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Copper tolerance of brown-rot fungi: Oxalic acid production in southern pine treated with arsenic-free preservatives ☆

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Abstract

The voluntary withdrawal of chromated copper arsenate (CCA)-treated wood from most residential applications has increased the use of non-arsenical copper-based organic wood preservatives. Because the arsenic component of CCA controlled copper-tolerant fungi, scientists have renewed interest in and concern about the decay capacity in the important copper-tolerant group of brown-rot fungi. We have demonstrated that the primary means of inactivating copper in preservatives is by excess production of oxalic acid (OA). Oxalic acid production is a key metabolic indicator of brown-rot decay, and our objective was to estimate the production of OA in five commercial or experimental arsenic-free preservatives. Ten aggressive brown-rot fungi, chosen from previous studies and representing the genera *Antrodia, Coniophora, Gloeophyllum, Postia, Serpula, Tyromyces*, and *Wolfiporia*, were tested against southern yellow pine (SYP) blocks that were vacuum-treated with ground contact retentions of copper naphthenate, amine copper azole, alkaline copper quat type D (ACQ-D), *N,N*-naphthaloylhydroxylamine (NHA), and copper borate in a 12-week soil-block test. After determination of block weight loss, blocks were also tested for the presence of OA. Weight loss ranged from 0.3% to 8.3% for treated blocks and from 16.4% to 59.6% for untreated controls. We conclude that SYP treated with these five preservatives limited OA production and prevented decay, and thus confirmed the efficacy of the co-biocides against copper-tolerant fungi.

Keywords: Brown-rot decay; Copper-tolerant fungi; Oxalic acid

1. Introduction

Copper-based biocides have provided protection against fungal decay and termite damage to wood since the 1930s. For most of the latter half of the twentieth century, the wood preservation industry was dominated by chromated copper arsenate (CCA). The copper provided broad-based protection against fungi and termites, and the chromium helped to fix the copper and arsenic into the wood. The arsenic provided supplemental protection against copper-tolerant fungi (Lebow et al., 2000), which have been shown to represent most of brown-rot genera tested so far (Green and Clausen, 2003).

Increasing environmental concerns led to voluntary withdrawal of arsenic-containing wood preservatives in the residential markets, lumberyards, and home supply stores, thus reducing the use of CCA-treated wood by 70% after 2003. CCA has been replaced in the home-owner maintenance market by a new generation of copper-organic preservatives often supplemented with water repellants. This new generation of preservative systems is arsenic- and chromium-free (Schultz and Nicholas, 2003).

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Removal of arsenic from residential wood preservatives raises concern about copper-tolerant wood decay fungi. Studies have shown that many copper-tolerant fungi are cupriphilic in a choice test; thus, inhibition depends upon the co-biocidal components (Illman et al., 2000). Nearly all these copper-tolerant fungi are likely to be brown-rot fungi, and their distribution and prevalence are unknown. We have been evaluating the relationship of oxalic acid (OA) production to the time course and severity of decay by this group of fungi and have shown that copper induces rapid OA production by copper-tolerant brown-rot fungi (Clausen et al., 2000; Clausen and Green, 2003; Green and Clausen, 2003). In laboratory studies, Pohleven and others (Pohleven et al., 2002) evaluated Norway spruce with commercial copper-based preservatives common to the European market. When wood treated with copper-based biocides was challenged with several copper-tolerant *Antrodia* spp., individual strains exhibited varying degrees of copper tolerance. Of the preservatives tested, copper amine and chromated copper boron inhibited decay by these fungi. The objective of our study was to estimate the potential of known copper-tolerant fungi to produce OA in southern yellow pine (SYP) treated with arsenic-free preservative formulations in the ASTM soil-block test.

2. Materials and methods

2.1. Fungal cultures

The strains of brown-rot fungi evaluated in this study (Table 1) were maintained on 2% malt extract agar (Difco Laboratories, Detroit, Michigan).

