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Development of environmentally-benign wood preservatives based on the combination of organic biocides with antioxidants and metal chelators

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Abstract

Wood extractives can be envisaged to protect heartwood by at least three different mechanisms, i.e. fungicide, free radical scavengers/antioxidants and as metal chelators. In short-term laboratory decay tests using two different wood species and decay fungi, the combination of different organic fungicides with various antioxidants and/or metal chelators gave enhanced activity as compared to the organic biocide alone, with the best results usually obtained with all three compounds. Outdoor ground-contact stakes treated with a biocide and antioxidant combination and exposed for 30 months also gave enhanced protection against both decay fungi and termites. It was concluded that the combination of an organic biocide with metal chelating and/or antioxidant additives gives enhanced protection to wood against fungi as compared to the biocide alone and, consequently, it may be possible to develop environmentally-benign wood preservative systems based on this idea.

Keywords: Antioxidants; Extractives; Heartwood; Metal chelators; Natural durability; Termites; Wood decaying fungi; Wood preservatives

1. Introduction

Lumber and other wood products are extensively used in residential construction, utility poles, railroad ties, decking, etc. As a natural organic material wood is degraded by many organisms, principally fungi and insects. Consequently, in certain applications such as ground-contact or above-ground applications where the wood is frequently wet, wood or wood-based composites should be treated with biocides for protection against wood destroying organisms (Preston, 2000). The three major USA wood preservative systems are the oil-borne, organic pentachlorophenol and creosote systems and the water-borne, inorganic "chromated copper arsenate" (CCA-type C) preservative. Most of the treated wood products in the USA, about 80% by wood volume, are treated with CCA (Micklewright, 1998).

CCA is highly effective in protecting wood against a wide variety of wood-destroying organisms, besides being inexpensive, water-soluble, and leach-resistant. However, the perceived environmental hazards of the disposal of wood which contain such metals may limit the future use of CCA in the US (Preston, 2000). Indeed, the availability of CCA-treated lumber has been greatly reduced in Hawaii, and CCA use has been restricted in many European countries and Japan. Also, while current US regulations permit disposal of CCA-treated wood by landfill burial, discarding CCA-treated products will likely become more expensive and onerous in the future. The State of Florida is in fact considering prohibiting the disposal of treated wood in public landfills, which is already mandated in some countries. Consequently, a need exists for developing alternative, environmentally-benign non-metallic wood preservatives.

One possible approach for developing new wood preservatives is to study the heartwood of naturally durable wood species, since an understanding of the cause(s) for natural durability might suggest alternative ways to protect lumber. Consequently, we have studied the role which extractives, particularly stilbenols, play in the natural durability of heartwood (Schultz et al., 1995). Based on our studies we suggested that extractives may protect heartwood by having some fungicidal activity

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and possibly other non-biocidal properties. One possibility is that extractives have both fungicidal and antioxidant properties, which work together to protect the heartwood against fungal colonization and degradation (Schultz and Nicholas, 2000).

In continuing this reasoning, phenolic extractives have the ability to complex with metals, i.e., extractives are metal chelators (Hillis and Sumimoto, 1989; Slabbert, 1992; Cooper-Driver and Bhattacharya, 1998). It is also well known that wood degradation by fungi often involves various metals, either in free form or as key components of enzymes (Green et al., 1997; Eriksson et al., 1990). Consequently, metal chelation may be an additional means by which extractives protect wood, and could act synergistically combination with the fungicidal and antioxidant properties studied earlier.

The objective of this work was to test the above trinary (fungicidal/antioxidant/metal chelator) protective hypothesis. Specifically, commercial organic biocides with no antioxidant or metal chelating properties were examined by two different laboratory decay tests using brown- and white-rot fungi and two wood species. All samples were treated with various levels of biocides, with some samples also co-treated with metal chelators and/or antioxidants to see if these nonfungicidal additives enhance biocide efficacy. Other antioxidants besides 2,6-di-*tert*-butyl-4-methylphenol (BHT), which was used in our earlier experiments, were also examined to further test our multiple-role hypothesis. In addition, results from outdoor exposure ground-contact field stakes are also reported.

