

Effects of Loblolly Pine Extract, Primary and Quaternary Alkyl Ammonium Chlorides Combined with Burgundy Oil from Eastern Red Cedar against Subterranean Termites and Wood-Decay Fungi

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Burgundy oil (BO) from Eastern red cedar provides resistance against termites and wood-decay fungi and is enhanced when combined with an amylose inclusion complex (AIC) containing hexadecylammonium chloride (HAC). Indirect evidence also indicated that a methanol Loblolly pine extract (LPE) was inhibitory against termites. This study compared the effects of HAC and didecyldimethylammonium chloride (DDAC) combined with LPE and BO on termites and wood-decay fungi. Southern pine was treated by vacuum/pressure impregnation and resistance evaluated after exposure to termites and decay fungi. The combination of BO and either HAC/AIC or DDAC/AIC reduced wood mass losses by termites, increased termite mortality, and inhibited all wood-decay fungi. The HAC/AIC and DDAC/AIC resulted in equivalent mass losses by termites and termite mortalities. The DDAC was slightly more inhibitory than the HAC against wood-decay fungi. Given the slight advantage of DDAC over HAC and because DDAC is currently used to preserve wood, DDAC might be preferred over HAC. The LPE had a very minor effect on mass loss by termites, termite mortality, and only a slight inhibitory effect on *G. trabeum* and *T. versicolor*, while *R. placenta* and *I. lacteus* were unaffected. Higher concentrations of DDAC and/or LPE might improve protection against termites and wood-decay fungi.

Keywords: *Juniperus virginiana*; Wood extract; Southern pine; *Reticulitermes flavipes*; Brown-rot fungi; White-rot fungi

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INTRODUCTION

Continually evolving wood protection methods emphasize the use of chemicals that minimize concerns with toxicity and harm to the environment, and there has been considerable effort to employ natural products with little or no mammalian toxicity (Singh and Singh 2012). Derivatives from a wide range of plant parts for wood protection have

been reviewed including phenolics, tannins, essential oils, resins, and lignans (Yang 2009; Singh and Singh 2012). The wood-decay antifungal activities of wood extractives, such as terpenes and metal scavengers, are reviewed by Valette *et al.* (2017).

The treatment of a non-durable wood with an extract from a durable wood species (*i.e.*, transferrable durability) is a promising technique (Kirker *et al.* 2013; 2016; Hassan *et al.* 2017). This method is particularly efficient if the extracts originate from a sustainable waste or by-product (Saha Tchinda *et al.* 2018). Extractives from heartwood of several tree species have been shown to be particularly effective. Representative species include black locust, Chinese coffin tree, Western red cedar, Alaska cypress, white cypress-pine, and Chinese incense-cedar (Smith *et al.* 1989; Chang *et al.* 1999, 2000; Taylor *et al.* 2002; Watanabe *et al.* 2005; Taylor *et al.* 2006; Yen *et al.* 2008; Wu *et al.* 2012). Heartwood extractives and durability has been reviewed by Taylor *et al.* (2002).

Numerous reports discuss the use of essential oils or extracts for wood preservation. There are many reports of antifungal activity (Morita *et al.* 1997; Chang *et al.* 1999, 2000; Cheng *et al.* 2005; Wang *et al.* 2005a,b; Wu *et al.* 2005; Cheng *et al.* 2006; Kazemi *et al.* 2006; Cheng *et al.* 2008; Yen *et al.* 2008; Cheng *et al.* 2011, 2012; Wu *et al.* 2012; Li *et al.* 2013; Tascioglu *et al.* 2013; Mansour and Salem 2015; Salem *et al.* 2016; Xie *et al.* 2017). Some report anti-termite activity (French *et al.* 1979; Zhu *et al.* 2001; Chang and Cheng 2002; Watanabe *et al.* 2005; Taylor *et al.* 2006; Gupta *et al.* 2011) and a few report both antifungal and anti-termite activity (Cheng *et al.* 2004; Pandey *et al.* 2012; Xie *et al.* 2015).

The plant family Cupressaceae, in particular, has several examples of active essential oils or extracts with anti-termite activity (French *et al.* 1979; Chang *et al.* 2001), antifungal activity (Morita *et al.* 1997; Cheng *et al.* 2005; Wu *et al.* 2005; Wan *et al.* 2007; Wang *et al.* 2011), or both (Chang *et al.* 2003; Taylor *et al.* 2006). In addition, sesquiterpenes from Cupressaceae have been identified to have both anti-termite (Watanabe *et al.* 2005; Mankowski *et al.* 2016; Hassan *et al.* 2017) and antifungal activities (Bauch *et al.* 2004; Cheng *et al.* 2005).

Eastern red cedar (ERC) (*Juniperus virginiana* L.) (Cupressaceae) is an abundant natural resource in the United States and represents an underutilized renewable natural product with several potential uses (Eller 2018). Eastern red cedar heartwood is resistant to subterranean termites (Carter and Smythe 1974; Arango *et al.* 2006; Kard *et al.* 2007; Kose and Taylor 2012; Konemann *et al.* 2014), and extracts of ERC sawdust have shown promise as a wood preservative by their termiticidal activity (Adams *et al.* 1988; Carter 1976; McDaniel *et al.* 1989; Eller *et al.* 2010, 2018, 2020) or reduction in termite attack (McDaniel and Dunn 1994; Eller *et al.* 2010, 2018, 2020). In addition, ERC extracts have shown antifungal activity against brown-rot and white rot decay fungi (Eller *et al.* 2010; Tumen *et al.* 2013; Eller *et al.* 2018, 2020). Using a different extraction method, Kirker *et al.* (2016) found only a marginal durability improvement for the ERC extract against wood decay fungi. The resistance of ERC heartwood to fungal decay is illustrated by Smith and Glaeser (2013) in an interesting photograph showing a skeletonized ERC tree with heartwood remaining after attack.

