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Field tests of the efficacy of zinc and fatty amine in preventing colonization by copper-tolerant fungi

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ABSTRACT

Ground-contact durability of stakes treated with acidic copper formulations was evaluated. All test formulations incorporated copper, dimethylcocoamine and propanoic acid; one set of formulations also included zinc. Sapwood stakes cut from the southern pine group were pressure-treated to a range of retentions with each formulation and placed into plots within Harrison Experimental Forest in Mississippi and compared with untreated stakes and chromated copper arsenate-treated stakes. Stakes were inspected and given a visual condition rating after 1, 2, 3, 4, 5, and 7 years. Most stakes at higher retentions remained in good condition after 7 years; sporadic failures occurred in all but the highest retention of the zinc formulation. The sporadic nature of fungal attack by a fungus thought to be a strain of *Antrodia radiculosa* indicates that failures were caused by copper-tolerant fungi. At the concentrations evaluated, neither the dimethylcocoamine nor the propanoic acid offered adequate protection against copper-tolerant fungi. Addition of zinc notably increased decay resistance, and absence of failures at the highest retention may indicate that zinc can help to protect against copper-tolerant fungi. However, the sporadic nature of copper tolerance makes this finding difficult to confirm. Inspection of theses stakes will continue.

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1. Introduction

Traditional water-based preservative formulations such as chromated copper arsenate (CCA) relied heavily on copper because of its excellent fungicidal properties. Although multiple chromium and arsenic-free alternative wood preservatives have been introduced in recent years, those intended to protect wood used in contact with soil continue to use copper as the primary fungicide. Acidic copper solutions without chromium can be highly corrosive to metal fasteners, and to lessen this concern, these alternative formulations use alkaline formulations of copper. Organic fungicides such as quaternary ammonium or azole compounds are included in these formulations to help protect the wood from attack by copper-tolerant fungi. These formulations also use higher concentrations of copper than did CCA, and there is some indication that this alkaline copper is less resistant to leaching than the acidic form used in CCA (Waldron et al., 2005; Temiz et al., 2006). Ideally, an acidic formulation would be effective at lower copper concentrations, more similar to that used in CCA. One rationale for the expected decay resistance of an acidic copper formulation is the performance of copper formate and acid copper chromate- (ACC-) treated stakes exposed at FPL's Harrison Experimental Forest test site. Although these formulations have not been as consistently effective in protecting wood as CCA, many stakes remained in good condition for over 40 years (Woodward et al., 2011). Some early failures did occur with these treatments, which may be attributable to attack by copper-tolerant fungi.

Colonization and decay caused by copper-tolerant fungi has long been recognized as a major concern for copper-based preservative treatments (Morrell, 1991). Numerous fungi have been shown to have some degree of copper tolerance in laboratory testing (Sutter et al., 1983; De Groot and Woodward, 1999; Green and Clausen, 2003), and attack of wood treated with copper formulations has also been reported in field testing (Schultz and Nicholas, 2009, 2010). The extent and severity of attack of copper-treated wood products in-service is not well understood, but commercial copper-based preservatives typically include a co-biocide to minimize this risk. Arsenic was an effective co-biocide in CCA, and most current commercial alkaline and dispersed copper formulations use quaternary amine or azole co-biocides.

In this study, we evaluated the durability of acidic copper formulations using copper concentrations below the level used in

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Table 1
Retention of individual preservative components in treated stakes.

Solution type	Solution (%)	Retention in stakes (kg/m^3)					
		CuO[a]	Dimethylcocoamine[b]	Propanoic acid[b]	ZnO[a]	B_2O_3[a]	Total
CDP	0.45	0.63	0.69	1.68	–	–	3.01
CDP	0.89	1.14	1.25	3.03	–	–	5.41
CDP	1.34	1.65	1.80	4.39	–	–	7.85
CDP	1.78	2.04	2.23	5.43	–	–	9.70
CDP	2.23	2.33	2.55	6.21	–	–	11.09
CDP	2.67	3.04	3.33	8.11	–	–	14.48
CZDP	0.33	0.30	0.27	1.09	0.28	–	1.94
CZDP	0.66	0.78	0.65	2.59	0.61	–	4.63
CZDP	0.99	0.91	0.77	3.10	0.75	–	5.53
CZDP	1.32	1.51	1.28	5.11	1.23	–	9.13
CZDP	1.65	1.46	1.23	4.92	1.17	–	8.79
CZDP	1.98	2.01	1.71	6.85	1.66	–	12.23
CZDP + B	0.66 + 0.28 B_2O_3	0.69	0.59	2.35	0.57	1.20	5.39
CZDP + B	1.32 + 0.56 B_2O_3	1.28	1.05	4.22	0.98	2.54	10.07

