CHARACTERIZATION OF COPPER IN LEACHATES FROM ACQ- AND MCQ-TREATED WOOD AND ITS EFFECT ON BASIDIOSPORE GERMINATION

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Abstract. The unpenetrated interior of wood with a shell of preservative treatment may be exposed when the wood is cut or when checks open up. Mobile copper from wood shell-treated with chromated copper arsenate (CCA) has been shown to protect cut ends and checks against basidiospore germination. However, recent observations found that leachates from alkaline copper quat (ACQ)-treated wood failed to prevent basidiospore germination on untreated wood although copper levels were higher than toxic thresholds previously identified. It was hypothesized that the copper in leachate from ACQ-treated wood may be coordinated with monoethanolamine and/or lignin-based ligands and that this may result in poorer performance against basidiospores. In this study, electron paramagnetic resonance spectroscopy was used to determine the form of copper in leachates from ACQ, micronized copper quat (MCQ), and copper-sulfate-treated wood. Leachates from ACQ-treated wood contained at least some degree of coordination with a nitrogen- and oxygen-containing ligand, probably monoethanolamine. This was not detected in

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leachates from MCQ and copper-sulfate-treated wood. These leachates were further evaluated for their ability to inhibit germination of *Tyromyces palustris* basidiospores. At low concentrations of copper, the $CuSO_4$ and MCQ leachates were more effective than the ACQ leachate. At high concentrations $CuSO_4$ and MCQ, leachates prevented germination in all samples, whereas ACQ leachates prevented germination in all but one sample.

Keywords: ACQ, basidiospores, copper, decay, EPR, ethanolamine, leaching, MCQ, *Tyromyces palustris*, wood preservation.

INTRODUCTION

One of the principal challenges of wood preservation is moving the preservative deep into wood that is resistant to treatment. Wide sapwood species, such as southern pine and red pine, are favored by the wood preservation industry for the relative ease of preservative penetration in their sapwood (Winandy et al 2001). Nevertheless, full sapwood penetration is not always achieved, nor is it required by AWPA standards (AWPA 2014). In North America, western species are generally much more difficult to treat and have lower requirements for preservative penetration in the Canadian and US standards (CSA 2012; AWPA 2014). The main concern with incomplete preservative penetration is that untreated wood may become exposed if cut ends are not protected or if checks and cracks open up to expose the untreated interior. In spite of these limitations, refractory wood species with a thin shell of chromated copper arsenate (CCA)-penetrated wood have been shown to give service lives of more than 20 yr in above-ground exposures with moderate decay hazards with or without application of field-cut treatment (Morris and Ingram 2012; Morris and Morrell 2014). Mobile copper, present as cupric ions in CCA shell-treated wood, has been shown to migrate into checks and on end cuts, thus protecting exposed untreated wood from germination of basidiospores (Choi et al 2004; Morris et al 2004). Spores from copper-tolerant basidiomycetes are not copper tolerant (Choi et al 2002; Woo and Morris 2010). Therefore, shell treatment was considered to be sufficient for above-ground applications in which spores were the primary means by which fungi came into contact with the wood. Based on the levels of copper leached from copper–amine- and basic copper-carbonate-based preservatives (Cooper and Ung 2009), it was assumed that these systems would also provide the same, if not better, protection against spores. However, in subsequent basidiospore germination tests on wood samples exposed to leachates from CCA-, ACQ-, and micronized copper quat (MCQ)-treated wood, the leachate from MCQ-treated wood performed similarly to the CCA reference, whereas the material exposed to the leachate from ACQ-treated wood exhibited significant levels of spore germination, similar to the untreated control (Stirling et al 2012). This was particularly surprising because the samples exposed to leachate from ACQ-treated wood had the highest levels of copper uptake.

In ACQ, copper coordination with monoethanolamine ligands results in a water soluble copper–monoethanolamine complex. Stable copper–amine complexes ensure good penetration, but this stability leads to lower reactivity with wood and can contribute to decreased copper leach resistance (Zhang and Kamdem 2000; Jiang and Ruddick 2004). The lack of effect on spore germination of copper present in the leachate from ACQ-treated wood suggested this may consist of a copper–amine complex or a copper–amine–lignin complex (Humar et al 2007). Such complexes have been reported to be less effective than cupric ions in controlling algae (Murray-Gulde et al 2002). Monoethanolamine does not decrease the efficacy of copper against mycelia of *Antrodia vaillantii*, *Gloeophyllum trabeum*, or *Trametes versicolor* (Humar and Lesar 2008). However, its effect on the efficacy of copper against spores has not been assessed.