The authors' laboratory has investigated the use of cedarwood oil (CWO) (CAS 8000-27-9), as a wood preservative against termites and wood-decay fungi (Eller *et al.* 2010; Tumen *et al.* 2013; Eller *et al.* 2018, 2020). Cedarwood oil is an essential oil mixture of sesquiterpenes with a high percentage of cedrol (CAS 77-53-2) (Adams 1991).

Non-polar solvents including hexane, supercritical, and liquid CO₂ have been used to extract CWO from ERC heartwood (Eller 2018). If a non-polar solvent is used first to

remove the CWO from ERC sawdust, a burgundy solid (BS) can subsequently be removed separately using a polar solvent like methanol (Tumen *et al.* 2013). A burgundy-colored oil (BO), which is a mixture of CWO and BS, can be extracted from ERC heartwood sawdust if a polar solvent such as methanol is used first (Tumen *et al.* 2013). All three of these extracts (*i.e.*, CWO, BO, and BS) have been tested for their biological activity against subterranean termites, brown-rot decay fungi, and white-rot decay fungi (Eller *et al.* 2010; Tumen *et al.* 2013; Eller *et al.* 2018, 2020). In general, these studies showed that CWO significantly reduced wood mass loss by termites and increased termite mortality. The BS has only very minor activity against termites, and the BO is equal to the CWO in bioactivity. For the decay fungi studied, CWO inhibited both the brown-rot and white-rot decay fungi. The BS inhibited the white-rot more than the brown-rot decay fungi. Accordingly, the BO was more active than CWO against the white-rot decay fungi. Cedrol, a major component of CWO and a sesquiterpene alcohol, has previously been reported to have anti-termite activity (McDaniel *et al.* 1989) and antifungal activity (Chang *et al.* 1999; Cheng *et al.* 2011; Mun and Prewitt 2011; Wang *et al.* 2011). The sesquiterpenes in CWO have also been reported to be responsible for the antifungal activity of CWO and BO against brown-rot fungi (Bauch *et al.* 2004; Mun and Prewitt 2011).

An amylose inclusion complex (AIC) was used as an aqueous emulsifier for CWO to pressure treat wood (Eller *et al.* 2018) because the AIC confers both emulsification properties and water resistance (Hay *et al.* 2019; Fanta *et al.* 2016). By chance, the AIC used contained hexadecylammonium chloride (HAC) (CAS 1602-97-7), and the HAC/AIC by itself significantly reduced wood mass loss by termites and was highly termiticidal but had only relatively minor effects on the wood-decay fungi (Eller *et al.* (2018). Subsequently, Eller *et al.* (2020) found the BO in combination with the HAC/AIC resulted in the highest termite mortality and the best overall protection against termites and decay fungi. Only after the study was completed, were the chemical similarities between HAC and didecyltrimethylammonium chloride (DDAC) (CAS 7173-51-5), a compound already used to pressure treat wood, realized. Although DDAC is currently used to treat wood, the relative bioactivities of HAC and DDAC are unknown. Both HAC and DDAC are ammonium chlorides (AC), but HAC is a primary alkyl amine and DDAC is a quaternary alkyl amine in addition to their differences in their alkyl chain lengths.

Loblolly pine blocks exposed to polar solvents including ethanol or methanol sustained more termite mass loss and lower termite mortality than blocks exposed only to water (Eller *et al.* 2018, 2020). This suggests that exposure of the test wood blocks to these polar solvents removes extractables from the pine blocks that are inhibitory to the termites and increases termite mortality. It was hypothesized that treating wood blocks with an extract of pine would result in both lower mass loss by termites and higher termite mortality.

The objective of this study was to investigate the relative effectiveness of AICs prepared with HAC or DDAC in combination with a loblolly pine extract (LPE) and burgundy oil (BO) extracted from ERC on subterranean termites and four species of wood decay fungi (two brown-rot and two white-rot). Wood blocks were treated by vacuum/pressure impregnation and subsequently exposed to termites and wood decay fungi. Treatment effectiveness was evaluated by measuring wood mass loss and termite mortality.

EXPERIMENTAL

Burgundy Oil

The burgundy oil (BO) solution used to treat the wood test samples consisted of a 1:1 mixture of CWO and burgundy solid (BS) dissolved in methanol (MeOH). The CWO was extracted using supercritical CO₂ (70 °C, 27.6 MPa) from ERC heartwood sawdust as previously described (Eller and King 2000), and the BS was extracted as described by Eller *et al.* (2020). Briefly, ERC heartwood sawdust was first extracted with refluxing n-hexane in a Soxhlet extractor to remove CWO. After allowing residual hexane to evaporate from the hexane-extracted sawdust, the sawdust was subsequently extracted with refluxing methanol (MeOH) in a Soxhlet extractor to yield the BS.

Soxhlet Extraction of Loblolly Pine

In order to investigate polar constituents in the loblolly pine, a Soxhlet extraction was performed on 100 g of loblolly pine sawdust with refluxing methanol (EMD Millipore Corp., Billerica, MA) for 10 cycles. The methanol was separated from the solution by rotary evaporation (Buchi Rotavapor RE 120 with a vertical condenser, New Castle, DE), and the loblolly pine extract (LPE) mass was determined and the dry-mass yield was calculated after drying the extracted sawdust overnight in a vacuum oven (105 °C and −0.088 MPa). There were three replications of Soxhlet extractions.

