IRG/WP 04-30333

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 3 Wood Protecting Chemicals

Multicomponent biocide systems protect wood from decay fungi, mold fungi, and termites for interior applications

Carol A. Clausen and Vina W. Yang

USDA Forest Service
Forest Products Laboratory
One Gifford Pinchot Drive
Madison, Wisconsin 53726 USA

Paper prepared for the 35th Annual Meeting
Ljubljana, Slovenia
6–10 June, 2004

IRG Secretariat
SE-100 44 Stockholm
Sweden

Multicomponent biocide systems protect wood from decay fungi, mold fungi, and termites for interior applications

Carol A. Clausen and Vina W. Yang
USDA Forest Service, Forest Products Laboratory, Madison, WI. USA 53726

Abstract

Concerns about indoor air quality due to mold growth have increased dramatically in the United States. In the absence of proper 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 be nontoxic, nonvolatile, odorless, and hypoallergenic. Multicomponent systems were tested in AWPA soil block tests for inhibition of brown-rot and white-rot fungi, and ASTM standard tests for inhibition of mold fungi and termites. Multicomponent biocide systems combining a borate-base supplemented with voriconazole or thiabendazole, and either thujaplicin, or ethanolamine performed well against two brown-rot fungi, *Postia placenta,* and *Gloeophyllum trabeum*, the white-rot fungus, *Coriolus versicolor*, three mold fungi, *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma viride*, and the subterranean termite, *Reticulotermes flavipes* (Kollar). We conclude that a multicomponent biocide system containing Bor-A+ supplemented with azoles and either ethanolamine or thujaplicin can protect wood from decay fungi, mold fungi, and termites for interior applications.

Key words: Mold fungi, decay fungi, borate, biocide, termidicide, mildewcide, azole

Introduction

From a wood protection perspective, increased public concern about indoor air quality (IAQ) and mold infestations simply means that chronic moisture problems in residential structures are finally receiving the attention they deserve. Decay fungi have been responsible for steady annual losses of over $1 billion in the United States to in-service wood (Scheffer 1973) and the combined damage from Eastern subterranean termites and Formosan subterranean termites exceeds 2 billion US dollars annually. Mold claims, including pre- and post-construction, exceeded $2.8 billion in 2002. Moisture is the key ingredient for all three types of biological damage to structures. Any number of reasons may be responsible for excess moisture in existing structures, such as original design, poor construction practices, poor site drainage, leaky roofs or plumbing, inadequate insulation, improper ventilation, etc (Clausen 2002). Unfortunately, new structures are equally susceptible to these problems. Chronic moisture in a structure can lead to a cascading biological succession from mold fungi to decay fungi to insect infestation.

Water vapor in humid air will not wet wood sufficiently to support the growth of decay fungi, but will permit mold growth. A chronic leak, however, can eventually increase the moisture content of wood above the fiber saturation point and initiate decay. Termites seek water and are not only attracted to conditions that might also be favorable for growth of decay and mold fungi, but there is evidence that they are attracted to wood that has been decayed by certain brown-rot fungi. Once established, it is possible for mold, decay fungi, and termites to independently increase the moisture in the infested area through water trapping or transporting, or the conversion of cellulose to carbon dioxide and water.

Ideally, determining and controlling the sources of moisture, as well as rapidly drying wetted building components will prevent or arrest fungal growth and insect infestations. 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. Since even the best moisture management practices cannot prevent eventual moisture intrusion, economical, non-toxic, stable, nonvolatile fungicides that are suitable for interior use are needed. 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 a biocide would lessen the impact of current IAQ issues. This strategy is already being used in the manufacture of some building materials, such as gypsum (Fogel and Lloyd 2000; 2002) and oriented strand board. Addition of zinc borate and DOT during the manufacture of wood composites has been shown to prevent termite damage (Grace 1997; Manning *et al.* 1997; Tsunoda *et al.* 2002). Other examples of incorporating biocides into products for interior use are mildewcides in paint formulations and household disinfectants. 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). This study expounds on the results from Clausen and Yang (2003) by developing a multicomponent biocide system suitable for protection against mold fungi, decay fungi, and termites for interior applications.