This study aims to identify the form of copper present in ACQ and MCQ leachates based on the electron paramagnetic resonance (EPR) methods developed by Xue et al (2010, 2012, 2013) and to evaluate these leachates for their ability to protect untreated wood from basidiospore germination.

MATERIALS AND METHODS

Characterization of Wood, Leachates, and Wood Exposed to Leachates

Ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) sapwood was chosen for its high treatability and was cut into 100-mm-long, 10- × 1-mm veneer strips. Approximately 100 g of these strips were selected and treated with ACQ-D, MCQ, or copper(II) sulfate to 1.2 kg/m^3 (copper metal basis) based on weight uptake or left untreated. After stabilization and air-drying for approximately 1 wk, veneer strips from each treatment group were placed into separate 2.5-L polyethylene buckets with the individual samples separated from one another by polypropylene mesh. Approximately 370 mL of distilled water was added to each bucket so that all samples were fully covered. One leachate sample was obtained by decanting from each treatment group after steeping for 6 h. Fresh distilled water was then added, and the samples were leached for an additional 18 h. The leachates were then decanted and retained. Subsequently, 25 mL of each leachate was removed for analysis.

White spruce (*Picea glauca* [Moench] Voss) was chosen for its low treatability and was cut into strips of 1-mm-thick veneer. Three grams of spruce veneer samples were steeped in the raw leachates for 24 h, air-dried, and ground to pass through a 40-mesh screen.

Copper concentrations in each leachate solution were determined using an ELAN6000 ICP-MS (PerkinElmer, Waltham, MA). pH of the leachate solutions was measured using a 350 pH/Temp/mV meter (Beckman Coulter, Inc., Brea, CA) equipped with an AccuTupH double junction electrode (ThermoFisher Scientific, Fitchburg, WI). Didecyldimethylammonium carbonate (DDACarbonate) was analyzed by liquid chromatography/mass spectrometry (LC/MS) using the method described by Stirling et al (2010). One leachate and one sawdust sample from each treatment group were analyzed by EPR at 77 K using a liquid N_2 cooled quartz cold finger dewar. Each EPR sample was analyzed in triplicate and averaged. EPR data were collected on a Bruker Elexsys E500 series (Fitchburg, WI) continuous wave EPR spectrometer at a frequency of 9.40 GHz (X-band) with 100-kHz field modulation and 1G modulation amplitude. Frequency calibration (Krzystek et al 1997) was independently verified using 2,2-diphenyl-1-picrylhydrazy (DPPH, $g = 2.0036$) as an external standard. Spectra recorded were simulated using SIMFONIA (Bruker BioSpin). OriginPro 8 (OriginLab Corp., Northampton, MA) was used to calculate spectral intensities for quantifying reacted copper contents in the sawdust (Xue et al 2013). The amounts of copper coordinated as CuN_2O_2 and CuO_4 were calculated using Excel Solver (Microsoft Corp., Redmond, WA).

Evaluation of Basidiospore Germination on Untreated Wood Exposed to Leachates

Additional spruce veneer strips were cut into 10- × 10- × 1-mm samples. To determine uptake, 20 samples were pressure-treated with distilled water. Leachate samples (previously described) were freeze-dried and reconstituted to make treating solutions to target copper contents of 0.4, 0.8, and 1.2 mg/g (copper metal basis) for ACQ, MCQ, and copper(II) sulfate, respectively. Veneer samples were pressure-treated, air-dried, and sterilized by 2.5 MRad of irradiation. Copper concentration was determined by solution uptake. Eight samples from each treatment group, plus untreated controls, were selected for spore germination testing.

Tyromyces palustris (Berk. & M.A. Curtis) Murrill P227B was obtained from FPInnovations' culture collection and grown on malt agar plates. *T. palustris* is a brown-rot fungus that has copper-tolerant mycelia (Green and Clausen 2003) but copper-susceptible spores (Woo and Morris 2010). Sporulating cultures were grown using methods outlined by Choi et al (2001) and Woo and Morris (2010). Spore suspensions were prepared and diluted to 250,000 spores/mL with sterile water. Eight veneer samples from each treatment group were inoculated with 30 μL of spore suspension, resulting in approximately 7500 spores being added to each sample. Inoculated veneer samples were placed in sterile 50-mL Falcon tubes stuffed with wet cotton wrapped in cheese cloth and topped with polypropylene mesh (Stirling et al 2012). Samples were incubated for 2 wk at 25°C and assessed visually, with the aid of a Zeiss (Carl Zeiss Meditec Group, Jena, Germany) dissecting microscope at magnification between 20× and 40×, to determine if spores had germinated on the sample surface.