Amylose Inclusion Complex (AIC)

The AICs were composed of 95% high-amylose corn starch and 5% alkyl ammonium chloride (AC). The primary alkyl AC, hexadecylammonium chloride (HAC) (CAS 1602-97-7), was prepared as detailed previously (Eller *et al.* 2018), and the quaternary AC, didecyldimethylammonium chloride (DDAC) (CAS 7173-51-5) was purchased (Santa Cruz Biotechnology, Dallas, TX, USA). Deionized water (1800 mL) and 100 g of high-amylose corn starch (~68% amylose, AmyloGel 03003, Cargill, Minneapolis, MN, USA) were mixed in a Waring blender (Torrington, CT, USA) and passed through a steam jet-cooker. To this hot starch dispersion, a solution of 5.25 g AC in 200 g of hot (90 °C) water was added and mixed for 1 min at high speed. The resulting alkyl AC/AIC colloidal suspensions were cooled and freeze dried. The impregnation treatment solutions contained 2% alkyl AC/AIC (HAC/AIC or DDAC/AIC) and 2% polyvinyl alcohol (PVOH) (MW 133,000, 99 mol% hydrolyzed, Polysciences, Warrington, PA, USA).

Treatment Descriptions

The seven wood block treatments tested were: Water Only Control (H₂O); Water after Methanol (MeOH>H₂O); Water after LPE in Methanol (LPE/MeOH>H₂O); DDAC/Amylose Inclusion Complex after BO in Methanol (BO/MeOH>DDAC/AIC); HAC/Amylose Inclusion Complex after BO in Methanol (BO/MeOH>HAC/AIC); DDAC/Amylose Inclusion Complex after BO+LPE in Methanol (BO+LPE/MeOH>DDAC/AIC); and HAC/Amylose Inclusion Complex after BO+LPE in Methanol (BO+LPE/MeOH>HAC/AIC).

The BO/MeOH solution contained 5.0% CWO and 5.0% BS by weight. The LPE/MeOH solution contained 3.4% LPE by weight. The BO+LPE/MeOH treatment contained 5.0% CWO, 5.0% BS and 3.4% LPE by weight.

Vacuum/Pressure Impregnation

Vacuum/pressure impregnation was used to treat the wood test blocks as described by Eller *et al.* (2018). Southern pine blocks (2.54 cm × 2.54 cm × 0.64 cm) were used for termite tests, and 1-cm³ southern pine and 1-cm³ yellow poplar blocks were used for the brown-rot and white-rot fungal tests, respectively. Wood was purchased locally and milled to size in the laboratory. Briefly, after conditioning (25 °C and 50% RH for termite blocks and 25 °C and 70% RH for fungal blocks) to a constant mass, blocks were submerged under a given treatment solution and held under vacuum (−0.088 MPa) for 30 min and then pressurized to 0.69 MPa for 60 min. Blocks were weighed immediately after impregnation. For treatments with two impregnations (*i.e.*, MeOH>H₂O; LPE/MeOH>H₂O; BO/MeOH>DDAC/AIC; BO/MeOH>HAC/AIC; BO+LPE/MeOH>DDAC/AIC; and BO+LPE/MeOH>HAC/AIC), the blocks were re-conditioned as described above to a constant mass after the first impregnation treatment. The blocks were then impregnated with the second treatment, immediately weighed, and re-conditioned to a constant mass a second time. This allowed the determination of the incorporation rates of the components of the first and second impregnations. All blocks were weighed prior to exposure to termites or wood-decay fungi.

Termite Resistance

Reticulitermes flavipes (Kollar) (Blattodea: Rhinotermitidae) were collected from a single colony found in a dead log at Sam D. Hamilton Noxubee National Wildlife Refuge (Starkville, Mississippi). Cut log sections containing the termites were kept in 30-gallon trashcans and maintained in the laboratory at 25 °C in darkness. The day of the test setup, termites were removed from the collected log sections by breaking the rotting wood open and shaking the termites out of the wood through a screen to catch large debris. Termites were placed in plastic tubs with moistened paper towels for 2 h before being counted with an aspirator.

A no-choice bioassay based on studies described by Kard and Mallette (1997), Konemann *et al.* (2014), Eller *et al.* (2018), and Lipeh *et al.* (2020) was used to evaluate resistance of the treated wood test samples. Resistance was evaluated based on wood mass loss and termite mortality. These tests used smaller numbers of termites (100 to 150 workers), containers, and substrate compared to methods like AWPA E1-17. As termites can be sometimes difficult to collect in large numbers and counting large numbers can be laborious, the authors prefer to use these types of test parameters. Cylindrical plastic containers (Pioneer Plastics 002C, 50.8 mm D x 36.5 mm H) were filled with 50 g of washed, dried, screened, sterilized sand. The sand used was American Countryside All Purpose Sand purchased from Lowes. The sand 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 minutes, allowed to cool overnight, and autoclaved a second time and allowed to cool. To the sand, 9 mL of sterile deionized water was added to create a moisture content of 18%. The containers with sand and water were allowed to sit for one hour, and a small plastic grid (25mm x 25mm Gutter Guard) was added on top of the wet sand. The test wood samples used in the test measured 2.54 cm × 2.54 cm × 0.64 cm. This is the sample size suggested in the AWPA E1-17 (AWPA 2020) and other studies (Kard and Mallette 1997; Konemann *et al.* 2014). Wood samples were placed on the plastic grid so they were not in contact with the damp sand – approximately 2 to 3 mm above sand surface. One-hundred fifty worker termites were added to each container (Kard and Mallette 1997). Worker termites were only used, as

very few soldier termites were found in the collected termite colony. Containers were kept in darkness at room temperature and relative humidity (21 °C, 55% RH) for 28 days. At the end of the test, living termites were counted, test sample blocks were cleaned and conditioned to a constant mass at 25 °C and 50% relative humidity (RH). Wood mass loss and termite mortality were then calculated. The seven treatments were replicated six times.

