Butylated Hydroxytoluene and Ethylenediaminetetraacetic Acid Combined with Cedarwood Oil as Wood Treatments for Protection from Subterranean Termites and Wood-Decaying Fungi

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The effects of the antioxidant, butylated hydroxytoluene (BHT), and the metal chelator, ethylenediaminetetraacetic acid (EDTA) in combination with cedarwood oil (CWO) were investigated for wood preservation against subterranean termites as well as two species of white-rot decay fungi and two species of brown-rot decay fungi. Vacuum pressure impregnation was used to treat wood blocks. Resistance of the treated wood test blocks was evaluated using a no-choice bioassay for termites and a soil bottle assay wood decay fungi. Eight treatments were tested: H_2O only; BHT only; EDTA only; BHT with EDTA; CWO only; CWO with EDTA; CWO with BHT; and CWO with BHT plus EDTA. For termites, the lowest percentage wood mass losses were for the EDTA, BHT, CWO, and CWO/EDTA treatments, all of which were statistically equivalent. Correspondingly, these treatments all had the highest termite mortalities at 100%. The four species of decay fungi were affected differently by the wood treatments; however, overall CWO and EDTA gave the best protection against wood mass loss. The addition of BHT did not decrease mass loss.

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INTRODUCTION

Eastern red cedar (ERC; *Juniperus virginiana* L.; Cupressaceae) is a common conifer found throughout the eastern United States. Its wood is valued for making cedar chests, paneling, decorative novelties, and fence posts. The heartwood of ERC is classified as "resistant to very resistant" to decay (U.S. Forest Service Research Note 1967). This resistance is attributable to the presence of cedarwood oil (CWO) (CAS 8000-27-9) in the heartwood (Carter 1976; Oda *et al.* 1977; Adams *et al.* 1988; Clark *et al.* 1990; McDaniel and Dunn 1994). There are many reports describing antifungal activity of essential oils

against wood-decaying fungi, which have been recently reviewed (Broda 2020; Goodell *et al.* 2020; Wozniak 2022).

Several organic solvents (*i.e.*, pentane, hexane, acetone, and methanol) have been used to extract antitermitic compounds from ERC heartwood (Carter and Smythe 1974; Carter 1976; Adams *et al.* 1988; McDaniel *et al.* 1989), although CWO is typically obtained by steam distillation. In addition, excellent yields of high quality CWO have been obtained by supercritical CO_2, liquid CO_2, and hexane extraction of ERC sawdust or chips. (Eller 2018).

Vacuum impregnation of an extract (acetone/hexane/water mix) of ERC reduced termite attack (McDaniel and Dunn 1994). In addition, an essential oil extract of ERC had antifungal activity (Mun and Prewitt 2011). Other research has shown CWO to have a wide variety of additional biological activities. For example, CWO has been shown to be repellent to ants (Eller *et al.* 2014, 2015), both repellent and toxic to ticks (Panella *et al.* 1997; Eller *et al.* 2014, 2015; Flor-Weiler *et al.* 2022), and toxic to houseflies and mosquitoes (Eller 2018). Vacuum pressure impregnation of CWO has been used successfully to protect otherwise susceptible wood against both termites and wood-decay fungi (Eller *et al.* 2010, 2018, 2020, 2021; Tumen *et al.* 2013).

Most wood preservatives are effective because of their biocidal (*e.g.*, termiticidal or fungicidal) activity. However, some materials do not have biocidal activity *per se* but have preservative activity. For example, antioxidants have been investigated as benign alternative wood preservatives. Reports have suggested that free radical species help disrupt the cell walls of wood and facilitate the penetration of both white- and brown-rot fungal enzymes (Backa *et al.* 1992, 1993; Flournoy *et al.* 1993). Research has shown that the combination of antioxidants and an organic biocide gives enhanced wood protection against fugal decay.

Schultz and Nicholas (2000) found that butylated hydroxytoluene (BHT) alone does not provide protection against fungal degradation but when combined with six different organic biocides (*i.e.*, propiconazole, didecyldimethylammonium chloride, Kathon 9302, tebuconazole, chlorothalonil, and 3-iodo-2-propynyl butyl carbamate) a much greater efficacy was obtained. Schultz and Nicholas (2002) report the addition of BHT and/or EDTA with the biocide tebuconazole gave greater protection than the biocide alone against brown- and white-rot fungi. They postulated that wood extractives can protect heartwood *via* at least three different mechanisms, including fungicidal activity, free radical scavenging/antioxidants, and metal chelation. The combination of an organic biocide with antioxidant additives and/or metal chelating may give enhanced protection to wood against fungi compared to the biocide alone.

After 52 months of field exposure, Schultz *et al.* (2004) found increased wood preservation efficacy against both fungal and termite degradation for samples with added BHT. Schultz *et al.* (2005) reported that the antioxidant BHT had 2 to 3-fold enhanced efficacy after four years of exposure against both fungal decay and termite degradation compared to the biocide only treatment. It is thought that BHT, or other free radical scavengers (*i.e.*, antioxidants), interfere with the fungal generated radicals that initiate fungal decay.