2. Results and discussion

Preliminary experiments were carried out using the procedures described earlier (Schultz and Nicholas, 2000). Specifically, four levels of the biocide 2*RS*, 4*RS*-2-(2,4-dichlorophenyl)-2-[1--1*H*-(1,2,4-triazole)methyl]-4-propyl-1,3-dioxolane (propiconazole), with and without an antioxidant (5% co-added BHT) and/or 3% of the metal chelator *N*,*N*′ - 1,2 - ethanediylbis[*N* - (carboxymethyl)glycine] trisodium salt (tri-Na EDTA, abbreviated EDTA). Wood samples were evaluated for strength loss using either the soil-block test with the brown-rot fungus *Gloeophyllum trabeum* and southern yellow pine (SYP) sapwood, or the agar-block test with the white-rot fungus *Trametes versicolor* (which we found to be more aggressive than the fungus *Irpex lacteus* used earlier) and aspen sapwood. For both tests the incubation period was 4 weeks, with the extent of degradation measured by loss in compression strength loss (Nicholas and Jin, 1996). Additional tests were conducted with propiconazole/*G. trabeum*/soil-block/SYP for 4 weeks with the antioxidants BHT or tannic acid (hydrolyzable tannin) (Su et al., 1988) and the metal chelator 1,10-phenanthroline.

In the first preliminary test (data not shown), BHT alone gave a slight protective effect. Consequently, later tests were conducted using longer incubation times for both the brown-rot/soil-block (5 weeks) and the white-rot/agar-block (6 weeks) tests to increase the test severity.

The results of the second preliminary experiment (*G. trabeum*/soil-block/SYP for 4 weeks) are shown in Table 1. Propiconazole alone had no effect except at the highest biocide level. With 5% co-added BHT or 3% co-added tannic acid, increased efficacy was observed, which was further improved by the addition of both 5% BHT and 3% tannic acid. The co-addition of 3% of the metal chelator 1,10-phenanthroline also increased the efficacy of propiconazole, while 3% phenanthroline and 5% BHT or 3% tannic acid provided further protection.

To determine if these positive results could be due to the additives having some fungicidal activity, agar-plate

Table 1
Average % strength loss for southern yellow pine samples treated with the biocide propiconazole (Prop.) and various antioxidants and/or metal chelators, then exposed to the brown-rot fungus *G. trabeum* for 4 weeks[a]

Treatment	Average retention (kg m^{-3})	Average% strength loss
0.01% Prop.	0.05	87.7
0.03% Prop.	0.13	86.3
0.06% Prop.	0.26	64.3
0.12% Prop.	0.51	23.4
0.01% Prop/5% BHT	0.05/22.1	42.8
0.03% Prop./5% BHT	0.13/21.8	25.8
0.06% Prop./5% BHT	0.26/21.5	23.8
0.12% Prop./5% BHT	0.53/22.2	12.5
0.01% Prop./3% TA	0.05/13.9	74.1
0.03% Prop./3% TA	0.14/13.8	63.8
0.06% Prop./3% TA	0.27/13.8	30.9
0.12% Prop./3% TA	0.54/13.5	9.9
0.01% Prop./3% Phen.	0.05/14.1	96.2
0.03% Prop./3% Phen.	0.14/13.9	20.0
0.06% Prop./3% Phen.	0.29/14.2	14.6
0.12% Prop./3% Phen.	0.56/14.1	16.4
0.01% Prop./5% BHT/3% TA	0.05/24.3/14.6	34.8
0.03% Prop./5% BHT/3% TA	0.14/24.6/14.7	15.7
0.06% Prop./5% BHT/3% TA	0.27/24.0/14.4	11.0
0.12% Prop./5% BHT/3% TA	0.58/23.9/14.4	5.2
0.01% Prop./5% BHT/3% Phen.	0.05/23.9/14.4	10.0
0.03% Prop./5% BHT/3% Phen.	0.14/24.8/14.9	10.1
0.06% Prop./5% BHT/3% Phen.	0.30/25.9/15.5	13.9
0.12% Prop./5% BHT/3% Phen.	0.61/25.2/15.1	10.2
5% BHT	22.6	51.2
3% Phen.	14.4	3.1
3% TA	14.5	85.4
Control		94.4

For samples treated with more than one compound, the concentrations (first column) and retentions (second column) are separated by slashes. TA is tannic acid, BHT is 2,6-di-*tert*-bytyl-4-methylphenol and Phen. is 1,10-phenanthroline.

