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Protecting wood from mould, decay, and termites with multi-component biocide systems

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Abstract

Biocides must be developed for controlling mould establishment on cellulose-based building materials. Accordingly, biocides intended for indoor applications must be non-toxic, non-volatile, odourless, hypoallergenic, and able to provide long-term protection under conditions of high humidity. Multi-component biocide systems were tested in American Wood-Preservers' Association soil block tests for inhibition of brown-rot and white-rot decay fungi and American Society for Testing and Materials standard tests for inhibition of mould fungi and termites. Multi-component systems combining a borate base supplemented with either 0.1% azole or 0.5% thujaplicin, performed well against the two brown-rot fungi *Postia placenta* and *Gloeophyllum trabeum*; the white-rot fungus *Coriolus versicolor*; the three mould fungi *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma viride*; and the subterranean termite *Reticulitermes flavipes* (Kollar). It was concluded that for interior applications borate-based multi-component biocide systems can protect wood from decay fungi, mould fungi, and termites, and that a system containing thiabendazole provided protection at a lower retention than the other biocides in this study. Synergy was observed between the borate base and voriconazole in inhibition of mould.
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Keywords: Mould fungi; Decay fungi; Termite; Biocide; Azole; Thujaplicin

1. Introduction

Replacement of biodeteriorated wood in service accounts for about 10% of the annual timber cut in the United States (Zabel and Morrell, 1992). Annual losses of over 1 billion US dollars (USD) result from fungal deterioration of untreated or inadequately treated wood (Scheffer, 1973). Insurance claims for pre- and post-construction mould problems exceeded 2.8 billion (USD) in 2002, and the combined damage from Eastern and Formosan subterranean termites exceeds $2 billion in the United States annually. Moisture is the key ingredient for all three types of biological damage to structures. Among problems that may contribute to excess moisture in existing structures are flawed design, poor construction practices or maintenance, poor site drainage, leaky roofs or plumbing, inadequate insulation, and improper ventilation (Clausen, 2002). Unfortunately, new structures are equally susceptible to these problems. Under the right circumstances, chronic moisture in a structure can lead to a cascading biological succession from mould fungi to decay fungi to insect infestation.

Water vapour in humid air will not wet wood sufficiently to support the growth of decay fungi but will permit mould growth (Highley, 1999). A chronic leak, however, can eventually increase the moisture content of wood above fibre saturation point and initiate decay. Termites seek water and are attracted to conditions that might also be favourable for growth of decay and mould fungi. Evidence also suggests that they are attracted to wood decayed by certain brown-rot fungi (Lenz et al., 1991; Kartal et al., 2003). Once established, mould, decay fungi and termites may independently increase the moisture in the infested area through water trapping or transporting, or the conversion of cellulose to carbon dioxide and water.

Determining and controlling the sources of moisture and rapidly drying wetted building components will limit or arrest fungal growth and insect infestations. No matter how meticulous the maintenance, eventually every

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structure will encounter moisture that may be as obvious as flooding or as subtle as a chronic leak inside a wall that becomes apparent only in advanced stages of biological activity. Because even the best moisture management practices cannot prevent eventual moisture intrusion, economical biocides suitable for interior use are needed. In addition to being effective against mould fungi, they must be non-toxic, non-volatile, environmentally acceptable, safe to handle, and low in solubility (Zabel and Morrell, 1992). Surface treatment of dimension lumber or engineered products with mould inhibitors would add an additional layer of protection for in-service wood products and lessen the effect of current indoor air quality issues. This strategy is being used to some degree in the manufacture of gypsum board (Fogel and Lloyd, 2000, 2002) and oriented strandboard. Addition of zinc borate and disodium octaborate tetrahydrate (DOT) during the manufacture of wood composites has been shown to prevent termite damage (Grace, 1997; Manning et al., 1997; Tsunoda et al., 2002).

The study described here expands on earlier work (Clausen and Yang, 2003), in which agricultural fungicides, pharmaceuticals and plant extractives with the ability to inhibit mould growth on solid wood and wood composites were identified. The moulds chosen for the tests were species of *Aspergillus*, *Penicillium* and *Trichoderma*. Members of the first two of these genera are prominent among "indoor moulds" reported in buildings in several parts of the world (Flannigan and Miller, 1993). Species in all three mould genera readily, and often preferentially, colonize solid wood and wood-based building materials under moisture and temperature conditions conducive to spore germination (Pasanen et al., 2000; Bech-Andersen and Elborne, 2003; Nielsen et al., 2004). The objectives of the present study were to (1) develop multi-component biocide systems to inhibit mould growth on wood, (2) evaluate these systems for their ability to inhibit decay fungi and termites, and (3) evaluate individual components of these systems for synergistic biocidal properties.

