 weeks, Douglas-fir inhibited all test fungi for 8 weeks but only *T. viride* for 12 weeks, kiln-dried pine only inhibited test fungi for 4 weeks, while unseasoned pine continued to inhibit *T. viride* for 12 weeks. For the thujaplicin treatment, aspen partially inhibited test fungi after 12 weeks, Douglas-fir inhibited all test fungi for 8 weeks, while kiln-dried and unseasoned pine only inhibited test fungi for 4 weeks. All treatments were least effective on pine despite an approximate four-fold increase in chemical retention compared with aspen and Douglas-fir. Douglas-fir and aspen appeared to have some inherent mold resistance, even though untreated controls failed to inhibit test fungi after 4 weeks. In the multicomponent borate-DMCA system, voriconazole and thiabendazole provided superior mold inhibition compared with tebuconazole and propiconazole.

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