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

Fred J. Eller,[a,]* Mark E. Mankowski,[b] Grant T. Kirker,[c] and Gordon W. Selling [d]

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.

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

bioresources.com

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-termitic 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-termitic 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-termitic 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-termitic (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 CO_2 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-termitic 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 didecyldimethylammonium 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 CO_2 (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 (H_2O); Water after Methanol (MeOH>H_2O); Water after LPE in Methanol (LPE/MeOH>H_2O); 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-cm^3 southern pine and 1-cm^3 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>H$_2$O; LPE/MeOH>H$_2$O; 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 AWPA 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-cm^3 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*$\sqrt{}$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 ≤ 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 H_2O (*i.e.*, -0.7%). For the MeOH>H_2O treatment, there was a small mass loss after both MeOH (*i.e.*, -0.8%) and H_2O (*i.e.*, -0.4%) exposures. The wood blocks treated with the LPE were slightly more yellow than the blocks exposed to either water only (H_2O) or methanol and water (MeOH>H_2O) 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

	Mean Percentage Change [a]		
Treatment	First Impregnation		Second Impregnation
H_2O	-0.7		na
MeOH > H_2O	-0.8	>	-0.4
LPE/MeOH > H_2O	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 H_2O 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 H_2O only control and MeOH>H_2O treatments had the highest mass losses and were statistically equivalent to one another. The LPE/MeOH>H_2O treatment had statistically lower mass loss than either the H_2O or MeOH>H_2O 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.
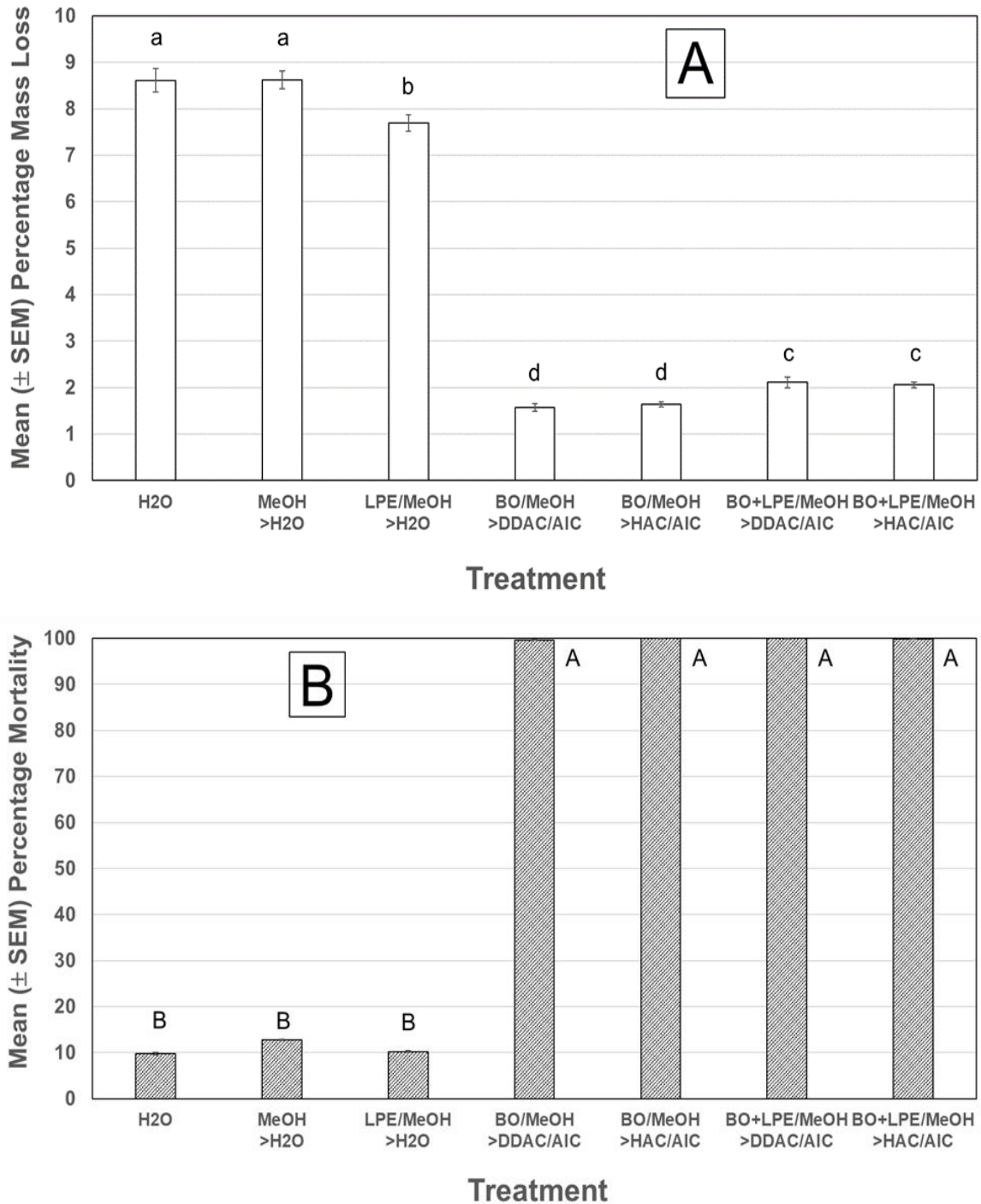
Fig. 1. (A) Mean (±SEM) percentage wood mass losses for treated loblolly pine samples exposed to subterranean termites (open bars with lower case letters) and (B) mean (±SEM) percentage subterranean termite mortality (shaded bars with upper case letters). Means without letters in common differ significantly using Least Significant Difference (P ≤ 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 H_2O, MeOH>H_2O and LPE/MeOH>H_2O 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 H_2O 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 H_2O, MeOH>H_2O and LPE/MeOH>H_2O 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 H_2O 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).
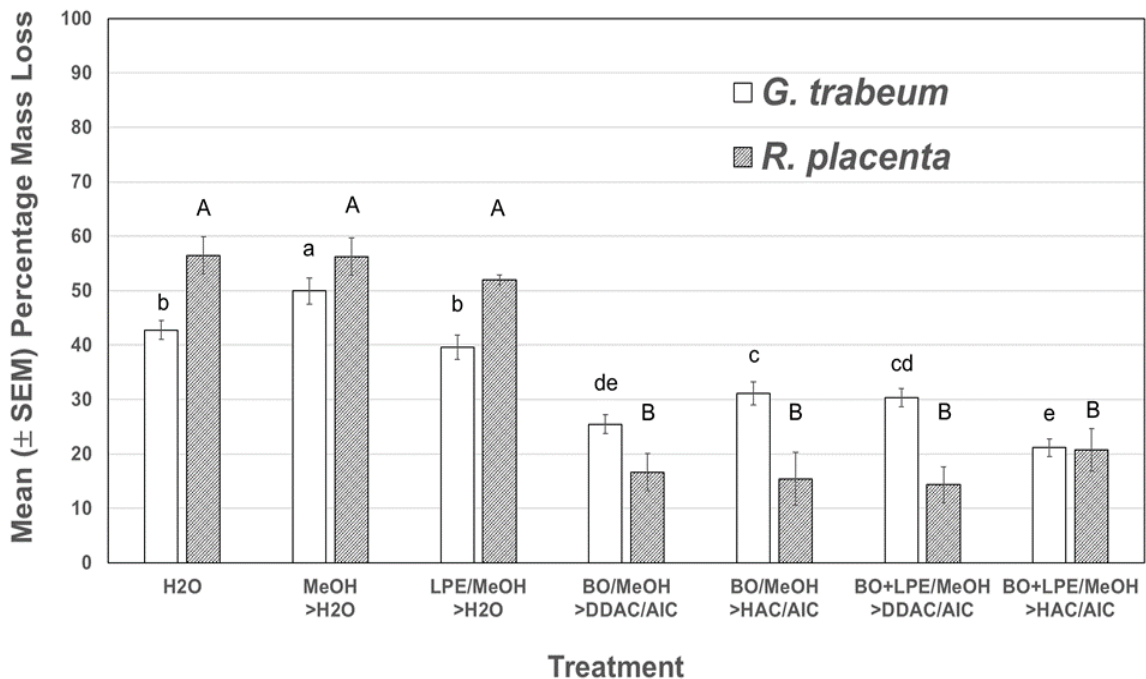
Fig. 2. Mean (±SEM) percentage mass losses for treated loblolly pine samples exposed to brown-rot decay fungi *Gloeophyllum trabeum* (open bars with lower case letters) and *Rhodonia placenta* (shaded bars with upper case letters). For a given fungal species, means without letters in common differ significantly using Least Significant Difference (P≤0.05).

