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Fungal degradation of wood treated with metal-based preservatives: 1. Fungal tolerance

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Fungal degradation of wood treated with metal-based preservatives:
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ABSTRACT

In recent years, concerns have arisen about the leaching of heavy metals from wood treated with chromated copper arsenate (CCA), particularly because of the large amount of CCA-treated wood that will be discarded in the coming years. The long term objectives of this work are to determine the fate of copper, chromium and arsenic with the aging and potential decay of CCA-treated wood, and to develop strategies for recycling and remediation of disposed wood. In this study, we determined the ability of various decay fungi to decompose southern yellow pine wood treated with CCA or other metal-based preservatives. Isolates of *Meruliporia incrassata* and an isolate of *Antrodia radiculosa* caused the highest weight losses in CCA-treated southern yellow pine. One isolate of *M. incrassata* produced similar weight losses in CCA-treated and untreated southern pine after 10 weeks. Pine samples treated with very high levels of copper sulphate were decayed by *M. incrassata*, but the fungus was unable to decay wood treated with copper napthenate or copper-8-quinolinolate.

Introduction

Chromated-copper-arsenate (CCA) is one of the most widely used wood preservatives. Of the 6,568 million board feet of lumber and timbers treated in 1993 in the United States, 98 percent were treated with waterborne preservatives (Micklewright, 1994). CCA is favored for lumber treatment because it is inexpensive, leaves a dry, paintable surface, and binds to become relatively leach-resistant. However, there is increasing concern about potential environmental contamination from leaching losses of Cu, Cr and As from treated wood in service and from wood removed from service and placed in landfills. The life cycle of treated wood is estimated to be about 25 years; the wood is then discarded as waste. While CCA-treatment is considered to be highly leach resistant, small amounts of contaminants can be measured in leach water and soils. However, the issue of widespread low-level contamination and of ultimate disposal can no longer be ignored considering the quantities of treated timber that will be in service in coming years.

Certain species of fungi are capable of decaying CCA-treated wood and wood treated with other copper-based preservatives. Most of these fungi are basidiomycetes that cause brown-rot decay of wood. For example, the brown-rot fungi *Poria cocos, P. incrassata* and *P. vailantii* have been reported to cause substantial weight losses to CCA-treated blocks (DeCosta and Kerruish, 1964). The purpose of this study was to determine the ability of various decay fungi to decompose CCA-treated wood and other metal-based treated wood.

Methods

Treatment of Wood and Exposure to Decay Fungi

CCA-treated blocks were cut from southern pine *(Pinus* sp.) treated to 6.4 kg/m^3 (.40 pounds/cubic foot) according to American Wood Preservers' Association (AWPA) standards (AWPA, 1991). Copper sulfate ($CuSO_4$-$5H_2O$), copper-8-quinolinolate (PQ-8, ISK Bioscience) and copper napthenate (copper-hydro-nap, Mooney) were used to treat southern pine blocks in water solution according to AWPA standards (1991). Five replicate blocks (2.5 by 2.5 by 0.9 cm) were exposed to each fungus in soil-block tests (AWPA, 1991). Control blocks were held in soil-block bottles without exposure to fungi. Following incubation at 80°F and 70 percent relative humidity, blocks were removed from bottles and weight losses determined as a measure of preservative efficacy.

The following brown-rot decay fungi were used: *Postia placenta (MAD-698), Meruliporia* (=Poria) *incrassata* (TFFH-294, TFFH-296, MAD-563) *Antrodia radiculosa* (FP-90848, HHB-11414), *Poria cocus* (90850-S), and *Antrodia sinuosa* (LRG-1, LRG-2, LRG-3, LRG-4, LRG-5).