2.2. Preservative treatments and decay test

SYP sapwood blocks (1 cm^3) were conditioned to 70% relative humidity (RH) at 27°C and weighed. The blocks were then vacuum treated according to modification of AWPA standard E10-01 (AWPA, 2003a) with one of five preservatives: water-borne copper naphthenate (0.15% elemental copper concentration prepared from a stock solution); amine copper azole (1% a.i., or active ingredient, with an actives composition of 96.1% Cu and 3.9% azole); alkaline copper quat (ACQ-D, 6.41 kgm^{-3} = 0.40 lb ft^{-3}, with an actives composition of 66.7% CuO and 33.6% didecyl dimethyl ammonium chloride (DDAC)); NHA-Na (1.0% a.i., as sodium N,N-naphthaloylhydroxylamine); and copper borate (2.0% ai, with an actives composition of 7.2% copper hydroxide and 92.8% sodium tetraborate decahydrate). Table 2 lists treatments, target retention, and actual assay retention for copper. After treatment, blocks were conditioned for 4 weeks, reweighed, and steam sterilized. Untreated (control) and treated blocks were challenged with 10 brown-rot fungi in a modified soil-block test. Soil-block bottles were incubated at 27°C and 70% RH for 12 weeks. Following incubation, test blocks were removed from soil bottles, brushed free of mycelium, dried at 60°C for 48 h, reconditioned at 27°C and 70% RH, and reweighed to determine weight losses for each fungus/treatment combination. Mean percentage weight loss was calculated for each group of five blocks and five untreated control blocks for each test fungus.

Table 1
Fungi strains tested

Organism	Designation
Antrodia vaillantii FP-90877-R	(DeCandolle: Fries) Ryvarden
Antrodia radiculosa FP-90848-T	(Peck) Gilbertson et Ryvarden
Postia placenta MAD-698	(Fries) Larsen et Lombard
Postia placenta TRL-2556	(Fries) Larsen et Lombard
Wolfiporia cocos MD-106-R	(Wolf) Ryvarden et Gilbertson
Meruliporia incrassata MAD-563	(Berkeley et Curtis) Murrill
Tyromyces palustris TYP-6137	(Berkeley et Curtis) Murrill
Meruliporia incrassata TFFH-294	(Berkeley et Curtis) Murrill
Tyromyces palustris L-15755-SP	(Berkeley et Curtis) Murrill
Gloeophyllum trabeum MAD-617	(Persoon: Fries) Murrill

2.3. Oxalic acid production

Each block exposed to decay fungi in the soil-block test was extracted in 3.0 mL 0.1 M phosphate buffer, pH 7.0, for 2 h with shaking. For each extracted sample, OA was determined by microassay using a commercial test kit (Trinity Biotech Co., Wicklow, Ireland). Units of OA were expressed as micromoles OA per gram of final dry-weight of wood.

2.4. Elemental analysis

Five oven-dried blocks per treatment were ground to pass a US standard 20 mesh screen (0.841 mm sieve), digested, and analyzed for copper content by inductively coupled plasma emission spectrometry according to American Wood-Preservers' Association Standard A-21-00 (AWPA, 2003b). Copper concentrations are expressed as kgm^{-3} of copper or copper oxide for each preservative (Table 2).

3. Results and discussion

3.1. Decay test

The decay capacity of the 10 brown-rot fungi on untreated SYP was compared to that on preservative-treated SYP (Fig. 1). Overall, mean weight losses of

Table 2
Preservative content of treated blocks

Treatmenta	Target retention (kg m^{-3})[b]	Assay retention (kg m^{-3})[c]	
			Co-biocide
Cu Naph	0.96	0.64 Cu	na
Cu azole	6.4	2.72 Cu	0.11 azole[d]
ACQ-D	6.4	3.68 CuO	1.76 DDAC[d]
CuBor	5.6	0.27 CuO	2.08 B_2O_3
NHA	6.4	3.2 NHA[e]	na

na: not applicable.
[a]Copper naphthenate (Cu Naph); copper azole (Cu azole); alkaline copper quaternary (ACQ-D); copper borate (Cu Bor); *N*, *N*-naphthaloylhydroxylamine (NHA); didecyldimethyl ammonium chloride (DDAC).
[b]Theoretical concentrations as computed from the treatment loads.
[c]Actual concentrations from assay analysis.
[d]Estimated from treatment solution composition.
[e]Method of Kartal et al. (2002).