[a] Determined by ICP analysis of milled wood.
[b] Estimated from solution composition and assayed concentration of CuO and/or ZnO.

conventional alkaline-copper formulations. A fatty amine (dimethylcocoamine), and in some cases zinc oxide, were included in the formulation to provide protection against copper-tolerant fungi and to lessen concerns with corrosion (Williams et al., 1994; West, 2004). Previous research has shown that fatty amine salts exhibit toxicity to microorganisms through cell membrane disruption (van Ginkel et al., 2008) and can inhibit decay by some types of fungi (Butcher et al., 1977; Preston, 1983). Although zinc is a somewhat less effective fungicide than copper (Barnes et al., 2004; Woodward et al., 2011) it has been widely used as a component of wood preservative formulations (Richardson, 1993). Propanoic (propionic) acid was also used in the formulations, primarily to improve solubility. However, propanoic acid does have efficacy against mold fungi in crop storage and food products (Sheaffer and Clark, 1975; Suhr and Nielsen, 2004), and may also contribute to the formulations' performance. To evaluate these formulations, stakes treated with a range of retentions were placed into the ground at a test site in Harrison Experimental Forest in southern Mississippi. The presence of copper-tolerant fungi at this site has been reported previously (Lebow et al., 2003; Schultz and Nicholas, 2010). This report discusses the decay and termite resistance of the treated stakes after 7 years of exposure.

2. Materials and methods

2.1. Preservative formulations

Two sets of formulations were tested. All formulations used copper hydroxide, dimethylcocoamine, and propanoic acid solubilized in water. One set of formulations also contained zinc oxide.

CDP: The CDP formulation had a composition of 21% copper (CuO basis), 23% dimethylcocoamine, and 56% propanoic acid. Specimens were treated with 0.45, 0.89, 1.34, 1.78, 2.23, and 2.67% solution concentrations.

CZDP: CZDP was composed of 18% copper (CuO basis), 12% zinc (ZnO basis), 14% dimethylcocoamine, and 56% propanoic acid. Specimens were treated with 0.33, 0.66, 0.99, 1.32, 1.65, and 1.98% solution concentrations. Two additional treatments were included to evaluate the value of adding boron to the CZDP. These were 0.66% CZDP + 0.28% B_2O_3 (0.5% boric acid) and 1.32% CZDP + 0.56% B_2O_3 (1.0% boric acid). In this paper these treatments are referred to CZDP + B.

Reference preservative: A 1% solution of chromated copper arsenate Type C (CCA-C) was included as an established reference preservative for both types of formulations. Based on AWPA Standard P5 (American Wood Protection Association Standards, 2011a), the CCA-C had an actives composition of 47.5% chromium (CrO_3 basis), 34.0% arsenic (As_2O_5 basis), and 18.5% copper (CuO basis).

2.2. Stake preparation and treatment

Twenty-five stakes (19 × 19 × 457 mm) per treatment group were cut from clear sapwood of the Southern Pine species group and conditioned to constant weight in a room maintained at 23 °C (74 °F) and 65% relative humidity. As described in AWPA Standard E7 (American Wood Protection Association Standards, 2003), the stakes were sorted by weight to minimize differences in density between treatment groups. Each set of 25 stakes was treated with one of the preservative formulations using a full-cell pressure process. The initial vacuum was maintained at −75 kPa (gauge) for 30 min; the pressure was maintained at 1.03 MPa for 1 h. Following treatment, the stakes were stacked in plastic bags for one week and then allowed to air-dry under room conditions. Five of the stakes from each of the test formulations were randomly selected and set aside for chemical analysis of preservative retention. Sections cut from these stakes were milled and digested in accordance with AWPA Standard A7 (American Wood Protection Association Standards, 2011b) and concentrations of copper and/or zinc and boron (as appropriate) were determined by inductively coupled plasma emission spectrometry (ICP–AES) (Table 1). The ICP analysis revealed that stakes treated with the 1.65% CZDP solutions had unexpectedly low copper and zinc retentions (Table 1). The cause of this anomaly is unclear, but it must be considered when reviewing the durability of this treatment group. An additional 20 untreated stakes were also prepared for installation in the plots.