2. Materials and methods

2.1. Test organisms

The decay fungi, two brown-rot species, *Postia placenta* MAD 698 (Fries) Lars. & Lomb. and *Gloeophyllum trabeum* MAD 617 (Pers: Fries) Murrill, and a white-rot species, *Coriolus versicolor* MAD 697 (L.: Fr.) Pilat, were maintained on 2% malt agar (Difco, Detroit, Michigan) as inoculum for decay tests.

Mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC20476, were grown on 2% malt extract agar for 2 weeks. A mixed mold spore suspension was prepared by washing the surface of one petri dish for each test organism with 10 mL of sterile deionized water (DI) according to ASTM standard D4445-91 (ASTM 1998). The surface of each plate was rubbed with a blunt glass rod to loose the spores. Collected spores were counted and equal numbers of spores for each test organism were transferred to a spray bottle. The spore mixture was diluted to 100 mL with DI water to yield 3×10^7 spores/mL. The spray bottle was adjusted to deliver 1 mL inoculum per spray and was mixed frequently during inoculation to ensure a homogeneous inoculum.

Subterranean termites, *Reticulitermes flavipes* (Kollar), were collected in Janesville, Wisconsin, for the termite bioassay.

2.2. Test chemicals

The borate base comprised 5% boric acid (National Borax, Cleveland, Ohio, USA), 25% propionic acid (J.T. Baker, Phillipsburg, New Jersey, USA), 55% dimethylcocoamine (Lonza Inc., Fair Lawn, New Jersey), and 15% 1,2-propanediol (Sigma-Aldrich, St. Louis, Missouri, USA).

Three multi-component systems were prepared from 2% borate base: biocide A contained 0.1% voriconazole (Pfizer Inc., NY, USA); biocide B contained 0.1% thiabendazole (Sigma-Aldrich) in 70% ethanol; and biocide C contained 0.5% thujaplicin (isopropyltropolone) (Cedarome Canada Inc., Brossard, Quebec) in 70% ethanol.

2.3. Mould test

Specimens (7×20 mm cross section by 7 cm long) were cut from southern pine mill ends obtained from a Mississippi sawmill and stored at 0 °C. The average moisture content of the pine was 48% by weight ($n = 3$). Five random replicate specimens were dip-treated for ~15 s in combination biocides A, B, or C (Table 1) and reweighed to determine biocide retention level. The borate base and additives were also evaluated separately. Treated specimens were held in a covered container overnight according to the ASTM standard test method D 4445-91 (1998a). Five specimens for each treatment 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 (150×25 mm) (B-D Falcon, Los Angeles, CA, USA). Untreated specimens dipped in DI water served as a control for water-based test chemicals. Specimens dipped in 70% ethanol served as a control for test chemicals of low aqueous solubility. After spraying with 1 mL mixed mould-spore inoculum, specimens were sealed in polyethylene bags to prevent drying, and incubated at 27 °C and 70% relative humidity (RH) for 4 weeks. Following incubation, specimens were individually rated for mould growth on a scale of 0–5, with 0 denoting clean specimens and 5 representing heavy mould growth.

2.4. Decay test

Soil block culture bottles were prepared according to AWPA E-10-01 (American Wood Preservers' Association (AWPA), 2003). Southern pine feeders were inoculated with the brown-rot fungi *P. placenta* and

Table 1
Average retention level and mould resistance rating for southern pine specimens treated with three combination biocides and individual components of the biocides ($n = 5$)

Treatment	Retention (kg m^{-3})	Mould resistance rating[b]
Combination[a]		
A	2.19	1.8
B	0.83	0
C	1.28	0
Individual components		
Borate base		2.8
Voriconazole		0.04
Thiabendazole		0
Thujaplicin		3.2
Untreated		5

[a]2% borate base plus 0.1% voriconazole (combination A), 0.1% thiabendazole (combination B), and 0.5% thujaplicin (combination C).
[b]Mould resistance rating scale: 0, clean; 1, 20%; 2, 40%; 3, 60%; 4, 80%; 5, 100% coverage.

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 southern pine blocks (1 × 1 × 1 cm) conditioned at 27 °C and 70% RH, were vacuum-treated for 40 min at 172 kPa with each biocide combination listed in Table 1 ($n = 5$ per 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 weeks. Following incubation, surface mycelium was brushed from each block before the blocks were oven-dried at 60 °C for 24 h, and reconditioned at 27 °C and 70% RH to a constant weight. Average percentage weight loss was calculated.