RESULTS AND DISCUSSION

Characterization of Wood, Leachates, and Wood Exposed to Leachates

Leachate pH varied from 3.9 for the 6-h leachate from copper-sulfate-treated wood to 8.9 for the 24-h leachate from ACQ-treated wood (Table 1). The alkaline leachate from ACQ-treated wood suggests loss of amine. As anticipated, copper concentrations were greater in the leachate from wood treated with ACQ than in the leachate from wood treated with MCQ (Zhang and Ziobro 2009). Only traces of copper were detected in the untreated controls. DDACarbonate was detected only in the leachates from MCQ-treated wood. This was probably a function of pH, because lower pH has been associated with decreased fixation of quaternary ammonium compounds in wood (Butcher and Drysdale 1978; Ruddick and Sam 1982). The ratios of copper to quat were much higher than in the treating solution, confirming that the copper was the more leachable component (Stook et al 2005). At this ratio, a maximum of 0.27 mg/g of DDACarbonate would be present in the samples treated with concentrated MCQ leachate. This was not expected to affect spore germination results because DDACarbonate does not inhibit germination of *Oligoporus placentus* and *Gloeophyllum sepiarium* at this concentration (Woo 2010).

The EPR spectrum of the untreated 6-h leachate solution had no copper signal, but an organic free radical signal was observed in the spectrum (Fig 1). Copper species in the copper-sulfate-treated wood leachates (Table 2) appeared to be simple hydrated copper (II) species, similar to those observed in aqueous $CuSO_4$ solutions (Ruddick 1992). Simulated EPR parameters for MCQ and ACQ leachates were similar to those obtained from the sawdust treated by the corresponding formulations (Xue et al 2012). However, the MCQ leachates had very low copper concentrations, and therefore, the simulations may be less accurate because of the low signal to noise ratio. The parameters of the ACQ leachates show that the copper species is likely to have two nitrogen atoms complexed to the copper (Peisach and Blumberg 1974).

The EPR spectrum of the spruce treated with leachate from untreated wood showed no visible copper signal (Table 3). Samples treated with MCQ and $CuSO_4$ leachates showed copper

Table 1. pH and copper and didecyldimethylammonium carbonate (DDACarbonate) concentrations in leachates from wood without treatment or treated with alkaline copper quat (ACQ), $CuSO_4$, or micronized copper quat (MCQ) prior to leaching.

Leachate	Total leaching time (h)	pH	Copper (mg/L)	DDACarbonate (mg/L)
Untreated	6	4.9	0.10	<0.25
	24	5.0	0.05	<0.25
ACQ	6	8.8	55	<0.25
	24	8.9	21	<0.25
$CuSO_4$	6	3.9	243	<0.25
	24	4.2	67	<0.25
MCQ	6	5.6	15	3.38
	24	5.8	6	0.57

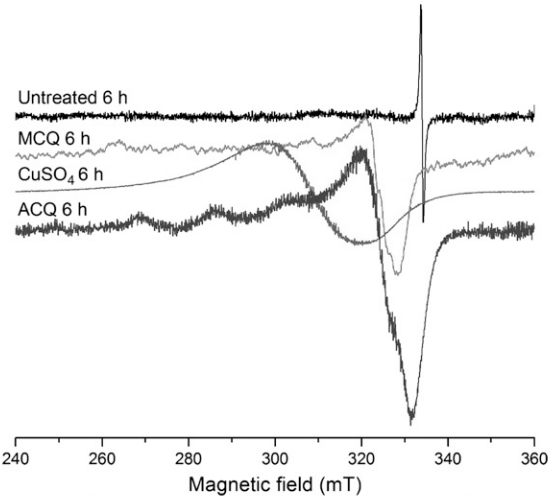

Figure 1. Electron paramagnetic resonance spectra of selected leachates at 77 K.

species similar to those in the MCQ and copper-sulfate-treated wood, which had only oxygen atoms complexed to the copper (Xue et al 2010). However, samples treated with the ACQ leachates appeared to have a mixture of CuN_2O_2 and CuO_4 species (Fig 2). In the wood treated with 6-h ACQ leachate, there was a 3:2 ratio of CuN_2O_2 to CuO_4 species. Copper concentrations in wood treated with 24-h ACQ leachate were below the calibration range used to generate the simulated spectra.