Ragon *et al.* (2008) found that BHT treated wood inhibited termite feeding as well as unexpectedly resulting in elevated termite mortality. Little *et al.* (2010) reported that wood treated with 0.5% of the antioxidant butylated hydroxy aniline (BHA) caused 100% termite mortality and there was less wood block mass loss than controls.

BHT is a low-cost antioxidant used in foods, personal care products and industrial oils, *etc.* BHT has been designated Generally Recognized as Safe by the US Food & Drug Administration (Code of Federal Regulations, Title 21, Chapter I, Subchapter B, Part 172, Subpart B, Section 172.115, 2023). Therefore, BHT appears a good choice for enhancing the effectiveness of some wood preservatives.

In addition to antioxidants such as BHT, metal chelators might also offer enhanced wood protection. In some decay fungi, the Fenton reaction (Fenton 1894) is thought to participate in wood degradation, in which hydrogen peroxide (H_2O_2) oxidizes ferrous iron, Fe (II) to yield ferric iron, Fe (III), hydroxide ions (OH-), and hydroxyl radicals (OH•). The highly reactive hydroxyl radicals degrade cell wall constituents, causing disruption of the lignocellulose matrix by oxidative cleavage of glycosidic bonds in the polysaccharides (Highley and Illman 1991; Goodell *et al.* 1997; Hosseinpourpia and Mai 2016). Brown-rot fungi, such as *Gloeophyllum trabeum* and *Poria placenta*, synthesize extracellular compounds that initiate the Fenton reaction and the production of hydroxyl radicals (Xu and Goodell 2001; Cohen *et al.* 2002; Diouf *et al.* 2002; Quian *et al.* 2002).

This reaction could be inhibited using iron chelators (Mabicka *et al.* 2005). The chelation of metal ions employed by decay fungi so that the metals are no longer available to participate in the degradation of wood *via* the Fenton reaction is important in natural durability (Schultz and Nicholas 2002; Henry 2003; Goodell *et al.* 2007; Binbuga *et al.* 2008). Schultz and Nicholas (2011) concluded that the addition of a benign antioxidant (*i.e.*, BHT) and/or metal-complexing additive (*i.e.*, propyl gallate) enhances the efficacy of organic biocides against wood-decay fungi in an accelerated soil-contact test. Mabricka *et al.* (2005) discuss the synergistic wood preservation effect of EDTA in combination with 2-hydroxypyridine-N-oxide (2-HPNO) even though EDTA has no antifungal activity of its own. Binbuga *et al.* (2008) report natural non-biocidal antioxidant/metal chelators enhance the efficacy of fungicidal biocides.

Thus far, there are no reports of using CWO in combination with BHT and/or EDTA additives against termites or wood decay fungi. The objective of this study was to investigate the effects of the antioxidant, BHT, and the metal chelator, EDTA, in combination with CWO for enhanced wood preservation against termites and two species of white-rot decay fungi and two species of brown-rot decay fungi.

EXPERIMENTAL

Cedarwood Oil

Supercritical carbon dioxide (SC-CO_2) was used to obtain the CWO extract from ERC heartwood sawdust as described by (Eller and King 2000). Briefly, SC-CO_2 at 70 °C and 27.6 MPa was used to extract the sawdust held within a stainless-steel vessel at a flowrate of ca. 2 L/min expanded gas.

Amylose Inclusion Complex (AIC)

The preparation of the AIC used to make the treatment suspension mixture is detailed by Eller *et al.* (2021). Briefly, the AIC consisted of 95% high-amylose corn starch (~68% amylose, AmyloGel 03003, Cargill, Minneapolis, MN, USA) and 5% didecyldimethylammonium chloride (DDAC). High-amylose corn starch (100 g) and 1800 mL deionized water were mixed and passed through a steam jet cooker. To this hot starch dispersion, a solution of 5.25 g DDAC in 200 g of 90 °C water was added, mixed, cooled,

and freeze dried. The final AIC suspensions contained 2% DDAC/AIC) and 2% polyvinyl alcohol (PVOH) (MW 133,000, 99 mol% hydrolyzed, Polysciences, Warrington, PA, USA).

Treatment Descriptions

The eight treatments tested were: Water only control (H_2O); butylated hydroxytoluene alone (BHT); ethylenediaminetetraacetic acid tetrasodium tetrahydrate salt alone (EDTA); BHT and EDTA (BHT/EDTA); cedarwood oil alone (CWO); CWO and EDTA (CWO/EDTA); CWO and BHT (CWO/BHT); and CWO combined with both BHT and EDTA (CWO/BHT/EDTA).