Wood-Decay Fungi Resistance

Soil bottle assays were conducted according to AWP A E10-16 (2012) to compare the efficacy of actives. 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 used in experiments with treated southern pine and yellow poplar, respectively. Cultures were grown and maintained on malt extract agar (MEA) plates prior to experiments. Two 5-mm plugs from the actively growing edge of petri dish cultures of brown-rot fungi were added to sterile soil bottles containing 40 x 30 x 3 mm southern pine feeder strips, while maple feeders were used for the white-rot fungi. Fungi were allowed to grow and colonize the feeders for three weeks at 27 °C and 70% humidity prior to adding 1-cm³ southern pine or yellow poplar test blocks. Test blocks were propylene gas sterilized overnight in vials separated by treatment, and then one or two blocks were added to each bottle in a sterile hood. Bottles were placed in the controlled humidity incubator for eight weeks, and then blocks were removed and scraped clean of fungal mycelium. Test blocks were oven dried at 60 °C for 4 h to stop fungal growth, followed by one week of conditioning at 27 °C and 30% humidity, and conditioned weights were recorded. Percent weight loss from the initial weight of conditioned blocks was calculated and reported. The seven treatments were replicated six times for each fungal species.

Statistical Analyses

Statistix™ 8.1 software (Analytical Software, Tallahassee, FL, USA) was used to perform statistical analyses of the data. Box and whisker plots were examined to identify and remove outliers. A Levene's homogeneity of variance test was performed to determine if values needed to be transformed in order to satisfy analyses of variance (ANOVA) assumptions. Single-factor ANOVA were performed on the percentage wood mass loss for the termites, percentage termite mortality, and percentage wood mass loss for each of the four fungal species tested after arcsine square-root transformation (*i.e.*, $\arcsin\sqrt{\text{proportion mass loss}}$) to stabilize the variance. Linear contrasts were performed to compare treatments with LPE against treatments without LPE as well as to compare treatments with DDAC against those with HAC. Statistical analyses were performed on transformed data but untransformed means are presented for ease of interpretation. Treatment means were compared using least significant difference (LSD) in case of a significant F-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Soxhlet Extraction of Loblolly Pine

The methanol Soxhlet extraction of the Loblolly pine yielded a semi-solid amber-colored material with a dry-mass yield of LPE of 3.35% and scent reminiscent of freshly cut pine wood.

Vacuum/Pressure Impregnation

After re-conditioning to a constant mass, the mean percentage mass changes for the treated wood samples are shown in Table 1. The alkyl AC/AIC solutions used to treat the wood samples contained 2% PVOH and 2% alkyl AC/AIC; therefore, half of the observed approximate 4.0% mass increase for the samples treated with alkyl AC/AIC is from the PVOH and half is from the alkyl AC/AIC. Because the alkyl AC/AIC itself contained 5% alkyl AC, the observed 4.0% percentage mass gains for the wood samples treated with alkyl AC/AIC contained *ca.* 0.1% alkyl AC (*i.e.*, 2% X 5% = 0.001 = 0.1%).

There was a small mass loss observed for H₂O (*i.e.*, -0.7%). For the MeOH>H₂O treatment, there was a small mass loss after both MeOH (*i.e.*, -0.8%) and H₂O (*i.e.*, -0.4%) exposures. The wood blocks treated with the LPE were slightly more yellow than the blocks exposed to either water only (H₂O) or methanol and water (MeOH>H₂O) indicating the amber color of the LPE was conferred to the wood during impregnation. All of the wood blocks treated with any of the four treatments containing BO were burgundy colored after impregnation.

Table 1. Mean Percentage Wood Block Mass Changes after Impregnation Treatments

Treatment	Mean Percentage Change ^a		
	First Impregnation		Second Impregnation
H ₂ O	-0.7		na
MeOH > H ₂ O	-0.8	>	-0.4
LPE/MeOH > H ₂ O	1.8	>	-0.3
BO/MeOH > DDAC/AIC	7.3	>	4.0
BO/MeOH > HAC/AIC	7.5	>	4.0
BO+LPE/MeOH > DDAC/AIC	10.4	>	3.9
BO+LPE/MeOH > HAC/AIC	10.2	>	4.0

^a Percentage (N=30) changes based on initial pre-impregnation conditioned wood block masses. na stands for not applicable.

Termite Resistance

Box and whisker plots identified one probable outlier replication in the H₂O treatment with both low percentage mass loss and 100% termite mortality, and this single outlier replication was removed from the data set prior to analysis. The percentage mass losses for the termites are shown in Fig. 1A. The ANOVA indicated the treatment effect for percentage mass loss was highly significant ($F_{6,34} = 606$; $P=0.0000$). The H₂O only control and MeOH>H₂O treatments had the highest mass losses and were statistically equivalent to one another. The LPE/MeOH>H₂O treatment had statistically lower mass loss than either the H₂O or MeOH>H₂O treatments, although by only a relatively small amount (*i.e.*, 7.7 *versus* 8.6 and 8.6%, respectively). The linear contrast was significant ($T = 2.24$; $P = 0.032$) for a mass loss difference between treatments with or without LPE. In a study with a similar methodology, Kard and Mallette (1997) saw much higher mass loss on southern pine wood exposed to 100 worker termites. Due to contamination issues in our laboratory incubators, the present study was run at a slightly lower temperature (21 °C) than earlier studies (23 to 24 °C). This may have led to a reduction in the amount of feeding in the control treatments. We believe in the validity of our test because, as will be discussed, termite mortality in the control treatments was very low indicating the termites were not having an issue feeding in the control treatments. We also used AWPA E1-17 sized blocks (large) for the number of termites tested.