[a] Results are an average of five replicates.

experiments (Archer et al. 1995) were conducted with four wood-destroying fungi on BHT, tannic acid, EDTA and 1,10-phenanthroline. High fungicidal activity ($IC_{50} < 25$ ppm) was observed with phenanthroline against all four fungi, minor activity (IC_{50} greater than 250 ppm) with three of the four fungi for BHT, and essentially no activity for EDTA or tannic acid. Thus, the promising results with phenanthroline observed by us and Green et al. (1997) may be due to phenanthroline having both metal chelating and fungicidal properties. While some limited activity was observed with BHT, samples treated with only 5% BHT showed a small protective effect in this or other lab experiments. Thus, the enhanced efficacy observed with propiconazole against *G. trabeum* by co-adding BHT and/or tannic acid suggests that the combination is synergistic.

Additional experiments were carried out using the commercial biocides (3*RS*) - 5 - (4 - chlorophenyl) - 2,2 - dimethylethyl - 3 - 1*H*-[1,2,4 - triazole]methyl) - 3 - pentanol (tebuconazole); and 3-iodo-2-propynylbutyl carbamate (IPBC) (Fig. 1). Samples were evaluated by the *G. trabeum*/soil-block/SYP method for 5 weeks, or *T. versicolor*/agar block/aspen for 6 weeks, with 5% BHT and/or 3% EDTA co-added. With the brown-rot/soil-block experiments, the addition of 3% EDTA had little effect, while in the white-rot/agar-block tests 3% EDTA appeared to have more of an influence. No fungicidal activity was found with EDTA in the agar-plate test against the two white-rot fungi examined, and therefore it is unlikely that the results with EDTA alone in the white-rot/agar-block experiments are due to EDTA being fungicidal. Green et al. (1997) also reported that metal chelators alone have some effectiveness in the agar-plate test. They suggested that the relatively high amount of minerals in a soil-block test might overwhelm the metal chelator, while the relatively low levels of minerals in malt-agar medium would result in a metal chelator being more effective. When white-rot fungus/aspen block tests were performed using the soil-block test, 3% EDTA had no effect, which supports the above suggestion.

In the brown-rot and white-rot tebuconazole experiments (Fig. 1), the addition of BHT and/or EDTA with tebuconazole gave greater protection than the biocide alone. For the brown-rot/IPBC test, essentially no protection was observed with IPBC alone at any of the four