Materials and Methods:

Test organisms

Decay fungi, *Postia placenta* MAD 698, *Gloeophyllum trabeum* MAD 617, and *Coriolus versicolor* MAD 697, were maintained on 2% malt agar (Difco, Detroit, MI, USA). 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 (1998). Spore suspensions were transferred to a spray bottle and diluted to 100 mL with DI water. The spray bottle was adjusted to deliver 1 mL inoculum/spray. Subterranean termites, *Reticulotermes flavipes* (Kollar), were collected in Janesville, WI., USA for the termite bioassay.

Test chemicals

Disodium octaborate tetrahydrate (DOT) (US Borax, Inc., Valencia, CA)
Boric acid (5%) and dimethylcocoamine (80%) (Bor-A+) (Copper Care Wood Preservatives, Inc., Columbus, NE)
Voriconazole (Pfizer Inc., NY, NY)
Thiabendazole (Sigma-Aldrich Chemical, St. Louis, MO)
Thujaplicin (isopropyltropolone) (Cedarome Canada, Inc., Montreal, Quebec)
Ethanolamine (Sigma Chemical, St. Louis, MO)

Mold test

Specimens (7 x 20 mm cross section by 7 cm long) were cut from unseasoned southern pine mill ends from a Mississippi sawmill and stored at 0^{o}C. Average moisture content of the specimens was 48 % by weight (n=3). Southern pine oriented strand board (OSB) specimens (11 x 20 mm cross section by 7 cm long) were cut from a full sheet of OSB and conditioned to 70% RH. Five random replicate stakes were dip-treated for ~15 seconds in each biocide combination (Table 1) and held in a covered container overnight according to the ASTM standard test method D4445-91 (1998). Specimens were arranged over 4 layers of blotting 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). Untreated stakes

Table 1. Mold resistance and soil block test results for various multicomponent biocide systems

Treatment (mg/mL)			Mold resistance Average rating [a]		Soil block test results Average % wt. loss ±SE		
Borate component	Azole component	Additional component (%)	Pine	OSB	*P. placenta*	*G. trabeum*	*C. versicolor*
DOT	**Voriconazole**	**Ethanolamine**					
20	1	5	5	5	0	0	0
20	0	5	5	5	0	0	0
Bor-A+ [b]							
20	**1**	**5**	**1.8**	**0**	**0**	**0**	**0**
20	0	5	2.8	1.4	0	0	7
None	1	5	5	5	0	0	6
"	0	5	2.6	4	0	1	2
DOT	**Thiabendazole**	**Thujaplicin**					
20	1	0.5	5	1	0	0	0
20	1	1	4	0.4	0	0	0
20	1	0	5	0.6	0	0	0
20	0	0.5	4.2	2.6	0	0	0
20	0	1	5	0.6	0	0	0
Bor-A+							
20	**1**	**0.5**	**0**	**0**	**1**	**1**	**0**
20	1	1	0	0	0	0	0
20	**1**	**0**	**0**	**0**	**0**	**7**	**0**
20	**0**	**0.5**	**0**	**0**	**1**	**1**	**2**
20	0	1	0	0	1	10	1
None	0	1	3.2	5	59±5.3	38.4±6.6	14.8±9.3
"	0	0.5	0.6	0.4	59.4±7.9	54.4±9.3	29.6±4.4
"	0	0	5	5	31±8	42.4±8.8	16.8±6.8
EtOH control	0	0	5	5			

[a]Average rating for 5 test specimens; 0=no growth; 1=20%; 2=40%; 3=60%; 4=80%; 5=100% coverage with mold fungi.
[b]20 mg/mL Bor-A+ is equivalent to 1 mg/mL boric acid.

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 0–5 with 5 representing heavy mold growth.