The treatment suspension mixtures with CWO all contained 5.0% CWO by weight and the AIC suspension was used to emulsify the CWO. The treatment mixtures with BHT all contained 1% BHT by weight. The BHT alone treatment was prepared by treating the wood blocks with a solution of 1% BHT in 70:30 ethanol:water by volume. For treatments with both CWO and BHT, the BHT was dissolved in the CWO in a 1:5 ratio by weight before being mixed into the AIC suspension. Because of the limited solubility of EDTA in water (ca. 0.5 g/L), the EDTA was dissolved in 5.5 M NaOH and then this solution was added to the AIC suspension to give a final concentration of 1% EDTA. The BHT/EDTA treatment was prepared by first treating the wood blocks with a solution of 1% BHT in 70:30 ethanol: water by volume, re-equilibrating the wood blocks to a constant mass and then treating the blocks with the solution of EDTA in NaOH added to the AIC suspension.

Vacuum Pressure Impregnation

For both termite and fungal bioassays, wooden test blocks were treated by vacuum pressure impregnation following AWPA E10-01 (2003). For termite bioassays, southern pine blocks (2.54 cm x 2.54 cm x 0.64 cm) were conditioned to 25 °C and 50% RH. For the fungal bioassays, 1 cm^3 blocks were conditioned to 25 °C and 70% RH. Yellow poplar blocks were used for white-rot fungi and southern pine blocks were used for brown-rot fungi. For a given treatment, blocks were submerged and held under vacuum (−0.088 MPa) for 30 min and then pressurized to 0.69 MPa for 60 min. Blocks were then patted dry and weighed to determine incorporation percentages as calculated by Eq. 1:

$$Incorporation\ \% = \frac{\text{Compound \% Concentration x Post−Impregnation Mass Gain}}{\text{Pre−Impregnation Mass}} \quad (1)$$

Treated wood blocks were then re-conditioned to a constant mass and their weights recorded prior to exposure to termites or wood-decay fungi.

Termite Resistance

Reticulitermes sp. (Blattodea: Rhinotermitidae) were collected from a single colony found in a dead log at Sam D. Hamilton Noxubee National Wildlife Refuge (Starkville, Mississippi) one week before the test was initiated. Cut log sections containing termites were kept in a 30-gallon trashcan and maintained in the laboratory at 25 °C in darkness. The day before test initiation, termites were removed from the collected log sections by breaking the rotting wood open and shaking the termites out of the wood over a screen to catch large debris. The screen was placed over a shallow plastic tub to catch falling termites. Termites were gently shaken from the large capture tubs into smaller plastic tubs containing 2 to 3 layers of moistened brown paper towels. These tubs were covered with a lid and allowed to sit overnight before test initiation.

A no-choice bioassay based on studies described by Kard and Mallette (1997), Kard *et al.* (2007), Konemann *et al.* (2014), Eller *et al.* (2018), and Lipeh *et al.* (2020) was used to evaluate resistance of the treated wood test blocks termites. This bioassay was also similar to AWPA E1-17 (AWPA 2022) standard test method with some modifications regarding substrate, container size, and number of termites used. The substrate used in the test consisted of a 10:1 mixture of sand and vermiculite as described by Kard *et al.* (2007). The sand used was American Countryside All Purpose Sand purchased from Lowes, which was washed and rinsed three times using deionized water, oven-dried at 100 °C overnight, then sifted using a 600-micron screen #30. Sifted sand was autoclaved at 121 °C and 15 psi for 45 min allowed to cool overnight and autoclaved a second time and allowed to cool. The vermiculite used was also purchased at Lowes. Vermiculite was dried in 500-g batches in a metal tray at 60 °C for 24 h. To make the substrate mixture homogenous, 300.0 g of sand was mixed with 30 g of vermiculite in a plastic jar and the dry mixture was stirred with a metal teaspoon for approximately 5 min. To this mixture 116 mL of sterile deionized water was added and the jar containing it was shaken for 3 to 5 min to homogenize the substrate. Cylindrical plastic containers (Pioneer Plastics 002C, 50.8 mm D x 36.5 mm H) were filled with 35 g of the substrate mixture described above. The containers with the substrate were allowed to sit for 1 h and a small plastic grid (25 mm x 25 mm Gutter Guard™) was added on top of the substrate. The wood blocks used in the test measured 2.54 cm × 2.54 cm x 0.64 cm, as specified in AWPA E1-17 (AWPA 2022) and other studies (Kard and Mallette 1997; Kard *et al.* 2007; Konemann *et al.* 2014). Wood blocks were placed on the plastic grid approximately 2 to 3 mm above sand surface, so they were not in contact with the damp substrate. Lids were placed on the containers holding the test wood blocks and allowed to sit overnight before the addition of the termites. The next day, 300 termites (297 workers and 3 soldiers) were counted with an aspirator and added to each container. Containers were kept in darkness at room temperature and relative humidity (22 °C, 55% RH) for the overnight wood conditioning and the duration of the test. After 21 days, 100% mortality was observed in all treatments except the water-treated control blocks exposed to termites. The test ceased at this point, and living termites were counted, test sample blocks were cleaned and conditioned to a constant mass at 25 °C and 50% RH. Percentage wood mass loss was based on the difference between the conditioned wood mass before and after exposure to termites. Percentage termite mortality was based on the number of dead termites out the 300 added. Mean percentage wood mass loss and mean percentage termite mortality were then calculated for each of the eight treatments based on six replicates.