These data suggest that copper redistributed into checks from ACQ-treated wood would be present as two species: one that is similar to regular hydrated copper in wood and is similar to that found in MCQ-treated wood and another that is coordinated with ethanolamine ligands, and as such would be chemically different from copper redistributed into checks from MCQ-treated wood. These different chemical interactions on check surfaces may explain the differences between the efficacy of ACQ and MCQ shell treatments against basidiospores (Stirling et al 2012).

Evaluation of Basidiospore Germination on Untreated Wood Exposed to Leachates

T. palustris basidiospore germination was observed on all untreated control samples after 2 wk of incubation (Fig 3). Spore germination was observed at the lowest copper concentration on all but one of the samples treated with ACQ leachate. At the same copper concentration, basidiospore germination was observed on less than half of the samples treated with $CuSO_4$ or MCQ leachates. Medium and high concentrations of copper inhibited basidiospore germination in most samples, regardless of the source of the copper, although even at the highest concentration, germination was observed on one sample treated with ACQ leachate. These results are consistent with previous basidiospore germination tests with *G. sepiarium* and *O. placentus*, which showed that leachate from ACQ-treated wood was less effective than leachate from MCQ-treated wood in inhibiting spore germination despite having higher concentrations of copper (Stirling et al 2012). Together, these data suggest that copper ethanolamine is less effective

Table 2. Simulated electron paramagnetic resonance parameters for leachate solutions from untreated wood and wood treated with alkaline copper quat (ACQ), $CuSO_4$, or micronized copper quat (MCQ) at 77 K.

		g-factor			Hyperfine coupling constant (10^{-4} cm^{-1})		
Leachate	Total leaching time (h)	g_x	g_y	g_z	A_x	A_y	A_z
Untreated	6	2.008	2.008	2.008	0	0	0
	24			No signal			
ACQ	6	2.064	2.064	2.278	5	37	180
	24	2.064	2.064	2.278	5	37	180
$CuSO_4$	6	2.151	2.151	2.265	40	40	85
	24	2.144	2.144	2.262	40	40	84
MCQ	6	2.066	2.066	2.365	5	24	144
	24	2.066	2.066	2.365	5	34	144

Table 3. Simulated electron paramagnetic resonance parameters and copper contents of CuN_2O_2 and CuO_4 in spruce treated with leachate solutions.

Leachate	Total leaching time (h)	g-Factor			Hyperfine coupling constant (10^{-4} cm^{-1})			Copper content (% w/w)	
		g_x	g_y	g_z	A_x	A_y	A_z	CuO_4	CuN_2O_2
Untreated	6				No copper signal				
Untreated	24				No copper signal				
ACQ	6	2.066	2.076	2.281	5	44	179	—	0.03
		2.075	2.075	2.370	5	29	147	0.02	—
ACQ	24	2.073	2.073	2.372	5	29	147	0.01	0
$CuSO_4$	6	2.081	2.081	2.385	5	29	152	0.27	0
$CuSO_4$	24	2.079	2.079	2.381	5	30	148	0.09	0
MCQ	6	2.078	2.078	2.378	5	29	152	0.02	0
MCQ	24	2.077	2.077	2.378	5	29	152	<0.01	0

ACQ, alkaline copper quat; MCQ, micronized copper quat.

against basidiospore germination than cupric ions. Although copper amine systems generally leach more copper than particulate copper systems (Zhang and Ziobro 2009), this does not mean they will be more effective in preventing spore germination on untreated check surfaces and cut ends, because the form of copper leached differs in the two systems.

Few field studies report the degree to which copper from shell-treated wood accumulates in untreated checks, and existing studies are limited in scope (Choi et al 2004; Stirling and Morris 2010). Although the effect of initial retention on redistribution of copper into checks has not been studied, the higher leaching rates observed from wood treated to higher retentions (Ung and Cooper 2005) suggests that greater amounts of copper would migrate into checks. Parallel field efficacy tests are also needed because the laboratory methods used expose samples to large numbers of spores and ideal germination conditions that would seldom be encountered in the field. The degree to which copper species fix to the check surfaces was not investigated. Further work is needed to monitor copper species on check surfaces and their effect on basidiospore germination with time. Formulations and treatment conditions need to be optimized to balance the need to minimize copper leaching into the environment with the need to ensure that adequate amounts of mobile copper are available to protect untreated wood exposed by checking.

