

Multi-component Biocide Protects Wood from Fungi and Insects in UC2 Applications

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ABSTRACT

Results that demonstrate wood protection from mold, decay and termites in laboratory and above ground field tests are presented for an experimental synergistic biocide, called Durazol.

Keywords: biocide, moldicide, termiticide, decay fungi, termite, synergy

INTRODUCTION

Development of synergistic biocides to protect wood in interior applications has been of particular interest since the recent increase of indoor mold infestations. Many products have been developed to address the recent influx of indoor mold infestations. Researchers must emphasize the use of environmentally benign chemicals due to the need for safety of human occupants. Some typical strategies employed include one or more of the following: naturally occurring antimicrobial or insecticidal chemicals, synergistic combinations of chemicals, chemicals with known performance (and previous EPA registration). Unfortunately, safely controlling fungal growth in the same environment as human occupants is difficult to accomplish. Since fungi and humans are both eukaryotic, (i.e. higher multi-cellular organisms with organized nuclei), metabolic inhibitors of fungi are likely to be toxic to humans (Clausen and Yang 2005a). Use of naturally occurring antimicrobials needs to be approached with caution, since many but not all naturally occurring compounds are toxic in tissue culture. Chemicals with previous EPA approval are limited, but certainly, new, unique combinations of such chemicals can create new formulations with antimicrobial or insecticidal properties (Clausen and Yang 2003). Occasionally, combinations of known fungal or insect inhibitors are synergistic, i.e. combined chemicals are more effective than the individual components at higher concentrations. One such synergistic combination, called Durazol, incorporates some known antimicrobials and insecticides, namely boric acid and propionic acid with a quaternary amine compound and an azole to provide protection against mold fungi, stain fungi, decay fungi, and termites at lower concentrations than any of the individual components alone (Clausen and Yang 2004; 2005b; 2005c; 2007). A patent application was filed with the US Patent and Trade Office (USPTO) on 3/21/07 (Clausen et al. 2007).

MATERIALS AND METHODS

Organisms

Mold fungi: Three mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02 and *Trichoderma viride* ATCC 20476 were grown and maintained on 2% malt agar (Difco, Detroit, MI, USA) at 27°C, and 80% relative humidity (RR). *Aureobasidium pullulans* MDX-18 was grown and maintained on 2% potato dextrose agar at *NOC*, and 80% relative humidity .. Spore suspensions of test fungi were prepared by washing the surface of individual 2-wk old Petri plate cultures with 10-15 ml of sterile deionized water (DI) according to ASTM standard D4445-91 (ASTM 1998). The wash water was transferred to a spray bottle and diluted to approximately 100 mL with DI water to yield $\sim 3 \times 10^7$ spores/ml. The spray bottle was adjusted to deliver 1 mL inoculum per spray. Individual spore suspensions were used to inoculate

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mono culture Petri plate tests or the mold test chamber, while *Aureobasidium pullulans* was used exclusively for inoculation of the mold chamber test.

Decay fungi: Two brown-rot fungi, *Gloeophyllum trabeum* Mad-563 and *Postia placenta* Mad-698 and one white-rot fungus, *Trametes versicolor* Mad-697 were grown and maintained on 2% malt extract agar at 27°C and 70% RH.

Termites: Subterranean termites, *Reticulitermes flavipes* were collected in Janesville, WI and maintained at the Forest Products Laboratory, Madison, WI. *Coptotermes formosanus* were collected and maintained at the Formosan Termite Research Facility in McNeill, MS.

Chemical formulation

Durazol's chemical formulation consists of 0.5% propionic acid, 0.1% boric acid, 1.1% dimethyl cocoamine, 0.1% thiabendazole and 0.3% propylene glycol in an aqueous solution, however, due to low solubility, thiabendazole must be solubilized in 70% ethanol prior to addition to the treatment solution.