Tolerance to Cu, Cr and As

Copper, chromium or arsenate were incorporated into 2% malt extract agar (MEA) in petri plates and inoculated in the center with *M. incrassata* (TFFH-294, TFFH-295). Cupric sulfate, potassium bichromate or sodium arsenate (Aldrich) were added to MEA at levels of 0,0.1, 110 and 100 mM of Cu, Cr or As. Periodic measurements of growth were made at day 3, 6, 10 and 14 to determine the inhibitory effects of the metals on fungal growth.

Results and Discussion

M. incrassata (TFFH-294) was the most aggressive of the isolates tested in ability to degrade CCA-treated pine, causing almost-as much weight loss in CCA-treated pine as in untreated pine (Table 1). *A. radiculosa* (FP-90877-R) was the second most aggressive.

Table 1. Screening decay fungi for ability to decay CCA-treated southern pine

Fungus	Weight loss[1] (%) Control	CCA-treated
Meruliporia incrassata (TFFH 294)	47.7±2.7	40.2±3.9
Meruliporia incrassata (TFFH 296)	15.2±1.6	9.1±4.6
Meruliporia incrassata (MAD-563)	55.5±1.7	9.1±3.2
Antrodia vaillantii (FP-90877-R)	18.0±2.3	6.2±1.5
Antrodia radiculosa (FP-90848)	60.5±2.3	17.2±1.2
Antrodia radiculosa (HHB-11414)	47.2±2.0	3.8±0.8
Poria cocos (90850-s)	62.3±5.3	0±0.1
Postia placenta (MAD-698)	62.3±2.0	0±0.1
Antrodia xantha (LRG-1)	47.6±3.2	0±0
Antrodia sinuosa (LRG-2)	32.1±0.7	0±0
Antrodia sinuosa (LRG-3)	35.1±1.0	0±0
Antrodia sinuosa (LRG-4)	4.2±5.5	0±0
Antrodia sinuosa (LRG-5)	33.3±0.8	0±0

[1]Exposed 10 weeks in soil-block test

There was considerable variation among isolates within a species to degrade CCA-treated pine (Table 1). For example, *M. incrassata* (TFFH-294) produced considerably more weight loss in the treated wood than *M. incrassata* (TFFH-296). *P. cocos is* reported to cause weight loss in CCA-treated wood (DeCosta and Kerrish, 1964) but the isolate (90850-s) used in this study did not degrade CCA-treated pine, Likewise, the copper tolerant fungi, *P. placenta* and *A. sinuosa* (all isolates) were unable to cause weight-loss in CCA-treated pine. Cowling (1957) determined the tolerances of several wood-destroying fungi to wood preservatives and also found considerable variation in tolerance to preservative materials within and among species of fungi that destroy wood. He suggests that because of this variation, isolates and species of fungi used in evaluation tests should be selected on the basis of their tolerance to preservatives as well as for their prevalence in causing decay. Therefore, to obtain the best estimate of the effectiveness of a toxicant, it is desirable to use the most tolerant fungi that frequently cause decay as test organisms.

M. incrassata isolates TFFH-294 and TFFH-296 produced substantial weight loss in pine blocks treated with very high levels of copper sulphate (Table 2). High tolerance of brown-rot fungi to copper has been suggested to be due to oxalic acid production by the fungi which precipitates the copper into the insoluble form of the oxalate. This renders the copper metabolize inert (Shimazono and Takubo, 1952). However, Young (1961) found that lowering the pH of the substrate from pH 6 to pH 2 strikingly increased the tolerance of brown-rot fungi to copper. He thus suggests that the low pH which brown-rot fungi generally develop, irrespective of the acid produced, is more important as a factor in their copper tolerance. Horsfall (1956) attributes the greater tolerance in acid substrates to the protection of amino acids against replacement of their hydrogen by chelating the copper.