Decay test

Soil block culture bottles were prepared according to American Wood Preservers' Association (AWPA) E-10-01 (2003). Southern pine feeders were inoculated with two brown-rot fungi, *P. placenta* and *G. trabeum* and maple feeders were inoculated with one white-rot fungus, *C. versicolor*. Bottles were incubated at 27°C and 70% RH for 3 weeks until the fungus completely colonized each feeder. Pre-weighed 1 x 1 x 1 cm southern pine blocks, conditioned at 27°C and 70% RH, were vacuum-treated for 40 minutes at 172 kPa with each biocide combination listed in Table 1 (n=5/fungus). Blocks were conditioned at 25°C for 7 days, propylene oxide-sterilized, placed on actively growing feeders, and incubated at 27°C, 70% RH for 12 wk. Following incubation, surface mycelium was brushed from each block, blocks were oven-dried at 60°C for 24 hr and reconditioned at 27°C and 70% RH to a constant weight. Blocks were weighed and average percentage weight loss was calculated.

Table 2. Visual rating, mortality rate and relative wood consumption in a termite bioassay against two multicomponent biocide systems

Treatment (mg/mL)		Additional component	Visual rating[a]	100% mortality	Mean mortality	Relative wood consumption	
Borate component	Azole component	(%)		# days	# days	(g/g termite)	Ave ± SE
DOT	**Voriconazole**	**Ethanolamine**					
20	1	5	10	13	13.6±3	0.5	0.6±0.1
20	1	5	10	19		0.5	
20	1	5	10	12		0.5	
20	1	5	10	12		0.7	
20	1	5	10	12		0.7	
Bor-A+							
20	1	5	10	12	10.8±1.6	0.9	0.9±0.1
20	1	5	10	12		1.0	
20	1	5	10	12		1.0	
20	1	5	10	9		0.8	
20	1	5	10	9		0.8	
None							
0	0	0	9	26	20±4.6	3.9	1.8±1.5
0	0	0	9	19		0.6	
0	0	0	9	21		0.0	
0	0	0	9	21		1.7	
0	0	0	9	13		0.8	

[a]Rating scale: 10=no attack; 9=light attack

Termite test
Pre-weighed and pre-conditioned southern pine blocks (1 x 1 x 1 cm) (n=5) were dip-treated with two multicomponent biocides (Table 2). Five control blocks were treated in DI water. All blocks were conditioned to ensure all solvent had dissipated prior to being subjected to a termite bioassay according to a no-choice test procedure (ASTM 1998). Each block was placed in a test dish with 1g termites. Damp filter paper in the lid of each dish helped maintain humidity during the 4-wk incubation at 27°C and 80% RH. Dishes were examined daily and mortality was recorded. At the end of four weeks, blocks were removed from the dishes, cleaned, dried, re-conditioned and weighed to determine weight loss. Termite survival rates were recorded during and after the test period. A visual rating of attack was recorded for each block.

Results and Discussion:

Mold test results
At moisture contents greater than 20%, mold establishment can occur on unseasoned wood in 24-48 hrs if temperatures permit and rapid drying of the wood does not occur. The high moisture content of the specimens used in this study might seem like an unusually rigorous test of the efficacy of a fungicide. Not only is green lumber commonly used in building applications in some regions of the United States, but pockets of very high moisture can be trapped and hidden from view within a structure. Therefore, if a biocide is efficacious on unseasoned wood, it is more likely to provide long term protection in high moisture situations.