Treatment method and chemical retention

Unseasoned southern pine specimens were brushed, sprayed, or immersed (15 sec) in treatment solution. Chemical treatment retentions were determined from the average difference in specimen weight before and after dipping and reported based on the volume of the specimen. Specimens were held in a closed container for 24 hr at 25°C prior to challenge with test organisms. Additionally, chemical retention in unseasoned pine was compared with kiln-dried pine. Retention rates were also compared for unseasoned pine, aspen, and Douglas-fir.

Mold test

Petri plate test: Five treated specimens and untreated controls were placed in a Petri dish (150x25mm) (B-D Falcon, Los Angeles, Calif.) containing four layers of blotting paper that was saturated with 30 mL DI water. A polyethylene mesh spacer was used to elevate specimens and prevent chemical leaching. Specimens were sprayed with 1 mL of individual spore suspension 24 hr post-treatment. Petri dishes were sealed in polyethylene bags to prevent drying, and incubated at 27°C, 70% RH. Specimens were individually rated for mold growth at 4 and 8 wk on a scale of 0 to 5 with 0 indicating no growth and 5 indicating heavy growth.

Environmental chamber test: A polypropylene mold chamber fabricated according to ASTM 3273-00 (ASTM 1986) was placed in a room maintained at 30°C and 70% RH. The mold chamber relies on a circulating fan above water to provide 100% humidity and non-sterile soil DJr a supplemental source of circulating mold spores. The soil was additionally inoculated with mold spores from three test fungi, *Aureobasidium pullulans*, *Aspergillus niger* and *Penicillium chrysogenum*, two weeks before placing the test specimens in the chamber. Five southern pine test specimens (75 x 100 mm by 12.5 mm-thick) were conditioned at *NOC*, 70% RH and weighed prior to immersion in Durazol for ~15 sec. Treated specimens were reweighed and held for 24 hr in a closed container. Treated specimens and untreated controls were vertically suspended across the width of the environmental chamber over inoculated soil. Specimens were sprayed with spore suspensions of *A. niger*, *P. chrysogenum* and *T. viride* and incubated at 30°C. After 8 wk incubation, specimens were individually rated for mold growth on a scale of 0 to 5 with 0 indicating no growth and 5 indicating 100% coverage.

Soil block decay test

Soil block culture bottles were prepared according to AWP A E10 (2006) with a modification of wood block size to 1 x 1 x 1 cm. In soil block bottles, southern yellow pine feeders were inoculated with brown

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rot fungi, *G. trabeum* and *P. placenta* and maple feeders were inoculated with the white-rot fungus, *T. versicolor*. Bottles were incubated at 27°C and 70% RH for 3 weeks until the fungus completely colonized each feeder. Pre-weighed southern yellow pine or maple blocks, conditioned at 27°C and 70% RH, were vacuum treated with 2 % Durazol for 2 x 20 min at 25 in Hg. Based on solution uptake, the boron retention in the blocks was 0.048 pcf (as B₂O₃). The total Durazol retention was 0,97 pcf. Treated blocks were conditioned for 2 wk at 27°C, 70% RH and reweighed before adding them to pre-grown soil block bottles and incubating at 27°C, 70% RH for 12 weeks. Untreated blocks served as controls. Following incubation, surface mycelia was removed and blocks were dried at 60°C overnight before reconditioning at 27°C, 70% RH for 2 wk. Blocks were reweighed and the average percent weight loss was calculated.

Termite test

A laboratory no-choice test was conducted according to AWP A El (2006) with *Reticulitermes jlavipes* at USDA Forest Products Laboratory in Madison, WI and on *Coptotermes formosanus* at the Formosan Termite Research Facility at McNeill, MS.

Above ground test methods

Ground proximity test: A standard test for evaluation of preservative treatments for lumber and timbers against subterranean termites in above-ground, protected applications (DC 1 and UC2) (AWPA E21) was initiated in July 2006 on southern yellow pine pressure-treated with 2% or 4% Durazol (0.97 and 1.93 pcf, respectively), disodium octaborate tetrahydrate (0.37 pet), and untreated controls (N=10). Specimens will be evaluated annually for termite and fungal decay.