For *G. trabeum*, wood mass loss was highest for the MeOH>H_2O treatment. Mass loss was significantly higher than both the H_2O and LPE/MeOH>H_2O treatments. However, the H_2O and LPE/MeOH>H_2O 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$_2$O, MeOH>H$_2$O and LPE/MeOH>H$_2$O 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$_2$O, MeOH>H$_2$O and LPE/MeOH>H$_2$O 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.
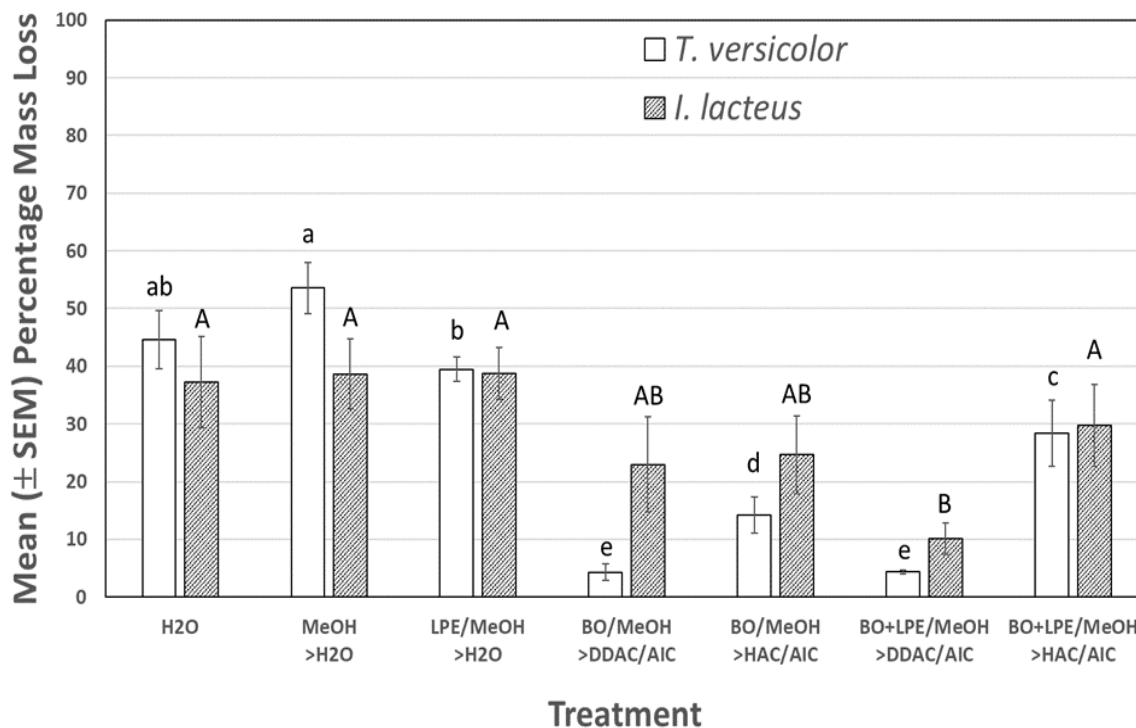
White-Rot Decay Fungi Resistance