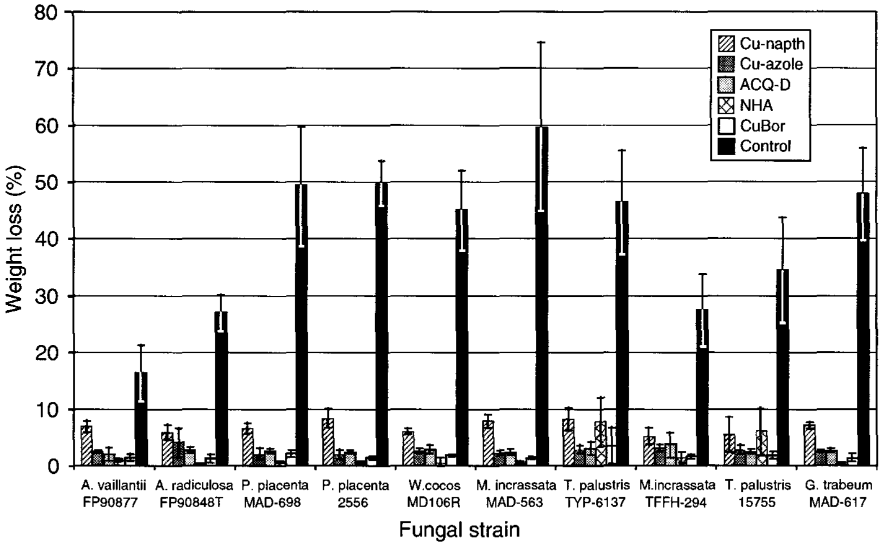

Fig. 1. Percentage weight loss of treated southern yellow pine following 12-week exposure to copper-tolerant brown-rot fungi.

treated wood blocks fell below 10%. Thus, no obvious failures of these preservatives were observed within the limits of this laboratory testing when compared to the mean weight loss of the control SYP at over 40%. In a previous paper, weight losses were 32%–57% in copper citrate (CC)-treated SYP after 10 weeks (Green and Clausen, 2003).

3.2. Oxalic acid analysis

In a previous paper, we showed that copper-tolerant brown-rot fungi produced 2–17 times more OA in CC-treated blocks than in untreated control SYP (Clausen and Green, 2003). Sub-lethal concentrations of Cu-based preservatives induced rapid OA production; 66–93% more OA was produced in 4 weeks in blocks treated with CCA, the ammoniacal copper quats ACQ-B and ACQ-D, and ammoniacal copper citrate (CC) than in untreated controls. Preservative retentions tested in the present study prevented appreciable fungal growth as well as OA production and accumulation (Fig. 2).

Antrodia vaillantii and related species form a group of interior brown-rot fungi associated with the decay of softwoods in buildings. In central Europe, these fungi are ranked below the dry rot fungus *Serpula lacrymans* and equal to *Coniophora* spp. as the most common internal decay fungi (Schmidt and Moreth, 2003). Thorton and Tighe (1987) demonstrated that two of eight strains of *S. lacrymans* caused the same weight loss in copper naphthenate-treated *Pinus radiata* as in water-treated controls. In a laboratory study, *A. vaillantii* was the most copper-tolerant fungus and had the highest OA production (Schmidt, 1995). In a previous study by Green and Clausen (2003), *A. vaillantii* also produced the highest OA on CC-treated SYP. In the same study,