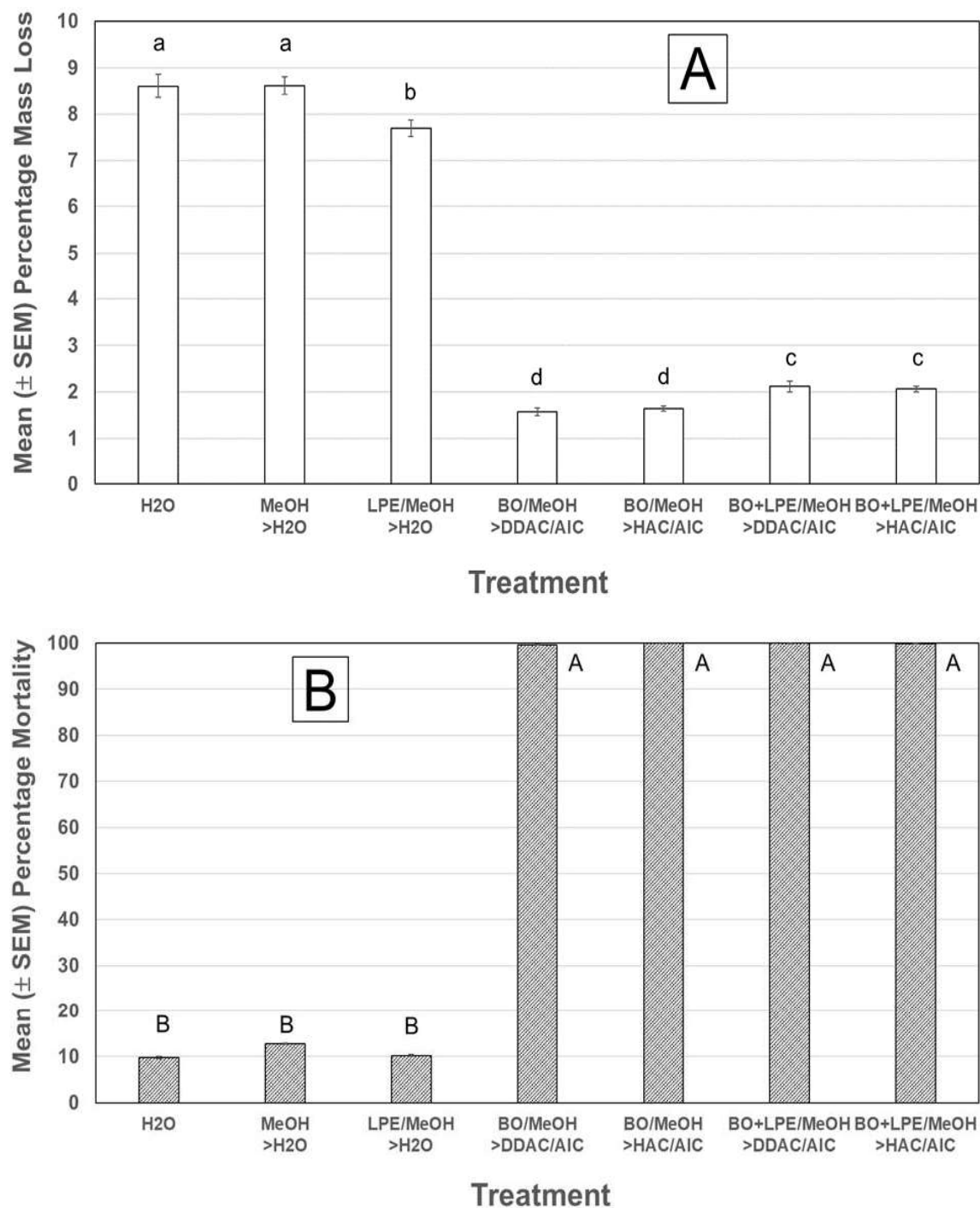


Fig. 1. (A) Mean (±SEM) percentage wood mass losses for treated loblolly pine samples exposed to subterranean termites (open bars with lower case letters) and (B) mean (±SEM) percentage subterranean termite mortality (shaded bars with upper case letters). Means without letters in common differ significantly using Least Significant Difference ($P \leq 0.05$).

The percentage termite mortalities are shown in Fig. 1B. The ANOVA indicated that the treatment effect for percentage termite mortality was highly significant ($F_{6,34} = 457$; $P=0.0000$). The H₂O, MeOH>H₂O and LPE/MeOH>H₂O treatments all had low termite mortalities and were statistically equivalent to one another. The linear contrast was

not significant ($T = 0.37$; $P = 0.716$) for a termite mortality difference between treatments with or without LPE. The very low mortality (9.73%) in the H₂O only treatment was indicative of excellent termite vigor for this test.

The treatments which included BO and either the DDAC/AIC or HAC/AIC (*i.e.*, BO/MeOH>DDAC/AIC, BO/MeOH>HAC/AIC, BO+LPE/MeOH>DDAC/AIC, and BO+LPE/MeOH>HAC/AIC) all had very high termite mortalities (*i.e.*, *ca.* 100%) and were statistically equivalent to one another. All four of these treatments were significantly higher than the H₂O, MeOH>H₂O and LPE/MeOH>H₂O treatments. Treatments BO/MeOH>DDAC/AIC and BO/MeOH>HAC/AIC were statistically equivalent to one another as were BO+LPE/MeOH>DDAC/AIC and BO+LPE/MeOH>HAC/AIC. The linear contrast was not significant ($T = 0.16$; $P = 0.870$) for termite mortality difference between treatments with DDAC or HAC.