Table 2
Typical rating scheme for evaluation of stakes.

Rating	Description of condition
10	No evidence of attack
9	Slight attack, up to 3% of cross-sectional area
8	Moderate attack, up to 10% of cross-sectional area
7	Moderate to severe attack, up to 30% of cross-sectional area
6	Severe attack, up to 50% of cross-sectional area
4	Very severe attack, up to 75% of cross-sectional area
0	Failure, can be broken easily by hand or the evaluation probe can penetrate through the stake

Table 3
Average ratings and number of failures noted for CDP- and CCA-treated stakes at each annual inspection.[a,b]

Formulation[c] and retention (kg m^{-3})	Year 1		Year 2		Year 3		Year 4		Year 5		Year 7	
	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite
CDP, 3.01	9.3	9.2	7.4 3 Failures	9.2	6.7 2 Failures	7.6	5.1 4 Failures	5.8	3.2 6 Failures	5.8	– 5 Failures	–
CDP, 5.41	9.7	9.8	9.2 1 Failure	9.5	8.6 1 Failure	9.6	7.7 1 Failure	9.3	6.3 2 Failures	9.4	2.2 12 Failures	7.7
CDP, 7.85	9.4 1 Failure	10.0	8.8 1 Failure	10.0	8.5	9.8	7.9 2 Failures	8.9	6.1 2 Failures	9.7	3.5 5 Failures	9.5
CDP, 9.70	9.9	10.0	9.4 1 Failure	10.0	9.3	9.9	7.4 2 Failures	9.8	7.9	9.8	6.7 2 Failures	9.5
CDP, 11.09	9.9	9.9	8.4 2 Failures	10.0	7.4 3 Failures	10.0	7.6	9.1	7.2	10.0	6.6 2 Failures	9.6
CDP, 14.48	9.9	10.0	9.4 1 Failure	9.3	8.9 1 Failure	9.5	8.9	10.0	7.7 2 Failures	9.5	4.7 6 Failures	9.4
CCA, 6.67	9.9 No failures occurred in this treatment group	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Untreated	4.2 10 Failures	6.0	0.5 9 Failures	3.4	– 1 Failure	–	– –	– –	– –	– –	– –	– –

[a] No ratings were taken for Year 6.
[b] Twenty replicate stakes were installed for each treatment.
[c] Refer to Table 1 for solution concentrations and formulation compositions.

2.3. Stake installation and inspection

The Mississippi test site is located in a forested area within Harrison Experimental Forest (approximately 24 km north of Gulfport-Biloxi). The location is within American Wood Protection Association Deterioration Zone 5 (severe deterioration hazard) (American Wood Protection Association Standards 2011c). Native subterranean termites are active at this site, but the presence or activity of Formosan subterranean termites has not been reported. The two formulations (CDP and CZDP) and the CCA-treated and untreated stakes were placed in two separate but adjacent plots. Plot stakes were placed in rows with 305-mm spacing between stakes within each row and 915 mm between rows. The stakes were randomly assigned locations within the plot and were buried vertically to one-half their length. An iron rod was used to create holes for stake placement. The stakes were installed in January and inspected annually after 1, 2, 3, 4, 5, and 7 years. At each inspection the stakes were removed, scraped lightly to remove soil, and given a visual rating for decay and/or termite attack according to the 10, 9, 8, 7, 6, 4, 0 scale as described in AWPA Standard E7-01 (American Wood Protection Association Standards, 2003) (see Table 2).

3. Results and discussion

3.1. CDP-treated stakes

All retentions of CDP, including the highest retention, suffered at least one stake failure within 2 years of exposure (Table 3). The lowest retention had the most failures, and also the lowest average ratings over time. Durability differences were less obvious for the five higher CDP retentions, and the three highest retentions had very similar average ratings and total numbers of failures. The timing of failures was not clearly linked with years of exposure, except for the lowest retention. In general, stakes treated with CDP exhibited a bimodal pattern of attack, which has been associated with colonization by copper-tolerant fungi (Schultz and Nicholas, 2009). This pattern of attack is typified by complete failure of a few stakes, while the remaining stakes show little or no evidence of attack (Schultz and Nicholas, 2009). The sporadic nature of the failures and weak correlation between retention and number of failures is also an indication of attack by copper-tolerant fungi (Lebow et al., 2003). Soil in this site is known to contain a copper-tolerant fungus that has been identified as *Antrodia radiculosa* (Peck) Gilb. & Ryvarden (Schultz and Nicholas, 2010). The fungus is characterized by a pronounced yellow color (Schultz and Nicholas, 2010) and by the presence of thick strands of yellow hyphae that are visible in the soil, and in some cases in the treated wood. Strains of *A. radiculosa* have been reported to colonize wood treated with copper (Green and Clausen, 2001, 2003), and some strains have shown the ability to colonize wood treated with copper, chromium, and arsenic (Illman and Yang, 2006). This fungus is either not uniformly distributed throughout the plots or does not consistently attack copper-treated wood. As a result, failures of copper-treated stakes are sporadic spatially and over time. In the case of CDP-treated stakes, this pattern was clearly observed for stakes treated to the three highest retentions. Only the lowest retention (3.01 kg m^{-3}) showed a deterioration pattern more typical of general decay and termite attack. This indicates that the lowest preservative retention was near the minimum threshold needed to prevent attack by both termites and decay fungi that are not copper