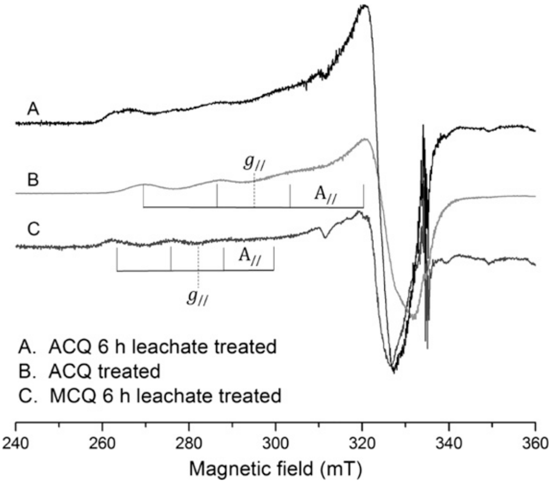

Figure 2. Smoothed (20 point) electron paramagnetic resonance spectra of ACQ and MCQ 6-h leachates at 77 K. The MCQ 6-h leachate spectrum has been enlarged 200%. The spectrum of the ACQ-treated sample was from a previous analysis and inserted here for comparison.

CONCLUSIONS

Leachates from copper-sulfate- and MCQ-treated wood were better able to protect untreated wood from *T. palustris* basidiospore germination than leachates from ACQ-treated wood.

Copper in leachate from ACQ-treated wood is coordinated by two ethanolamine ligands.

There were two copper species in wood treated with leachate from ACQ-treated wood, one of which was coordinated with both oxygen and

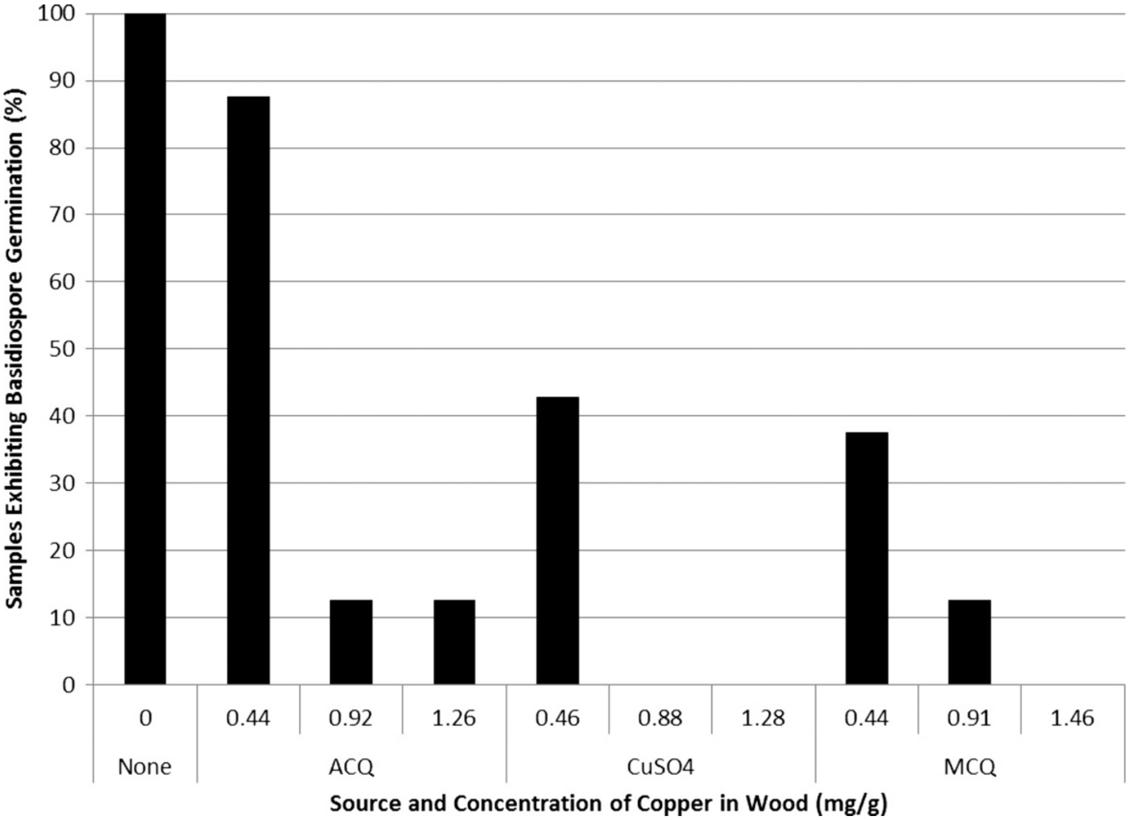

Figure 3. Incidence of basidiospore germination on spruce veneer samples untreated and treated with leachates from alkaline copper quat (ACQ), copper(II) sulfate, and micronized copper quat (MCQ).

nitrogen bonding, whereas the second contained only copper oxygen bonding.

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