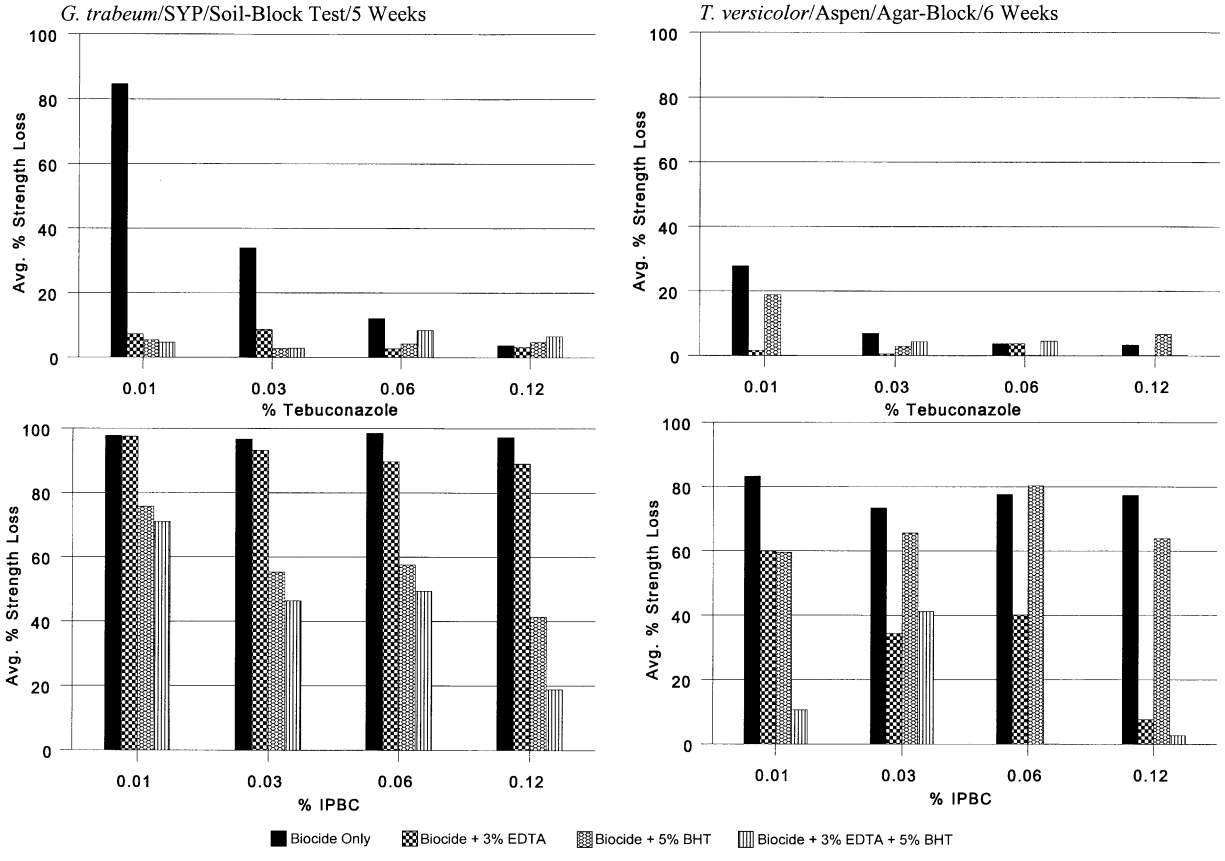

Fig. 1. Average % strength loss of five replicates for sapwood samples treated with four levels of organic biocides. The SYP samples were exposed to *G. trabeum* (left column) and the aspen samples to *T. versicolor* (right column). For the *G. trabeum* samples, untreated control strength loss ranged from 97.0 to 100%, samples treated with only 3% EDTA had 93.5 and 95.7% strength loss, and samples treated with only 5% BHT had 68.7 and 78.6% strength loss. *T. versicolor* controls had 70.5–80.6% strength loss, samples treated with only 3% EDTA had 32.4 and 65.1% strength loss, and samples treated with only 5% BHT had 62.5 and 64.7% average strength loss.

retentions examined, while the combination of all three components gave the greatest benefit. With the white-rot/IPBC experiment, the addition of EDTA provided some benefit while the best results were obtained with all three components. Since the agar-plate experiments showed that EDTA and BHT had little or no fungicidal activities, these results add further support to our contention that the combination of a fungicide with the antioxidant BHT and/or metal chelator EDTA is synergistic.