Fungal Decay Resistance

The effectiveness of the treatments was evaluated using soil bottle assays according to AWPA E10-22 (2022) as detailed by Eller *et al.* (2021). These tests were performed using 10 mm blocks (*i.e.*, 1 cm^3) instead of 14 mm blocks to shorten the duration of the test. Two brown-rot fungi, *Gloeophyllum trabeum* (Pers.) Murrill (1908) (MAD-617) and *Rhodonia (Postia) placenta* (Fr.) Niemelä, K.H.Larss. & Schigel (2005) (MAD-698), and two white-rot fungi, *Trametes versicolor* (L.) Lloyd (1920) (MAD-697) and *Irpex lacteus* (Fr.) Fr. (1828) (HHB-7328), were tested. Soil bottles with the test blocks and fungal cultures were held in a controlled humidity incubator for eight weeks. The blocks were removed and scraped clean of fungal mycelium, oven dried at 60 °C for 4 h to stop fungal growth, and re-conditioned for one week at 27 °C and 30% humidity. The re-conditioned

weights were used to calculate percent weight loss. The eight treatments were replicated six times for each fungal species.

Chemicals

Butylated hydroxytoluene (CAS 128-37-0) and ethylenediaminetetraacetic acid (CAS 60-00-4) were purchased from Sigma Aldrich Chemical Co., St. Louis, MO, USA. Sodium hydroxide (CAS 1310-73-2) was purchased from Fisher Scientific, Fair Lawn, NJ, USA. Didecyldimethylammonium chloride (CAS 7173-51-5) was purchased from Santa Cruz Biotechnology, Dallas, TX, USA.

Statistical Analyses

Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA) was used to perform all statistical analyses of the data. Box and Whisker plots were used to identify and remove outliers. Single factor analyses of variance (ANOVA) were performed on the percentage wood mass loss for the termites, percentage termite mortality, and percentage mass loss for each of the four fungal species evaluated. Treatment means were compared using least significant difference (LSD) after obtaining a significant F-test ($P \leq 0.05$). The combined mass loss data for all four species of decay fungi were analyzed together to compare the individual effects of CWO, BHT, and EDTA using linear contrasts (T-test).

RESULTS AND DISCUSSION

Vacuum Pressure Impregnation

Mean mass incorporation percentages for the treatments are shown in Table 1.

Table 1. Mean Mass Incorporation Percentages for Wood Blocks after Vacuum Pressure Treatment

Treatment	Mean Percentage Mass Incorporation [a]
H_2O	0.0
EDTA	1.2
BHT	1.0
BHT/EDTA	2.3 (1.1, 1.2) [b]
CWO	6.1
CWO/EDTA	7.0
CWO/BHT	7.4
CWO/BHT/EDTA	7.3

[a] Percentage (N = 30) changes based on initial pre-impregnation conditioned wood block masses.
[b] Percentages in brackets represent the separate amounts of BHT and EDTA, respectively.

The EDTA and BHT treatments had incorporation percentages (by mass) of 1.2 and 1%, respectively. The BHT/EDTA treatment had separate incorporation percentages of 1.1 and 1.2%, respectively, for a total of 2.3%. The CWO alone had an incorporation percentage of 6.1% and as expected, the CWO/EDTA and CWO/BHT had higher total incorporations of 7.0 and 7.4%, respectively. The CWO/BHT/EDTA had a total incorporation percentage of 7.3%. This was lower than would be predicted based on

totaling 6.1% for CWO, 1.0% for BHT, and 1.2% for EDTA (*i.e.*, 8.3%) and might indicate some interference between these components when they are incorporated together.

Termite Resistance

Percentage wood mass losses for termites are shown in Fig. 1A. ANOVA indicated the treatment effect for mass loss was highly significant ($F_{7,47}$ = 69.9; P = 0.0000).

