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Hexadecyl ammonium chloride amylose inclusion complex to emulsify cedarwood oil and treat wood against termites and wood-decay fungi☆

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ABSTRACT

Cedarwood oil (CWO) has a wide range of bioactivities, including insect repellency and toxicity, as well as conferring resistance against termites and wood-decay fungi. In previous work examining pressure treatment of wood, ethanol was used as the diluent/carrier for CWO. However, it is preferable to use a water-based carrier for environmental, safety and cost considerations. In this research, we describe the use of a hexadecyl ammonium chloride amylose inclusion complex/polyvinyl alcohol (AIC/PVOH) as an emulsifier for CWO to pressure treat wood. Wood samples were subsequently tested for resistance to termites and four species of wood-decay fungi. Wood was also compared for water absorption and swelling. In the termite test, the lowest wood mass losses were for the AIC/PVOH/CWO (5.4%) and EtOH/CWO (5.4%) treatments, which also had the highest termite mortalities (i.e., 100% and 97.6%, respectively). In general, for wood-decay fungi, wood mass losses were lowest for the EtOH/CWO and AIC/PVOH/CWO treatments and were highest for the Water, EtOH, and AIC/PVOH treatments. Wood blocks treated with AIC/PVOH repelled water as evidenced by higher contact angle, lower mass gain (both by submersion and water saturation) and lower swelling. The results indicated that the amylose inclusion complex makes an excellent emulsifier and the AIC/PVOH/CWO mixture inhibits both termites and wood-decay fungi. The amylose inclusion complex alone was as inhibitory as CWO against termites and also inhibits both water absorption and swelling in treated wood.

1. Introduction

There are numerous examples of woods that are resistant to termites and/or wood-decay fungi as well as many examples of extracts from resistant woods that confer resistance to termites and/or wood-decay fungi (Watanabe et al., 2005a,b; Cheng et al., 2007; Abdul Khalil et al., 2009; Santana et al., 2010; Wang et al., 2011; Wahyudi et al., 2012; Kadir et al., 2014; Brocco et al., 2017; Hassan et al., 2017). Eastern red cedar (ERC) (*Juniperus virginiana*) (Cupressaceae) has been demonstrated to be resistant to termites (Kard et al., 2007; Konemann et al., 2014) and extracts from ERC have been shown to decrease mass losses from wood treated with these extracts (Eller et al., 2010; Tumen et al., 2013) for both termites and wood-decay fungi.

Eastern red cedar is an abundant natural resource in the United States and it is the domestic source of cedarwood oil (CWO) (Schmidt and Leatherberry, 1995; Adams, 1987). High quality and yields of CWO have been obtained by CO_2 extraction, using both supercritical CO_2 (Eller and King, 2000) and liquid CO_2 (Eller and Taylor, 2004).

Junipers are well-known for their resistance to both termite attack and microbial decay (Adams et al., 1988) and extracts from junipers may serve as a source of safe, natural wood preservatives from this abundant renewable resource. Previously, CWO has been demonstrated to confer resistance to susceptible wood species against both termites and wood-decay fungi (Eller et al., 2010; Tumen et al., 2013).

To control the concentration of the preservative used during the pressure treatment of wood, the preservative must be diluted and uniformly dispersed into a suitable liquid carrier. The laboratory pressure treatment procedure also requires 90 min to complete (AWPA, 2012). Therefore, the liquid dispersion must be stable for at least this long. However, after the pressure treatment is completed, it is desirable to

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alter the carrier properties to decrease the likelihood of the preservative subsequently being removed (i.e., leached) from the wood. Previously, we used ethanol as the carrier which evaporated and left the CWO behind in the treated wood (Eller et al., 2010; Tumen et al., 2013). Although ethanol was suitable in several ways (i.e., miscible with CWO; readily available; relatively inexpensive; and "green"), an aqueous based carrier is preferrable due to its lower cost and safety advantages. In this study, we investigated the use of an amylose (i.e., corn starch) inclusion complex as an emulsifier in an aqueous carrier to make a better (e.g., nonflammable, less expensive and safer) alternative to ethanol for CWO treatment of wood.

The purpose of this study was to determine if an amylose inclusion complex could be used as an emulsifier for CWO to vacuum impregnate wood and determine how the dispersion components compared for imparting resistance to termites and wood-decay fungi. In addition, we studied how the test materials affect the interaction of water with wood, specifically contact angle, water mass gain and dimensional stability and how these characteristics are related to resistance against termites and wood-decay fungi.