Additional *G. trabeum*/SYP/soil-block and *T. versicolor*/aspen/agar-block experiments were carried out using the biocide propiconazole and the antioxidants propyl gallate and *t*-butylhydroquinone at 5% concentration levels (Fig. 2). Excellent results were observed in all cases, but it was also observed that samples treated with 5% of either antioxidant alone had less than 10% strength loss in all cases but for the *T. versicolor*/aspen/agar-block test. For this one test, the addition of either 3% EDTA, 5% *t*-butylhydroquinone, or both co-additives, with propiconazole gave better results than propiconazole used alone. To determine if the good results using propyl gallate alone against the brown- and white-rot fungi, or *t*-butylhydroquinone against *G. trabeum*, were due to these two antioxidants having fungicidal activity, these compounds were evaluated using the agar plate test against four fungi. Propyl gallate had no activity (IC_{50} > than 600 ppm for all four fungi), while the quinone had moderate activity ($IC_{50} < 100$ ppm) against two fungi and some activity ($IC_{50} < 200$ ppm) for the other two fungi. Thus, the good results with the quinone in the *G. trabeum*/SYP/soil-block test may be due to that antioxidant also having some fungicidal activity. Since no fungicidal activity was found with propyl gallate using the agar-plate test, the excellent results obtained against both fungi with propyl gallate (Fig. 2) are of particular interest. One possible explanation is that the vicinal triphenolics give gallate derivatives both antioxidant and metal chelating properties (Hillis and Sumimoto, 1989), and the dual properties result in enhanced wood protection against fungi.

To provide additional information on the efficacy of biocide/antioxidant combinations, ground-contact field stakes treated with three different retentions of 2, 4, 5,