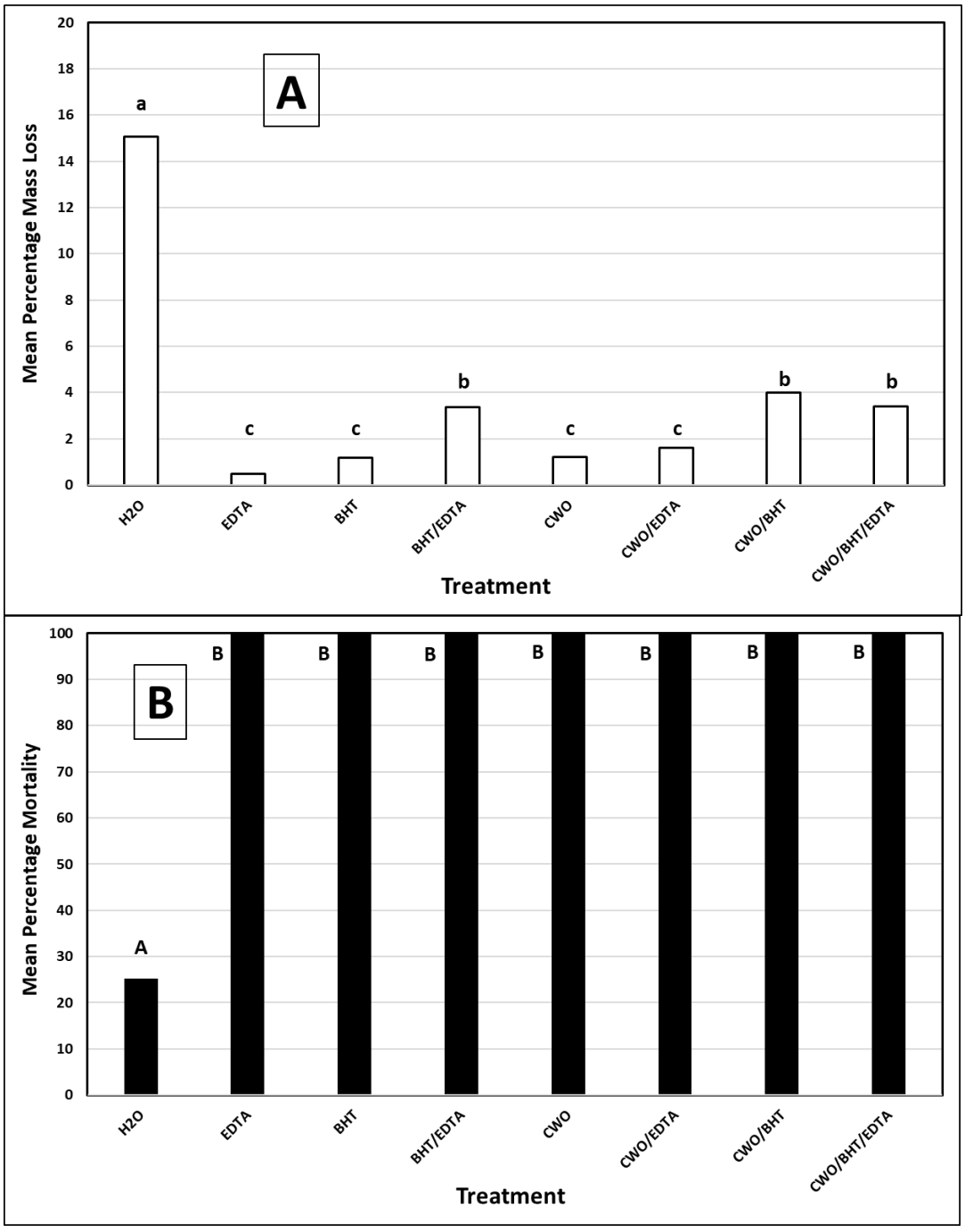

Fig. 1. (A) Mean (N = 6) percentage mass losses for treated southern pine blocks exposed to subterranean termites (open bars with lower case letters) and (B) mean percentage subterranean termite mortality (shaded bars with upper case letters). Means without letters in common differ significantly using Least Significant Difference (P ≤ 0.05).

The H_2O control treatment had the highest mass loss and was statistically higher than all the other treatments. Lowest percentage mass losses were for the EDTA, BHT, CWO, and CWO/EDTA treatments, all of which were statistically equivalent. Percentage mass losses for BHT/EDTA, CWO/BHT, and CWO/BHT/EDTA treatments were statistically equivalent to one another, lower than the H_2O treatment but higher than the EDTA, BHT, CWO, and CWO/EDTA treatments. These results suggest BHT was significantly less effective when used in combination with either EDTA or CWO or both together. In contrast, Schultz and Nicholas (2002) found that BHT alone had inhibitory activity and increased the activity of chlorothalonil against termites in a field study.

Percentage termite mortalities are shown in Fig. 1B. ANOVA indicated the treatment effect for percentage termite mortality was highly significant ($F_{7,47} = 77.12$; $P = 0.0000$). The H_2O control treatment had the lowest mortality (*i.e.*, 25%) and was statistically lower than all the other treatments, all of which were statistically equivalent to one another at 100% mortality.

Brown-Rot Fungi Decay Resistance

Percentage wood mass losses for the brown-rot fungi are shown in Fig. 2. For *G. trabeum*, the box and whisker plots identified two probable outlier replications, one in the H_2O treatment and the other in the BHT treatment. These two replications were removed from the data set prior to statistical analysis. ANOVA indicated a significant treatment effect for *G. trabeum* ($F_{7,45} = 16.0$; $P = 0.0000$). For *G. trabeum*, the numerically highest mass loss was for the BHT treatment, which was statistically equivalent to the H_2O treatment. Lowest mass losses were for the EDTA and BHT/EDTA treatments. Apart from the BHT/EDTA treatment, the inclusion of BHT resulted in higher mass losses than the corresponding treatments without BHT. In this experiment, the CWO and CWO/BHT treatments were statistically equivalent to the H_2O treatment for *G. trabeum*.