2. Materials and methods

2.1. Cedarwood oil

Heartwood samples were cut from freshly felled Eastern red cedar (Tazewell Co., Illinois) and sawdust was prepared from the heartwood as described by Eller et al. (2014). Cedarwood oil was extracted from this sawdust using supercritical carbon dioxide (70 °C, 27.6 MPa) as described by Eller and King (2000).

All CWO carrier mixtures were formulated to contain 5% CWO by weight. The carrier mixture treatments were prepared using an electric hand blender by mixing on high for approximately 30 s. The five treatments tested were: Water Only; Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); EtOH/CWO; and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO). The dispersion of CWO in the AIC/PVOH solution was observed to remain uniformly mixed for over 24 h, which was significantly longer than the 90 min needed for the vacuum impregnation of the wood samples described below.

2.2. Preparation of amylose-hexadecylammonium chloride inclusion complexes

High-amylose corn starch (~68% amylose, AmyloGel 03003) was a product of Cargill (Minneapolis, MN). Hexadecylamine (98%); and hydrochloric acid (HCl, 37%) were purchased from Sigma (St. Louis, MO); Polyvinyl alcohol (PVOH) (MW 133,000, 99 mol% hydrolyzed) was purchased from Polysciences, Warrington, PA; Ultrapure water was used for all solutions and was obtained from a Barnstead Nanopure System (ThermoScientific, Asheville, NC).

The procedure for producing the amylose-inclusion complexes was the same as that reported earlier (Hay et al., 2017a). The high-amylose corn starch (100 g) was dispersed in 1800 mL of deionized water within a 2-L stainless steel Waring blender (Waring Products division, New Hartford, CT). After high shear mixing the dispersion was subsequently passed through a Penick and Ford (Penford Corp., Englewood, CO) laboratory model steam jet-cooker operating at excess steam conditions (Klem and Brogly, 1981). The temperature in the hydroheater was 140 °C, steam back pressure was 380 kPa, and the steam line pressure was 550 kPa and the dispersion was pumped through at a rate of 1 L/min. The jet cooked solution was collected in a 4-L stainless steel Waring blender container (Waring Products division, New Hartford, CT). A solution of hexadecylammonium chloride was prepared, 5.25 g of hexadecylamine was dispersed in 217.42 g of 0.1 N HCl and fully dissolved by heating to 90 °C. The hexadecylammonium chloride solution was then immediately added to the hot starch dispersion after jet-cooking. The solution was sheared in the Waring blender for 1 min, and then quickly cooled in an ice bath to 25 °C. The solution was then freeze dried using a Labconco Freezone 6 L freeze dryer (Labconco, Kansas City, MO).

A solution containing 1% amylose-hexadecylammonium chloride complexes and 1% PVOH was prepared as reported previously by dispersing the polymers in nanopure water and heating the dispersions to 80 °C (Hay et al., 2017b).

2.3. Termite resistance

Using a no-choice test (i.e., only one treatment per container), vacuum impregnated wood blocks were tested for resistance to eastern subterranean termites, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) using Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites E1-06 (AWPA, 2016). Workers and soldiers of *R. flavipes* were collected from dead logs present at Sam D. Hamilton Noxubee National Wildlife Refuge (Starkville, Mississippi) and maintained in the laboratory at 25 °C in the darkness in cut sections of the collected logs in 30-gallon trashcans. 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. Screw top jars were filled with 150 g sand along with 27 mL distilled water and held for 2 h to equilibrate. For the no choice test, blocks were conditioned (33 °C, 62 ± 3%), weighed and placed on a square of foil on top of the damp sand with one block in each jar. A total of 400 termites (396 workers and 4 soldiers) were released in each jar (Fig. 1) and jars were kept in the conditioning chamber at 27 °C and 75 ± 2% relative humidity for 28 days. After four weeks, the number of live termites were counted. Blocks were brushed to remove sand, conditioned for one week, and re-weighed to determine weight loss as described in the AWPA E1 standard.

Spruce/Pine/Fir (SPF) blocks were prepared from a board milled to 2.54 cm × 2.54 cm × 0.64 cm. The wood blocks were conditioned to a constant mass at 25 °C and 50% relative humidity (RH) and weighed prior to vacuum impregnation. Wood samples 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. After impregnation, wood samples were reweighed, the solvent was allowed to evaporate and the blocks re-conditioned to a constant mass at 25 °C and 50% RH. Wood weight loss and termite mortality were determined after a 28 day exposure to the termites. There were six replications of each treatment. The five treatments tested were the same as described above.