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$_2$O treatment and was significantly higher than the LPE/MeOH>H$_2$O treatment, although the H$_2$O and LPE/MeOH>H$_2$O 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$_2$O, MeOH>H$_2$O and LPE/MeOH>H$_2$O 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$_2$O, MeOH>H$_2$O and LPE/MeOH>H$_2$O 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 (±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≤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. AWPA Standard T1 (2020) and AWPA 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/m^3. 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.

ACKNOWLEDGEMENTS

REFERENCES CITED

Adams, R. P. (1991). "Cedarwood oil: Analyses and properties," *Mod. Meth. Plant Anal.* 12, 159-173. DOI: 10.1007/978-3-642-84023-4_8

Adams, R. P., McDaniel, C. A., and Carter, F. L. (1988). "Termiticidal activities in the heartwood, bark/sapwood and leaves of *Juniperus* species from the United States," *Biochem. Sys. Ecol.* 16, 453-456. DOI: 10.1016/0305-1978(88)90043-9

ASTM D1105-96 (2014). "Standard test method for preparation of extractive-free wood," ASTM International, West Conshocken, PA, USA.

AWPA E10-12 (2016). "Standard method of testing wood preservatives by laboratory soil block cultures," American Wood Protection Association, Birmingham, AL, USA.

AWPA A16-16 (2016). "Standard method for determining didecyldimethyl ammonium compounds in treated wood by HPLC," American Wood Protection Association, Birmingham, AL, USA.

AWPA E1-17 (2020). "Laboratory method for evaluating the termite resistance of wood-based materials: Choice and no-choice tests," American Wood Protection Association, Birmingham, AL, USA.

AWPA Standard T1 (2020). "Table 3.2," American Wood Protection Association, Birmingham, AL, USA.

Arango, R. A., Green III, F., Hintz, K., Lebow, P. K., and Miller, R. B. (2006). "Natural durability of tropical and native woods against termite damage by *Reticulitermes*

bioresources.com

flavipes (Kollar)," *Inter. Biodeterior. Biodegrad*. 57, 146-150. DOI: 10.1016/j.ibiod.2006.01.007

Bauch, J., Puls, J., Klupsch, R., and Vogel, C. (2004). "Biological and chemical characteristics of "included sapwood" of *Juniperus virginiana* L.," *Holzforschung* 58, 74-81. DOI: 10.1515/HF.2004.010

Carter, F. (1976). "Responses of subterranean termites to wood extractives," *Mater. Organ. Beiheft*. 3, 357-364.

Carter, F. L., and Smythe, R. V. (1974). "Feeding and survival responses of *Reticulitermes flavipes* (Kollar) to extractives of wood from 11 coniferous genera," *Holzforschung* 28, 41-45. DOI: 10.1515/hfsg.1974.28.2.41

Chang, S. T., and Cheng, S. S. (2002). "Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophelum*," *J. Agric. Food Chem*. 50, 1389-1392. DOI: 10.1021/jf010944n

Chang, S. T., Cheng, S. S., and Wang, S. Y. (2001). "Antitermitic activity of essential oils and components from *Taiwania* (*Taiwania cryptomerioides*)," *J. Chem. Ecol*. 27, 717-724. DOI: 10.1023/A:1010397801826

Chang S. T., Wang S. Y., and Kuo, Y. H. (2003). "Resource and bioactive substances from *Taiwania* (*Taiwania cryptomeriodes*)," *J. Wood Sci*. 49, 1-4. DOI: 10.1007/s100860300000

Chang, S. T., Wang, S. Y., Wu, C. L., Chen, P. F., and Kuo, Y. H. (2000). "Comparisons of the antifungal activities of cadinane skeletal sesquiterpenoids from *Taiwania* (*Taiwania cryptomerioides* Hayata) heartwood," *Holzforschung* 54, 241-245. DOI: 10.1515/HF.2000.041