Brown-Rot Decay Fungi Resistance

There were two contaminated replications in the *R. placenta* data, one in the H₂O treatment and one in the BO/MeOH>DDAC/AIC treatment, and these two replications were omitted from the statistical analysis. The percentage mass losses for the brown-rot fungi are shown in Fig. 2. The ANOVAs indicated significant treatment effects for both *G. trabeum* and *R. placenta* ($F_{6,35} = 27.1$; $P = 0.0000$ and $F_{6,33} = 28.4$; $P = 0.0000$, respectively).

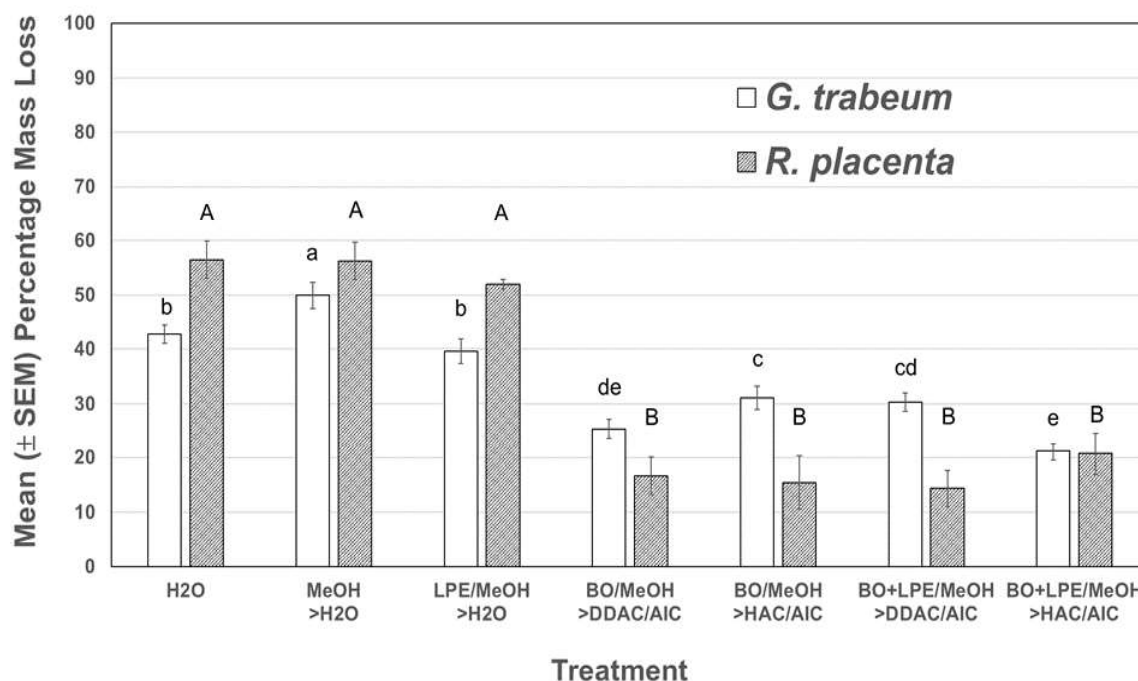


Fig. 2. Mean (±SEM) percentage mass losses for treated loblolly pine samples exposed to brown-rot decay fungi *Gloeophyllum trabeum* (open bars with lower case letters) and *Rhodonia placenta* (shaded bars with upper case letters). For a given fungal species, means without letters in common differ significantly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

For *G. trabeum*, wood mass loss was highest for the MeOH>H₂O treatment. Mass loss was significantly higher than both the H₂O and LPE/MeOH>H₂O treatments. However, the H₂O and LPE/MeOH>H₂O treatments were statistically equivalent. For *G.*

trabeum, the linear contrast was not significant ($T = 1.78$; $P = 0.083$) for a mass loss difference between treatments with or without LPE. For *R. placenta*, on the other hand, the H_2O , $MeOH > H_2O$ and $LPE/MeOH > H_2O$ treatments were statistically equivalent, and the contrast was not significant ($T = 0.01$; $P = 0.991$) for a mass loss difference between treatments with or without LPE.

For both *G. trabeum* and *R. placenta*, treatments which included BO and either AC (*i.e.*, $BO/MeOH > DDAC/AIC$, $BO/MeOH > HAC/AIC$, $BO+LPE/MeOH > DDAC/AIC$, and $BO+LPE/MeOH > HAC/AIC$) had significantly less mass loss than the H_2O , $MeOH > H_2O$ and $LPE/MeOH > H_2O$ treatments. For the brown-rot decay fungi, neither AC was consistently more effective than the other. For *G. trabeum*, without LPE, the $BO/MeOH > DDAC/AIC$ had less mass loss than $BO/MeOH > HAC/AIC$. However, with LPE, $BO+LPE/MeOH > DDAC/AIC$ had more mass loss than $BO+LPE/MeOH > HAC/AIC$. For *R. placenta*, there were no significant differences between any of these four treatments. The linear contrasts were not significant for a mass loss difference between treatments with DDAC or treatments with HAC for either *G. trabeum* or *R. placenta* ($T = 1.06$; $P = 0.296$ and $T = 0.48$; $P = 0.631$, respectively).

The inclusion of LPE in addition to the BO and either AC/AIC (*i.e.*, $DDAC/AIC$ or HAC/AIC) did not lead to a consistently lower mass loss. For *G. trabeum*, the inclusion of LPE led to a lower mass loss when HAC was used; however, the treatments $BO/MeOH > DDAC/AIC$ and $BO+LPE/MeOH > DDAC/AIC$ were statistically equivalent.