2.5. Termite test

Sets of five pre-weighed and pre-conditioned southern pine blocks (1 × 1 × 1 cm) were dip-treated with biocide combinations A, B or C (Table 1), and five control blocks were dipped 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, 1998b). Each block was placed in a lidded test dish with 50 g sand, 8.5 mL DI water, and 1 g termites. Dishes were incubated at 27 °C and 80% RH and examined after 1 and 4 weeks for evidence of tunnelling and termite mortality. After 4 weeks, the blocks were removed from the dishes, cleaned, dried, re-conditioned and weighed to determine weight loss. A visual rating of attack was recorded for each block.

3. Results and discussion

3.1. Mould

Borate base supplemented with 0.1% voriconazole, 0.1% thiabendazole or 0.5% thujaplicin inhibited development of mould on southern pine (Table 1). Dimethylcocoamine, an ingredient in the borate base, is reported to have antifungal properties. Borates alone, at higher loadings, are only marginally effective at controlling mould fungi (Barnes et al., 1989). Indeed, 5% DOT was unable to substantially inhibit mould fungi (Clausen and Yang, 2003), and the minimum fungicidal concentration (MFC_{90}) has been estimated as 7.6% for the borate base alone ($= 3.7\,mg\,mL^{-1}$ boric acid) (Clausen and Yang, 2005). The estimated MFC_{90} for voriconazole, thiabendazole, and thujaplicin are 0.043%, 0.016%, and 0.78%, respectively. Thiabendazole efficacy is reflected in the ability of biocide B to inhibit test fungi and termites at a lower retention than the other biocides in this study. With the exception of the two azoles, none of the individual biocide components at concentration levels used in the combination biocide was able to inhibit growth of test fungi. When retention levels of chemical were calculated for biocides A, B and C based on uptake of test chemical solution during the 15-s dip treatment, specimens treated with the water-based formulation, biocide A, were found to retain 62% more test chemical than biocide B and 42% more than biocide C, both of which were ethanol-based formulations.

Flannigan and Miller(1993) reported that species of the genus *Aspergillus* and *Penicillium* make up an appreciable percentage of the group of "indoor molds" reported in buildings in several parts of the world. *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. readily, and often preferentially, colonize solid wood and wood-based building materials when they are subjected to moisture and temperature conditions conducive to spore germination (Pasanen et al., 2000; Bech-Andersen and Elborne, 2003; Nielsen et al., 2004).

At moisture contents >20%, mould establishment can occur on unseasoned wood in 24–48 h if temperatures permit and rapid drying of the wood does not occur. The high moisture content of the specimens used in this study provides a necessarily rigorous efficacy test for a biocide. Rationale for this methodology is three-fold. First, if a biocide performs well on unseasoned wood, it will perform as well or better on kiln-dried (K-D) wood. Second, reports of K-D lumber with moisture content >19% are common. Third, unseasoned wood is an acceptable construction material in some regions of the United States.

3.2. Decay fungi

All three combination biocides, the borate base, and voriconazole alone, inhibited decay by *P. placenta*, *G. trabeum*, and *C. versicolor* at the concentrations tested in this study (Fig. 1). Weight losses ⩽10% indicate high resistance to fungal decay (ASTM, 1998c). Laboratory tests have demonstrated that borate treatment of wood is

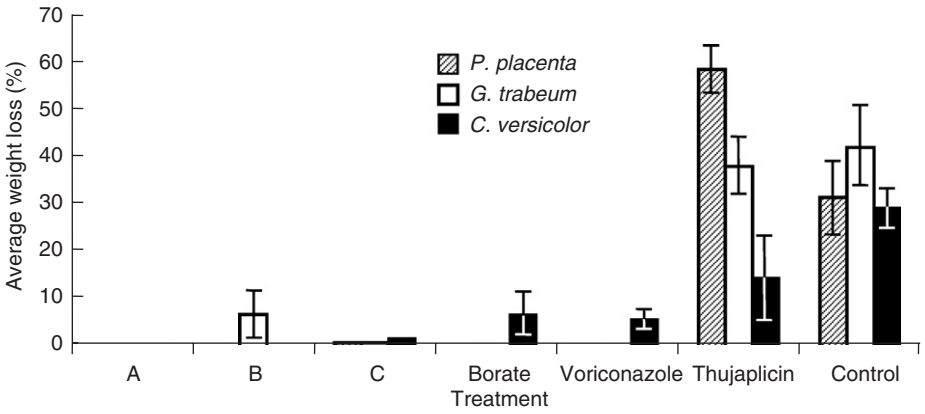

Fig. 1. Soil block test results for southern pine treated with combination biocides and individual components exposed to three decay fungi.