Chang, S. T., Wang, S. Y., Wu, C. L., Su,Y. C., and Kuo, Y. H. (1999). "Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood," *Holzforschung* 53, 487-490. DOI: 10.1515/HF.1999.080

Cheng, S. S., Chung, M. J., Lin, C. Y., Wang, Y. N., and Chang, S. T. (2012). "Phytochemicals from *Cunninghamia konishii* Hayata act as antifungal agents," *J. Agric. Food Chem*. 60, 124-128. DOI: 10.1021/jf2042196

Cheng, S. S., Lin, H. Y., and Chang, S. T. (2005). "Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica*)," *J. Agric. Food Chem*. 53, 614-619. DOI: 10.1021/jf0484529

Cheng, S. S., Liu, J. Y., Chang, E. H., and Chang, S. T. (2008). "Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi," *Bioresource Technol*. 99, 5145-5149. DOI: 10.1016/j.biortech.2007.09.013

Cheng, S. S., Lin, C. Y., Gu, H. J., and Chang, S.T. (2011). "Chemical composition and antifungal activities of wood and leaf essential oils from *Cunninghamia konishii* Hayata," *J. Wood Chem. Technol*. 31, 204-217. DOI: 10.1080/02773813.2010.515049

Cheng, S. S., Liu, J. Y., Hsui, Y. R., and Chang, S. T. (2006). "Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*)," *Bioresource Technol*. 97, 306-312. DOI: 10.1016/j.biortech.2005.02.030

Cheng, S. S., Wu, C. L., Chang, H. T., Kao, Y. T., and Chang, S. T. (2004). "Antitermitic and antifungal activities of essential oil of *Calocedrus fromosana* leaf and its compounds," *J. Chem. Ecol*. 30, 1957-1967. DOI: 10.1023/B:JOEC.0000045588.67710.74

EU Directive 98/8/EC (2012). "Directive 98/8/EC concerning the placing biocidal products on the market, Assessment Report, Didecyldimethylammonium chloride Product-type PT * (Wood preservative), European Union, Brussels, Belgium.

Eller, F. J. (2018). "Eastern red cedar: Cedarwood oil extraction and bioactivity," *International News on Fats, Oils, and Related Materials (INFORM)* May 2018, 29, 18-21. DOI: 10.21748/inform.05.2018.18

Eller, F. J., Clausen, C. A., Green, F., and Taylor, S. L. (2010). "Critical fluid extraction of *Juniperus virginiana* L. and bioactivity of extracts against subterranean termites and wood-rot fungi," *Ind. Crops Prod*. 32, 481-485. DOI: 10.1016/j.indcrop.2010.06.018

Eller, F. J., Hay, W. T., Kirker, G. T., Mankowski, M. E., and Selling, G. W. (2018). "Hexadecyl ammonium chloride amylose inclusion complex to emulsify cedarwood oil and treat wood against termites and wood-decay fungi," *Inter. Biodeterior. Biodegrad*. 129, 95-101. DOI: 10.1016/j.ibiod.2018.01.010

Eller, F. J., and King, J. W. (2000). "Supercritical carbon dioxide extraction of cedarwood oil: A study of extraction parameters and oil characteristics," *Phytochem. Anal*. 11, 226-231. DOI: 10.1002/1099-1565(200007/08)11:4<226::AID-PCA524>3.0.CO;2-7

Eller, F. J., Kirker, G. T., Mankowski, M. E., Hay, W. T., and Palmquist, D. E. (2020). "Effect of burgundy solid extracted from Eastern red cedar heartwood on subterranean termites and wood-decay fungi," *Ind. Crops Prod*. 144, 112023. DOI: 10.1016/j.indcrop.2019.112023