Results show that treatments containing 20 mg/mL Bor-A+ (=1 mg/mL boric acid) with 1 mg/mL voriconazole and a 5% ethanolamine supplement completely inhibited a mixed mold suspension on southern pine and pine OSB (Table 1). Likewise, Bor-A+ with 1 mg/mL thiabendazole or 0.5 mg/mL thujaplicin completely inhibited a mixed mold suspension on both pine and OSB. Dimethylcocoamine, an ingredient in Bor-A+ is reported to have antifungal properties. Borates alone are only marginally effective at controlling mold fungi (Barnes *et al.* 1989). Indeed, 5% DOT was unable to substantially inhibit mold fungi (Clausen and Yang 2003) and the minimum fungicidal concentration (MFC_{90}) has been estimated as 7.6% for Bor-A+ alone (=3.7 mg/mL boric acid) (Clausen *et al.*, in press). The estimated MFC_{90} for voriconazole, thiabendazole, and thujaplicin are 0.043, 0.016%, and 0.78%, respectively. Ethanolamine alone marginally inhibits molds (MFC_{90}=24.2%), but may act synergistically with the other components in a multicomponent system.

Soil-block culture results

Table 1 shows that all individual biocide components, except thujaplicin and all biocide combinations completely inhibited decay by *P. placenta, G. trabeum,* and *C. versicolor* at the concentrations tested in this study. Laboratory tests have demonstrated that borate-treated wood is very effective in preventing brown-rot and white-rot fungi (Williams and Amburgey 1987), with total protection indicated at 0.5% (w/w) boric acid equivalents (BAE).

Termite test results

Wood consumption (i.e. mean percentage weight loss) caused by *R. flavipes*, percent mortality, and visual attack ratings are shown in Table 2. Untreated controls died before the test concluded, but termites in control tests were healthy and active until day 13. After 1 week incubation, controls showing virtually complete survival are indicative that vigorous termites had been used in the bioassay (ASTM 1998). The average number of days to 100% mortality was 13.6 for the DOT-based biocide, 10.8 for the biocide containing Bor-A+, and 20 days for the untreated controls. Mauldin and Kard (1996) showed that 0.30% BAE will protect pine from significant damage by *Reticulotermes* sp. for 16-18 months in nonleaching conditions. In no-choice feeding assays conducted by Grace *et al.* (1990), 1% DOT-impregnated filter paper caused 100% mortality in 15 d. In this study, all test blocks were visually sound (rated 10), while control blocks showed signs of light attack (rated 9). Weight losses were nil in all test blocks and averaged 1.75% in the untreated controls.

Conclusions:

Multicomponent biocide systems containing a borate component (Bor-A+), an azole (voriconazole or thiabendazole), and an additional component (ethanolamine or thujaplicin) completely inhibited two brown-rot fungi, *P. placenta* and *G trabeum*, the white-rot fungus, *C. versicolor*, and a mixed mold spore suspension. All individual biocidal components, except thujaplicin, inhibited the decay fungi as well. A termite bioassay on a DOT/voriconazole/ethanolamine combination and a Bor-A+/voriconazole/ ethanolamine combination resulted in 100% mortality in 14 and 11 days, respectively. Relative inhibition of decay fungi, mold fungi, and termites to the biocide components is shown in Table 3. Although it is well documented that low concentrations of borates inhibit decay fungi and termites, multicomponent systems containing borates supplemented with azoles are necessary in order to inhibit mold fungi, because mold spores are the most chemically resistant of these three pests.

Table 3. Relative inhibition of decay fungi, mold fungi, and termites.

Biocide	Decay fungi	Mold fungi	Termites
Borate component			
DOT	Yes	No	Yes
Bor-A+	Yes	Yes	Yes
Azole component[a]			
Voriconazole	Yes	Yes	ND
Thiabendazole	Yes	Yes	ND
Additional component			
Ethanolamine	Yes	No	ND
Thujaplicin	No	No	ND
Combination			
Borate/Azole/Ethan.	Yes	Yes	Yes

[a]Clausen and Yang (2003)

Acknowledgements:

The authors would like to thank Dr. Frederick Green III, Microbiologist, for his expertise on the termite bioassay, and Brian Schlitt and Rachael Arango for their technical assistance.

References:

American Society for Testing & Materials. 1998. Standard test method for laboratory evaluation of wood and other cellulosic materials for resistance to termites. D3345–74. *In:* Annual Book of Standards, Vol. 4.10. ASTM, West Conshohocken, PA. pp. 430–432.