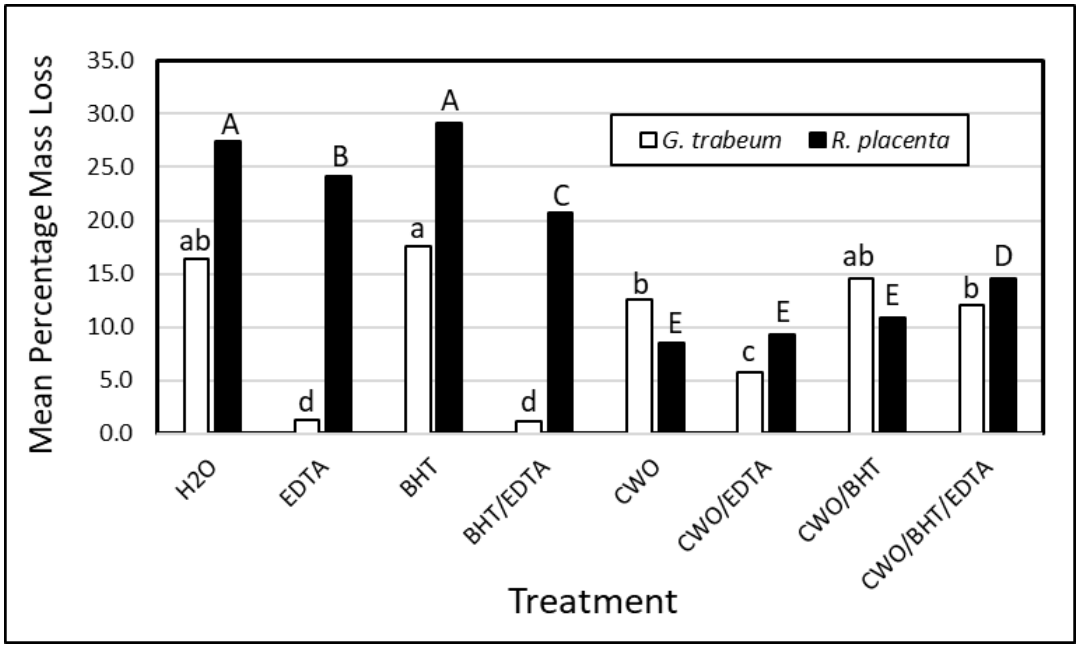

Fig. 2. Mean (N = 12) percentage mass losses for treated southern pine blocks exposed to brown-rot decay fungi *Gloeophyllum trabeum* (open bars with lower case letters) and *Rhodonia placenta* (solid bars with upper case letters). For a given fungal species, means without letters in common differ significantly using Least Significant Difference ($P \leq 0.05$).

ANOVA also indicated a significant treatment effect for *R. placenta* ($F_{7,47}$ = 53.8; P = 0.0000). *Rhodonia placenta* was the most aggressive fungus, and it caused the highest mass losses in this study. For *R. placenta*, highest mass losses were for the H_2O (*ca*. 27%) and BHT (*ca*. 29%) treatments, which were statistically equivalent. Mass loss for the EDTA (*ca*. 24%) was significantly less than the H_2O treatment. Lowest mass losses were for the CWO, CWO/EDTA, and CWO/BHT treatments, all of which statistically equivalent. The tertiary mix of CWO/BHT/EDTA had significantly greater mass loss than either the CWO/EDTA or CWO/BHT treatments.

White-Rot Fungi Decay Resistance

The percentage wood mass losses for the white-rot fungi are shown in Fig. 3. ANOVA indicated a significant treatment effect for *T. versicolor* ($F_{7,47}$ = 19.2; P = 0.0000). For *T. versicolor*, the highest mass losses were for the treatments without CWO (*i.e.*, H_2O, EDTA, BHT, and BHT/EDTA) and these four treatments were statistically equivalent to one another. Lowest mass losses were for the four treatments containing CWO (*i.e.*, CWO, CWO/EDTA, CWO/BHT, and CWO/BHT/EDTA). Mass losses for these four treatments were statistically equivalent to one another and significantly less than for the treatments without CWO.