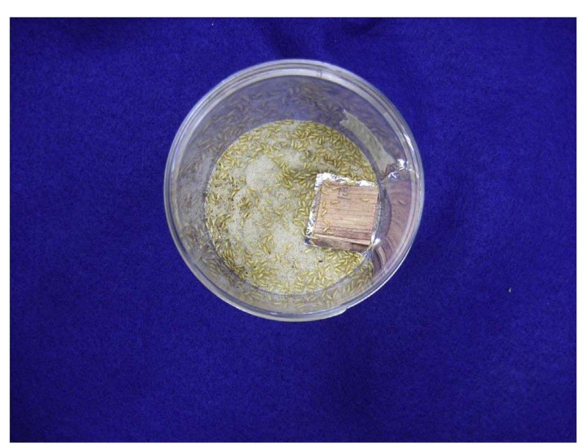

Fig. 1. Photograph showing termite resistance bioassay.

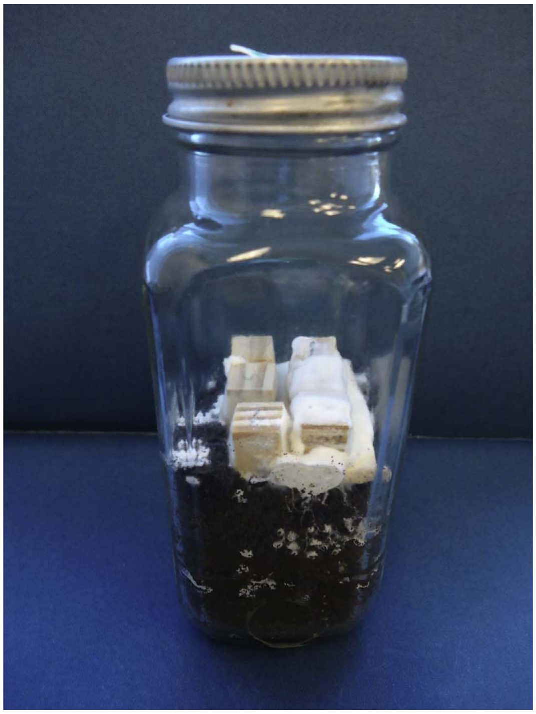

Fig. 2. Photograph showing wood-decay resistance bioassay.

2.4. Wood-decay fungi resistance

Vacuum impregnated wood cubes were tested for resistance to wood-decay fungi using Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures E10-06 (AWPA, 2012). This method calls for using Spruce/Pine/Fir (SPF) cubes for tests utilizing brown-rot fungi and Yellow Poplar (YP) cubes for tests utilizing white-rot fungi. Two brown-rot fungi (*Gloeophyllum trabeum* (Pers.: Fr.) Murr (MAD 617) and *Postia placenta* (Fr.) M. Lars., et Lomb (MAD 698) and two white-rot fungi (*Trametes versicolor* (L. Fr.) Pil. (MAD 697) and *Irpex lacteus* (Fr.: Fr.) Fr. (HHB 7328) were tested. Using a custom-made environment room (Environmental Growth Chambers, Chagrin Falls, OH), the 1-cm^3 wood cubes were conditioned to a constant mass at 27 °C and 70% RH and weighed prior to vacuum impregnation. Wood samples were vacuum impregnated as described for the termites. After impregnation, wood samples were reweighed, air-dried overnight and the blocks re-conditioned to a constant mass at 27 °C and 70% RH. Treated cubes were gas-sterilized with propylene oxide prior to exposure to test fungi in the soil block test (Fig. 2). Weight loss was determined after an 8-week exposure to the fungi at 27 °C and 70% RH. There were six replications of each treatment per test fungus. The five treatments tested were the same as described above.

2.5. Interaction of water and wood

Interactions of water and wood were determined using water contact angle, water mass gain and dimensional stability. Contact angles were determined using a DSA25 drop shape analyzer (Krüss, Matthews, NC), and the instrument was equipped with ADVANCE v1.4 software. The procedure was similar to that described previously (Fanta et al., 2016b). Contact angles were determined for Water controls and AIC/PVOH treated SPF cubes.

Water mass gain and dimensional stability were compared using a method modified from water soak test and test of dimensional stability (Rowell and Banks, 1985). The five treatments tested were the same as described above. The SPF wood cubes were equilibrated for two weeks in a desiccator at 30% RH, and humidity was maintained using a saturated calcium chloride solution. The radial, tangential, and longitudinal dimensions of five wood cubes per treatment were determined using a micrometer and their equilibrium mass was determined gravimetrically. Cubes were then submerged in 50 mL of deionized water for an hour. Every 10 min the cubes were removed and excess surface water was blotted off, samples were quickly weighed and changes in their radial, tangential and longitudinal dimensions were determined. Water mass gain was determined gravimetrically at each time point, and cube swelling was determined by the change in area of the wood cube based off the dimensional measurements. After an hour, the submerged wood cubes were exposed to a 44 mbar vacuum for 30 min to fully saturate the wood cubes with water. The mass and volume of the saturated wood blocks were determined following the method previously described. The water holding capacity is defined as being the water mass minus the dry mass.