American Society for Testing & Materials. 1998. Standard test method for fungicides for controlling sapstain and mold on unseasoned lumber (laboratory method). D4445–91. *In:* Annual Book of Standards, Vol. 4.10. ASTM, West Conshohocken, PA. pp. 497–500.

American Wood Preservers' Association Standards. 2003. Standard method of testing wood preservatives by laboratory soil block cultures. E10–01. *In:* Annual Book of AWPA Standards, Selma, Al. pp. 419–429.

Barnes H.M. Amburgey, T.L., Williams, L.H. and Morrell, J.J. 1989. Borates as wood preserving compounds: The status of research in the United States. International Research Group on Wood Preservation IRG/WP/3542. 16 p.

Clausen, C.A. 2002. Recognize, remove, and remediate mold and mildew. *In:* Proceedings of the 2nd Annual Conference on Durability and disaster mitigation in wood-fame housing. Forest Products Society, Madison, WI. Nov. 6–8, 2000, pp. 231–234.

Clausen, C.A. and Yang, V.W. 2003. Mold inhibition on unseasoned southern pine. International Research Group on Wood Preservation IRG/WP/03–10465. 9 p.

Clausen, C.A., Yang, V.W., and Lebow, P.K. (in press). Azole-based antimycotic agents inhibit mold growth on unseasoned pine. Journal of Applied Microbiology.

Fogel J.L. and Lloyd, J.D. 2000. Biological performance of gypsum products containing borates. International Research Group on Wood Preservation IRG/WP/00–30237. 13 p.

Fogel, J.L., Lloyd, J.D. 2002. Mold performance of some construction products with and without borates. Forest Products Journal. 52(2):38–43.

Grace, K.J., Abdallay, A. and Sisson, J.M. 1990. Preliminary evaluation of borate baits and dusts for Eastern Subterranean termite control. International Research Group on Wood Preservation IRG/WP/1433. 7 p.

Grace, J.K. 1997. Review of recent research on the use of borates for termite prevention. 2nd International Conference on Wood Protection by Diffusible Preservatives and Pesticides. Nov. 6–8, 1996. Mobile, AL. pp 85–92.

Laks, P.E., Richter, D.L. and Larkin, G.M. 2002. Fungal susceptibility of interior commercial building panels. Forest Products Journal 52(5): 41–44.

Manning, M.J., Lloyd, J., and Schoeman, M. 1997. The future of diffusible preservative and pesticide systems. 2nd International Conference on Wood Protection with Diffusible Preservatives and Pesticides. Nov. 6–8, 1996. Mobile, AL. pp. 157–168.

Mauldin, J.K. and Kard, B.M. 1996. Disodium octaborate tetrahydrate treatments to slash pine for protection against formosan subterranean termite and eastern subterranean termite: (Isopera: Rhinotermitidae). J. Econ. Entomol. 89:682–688.

Scheffer, T.C. 1973. Microbiological degradation and the causal organisms. *In:* Degradation and Protection of Wood, Vol. I., D.D. Nicholas, ed. Syracuse University Press, Syracuse, NY.

Tsunoda, K., Watanabe, H., Fukuda, K., and Hagio, K.K. 2002. Effects of zinc borate on the properties of medium density fiberboard. Forest Products Journal. 52:62–65.

Williams, L.H. and Amburgey T.L. 1987. Integrated protection against lyctid beetle infestations. IV. Resistance of boron-treated wood (*Virola* sp.) to insect and fungal attack. Forest Products Journal 37(2):10–17.

Zabel, R.A.; and Morrell, J.J. 1992. *In:* Wood Microbiology: Decay and its prevention. San Diego, CA: Academic Press.

IRG documents 2004: IRG 35, 6-10 June 2004, Ljubljana, Slovenia. Stockholm, Sweden: IRG Secretariat, 2004. 1 CD-ROM.