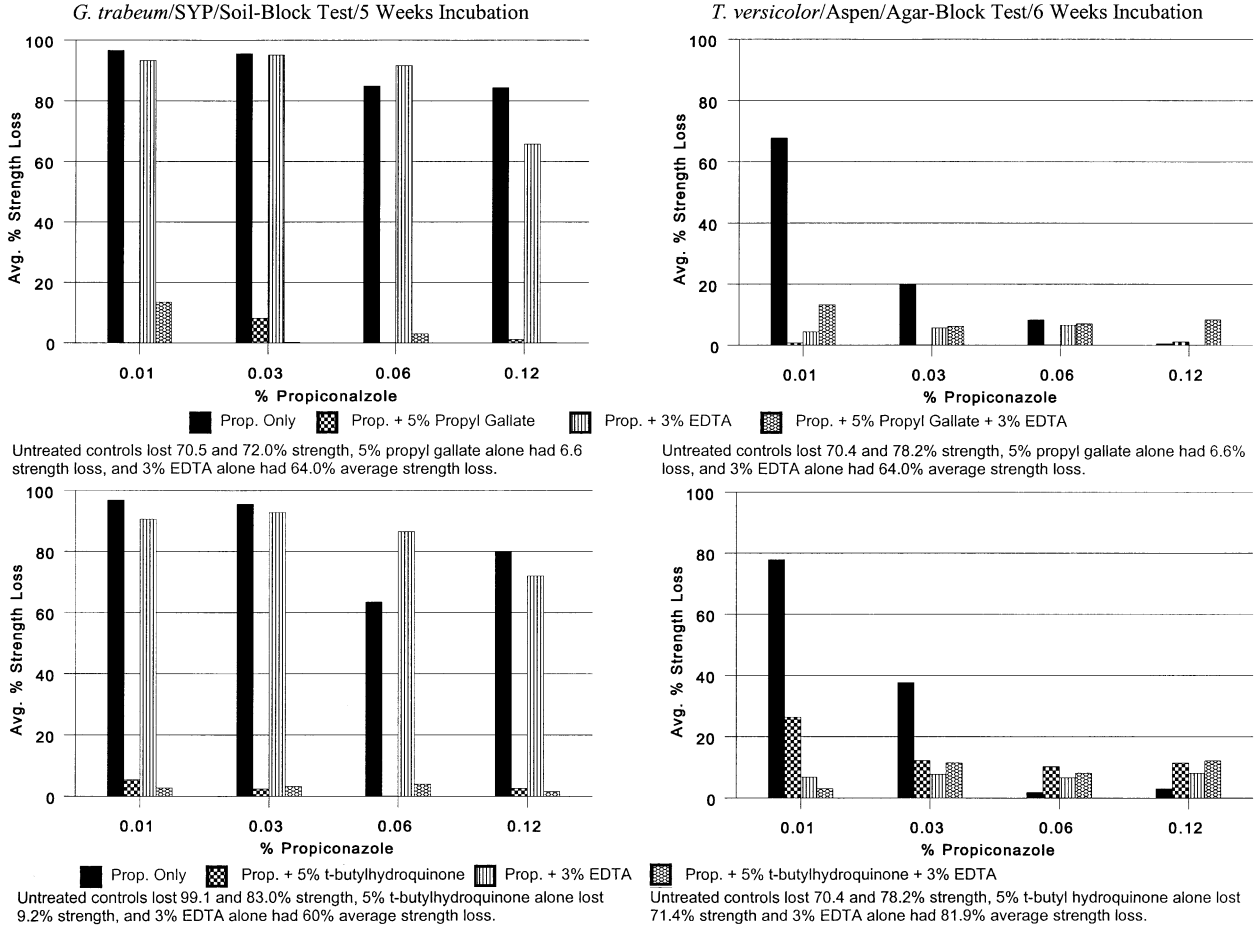

Fig. 2. Average % strength loss of five replicates for sapwood samples treated with four levels of propiconazole. The top graphs show samples co-treated with 5% propyl gallate and/or 3% EDTA, and the bottom graphs show samples co-treated with 5% *t*-butylhydroquinone and/or 3% EDTA. The SYP samples were exposed to *G. trabeum* (left column), and the aspen samples to *T. versicolor* (right column).

6-tetrachloroisophthalonitrile (chlorothalonil), either alone or with 2 or 4% BHT co-added, were installed at two test plots (Dorman Lake, MS and Saucier, MS) and the average decay (fungal) and termite ratings (Table 2) were determined after 30 months of exposure.

At both sites, when samples treated with chlorothalonil alone are compared to samples co-treated with either 3 or 5% BHT and chlorothalonil, samples treated with the combination performed better against fungi (Table 2). A positive effect was also observed with the combination in protecting the SYP sapwood against termites in all but one set (Saucier plot, lowest chlorothalonil level). This was an unexpected benefit, since our original idea was based only upon the protective effect of antioxidants against wood-decaying fungi alone.

Wood treated with 4% BHT alone performed significantly better than untreated controls after 30 months exposure. In contrast, wood treated with 5% BHT alone is as degraded as untreated wood in 5 weeks in a lab decay test. This demonstrates the unrealistically harsh conditions of lab decay tests, and also the advantages and limitations of both lab and field exposure tests. Since these outdoor exposure results, although positive, are based on a relatively short period (30 months), data from longer term exposures will be required before definitive conclusions are reached.

Based on laboratory and field data above we suggest that the combination of an organic fungicide with an antioxidant and/or metal chelator can give synergistic protection to wood against fungi. Furthermore, since heartwood extractives are well known to have fungicidal, antioxidant and metal chelating properties, it is not unreasonable that heartwood is protected against fungal attack and degradation by these multiple properties of extractives.

Our earlier article (Schultz and Nicholas, 2000) suggested that natural durability is extremely complex and proposed that additional properties of extractives may be involved in heartwood durability. For example, extractives—especially terpenoids—would impart water repellency which should enhance the durability of wood in an above-ground exposure (Preston, 2000). Thus, we feel that it is unlikely that all the factors involved in natural durability have been recognized.

3. Experimental

For the laboratory tests, sapwood portions of kiln-dried, defect-free southern yellow pine (SYP) (*Pinus* spp.) or aspen (*Populus* spp.) lumber were cut into 19×19 mm ($r\times t$) sticks, and then 5 mm thick (l) wafers were cut sequentially from these sticks and numbered. Ten sequential wafers were used for each biocide treatment, with the even-numbered samples serving as controls and the matched, odd-numbered samples exposed to the fungus. Wood wafers were treated using a full-cell process in a mini-treating cylinder, as described earlier (Schultz and Nicholas, 2000), with four concentrations of the biocide alone, with the same biocide concentrations used with the metal chelators and/or antioxidant. SYP samples were evaluated using the soil-block test

Table 2
Average decay and termite ratings for southern yellow pine field stakes treated with chlorothalonil (CTN), or a mixture of CTN and 2,6-di-*tert*-butyl-4-methylphenol (BHT), after 30 months exposure[a]