2.6. Statistical analyses

One way analyses of variance (ANOVA) were conducted on the percentage weight loss data after arcsine square-root transformation to stabilize the variance using Statistix 7 software (Analytical Software, Tallahassee, FL, USA). A few of the fungal soil-block culture jars became contaminated with an unidentified mold and these jars were excluded from the data analysis. Results for wood cube water mass gain, volume increase and differences in maximum water capacity were evaluated by a two factor repeated measure ANOVA, water mass gain was transformed to the $\sqrt[5]{x}$ to stabilize the variance in Proc Mixed SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Treatment means were compared using least significant difference (LSD) at P = 0.05 after obtaining a significant F-test.

3. Results

The mean percentage mass changes for the treated wood samples after being re-conditioned to a constant mass were as follows: Water −0.8%; EtOH −1.0%; EtOH/CWO 5.1%; AIC/PVOH 5.5%; and AIC/PVOH/CWO 11.0%. These values indicate essentially no mass change after treatment with Water or EtOH alone, but additive mass changes with CWO and/or AIC/PVOH.

3.1. Termite resistance

The results of the termite resistance tests are shown in Table 1. Percentage mass losses were highest for the EtOH (27.8%) and Water (22.8%) treatments and these two treatments were statistically equivalent. The lowest observed mean mass losses were for the AIC/PVOH/CWO (5.4%) and EtOH/CWO (5.4%) treatments which were statistically equivalent to each other and the AIC/PVOH (10.2%) treatment. These three treatments all had significantly lower mass losses than both the EtOH and Water treatments. The AIC/PVOH/CWO

Table 1
Subterranean termite mean (SEM) percentage wood block mass loss and mean (SEM) percentage mortality after exposure to treated wood blocks.

Treatment [a]	Mean (SEM)	Mean (SEM)
	Percentage Mass Loss [b]	Percentage Mortality [b]
Water Only	22.8 (3.7) a	54.3 (9.5) b
EtOH	27.8 (2.8) a	41.2 (6.6) b
AIC/PVOH	10.2 (1.1) b	94.5 (4.7) a
EtOH/CWO	5.4 (1.6) b	97.6 (2.4) a
AIC/PVOH/CWO	5.4 (0.9) b	100.0 (0.0) a

[a] Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

[b] Within a column, means without letters in common differ significantly using LSD (P = 0.05).

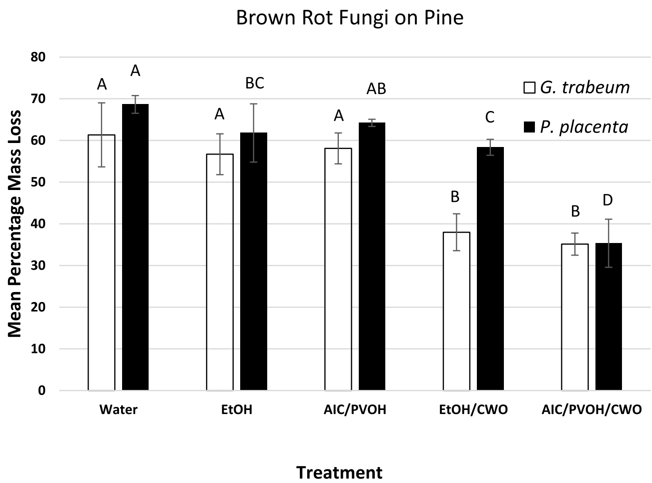

Fig. 3. Mean (± SE) percentage mass loss for wood exposed to brown-rot fungi. For a given fungus, treatment means without letters in common differ significantly by LSD (P = 0.05). Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

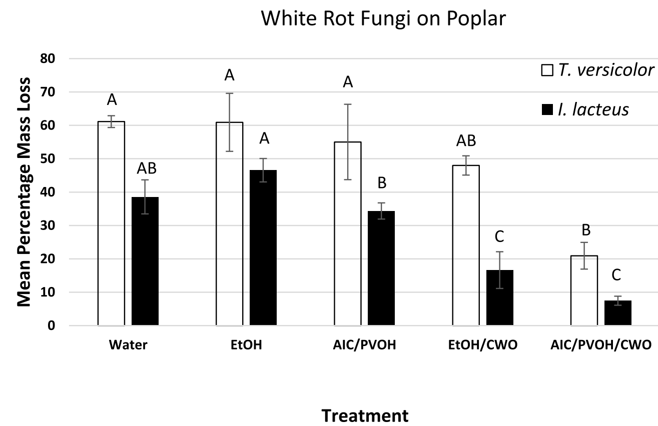

Fig. 4. Mean (± SE) percentage mass loss for wood exposed to white-rot fungi. For a given fungus, treatment means without letters in common differ significantly by LSD (P = 0.05). Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

treatment had approximately 75% less mass loss than the Water control.