White-Rot Decay Fungi Resistance

The percentage mass losses for the white-rot fungi are shown in Fig. 3. The ANOVAs indicated significant treatment effects for both *T. versicolor* and *I. lacteus* ($F_{6,35} = 31.5$; $P = 0.0000$ and $F_{6,35} = 3.14$; $P = 0.014$, respectively).

For *T. versicolor*, wood mass loss was highest for the $MeOH > H_2O$ treatment and was significantly higher than the $LPE/MeOH > H_2O$ treatment, although the H_2O and $LPE/MeOH > H_2O$ treatments were statistically equivalent. For *T. versicolor*, the overall linear contrast was not significant ($T = 1.19$; $P = 0.244$) for a mass loss difference between treatments with or without LPE. For *I. lacteus*, the H_2O , $MeOH > H_2O$ and $LPE/MeOH > H_2O$ treatments were statistically equivalent. The overall linear contrast was not significant ($T = 0.40$; $P = 0.694$) for a mass loss difference between treatments with or without LPE for *I. lacteus*.

Although treatments that included BO and either AC (*i.e.*, $BO/MeOH > DDAC/AIC$, $BO/MeOH > HAC/AIC$, $BO+LPE/MeOH > DDAC/AIC$, and $BO+LPE/MeOH > HAC/AIC$) had significantly less mass loss than the H_2O , $MeOH > H_2O$ and $LPE/MeOH > H_2O$ treatments for *T. versicolor*, only the $BO+LPE/MeOH > DDAC/AIC$ had an inhibitory effect against *I. lacteus*. For *T. versicolor*, the DDAC treatments had less mass loss than did the HAC treatments. For *I. lacteus*, the $BO+LPE/MeOH > DDAC$ treatment had significantly less mass loss than did the $BO+LPE/MeOH > HAC/AIC$ treatment. The linear contrast was significant for a mass loss difference between treatments with DDAC or treatments with HAC for *T. versicolor* ($T = 5.69$; $P = 0.000$); however, this contrast was not significant for *I. lacteus* ($T = 0.40$; $P = 0.694$).

The inclusion of LPE in addition to the BO and either AC/AIC (*i.e.*, $DDAC/AIC$ or HAC/AIC) did not lead to a significant lower mass loss for either *T. versicolor* or *I. lacteus*. In fact, for *T. versicolor*, the mass loss for the $BO+LPE/MeOH > HAC$ treatment (*i.e.*, 28.4%) was significantly higher than the mass loss for the $BO/MeOH > HAC$ treatment (*i.e.*, 14.2%).

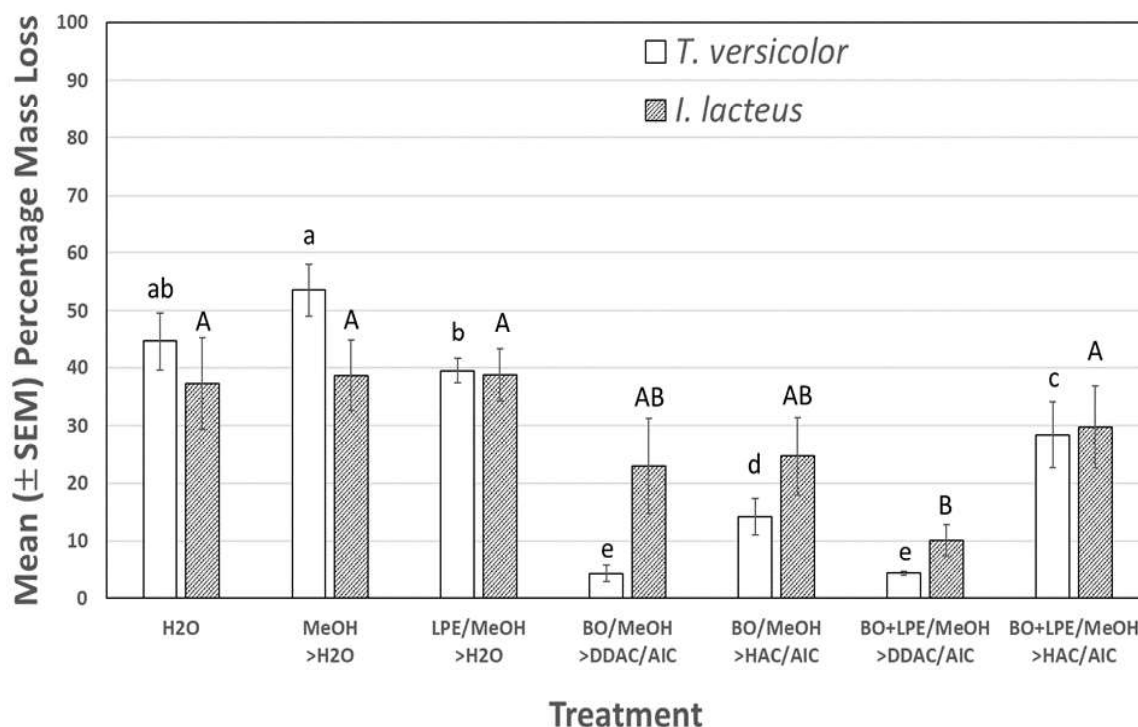


Fig. 3. Mean ($\pm$ SEM) percentage mass losses for treated yellow poplar samples exposed to white-rot decay fungi *Trametes versicolor* (open bars with lower case letters) and *Irpex lacteus* (shaded bars with upper case letters). For a given fungal species, means without letters in common differ significantly using Least Significant Difference ($P \leq 0.05$).