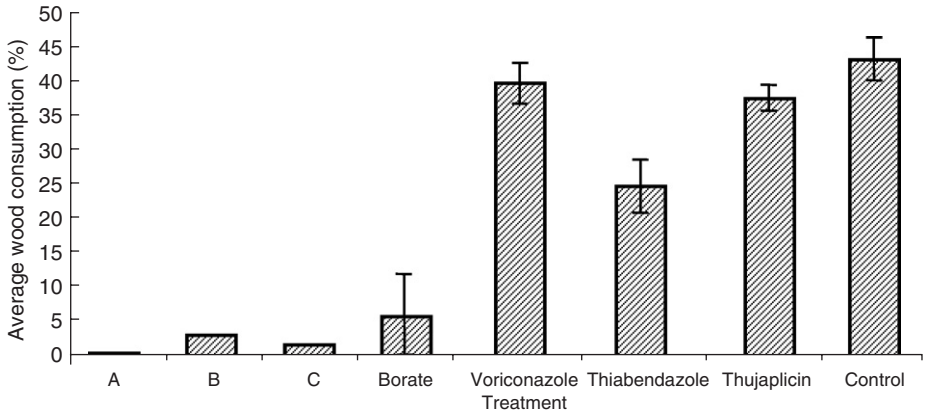

Fig. 2. Consumption by *Reticulitermes flavipes* of wood treated with three combination biocides and individual components of the biocides.

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). Thiabendazole was not individually tested. Thujaplicin, the western red cedar extract partially responsible for the durability of the heartwood of the cedar, has been shown to possess fungicidal activity to *P. placenta*, *G. trabeum*, and *C. versicolor* that is comparable to that of tebuconazole, azaconazoles, or copper oxine (Baya et al., 2001). However, under the conditions of this study, 0.5% thujaplicin failed to inhibit the test fungi.

3.3. Termite results

Wood consumption (as mean percentage weight loss) of treated and untreated specimens caused by *R. flavipes* is shown in Fig. 2 and mortality and visual attack ratings are given in Table 2. Multi-component biocides A, B, and C acted as repellents, causing moderate mortality rates, weight losses of 0.6–3.3%, and an average attack rating of 8.8 or higher, with 10 indicating no attack. Of the individual components tested, only the borate base had an acceptable attack rating of 8.6. Specimens treated with the borate base, which contained a final concentration of 0.1% boric acid, had an average weight loss of 6.1% and caused only limited mortality (0–33%). Mauldin and Kard (1996) showed that 0.30% BAE will protect pine from significant damage by *Reticulitermes* sp. for 16–18 months in non-leaching conditions. In no-choice feeding assays conducted by Grace et al. (1990), 1% DOT-impregnated filter paper caused 100% mortality in 15 days. The two azoles and thujaplicin were ineffective as termite inhibitors; results were virtually identical to the untreated controls, with weight losses of 26–44% and the springwood completely destroyed in all specimens. Thus, termite resistance was the result of synergy of the combination biocide components (Fig. 2).

In summary, the multi-component biocide systems containing a borate base and either voriconazole, thiabendazole, or thujaplicin resisted attack by *R. flavipes* and inhibited the brown-rot fungi *P. placenta* and *G. trabeum*, the white-rot fungus *C. versicolor*, and a mixture of moulds applied as a spore suspension. Synergistic effects were noted for the multi-component systems. At the concentrations used in this study, the borate base could not inhibit the mould, but was effective against termites and decay fungi. Thujaplicin alone was not effective against decay fungi, moulds or termites. Thiabendazole and voriconazole inhibited mould fungi, but neither azole could resist termite attack. In combination, however, the individual components performed well at lower concentrations against all test fungi and termites.

Table 2
Average termite attack and mortality rating for southern pine specimens treated with three combination biocides and individual components

Treatment	Termite bioassay	
	Attack rating[b]	Mortality rating[c]
Combination[a]		
A	9.8	Moderate
B	9.8	Moderate
C	9.8	Moderate
Individual components		
Borate base	8.6	Slight
Voriconazole	0	None
Thiabendazole	0	None
Thujaplicin	0	None
Untreated	0	None

[a]2% borate base plus 0.1% voriconazole (combination A), 0.1% thiabendazole (combination B), and 0.5% thujaplicin (combination C).
[b]Termite attack rating scale: 0, failure; 4, heavy; 7, moderate; 9, light; and 10, sound.
[c]Mortality rating: 0–33%, slight; 34–66%, moderate; 67–99%, heavy; and 100%, complete.

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