Percentage termite mortalities were lowest for the EtOH (41.2%) and Water (54.3%) treatments and these two treatments were statistically equivalent. The highest termite mortalities were for the AIC/PVOH/CWO (100%), EtOH/CWO (97.6%) and AIC/PVOH (94.5%) treatments. Termite mortalities for these three treatments were statistically equivalent to one another but were significantly higher than both the mortalities for the Water and EtOH treatments.

3.2. Wood-decay fungi resistance

The results of the resistance tests for the brown-rot fungi on SPF cubes are shown in Fig. 3. For *G. trabeum*, mass losses were highest for the Water, EtOH, and AIC/PVOH treatments and were lowest for the EtOH/CWO and AIC/PVOH/CWO treatments. For *P. placenta*, mass loss was highest for the Water treatment and lowest for the AIC/PVOH/CWO treatment. The EtOH, AIC/PVOH and EtOH/CWO treatments were intermediate between the Water treatment and the AIC/PVOH/CWO treatment.

The results of the resistance tests for the white-rot fungi on poplar are shown in Fig. 4. For *T. versicolor*, mass losses were highest for the Water, EtOH, and AIC/PVOH treatments and were lowest for the AIC/PVOH/CWO and EtOH/CWO treatments. For *I. lacteus*, mass losses were highest for the Water and EtOH treatments and lowest for the AIC/PVOH/CWO and EtOH/CWO treatments. The AIC/PVOH treatment was intermediate between the Water treatment and the EtOH/CWO treatment.

3.3. Interaction of wood and water

Fig. 5 shows a representative photograph of the contact angles for SPF wood blocks treated with the AIC/PVOH and water control wood blocks. After 30 s, the water control had a contact angle of 63.28° ± 9.63 while the treated sample was 125.60° ± 6.58. These two treatments were determined to be significantly different with a *t*-test, P = 5.6E-06.

The mass gains for the wood blocks soaked in water are shown in Fig. 6. The wood blocks treated with AIC/PVOH (i.e., AIC/PVOH/CWO and AIC/PVOH) gained significantly less water than wood blocks treated with Water or EtOH. Wood blocks treated with CWO (i.e., AIC/PVOH/CWO and EtOH/CWO) were statistically equivalent to each other and had water mass gains numerically between those for the Water or EtOH control treatments and the AIC/PVOH treatment. The EtOH/CWO treatment was statistically equivalent to the EtOH treatment. The AIC/PVOH/CWO treatment was statistically equivalent to the AIC/PVOH treatment.

The swelling (i.e., percentage volume increase) for the submerged wood blocks are shown in Fig. 7. The wood blocks treated with AIC/PVOH alone had the least amount of swelling, while the EtOH control treatment had the most swelling. The blocks treated with amylose-complexes and CWO resisted swelling (i.e., 55% of control) but not to the degree seen by the amylose-complexes only (i.e., 75% less than control). Treatment with EtOH/CWO, of which CWO is a hydrophobic mixture of compounds, did not significantly reduce the water mass gain compared to control but did reduce the overall swelling (i.e., ca. 14% less than control) and improved the dimensional stability of the wood compared to the EtOH control.

The water-saturated masses for the wood blocks are shown in Fig. 8. Under this severe test, the amylose-complex treated wood (i.e., AIC/PVOH) was observed to have a significantly reduced water holding capacity (i.e., 42% less). The amylose-complex with CWO (i.e., AIC/PVOH/CWO) was also observed to have a significantly reduced capacity for water (i.e., 32% less), though not as great as that seen for the amylose-complex only treated blocks.

4. Discussion

The use of the amylose inclusion complex as an emulsifier for CWO in an aqueous dispersion, rather than using ethanol as the solvent, was observed to significantly improve the properties of the treated wood. Treatments utilizing aqueous dispersions as a carriers for CWO have the advantage of being nonflammable, and safer when compared to ethanol. Additionally, the treatment should be low cost due to the use of the amylose inclusion complex, which is formed from high amylose corn starch and fatty amine salts utilizing the common industrial method of steam jet cooking and spray drying (Fanta et al., 2013; Hay et al., 2017a).