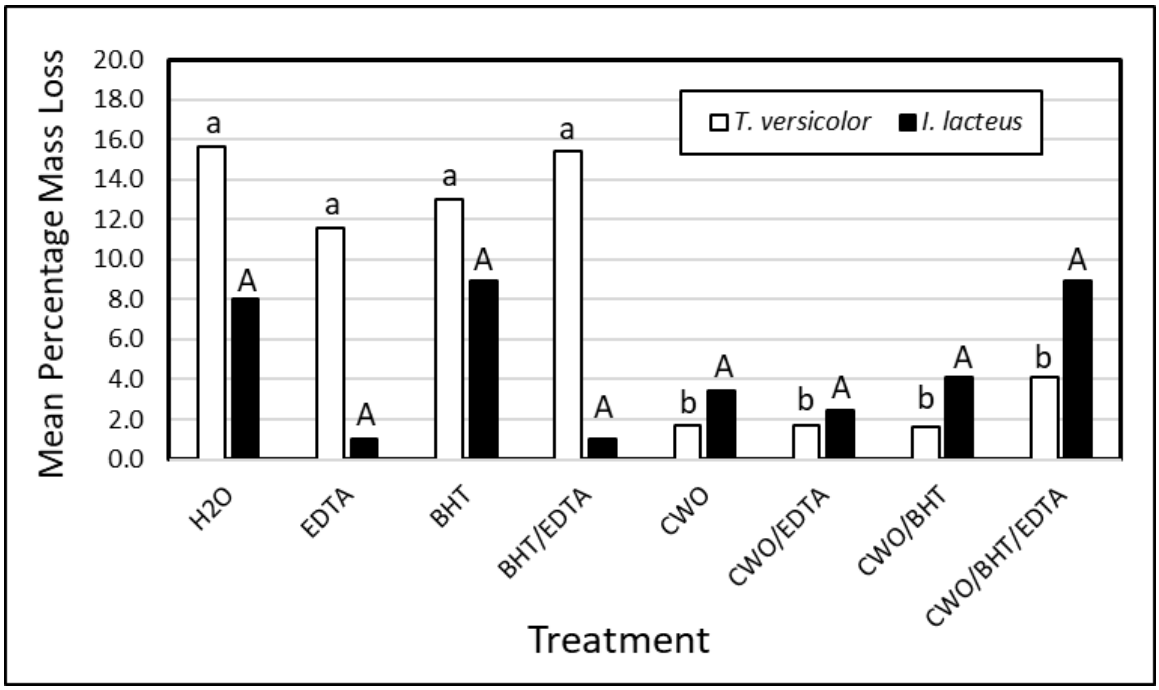

Fig. 3. Mean (N = 12) percentage mass losses for treated yellow poplar blocks exposed to white-rot decay fungi *Trametes versicolor* (open bars with lower case letters) and *Irpex lacteus* (solid bars with upper case letters). For a given fungal species, means without letters in common differ significantly using Least Significant Difference (P ≤ 0.05).

For *I. lacteus*, box and whisker plots identified two probable outlier replications, one in the EDTA treatment and the other in the BHT/EDTA treatment, and these two replications were removed from the data set prior to statistical analysis. ANOVA did not indicate a significant treatment effect for *I. lacteus* ($F_{7,45}$ = 1.1; P = 0.37), and for *I. lacteus* there were no significant statistical differences in mass loss between any treatments. *Irpex lacteus* was the least aggressive fungus, causing only relatively low mass losses in all

treatments, none of which exceeded 9% mass loss. Highest mass losses observed were for the BHT, H_2O, and CWO/BHT/EDTA treatments. Although not statistically significant, the tertiary mix of CWO/BHT/EDTA had nearly double the mass loss of CWO, CWO/BHT, or CWO/EDTA. The EDTA and BHT/EDTA treatments had mass losses of only 1%.

As reported by Schultz and Nicholas (2002), the white-rot fungus *T. versicolor* was more aggressive than *I. lacteus* particularly for the H_2O, BHT, EDTA, and BHT/EDTA treatments. However, *T. versicolor* was inhibited to a greater degree by CWO than was *I. lacteus*.

To simplify the overall decay fungi results, mass loss data for the four species of decay fungi were analyzed together and the significance of the individual effects of CWO, BHT, and EDTA determined from their linear contrasts. Overall mean mass losses over fungal species are shown in Fig. 4. Linear contrasts indicated significant effects of CWO (T = -6.64, P = 0.0000) and EDTA (T = -3.91, P = 0.0001) but not for BHT (T = 1.84, P = 0.0672). Interestingly, the BHT treatment had the highest mass loss and was statistically equivalent to the H_2O. Apart from the tertiary mix of CWO/BHT/EDTA, the other treatments containing CWO (*i.e.*, CWO/BHT, CWO, and CWO/EDTA) had the lowest mass losses with CWO/EDTA yielding the lowest overall mass loss. EDTA alone had lower mass loss than did the H_2O treatment. Inclusion of BHT never resulted in lower mass losses than corresponding treatments without BHT.