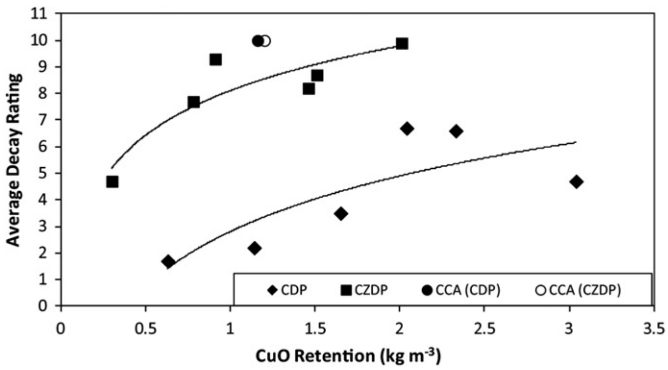

Fig. 1. Average decay rating versus CuO retention for stakes treated with CDP, CZDP or their respective CCA-treated positive controls.

tolerant for wood in soil contact. The relatively large decrease in average ratings between years 5 and 7 for the lower retentions might also be attributable to leaching (depletion) of preservative from the stakes, allowing colonization by non-tolerant fungi.

The CDP durability results indicate that neither the dimethylcocoamine nor the propanoic acid offered significant protection against copper-tolerant fungi at the concentrations evaluated. The CCA-treated stakes remained in excellent condition after 7 years, even though the copper concentration in the CCA stakes was below that of several of the CDP treatments (Fig. 1). This indicates that either the strain of *A. radiculosa* at the site is not CCA-tolerant, or that the conditions that allowed the CCA tolerance reported in laboratory testing (Illman and Yang, 2006) are less likely to occur in field exposures.

3.2. CZDP-treated stakes

The average decay resistance of the CZDP-treated stakes was superior to that of the CDP-treated stakes, especially when compared at similar copper concentrations (Table 4, Fig. 1). The CZDP-treated stakes also had fewer failures, and no failures occurred at the highest (12.23 kg m^{-3}) retention. The combined retentions of copper and zinc in the CZDP formulation were greater than the copper retention in the CDP formulations, with zinc replacing a portion of the dimethylcocoamine. This indicates that the addition of the zinc to the formulation may provide some benefit in decay resistance, and possibly some resistance against copper-tolerant fungi.

Although zinc has been used as a component of wood preservative formulations for over a century (Richardson, 1993), there is little information on zinc tolerance in wood decay fungi, and more specifically on zinc tolerance in copper-tolerant wood decay fungi. Copper and zinc are thought to share some mechanisms of toxicity toward microorganisms, but mechanisms of metal toxicity in fungi can vary greatly (Gadd, 1993). In addition, zinc does not exhibit some mechanisms of copper toxicity, such as oxidative injury to cell membranes (Colpaert et al., 2005). Tolerance mechanisms of microorganism tend to be metal specific (Hall, 2002; Bellion et al., 2005), and there is evidence that the organic acid exudation and metal chelation mechanism proposed for copper tolerance in fungi (Green and Clausen, 2001) is less important in zinc tolerance (Bellion et al., 2005). Thus, it is difficult to conclude that fungi exhibiting copper tolerance would necessarily demonstrate zinc tolerance.