Although leaching studies have not been performed for these treatments, it is unlikely that these test compounds would be easily leached from the treated wood. Both CWO and BS are insoluble in water, and HAC/AIC has previously been demonstrated to inhibit both water absorption and wood swelling (Eller *et al.* 2018). In addition, the LPE is not water soluble either. Therefore, exposure of wood with these treatments to water will not cause them to be removed from the wood.

The four treatments that included BO and either the DDAC/AIC or HAC/AIC all had both very low termite percentage mass loss as well as very high termite mortality, and the two alkyl AC/AICs (*i.e.*, DDAC/AIC and HAC/AIC) were effectively equivalent. The combination of an alkyl AC and BO gave excellent protection against termites. Similar results were reported for BO>HAC/AIC (Eller *et al.* 2020). As far as is known, Eller *et al.* (2020) and this report are the only studies testing an essential oil and an alkyl AC together against termites and wood decay fungi.

Previously, because the CWO>HAC/AIC and BO>HAC/AIC treatments were statistically equivalent, it was concluded that the CWO alone is largely responsible for both the lower mass loss and the higher termite mortality observed and that the effect of the BS on termites is relatively minor (Eller *et al.* 2020). Therefore, for protection against termites, CWO alone could be used instead of BO in conjunction with either DDAC/AIC or HAC/AIC.

The anti-fungal activities of the two alkyl ACs were very similar in their effectiveness. In only one case did the HAC have greater antifungal activity than DDAC; mass loss for *G. trabeum* on wood treated with BO+LPE/MeOH>HAC/AIC was

significantly less than mass loss on wood treated with BO+LPE/MeOH>DDAC/AIC. Otherwise, the two ACs were either statistically equivalent or the DDAC was more inhibitory than the HAC.

Exposure of the wood blocks to MeOH caused a slight decrease in wood block mass and indicates that MeOH extracts something from the wood, which is consistent to previous reports (Eller *et al.* 2018, 2020). In addition, treating wood blocks with the LPE led to a slight decrease in percentage wood mass loss by termites, although the addition of the LPE did not increase termite mortality. These results are similar to earlier reports that polar solvents such as ethanol or methanol can extract inhibitory materials from the loblolly pine test wood blocks making them both more palatable and less toxic (Eller *et al.* 2018, 2020). However, even though the observed LPE effect on termite mass loss was statistically significant, it was very minor and probably of little practical use.

The wood-decay fungal data indicate that the MeOH removed something that was inhibitory against *G. trabeum* and *T. versicolor* and that the LPE could add back this inhibition for these two species. It is hypothesized that the MeOH extracts compounds such as terpenes, resins, and/or lignans, which might otherwise be inhibitory. However, *R. placenta* and *I. lacteus* were not affected by the LPE. Using a higher concentration of LPE might lead to a higher level of protection against termites and wood-decay fungi. It also possible that other materials not tested in combination with the BO and AC/AIC such as the metal chelator, EDTA (Schultz and Nicholas 2002), or a radical or oxidant scavenger (Singh and Singh 2012) could increase treatment effectiveness.

Both the LPE and BS required a polar solvent such as MeOH as the diluent/carrier to solubilize these compounds during impregnation. If the LPE and BS were not included in an impregnation treatment, the potential hazards of using a toxic and flammable solvent such as MeOH could be avoided. If used alone, CWO could be formulated in an aqueous emulsion (*e.g.*, with HAC/AIC or DDAC/AIC) to pressure treat wood. The water-based carrier method would be non-flammable and less costly.

CONCLUSIONS

1. The combination of burgundy oil (BO) and either the didecyldimethylammonium chloride amylose inclusion complex (DDAC/AIC) or the hexadecylammonium chloride amylose inclusion complex (HAC/AIC) resulted in low percentage wood mass losses by termites (*i.e.*, *ca.* 80% less than Control), nearly 100% termite mortality, and this combination represents a very effective treatment against termites. In addition, this combination inhibited all four wood decay fungi species studied. The antifungal effects were more varied for the wood-decay fungi studied, and the combination of BO and either AC/AIC resulted in between 20 and 90% less mass loss by wood-decay fungi, depending on the species.
2. The didecyldimethylammonium chloride amylose inclusion complex (DDAC/AIC) and hexadecylammonium chloride amylose inclusion complex (HAC/AIC) resulted in equivalent mass losses by termites as well as equivalent termite mortalities. Therefore, either could be used and similar protection against termites would be expected. For the wood-decay fungi, the DDAC was slightly more inhibitory than the HAC. Because of their similar activities, the choice of one alkyl AC over the other could be based on the relative costs of the two ACs. However, given the slight overall advantage of DDAC

over HAC and the fact that DDAC is already used to preserve wood, DDAC might be preferred.

3. The concentration of AC used in this experiment was only 0.1%, which is lower than what is commonly used to treat wood. AWP A Standard T1 (2020) and AWP A16-16 (2016) give minimum quaternary alkyl ammonium chloride retentions ranging from 0.18 to 0.86% for southern pine with a standard density of 500 kg/m³. In addition, EU Directive 98/8/EC (2012) reports a final concentration of DDAC of between 0.3 and 1.8%. These concentrations represent an approximate 2-fold to 18-fold higher concentration than the 0.1% we used in this study. A higher concentration of AC would undoubtedly lead to more effective inhibition of the wood-decay fungi.
4. The addition of the LPE did not lead to a large increase in protection of treated wood against termites. Also, at the concentration of LPE tested, only a slight inhibitory effect of LPE on *G. trabeum* and *T. versicolor* was observed. The other two species of wood-decay fungi (*i.e.*, *R. placenta* and *I. lacteus*) were unaffected.

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