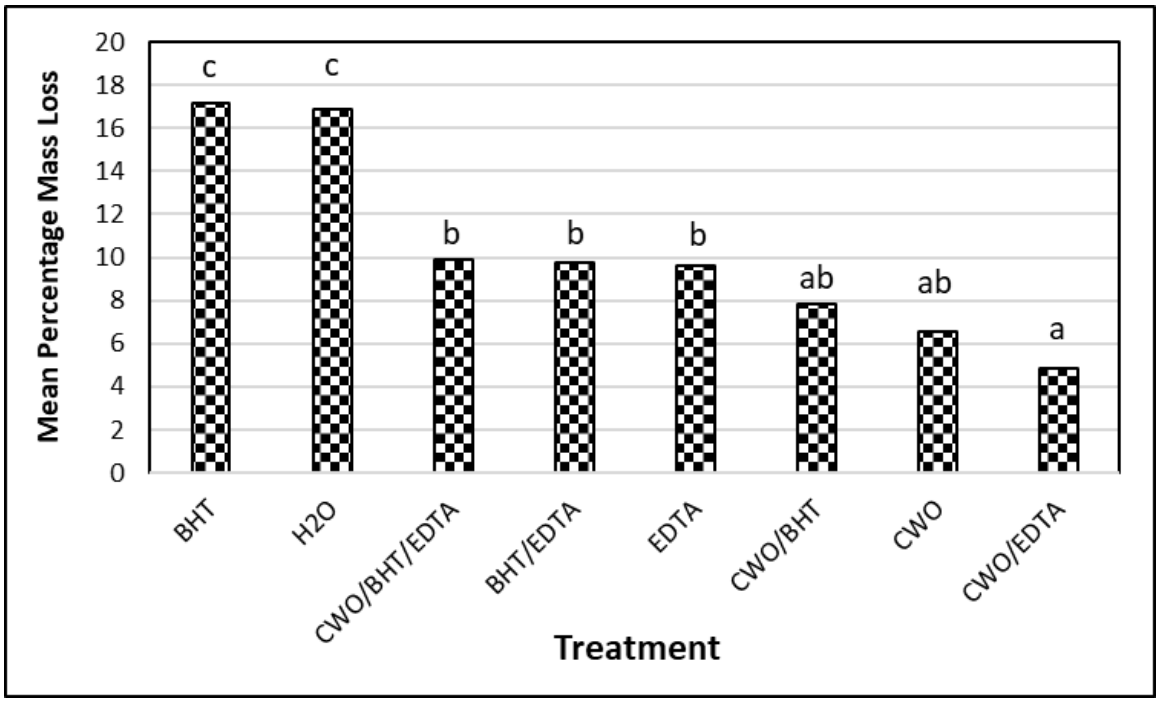

Fig. 4. Mean (N = 48) percentage mass losses over all four species of decay fungi; Means without letters in common differ significantly using Least Significant Difference (P ≤ 0.05)

Schultz and Nicholas (2002) studied the effects of both BHT and EDTA in combination with biocidal wood preservatives against wood decay fungi and termites. While they reported that in some cases BHT and EDTA had little or no effect alone, BHT and EDTA could synergize the effectiveness of the biocide. However, their results varied with the species tested, assay method and biocide used. In some cases, BHT or EDTA was

synergistic with the biocide, while other times there was little or no effect. The authors' results showed similar variability. Therefore, it is not possible to make broad generalizations about the effects of antioxidants or metal chelators in combination with biocides. Specific combinations will have to be tested before their effects can truly be determined.

Interestingly, Schultz and Nicholas (2002) reported that wood treated with 5% BHT alone was as degraded as untreated wood after 5 weeks in a lab decay test, but that wood treated with 4% BHT alone performed significantly better than untreated wood after 30 months exposure in a field trial. They pointed out that lab decay tests can be unrealistically harsh while noting the advantages and limitations of lab and field exposure tests. It is quite possible that some of the treatments in this study might have performed relatively better under longer, less harsh field trial conditions.

Treating wood for protection against decay organisms is very important. However, adverse interactions of treated wood with metal fasteners is also a serious concern. The corrosion of metals in preservative-treated wood has been reviewed (Baker 1988; Zelinka 2014). It is recommended that galvanized steel, copper, or stainless-steel fasteners be used in alkaline copper quaternary (ACQ)-treated wood because the copper accelerates fastener corrosion (Groenier and Lebow 2006; Kear *et al.* 2009). The absence of copper in the formulations described in this research might decrease the need for corrosion-resistant fasteners as is required for wood treated with ACQ-treated wood.

CONCLUSIONS

1. For termites, cedarwood oil (CWO), the oil with chelator (CWO/EDTA), butylated hydroxytoluene (BHT), and EDTA alone treatments all resulted in low wood mass losses and caused 100% mortality.
2. The CWO alone and CWO/EDTA significantly decreased wood mass loss by wood decay fungi.
3. The tertiary mixture of CWO/BHT/EDTA had both a lower-than-expected incorporation and resulted in higher wood mass losses than either CWO alone or CWO/EDTA.
4. The EDTA alone was effective at preventing fungal mass loss and the CWO/EDTA combination yielded the lowest overall fungal mass losses.
5. The inclusion of BHT resulted in higher wood mass losses by fungi than the corresponding treatments without BHT.

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