However, it is possible that copper-tolerant fungi are responsible for the failures in the 8.79 and 9.13 kg m^{-3} retentions, as yellow hyphae similar to those associated with *A. radiculosa* were observed on the stakes. As is typical of attack by copper-tolerant fungi, most other stakes in those groups were rated as 9 or 10. The retentions of these two groups are similar, and it is likely that the difference in the number of failures (a total of two failures at the 9.13 kg m^{-3} retention and five failures at the 8.79 kg m^{-3} retention) reflects the localized nature of attack by copper-tolerant fungi. Most of the stakes that failed were within a small area of the plot. This localized nature of the attack raises the possibility that the differences in the number of failures between the CDP- and CZDP-treated stakes are partly or wholly a function of the location of the stakes within the plots. Similarly, the absence of failures at the highest CZDP retention may simply be a function of the location of those stakes within the plot. Because of the inconsistent nature of attack by copper-tolerant fungi, it is difficult to reach strong conclusions about the ability of co-biocides to provide protection. It is interesting to note that termite ratings of the CZDP stakes appear to be slightly lower than those of the CDP stakes, and that termite damage was severe in many of the treatment groups at the 7-year inspection. It is unclear why the CDP treatment would provide greater termite protection, although it may be a function of the higher copper and dimethylcocoamine retentions in the CDP stakes. Addition of boric acid to the CZDP formulation did not provide an obvious benefit. The average decay ratings of the 0.66%

Table 4
Average ratings and number of failures noted for CZDP-, CZDP + B-, and CCA-treated stakes at each annual inspection.[a,b]

Formulation[c] and retention (kg m^{-3})	Year 1		Year 2		Year 3		Year 4		Year 5		Year 7	
	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite	Decay	Termite
CZDP, 1.94	9.8	9.6	9.9	8.0	6.8	4.7	5.2	3.6	4.7	2.0	–	–
					8 Failures		4 Failures		5 Failures		3 Failures	
CZDP, 4.63	10.0	10.0	9.8	9.6	9.5	9.5	8.9	8.9	7.8	7.4	7.7	4.3
							1 Failure		2 Failures		7 Failures	
CZDP, 5.53	10.0	10.0	10.0	9.8	9.7	9.7	9.6	9.3	9.4	8.5	9.3	6.5
											3 Failures	
CZDP, 9.13	10.0	10.0	10.0	10.0	9.9	10.0	9.3	9.8	8.7	9.5	8.7	8.7
							1 Failure		1 Failure			
CZDP, 8.79	10.0	10.0	10.0	9.9	9.8	10.0	9.0	9.7	8.8	9.4	8.2	7.3
							2 Failures				3 Failures	
CZDP, 12.23	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.9	9.8	9.9	9.2
	No failures occurred in this treatment group											
CZDP + B, 5.39	10.0	10.0	10.0	9.4	9.6	9.1	9.5	8.6	8.8	7.7	8.9	3.4
									1 Failure		9 Failures	
CZDP + B, 10.07	10.0	10.0	10.0	10.0	10.0	9.8	9.3	9.7	8.9	9.5	8.9	7.9
							1 Failure		1 Failure		1 Failure	
CCA, 6.47	10.0	10.0	10.0	10.0	9.9	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	No failures occurred in this treatment group											
Untreated	7.0	3.3	7.2	0.0	–	–	–	–	–	–	–	–
	12 Failures		8 Failures		–	–	–	–	–	–	–	–

[a] No ratings were taken for Year 6.
[b] Twenty replicate stakes were installed for each treatment.
[c] Refer to Table 1 for solution concentrations and formulation compositions.

CZDP treatment with boric acid were slightly higher than 0.66% treatment without boric acid, but both groups had the same number of failures after 7 years. Little difference in durability was observed in the 1.32% CZDP treatments with and without boric acid.

4. Conclusions

The acidic low-copper formulations evaluated in this study are exhibiting evidence of vulnerability to attack by copper-tolerant fungi. This indicates that neither the dimethylcocoamine nor the propanoic acid offers complete protection against copper-tolerant fungi at the concentrations evaluated. The addition of zinc does appear to have benefited the CZDP treatments, as they have higher average ratings and fewer failures. The highest CZDP retention had no failures after 7 years of exposure and has resisted decay nearly as well as CCA. This may indicate that CZDP can protect against both copper-tolerant and non-copper-tolerant fungi at retentions above 12 kg m^{-3}. However, the sporadic nature of attack makes it difficult to determine if a formulation is resistant to copper-tolerant fungi. No obvious durability benefit was obtained by adding boric acid to the CZDP formulation, and some termite attack was noted in formulations with and without boron. Above-ground testing has also been initiated with the CZDP formulation and will be reported after a longer exposure period.

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