Table 2. Ability of *Meruliporia incrassata* (TFFH-296 and TFFH-294) to degrade southern pine treated to different retentions of $CuSO_4$

Copper sulfate retention[1] kg/m³ (lb/ft³)	Weight loss with standard deviation[2] TFFH-296	TFFH294
0	23.4±2.6	21.2±3.1
.96 (.06)	31.2±1.6	28.7±4.7
1.92 (.12)	33.3±2.0	31.1±4.0
2.80 (.18)	28.7±5.8	29.0±6.9
5.6 (.35)	29.8±3.5	30.9±4.3
11.04 (.69)	12.8±5.4	22.0±6.1
20.96 (1.31)	13.0±1.7	12.8±1.2
39.69 (2.48)	18.3±0.2	17.9±0.8

[1]based on CuO.
[2]10 weeks exposure in soil-block test

The copper containing organic preservatives, copper naphthenate and copper-8-quinolinolate, protected pine against degradation by the two *M. incrassata* isolates (TFFH-294 and TFFH-296) and the four isolates of *A. sinuosa* (Table 3). Blocks were treated to a high retention of these materials and were not weathered. Naphthenic acids are fungitoxic and probably contributed to the effectiveness of copper-naphthenate against the brown-rot fungi. Previous work, however, has shown certain brown-rot fungi to be tolerant to copper napthenate (Zabel, 1954; Cowling, 1957; Duncan, 1957, 1958, & b; Thornton and Tighe) at retentions higher than we used in this study. Much of the previous work with copper napthenate used *P. placenta* as the test organism (Zabel, 1954; Duncan, 1957, 1958 a, b), which we did not use. Duncan (1958a, b) reported tolerance of *P. placenta* to weathered, copper-8-quinolinolate treated blocks. Cowling (1957), however, found several copper intolerant white-rot fungi to be more tolerant of copper-8-quinolinolate than copper-tolerant brown-rot fungi, suggesting that mechanisms in addition to copper toxicity contribute to the efficacy of this material, Oxalic acid produced by the brown-rot fungi would liberate free oxine (Byrde *et al.*, 1961). Oxine inhibits metabolic processes in microorganisms such as by competing with coenzymes for metal-binding sites on enzymes (Lukens, 1969) and, thus, may contribute to the toxicity of copper-8-quinolinolate.

Table 3. Ability of isolates from copper-treated wood to cause weight loss in pine treated with copper preservatives.

	Weight loss by fungal isolates[1] (%)					
Preservative retention kg/m³ (lb/ft³)	LRG-1	LRG-2	LRG-3	LRG-5	TFFH-294	TFFH-296
Untreated Control	57.5±1.0	46.8±1.9	47.1±6.9	41.5±2.6	44.4±2.6	16.5±2.6
Copper napthenate 34.2 (2.14)	0±0	1.0±0.1	0±0	0±0	2.9±2.9	3.4±3.1
Copper-8-quinolinolate 3.84 (.24)	1.5±0.02	1.7±0.1	1.7±0.1	1.2±0.2	3.3±0.1	3.3±0.3
CCA 6.4 (.40)	0±0	0±0	0±0	0±0	29.0±0.5	18.9±2.5

[1]Exposed 10 weeks in soil-block test

In malt-agar medium, *M. incrassata* 563 and TFFH-294 displayed similar tolerance to Cu, As and Cr despite the isolates having different capacity to produce weight loss in CCA treated wood (Table 4). This suggests that the metals are affecting a similar metabolic activity required for growth.

Table 4. Effect of copper, chromium and arsenic concentration on growth of *Meruliporia incrassata* on malt extract agar.

	Inhibition of growth (%)									
	M. incrassata (TFFH-294)					*M. incrassata* (MAD-563)				
Metal (mM)	0	0.1	1.0	10	100	0	0.1	1.0	10	100
Copper	0	0	0	100	100	0	0	0	100	100
Chromium	0	0	0	100	100	0	0	0	100	100
Arsenic	0	0	13.3	80.3	100	0	0	0	100	100

In summary, this work indicates that strains of brown-rot fungi exist in nature that are very aggressive degraders of CCA-treated wood, and thus have the potential of fungal bioleaching for detoxifying discarded CCA-treated wood.

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