Treatment	Average retention, (kg m^{-3})	Dorman Lake		Saucier	
		Decay	Termite	Decay	Termite
0.15% CTN	0.74	7.2	7.4	8.5	7.9
0.15% CTN/2% BHT	0.72/9.5	9.7	9.6	9.7	7.6
0.15% CTN/4% BHT	0.70/18.8	9.8	9.9	9.5	8.6
0.30% CTN	1.47	8.8	9.9	8.9	8.3
0.30% CTN/2% BHT	1.54/10.1	10	9.8	10	9.7
0.30% CTN/4% BHT	1.41/18.8	10	9.8	9.8	8.8
0.50% CTN	2.42	10	10	7.9	8.7
0.50% CTN/2% BHT	2.41/9.6	10	10	10	9.9
0.50% CTN/4% BHT	2.43/19.5	9.9	10	10	9.9
4% BHT	19.4	8.7	9.5	7.5	6.4
Controls	–	0.7	1.6	0.7	0

For samples treated with the CTN/BHT mixture, the treating solution concentration and average retention are shown for both compounds.

[a] Average of 10 stakes per treatment per site. A "10" rating is no attack, "9" trace of attack, etc., as per AWPA Standard E7-93.

with the brown-rot fungus *G. trabeum* (ATCC 11539), and aspen samples run using the agar-block test with the white-rot fungus *T. versicolor* (ATCC 12679) using a similar procedure as described (Schultz and Nicholas, 2000). The incubation time for the preliminary experiments was four weeks, all other decay tests were run for 5 weeks for the brown-rot/soil-block tests and 6 weeks for the white-rot/agar-block tests. One set of white-rot/*T. versicolor* samples were run using the soil-block test with only untreated or samples treated with 3% EDTA, using both aspen and yellow-poplar (*Liriodendron tulipifera*) sapwood. At the end of the experiment the samples were saturated with water and the compression strength of the exposed and unexposed matched samples measured and used to determine strength loss (Nicholas and Jin, 1996) in the radial direction. The average strength loss of five replicate samples was used as a measure of the extent of decay.

Field ground-contact stakes were prepared from defect-free, kiln-dried SYP sapwood. Field stake (AWPA Standard E7-93) samples, $19\times19\times1118$ mm ($r\times t\times l$), were treated using a full-cell process (30 min at 29 in Hg vacuum followed by 60 min pressure at 150 psig), using toluene as the solvent for chlorothalonil and the BHT/chlorothalonil mixture. After air-drying, a 152 mm piece was cut from the center of each stake and stored for future analysis, with the remaining two matched end pieces, $19\times19\times457$ mm, installed at the Dorman Lake and Saucier test plots. Each treatment set had ten replicates per treatment per test site. Dorman Lake is located in northeast Mississippi, and has a heavy clay soil (pH of 4.8). The Saucier test plot is located in the Harrison National Forest near the Gulf Coast, and has a sandy loam soil (pH of 5.1). The Harrison site represents a severe hazard zone (AWPA zone 5), while the Dorman site is representative of a high hazard zone (AWPA zone 4). Since the Saucier plot is near the Gulf Coast it has a relatively mild winter and wet summer as compared to Dorman Lake. The stakes were visually inspected yearly, with separate decay and termite ratings based on a semi-log system of 10 to 0 using AWPA Standard E7-93 AWPA (2000) (10 rating, sound to trace of degradation; 9 rating, trace to 3% degradation; etc.).

The agar-plate testing procedure was as described previously (Archer et al., 1995). Each fungus/concentration was replicated five times, with two brown-rot fungi [*G. trabeum* and *Postia placenta* (ATCC 11538)] and two white-rot fungi [*Irpex lacteus* (ATCC 11245) and *T. versicolor*]. Concentrations were 25, 50, 100, 250 and 500 ppm. The radial fungal growth was normalized to the fungal growth on the control plates that contained the same solvent (water or acetone). IC_{50} values were calculated by regression of the relative growth versus the log of the concentration.

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