For the termite tests, the mass loss data and mortality data are clearly inversely proportional. For example, the AIC/PVOH/CWO treatment gave the lowest percentage mass loss observed and resulted in the highest termite mortality. It is possible that the AIC/PVOH/CWO treatment was toxic to the termites and caused their death resulting in the low percentage mass loss. However, it is also possible that the treatment inhibited feeding and this resulted in death by starvation. With termites, resistance is generally demonstrated by a decrease in mass loss (Kard et al., 2007; Abdul Khalil et al., 2009; Wahyudi et al., 2012; Konemann et al., 2014). In most cases, decreased wood mass loss

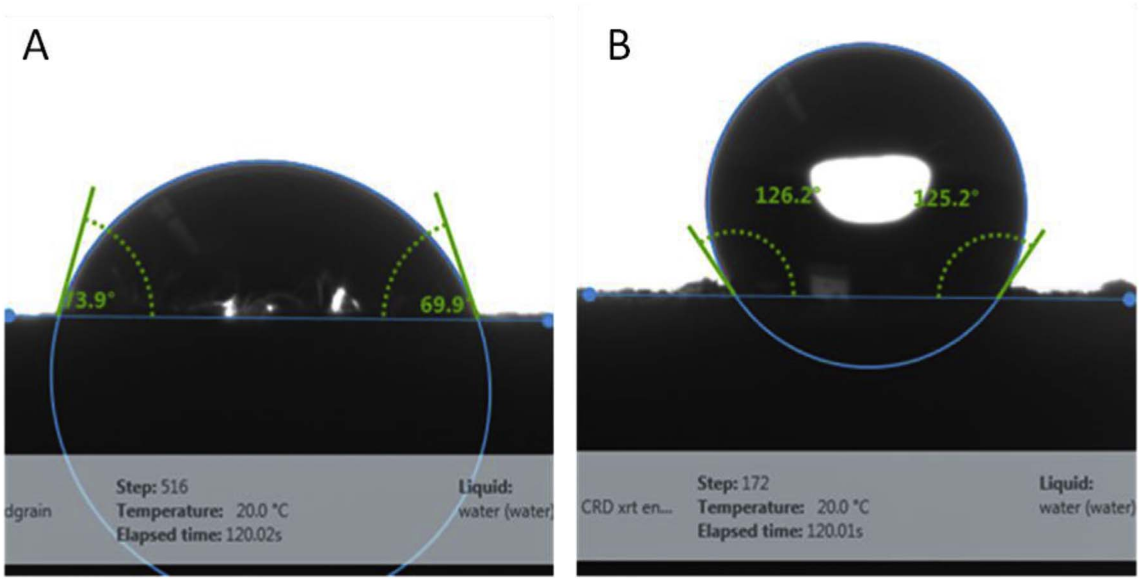

Fig. 5. Photograph of contact angle determination for water control wood (left) and wood treated with amylose inclusion complex (right).

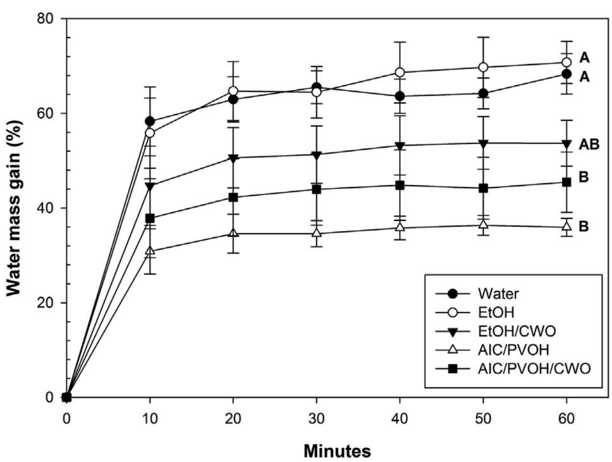

Fig. 6. Mean (± SE) percentage mass gain of wood after soaking in water. At 60 min, treatment means without letters in common differ significantly by LSD (P = 0.05). Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

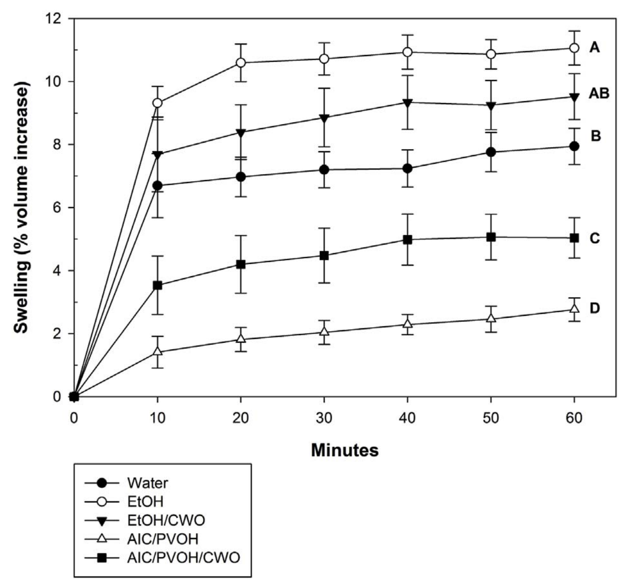

Fig. 7. Mean (± SE) percentage volume increase after submersion in water over time. At 60 min, treatment means without letters in common differ significantly by LSD (P = 0.05). Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