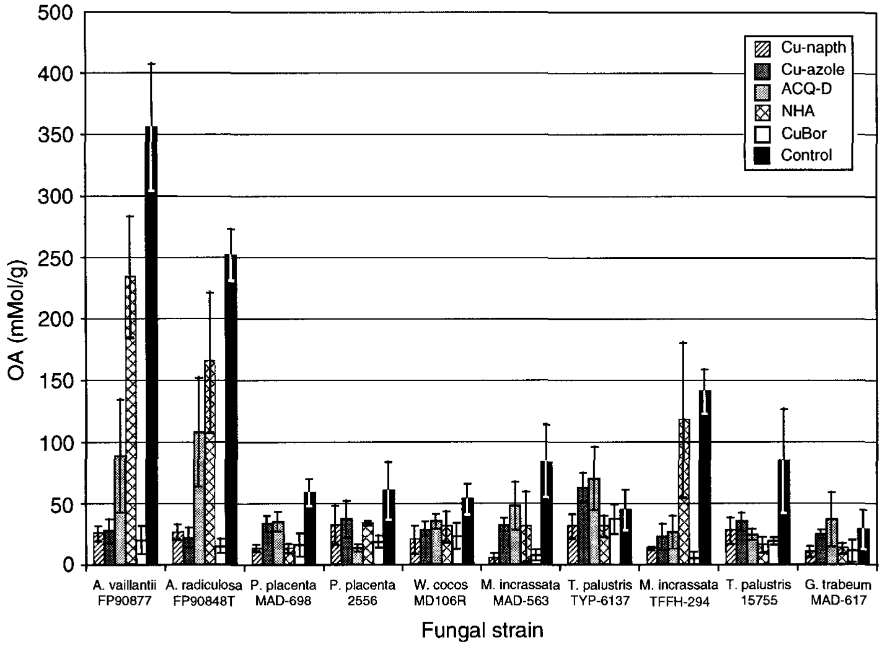

Fig. 2. Oxalic acid (OA) production by copper-tolerant brown-rot fungi after 12-week exposure to wood treated with various preservatives. OA is expressed as micromoles OA per gram of final oven-dry wood weight.

it was concluded that *Coniophora puteana* MAD-515 was not copper tolerant. In spite of demonstrated copper tolerance, *A. vaillantii* was unable to cause significant weight loss of treated wood against the copper-based preservative treatments used in this study (Fig. 1).

Wolfiporia cocos has also been shown to be copper-tolerant (Woodward and DeGroot, 1999). The *W. cocos* strain (106R) that caused the most weight loss in a previous study was a prolific accumulator of OA and was included in this evaluation of replacement copper-organic preservative formulations (Green and Clausen, 2003). No degradation of treated blocks was observed for any of the test preservatives. DeGroot and Woodward (1999) showed that two *W. cocos* isolates, MD-104 and L (61) 1-8-A, caused weight losses with CC and ACQ-B that were significantly greater than in untreated SYP. Retentions of ACQ-B were less than 1% a.i. However, results of this study showed that even low retentions of ACQ-D (3.7 kgm^{-3}) minimized weight losses (< 1%).

NHA is an experimental wood preservative without any heavy metals developed at the Forest Products Laboratory in Madison, Wisconsin (Green et al., 1997). Recent studies have demonstrated resistance to leaching (Kartal et al., 2002) and long-term survival of field stakes in Gulfport, Mississippi (Crawford and Green, 1999). A 1% aqueous treatment with NHA also inhibited weight loss by all 10 copper-tolerant fungi tested in this study, although NHA (3.2 $kg\ m^{-3}$) did not completely inhibit OA production in the two *Antrodia* isolates (Table 2).

4. Conclusions

Copper organic-containing preservatives (especially ACQ-D and Cu-azole) are currently the primary replacements for CCA in residential lumber markets. This immediately raises concern about the effect of copper-tolerant brown-rot fungi in the service life of treated wood. The results of this paper indicate that arsenic-free preservatives adequately inhibit our most aggressive isolates of copper-tolerant fungi under conditions favouring the fungi in laboratory tests.

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