Fanta, G. F., Felker, F. C., Selling. G. W., Hay, W. T., and Biswas, A. (2016). "Poly(vinyl alcohol) composite films with high percent elongation prepared from amylose-fatty ammonium salt inclusion complexes," *J. Appl. Polym. Sci*. 133, 44110. DOI: 10.1002/app.44110

French, J. R. J., Robinson, P. J., Yazaki, Y., and Hillis, W. E. (1979). "Bioassays of extracts from white cypress pine (*Callitris columellaris* F Muell) against subterranean termites," *Holzforschung* 33, 144-148. DOI: 10.1515/hfsg.1979.33.5.144

Gupta, A., Sharma, S., and Naik, S. N. (2011). "Biopesticidal value of selected essential oils against pathogenic fungus, termites, and nematodes," *Inter. Biodeter. Biodeg*. 65, 703-707. DOI: 10.1016/j.ibiod.2010.11.018

Hassan, B., Mankowski, M. E., Kirker, G. T., and Ahmed, S. (2017). "Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species," *Inter. Biodeter. Biodeg*. 123, 27-36. DOI: 10.1016/j.ibiod.2017.05.023

Hay, W. T., Fanta, G. F., Felker, F. C., Peterson, S. C., Skory, C. D., Hojilla-Evangelista, M. P., Biresaw, G., and Selling, G. W. (2019). "Emulsification properties of amylose-fatty sodium salt inclusion complexes," *Food Hydrocolloids* 90, 490-499. DOI: 10.1016/j.foodhyd.2018.12.038

Kard, B., Hiziroglu, S., and Payton, M. E. (2007). "Resistance of redcedar panels to damage by subterranean termites (Isoptera: Rhinotermitidae)," *For. Prod. J.* 57, 74-79.

Kard, B. M., and Mallette, E. J. (1997). "Resistance of six wood products used in paneling to *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)" *J. Econ. Entomol.* 90, 178-182. DOI: 10.1093/jee/90.1.178

Kazemi, S. M., Hosseinzadeh, A., and Rezaei, M. B. (2006). "The effect of woody and non woody plants extractives on microbial resistance of non-durable species," *J. Agric. Sci. Nat. Resour.* 13, 183-193.

Kirker, G. T., Bishell, A. B., and Lebow, P. K. (2016). "Laboratory evaluations of durability of southern pine pressure treated with extractives from durable wood species," *J. Econ. Entomol.* 109, 259-266. DOI: 10.1093/jee/tov286

Kirker, G. T., Blodgett, A. B., Arango, R. A., Lebow, P. K., and Clausen, C. A. (2013). "The role of extractives in naturally durable wood species," *Inter. Biodeterior. Biodegrad.* 82, 53-58. DOI: 10.1016/j.ibiod.2013.03.007

Konemann, C. E., Kard, B. M., and Payton, M. E. (2014). "Palatability of field-collected eastern redcedar, *Juniperus virginiana* L., components to subterranean termites (Isoptera: Rhinotermitidae)," *J. Kansas Entomol. Soc.* 87, 2014, 269-279. DOI: 10.2317/JKES130619.1

Kose, C., and Taylor, A. M. (2012). "Evaluation of mold and termite resistance of included sapwood in eastern redcedar," *Wood Fiber Sci.* 44, 319-324.

Li, Q., Lin, J. G., and Liu, J. (2013). "Decay resistance of wood treated with extracts of *Cinnamomum camphora* xylem," *BioResources* 8(3), 4208-4217. DOI: 10.15376/biores.8.3.4208-4217

Lipeh, S., Schimleck, L. R., Mankowski, M. E., McDonald, A. G. and Morrell, J. J. (2020). "Relationship between attenuated total reflectance Fourier transform infrared spectroscopy of western juniper and natural resistance to fungal and termite attack," *Holzforschung* 74, 246-259. DOI: 10.1515/hf-2019-0096

Mankowski, M. E., Boyd, B., Hassan, B., and Kirker, G. T. (2016). "GC-MS characterizations of termiticidal heartwood extractives from wood species utilized in Pakistan," in: *Proceedings IRG Annual Meeting, (ISSN 2000-8953) IRG/WP 16-10857*, The International Research Group on Wood Protection, Lisbon, Portugal.