is attributed to termite mortality (Watanabe et al., 2005a; Cheng et al., 2007; Kard et al., 2007; Santana et al., 2010; Wahyudi et al., 2012; Alavijeh et al., 2014; Kadir et al., 2014; Hassan et al., 2017). However, in a few cases, the effect is due to repellency or antifeedent effects (Watanabe et al., 2005b; Cheng et al., 2007; Wahyudi et al., 2012; Alavijeh et al., 2014). Interestingly, the presence of either CWO or AIC/PVOH alone both resulted in lower mass loss and higher termite mortality. Apparently, either one alone can cause termite mortality and reduce termite damage. When they are present together (i.e., AIC/PVOH/CWO), it resulted in the lowest mass loss and highest termite mortality.

Unexpectedly, the amylose inclusion complex alone (i.e., AIC/PVOH) had some inhibitory effects against the termites. The results of the water interaction studies indicated that the amylose inclusion complex inhibited the absorption of water by treated wood. The lower water content may have made the treated wood less palatable to the termites, possibly causing starvation leading to death. It is also possible that something in the amylose inclusion complex was toxic. The hexadecyl ammonium chloride used in the amylose inclusion complex is chemically similar to dimethyl didecyl ammonium chloride which is currently used to pressure treat wood (Kartal et al., 2005). This similarity might partially explain the activity we observed for treatments with this compound. When both the AIC/PVOH and CWO were together in the AIC/PVOH/CWO treatment, their combined inhibitory effects resulted in the lowest mass loss from termite feeding.

As observed for the termites, when both the AIC/PVOH and CWO were together in the AIC/PVOH/CWO treatment, their combined inhibitory effects resulted in the lowest mass loss from wood-decay fungi as well. Generally, for the wood-decay tests, the treatments containing CWO did inhibit mass loss compared to the controls (Figs. 3 and 4). Similar results have been previously reported for CWO-treated wood (Eller et al., 2010; Tumen et al., 2013). In addition, Mun and Prewitt (2011) investigated the antifungal activity of extracts of *J. virginiana* and individual components of the essential oil against *T. versicolor* and *G. trabeum*. They reported thujopsene and cedrol as the most active components against *T. versicolor* and *G. trabeum*. Interestingly, Wang

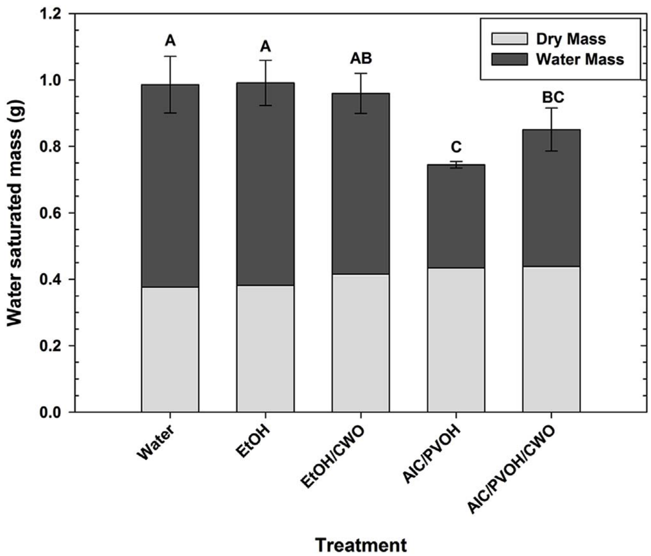

Fig. 8. Mean (± SE) mass of wood SPF cubes, before and after water-saturation. Treatment means without letters in common differ significantly by LSD (P = 0.05). Abbreviations are as follows: Ethanol Only (EtOH); Amylose Inclusion Complex/Polyvinyl Alcohol (AIC/PVOH); Ethanol/Cedarwood Oil (EtOH/CWO); and Amylose Inclusion Complex/Polyvinyl Alcohol/CWO (AIC/PVOH/CWO).

et al., 2011 also reported that cedrol was responsible for resistance to several wood-decay fungi. Cedrol, a sesquiterpene alcohol, it is the most abundant component of CWO followed by thujopsene, a sesquiterpene hydrocarbon (Eller and King, 2000). Watanabe et al., 2005a reported a different sesquiterpene alcohol was termiticidal and Watanabe et al., 2005b reported a sesquiterpene hydrocarbon was termiticidal. It is likely other sesquiterpenes will be found to inhibit wood-decay fungi or be termiticidal.