Above ground deck test: Five 2" x 6" x 30" long southern pine deck boards were pressure treated with 2% Durazol and installed in a moderate decay hazard zone in Madison, WI. After 2 years, there are no signs of decay, mold, or stain on any surface of the treated specimens. They are rated as sound with no evidence of weakening, softening, or discoloration from deteriorating organisms.

RESULT AND DISCUSSION

Chemical Retention

Comparative retention rates for Durazol applied by spray, brush and dip are shown in Table 1. Brushing and immersion provide roughly twice the treatment that spraying does. Retention rates for different wood types dip-treated with Durazol and the comparison of kiln-dried with unseasoned SYP are shown in Table 2. Pine retains the most treatment whether it is kiln-dried or unseasoned. Unseasoned Douglas-fir and aspen demonstrate similar retention rates, although they both retained approximately 3.4 times less than kiln-dried pine and 1.5 times less than unseasoned pine.

Table 1. Comparison of Durazol retention rate for brush, spray and dip application on southern yellow pine.

Application method	Ave. retention (kg/m ³)
Spray	0.54
Brush	0.95
Dip	0.83

Table 2. Comparative retention of different wood types and seasoning conditions dip-treated with 2% Durazol.

Treated wood type	Retention (kg/m ³)
Kiln-dried	
Pine	1.89
Unseasoned	
Pine	0.83
Aspen	0.56
Doug-fir	0.54

Mold results

Dip treatment of specimens with 2% Durazol resulted in complete inhibition of all test molds in the Petri plate test (rating of 0 = clean) after 4 weeks compared to untreated controls (rating of 5 = 100% mold growth). Likewise, specimens dip-treated with Durazol provided complete inhibition of mold growth (both inoculated organisms and those prevalent in the soil) in the environmental chamber method for 8 wk (average rating of 0) while untreated controls had an average rating of 4.7 on a scale of 0-5. Average retention of the test specimens in the environmental chamber was 0.04 g/cm³.

Decay results

Southern pine vacuum treated with 2% Durazol (0.97 pcf) effectively inhibited decay by two brown-rot fungi, *Postia placenta* and *Gloeophyllum trabeum* and one white-rot fungus, *Trametes versicolor* in a 12 wk soil block test (Table 3).

Table 3. Durazol efficacy against two brown-rot fungi and one white-rot fungus.

Treatment	Average Weight Loss (%)		
	<i>P.placenta</i>	<i>G. trabeum</i>	
Durazol	0	7	0
None	31 ± 8	42 ± 9	30 ± 4

Termite results

Durazol treated southern pine resisted attack and consumption by both *Reticulitermes flavipes* and *Coptotermes formosanus* (Table 4). The lower concentration of borate in Durazol compared to the commercial retention of DOT did not kill termites in the laboratory tests. *Reticulitermes* sp. is the principle termite pest in the Northern hemisphere: Canada, USA, Mexico, Europe, Middle East, Northern Africa, India, Southern Russia, Korea, Japan and China. However, the invasive Formosan subterranean termite, *Coptotermes formosanus* is present in 14 southern states, and accounts for ~1/3 of the total US subterranean termite control and repair costs annually. New wood protection systems for UC2 applications will attract national marketability if they are able to inhibit infestation of both types of subterranean termites.

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Table 4. Resistance of Durazol-treated pine to subterranean termite attack in a no-choice test.

Treatment	<i>Reticulitermes</i> Bioassay			<i>Coptotermes</i> Bioassay		
	Attack rating	% wood consumption	mortality rating	Attack rating	% wood consumption	mortality rating
Durazol	8.8	3.3	moderate	9.5	4.4	none
DOT	ND	ND	ND	9.5	4.2	100%
None	0	44.3	none	0	38.2	none

CONCLUSIONS

Durazol, a synergistic combination biocide, was effective as a surface treatment to protect wood from mold growth. It was equally effective as a pressure treatment against decay fungi and termites in laboratory tests, although the retention evaluated inhibited feeding but did not kill *Coptotermes formosanus*. Above ground deck test and ground proximity tests are in progress. Patent application #11/726,359 was filed with USPTO on 3/21/07 (Clausen et al. 2007).

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