Mansour, M. M., and Salem, M. Z. M. (2015). "Evaluation of wood treated with some natural extracts and Paraloid B-72 against the fungus *Trichoderma harzianum*: wood elemental composition, in-vitro and application evidence," *Inter. Biodeterior. Biodegr.* 100, 62-69. DOI: 10.1016/j.ibiod.2015.02.009

McDaniel, C. A., and Dunn, B. S. (1994). "Can wood extractives be used as wood protectants?," *Proc. Symp. Curr. Res. Chem. Sci. Gen. Tech. Rep. Soc.* 101, 61-63.

McDaniel, C. A., Klocke, J. A., and Balandrin, M .F. (1989). "Major antitermitic wood extractive components from eastern red cedar, *Juniperus virginiana*," *Mater. Org.* 24, 301-331.

Morita, S. I., Hidaka, T., and Yatagai, M. (1997). "Antifungal compounds of the extractives of Yakusugi (*Cryptomeria japonica* D. Don)," *Wood Preserv.* 23, 11-19. DOI: 10.5990/jwpa.23.61

Mun, S. P., and Prewitt, L. (2011). "Antifungal activity of organic extracts from *Juniperus virginiana* heartwood against wood decay fungi," *For. Prod. J.* 61, 443-449. DOI: 10.13073/0015-7473-61.6.443

Pandey, A., Chattopadhyay, P., Banerjee, S., Pakshirajan, K., and Singh, L. (2012). "Antitermitic activity of plant essential oils and their major constituents against termite *Odontotermes assamensis* Holmgren (Isoptera: Termitidae) of North East India," *Inter. Biodeter. Biodeg.* 75, 63-67. DOI: 10.1016/j.ibiod.2012.09.004

Saha Tchinda, J.-B., Ndikontar, M. K., Belinga, A. D. F., Mounguengui, S., Njankouo, J. M., Durmacay, S., and Gerardin, P. (2018). "Inhibition of fungi with wood extractives

and natural durability of five Cameroonian wood species," *Ind. Crops Prod.* 123:183-191. DOI: 10.1016/j.indcrop.2018.06.078

Salem, M. Z. M., Zidan, Y. E., Mansour, M. M. A., El-Hadidi, N. M. N., and Abo-Elgat, W. A. A. (2016). "Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi," *Inter. Biodeter. Biodeg.* 106, 88-96. DOI: 10.1016/j.ibiod.2015.10.010

Schultz, T. P., and Nicholas, D. D. (2002). "Development of environmentally-benign wood preservatives based on the combination of organic biocides with antioxidants and metal chelators," *Phytochemistry* 61, 555-560. DOI: 10.1016/S0031-9422(02)00267-4

Singh, T., and Singh, A. P. (2012). "A review on natural products as wood protectant," *Wood Sci. Technol.* 46, 851-70. DOI: 10.1007/s00226-011-0448-5

Smith, A. L., Campbell, C. L., Walker, D. B., and Hanover, J. W. (1989). "Extracts from black locust as wood preservatives: Extraction of decay resistance from black locust heartwood," *Holzforshung* 43, 293-296. DOI: 10.1515/hfsg.1989.43.5.293

Smith, K. T., and Glaeser, J. A. (2013). "Skeleton decay in eastern Red Cedar," *Arborist* News 22:32-34.

Tascioglu, C., Yalcin, M., Sen, S., and Akcay, C. (2013). "Antifungal properties of some plant extracts used as wood preservatives," *Inter. Biodeter. Biodeg.* 85, 23-28. DOI: 10.1016/j.ibiod.2013.06.004

Taylor, A. M., Gartner, B. L, and Morrell, J. J. (2002). "Heartwood formation and natural durability – A review," *Wood Fiber Sci.* 34, 587-611.

Taylor, A. M., Gartner, B. L., Morrell, J. J., and Tsunoda, K. (2006). "Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi," *J. Wood Sci.* 52, 147-153. DOI: 10.1007/s10086-005-0743-6

Tumen, I., Eller, F. J., Clausen, C. A., and Teel, J. A. (2013). "Antifungal activity of heartwood extracts from three *Juniperus* species," *BioResources* 8(1), 12-20. DOI: 10.15376/biores.8.1.12-20

Valette, N., Perrot, T., Sormani, R., Gelhaye, E, and Morel-Rouhier, M. (2017). "Antifungal activities of wood extractives," *Fungal Bio. Rev.* 31, 113-23. DOI: 10.1016/j.fbr.2017.01.002

Wan, H., Wang, X. M., and Yang, D. Q. (2007). "Utilizing eastern white cedar to improve the resistance of strand boards to mould and decay fungi," *For. Prod. J.* 57, 54-59.

Wang, S. Y., Chen, P. F., and Chang, S. T. (2005a). "Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi," *Bioresource Technol.* 96, 813-818. DOI: 10.1016/j.biortech.2004.07.010

Wang, S. Y., Wu, C. L., Chu, F .H., Chien, S. C., Kuo, Y. H., Shyur, L. F., and Chang, S. T. (2005b). "Chemical composition and antifungal activity of essential oil from *Chamaecyparis formosensis* wood," *Holzforschung* 59, 295-299. DOI: 10.1515/HF.2005.049

Wang, J., Li, J., Li, S., Freitang, C., and Morrell, J. J. (2011). "Antifungal activities of *Cunninghamia lanceolate* heartwood extractives," *BioResources* 6(1), 606-614. DOI: 10.15376/biores.6.1.606-614

Watanabe, Y., Mihara, R., Mitsunaga, T., and Yoshimura, T. (2005). "Termite repellent sesquiterpenoids from *Callitris glaucophylla* heartwood," *J. Wood Sci.* 51, 514-519. DOI: 10.1007/s10086-004-0683-6

Wu, C. L., Chien, S. C., Wang, S. Y., Ku, Y. H., and Chang, S. T. (2005). "Structure-activity relationships of cadinane-type sesquiterpene derivatives against wood-decay fungi," *Holzforschung* 59, 620-627. DOI: 10.1515/HF.2005.100

Wu, C. C., Wu, C. L., Huang, S. L., and Chang, H. T. (2012). "Antifungal activity of liriodenine from *Michelia formosana* heartwood against wood-rotting fungi," *Wood Sci. Technol.* 46, 737-747. DOI: 10.1007/s00226-011-0428-9

Xie, Y. J., Yang, Z. L., Cao, D. Y., Rong, F., Ding, H., and Zhang, D. Y. (2015). "Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (Clove)," *Ind. Crops Prod.* 77, 780-786. DOI: 10.1016/j.indcrop.2015.09.044

Xie, Y., Wang, Z., Huang, Q., and Zhang, D. (2017). "Antifungal activity of several essential oils and major components against wood-rot fungi," *Ind. Crops Prod.* 2017. 108, 278-85. DOI: 10.1016/j.indcrop.2017.06.041

Yang, D. Q. (2009). "Potential utilization of plant and fungal extracts for wood protection," *For. Prod. J.* 59, 97-103.

Yen, T. B., Chang, H. T., Hsieh, C. C., and Chang, S. T. (2008). "Antifungal properties of ethanolic extract and its active compounds from *Calocedrus macrolepis* var. *formosana* (Florin) heartwood," *Bioresource Technol.* 99, 4871-4877. DOI: 10.1016/j.biortech.2007.09.037

Zhu, B. C., Henderson, G., Chen, F., Fei, H., and Laine, R. A. (2001). "Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite," *J. Chem. Ecol.* 27, 1617-1625. DOI: 10.1023/A:1010410325174