Readily available free water, above the fiber saturation point, is necessary for the significant wood decay to occur from fungal attack (Scheffer, 1966). Reducing the free water within the wood blocks should reduce the rate and extent of fungal degradation. This would suggest that the HexAM/PVOH samples may have higher value in retarding fungal damage. Improved water resistance was also observed in the decrease in swelling or changes in wood volume as determined by changes in the radial, longitudinal and tangential dimensions for the AIC/PVOH treatment (Fig. 7). The dimensional stability of wood is important in reducing checking and separation of cellulose fibers, as the integrity of the wood fails the susceptibility to fungal decay increases (Rowell and Banks, 1985).

Treatment of the wood blocks with the amylose inclusion complex significantly improved water resistance of the wood as observed by the increase in water contact angle, decreased water mass gain and decreased swelling (Figs. 5–8). Treatment of cellulosic materials with amylose-fatty ammonium salt inclusion complexes has been previously demonstrated to improve the water contact angle and decrease water uptake due to ionic binding of the complexes to the cellulose fibers (Fanta et al., 2017). This is similar to altered sorption characteristics of other wood modification techniques (acetylation, thermal treatment), which have been found to change the dimensional stability of wood following treatment.

Treatment with EtOH/CWO, which contains hydrophobic CWO, did not significantly reduce the water mass gain compared to control but did reduce the overall swelling and improve the dimensional stability of the wood, which is consistent with wood treatments utilizing oil emulsions (Evans et al., 2009; Hyvönen et al.,2006). The AIC/PVOH/CWO emulsion may have had reduced water resistance due to partially inhibiting the amylose complexes from forming an ionic bond with the cellulose fibers (Fanta et al., 2017).

The most significant increase in dimensional stability was observed in the treatments containing the amylose inclusion complex (i.e., cationic amylose-complex). The treatment with only the amylose inclusion complex (i.e., AIC/PVOH) had the most significant increase in dimensional stability and water resistance, with approximately 75% less swelling than that observed in the ethanol treated sample (Fig. 7). The second best treatment in terms of dimensional stability was the AIC/PVOH/CWO emulsion, which significantly outperformed the control treatments, but was not as good as the AIC/PVOH alone.

While treatment of the wood blocks with the amylose inclusion complex improved the water resistance of the cellulose, it may also have provided a bulking treatment to reduce the available volume for water to occupy. Films formed from amylose-fatty ammonium inclusion complexes and PVOH exhibited a high degree of surface hydrophobicity (Fanta et al., 2016a). This demonstrates that treatment of the wood with the amylose inclusion complex does not simply slow the absorption of water, but significantly increases water resistance and reduces free water in the wood (Fig. 8).

It has been suggested that waste material may be a source of extracts that could be used against wood-decay fungi (Brocco et al., 2017) or against termites (Mishra et al., 2017). Sawdust from sawmills processing ERC could be used in this way. In addition, the vast quantity of junipers in the United States is an underutilized resource that could provide natural compounds to control wood-decay fungi and termites.

5. Conclusions

The amylose inclusion complex gave a stable and uniform dispersion of CWO in water and this mixture was suitable for preparing pressure treated wood samples. The AIC/CWO dispersions conferred resistance against both termites and wood-decay fungi. The amylose inclusion complex decreased water absorption and swelling and these effects may be partly responsible for its activity against termites and wood-decay fungi. The amylose inclusion complex and CWO have different mechanisms of activity but function together to provide resistance against termites and wood-decay fungi. The amylose inclusion complex holds promise as an emulsifier for CWO as an aqueous carrier to make a nonflammable, inexpensive and safer alternative to ethanol for CWO treatment of wood using an underutilized, natural renewable resource.

Conflicts of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication.

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References

Abdul Khalil, H.P.S., Kong, N.H., Ahmad, M.N., Bhat, A.H., Jawaid, M., Jumat, S., 2009. Selective solvent extraction of the bark of *Rhizophora apiculata* as an anti-termite agent against *Coptotermes gestroi*. J. Wood Chem. Technol. 29, 286–304.
Adams, R.P., 1987. Investigation of *Juniperus* species of the United States for new sources of cedarwood oil. Econ. Bot. 41, 48–54.
Adams, R.P., McDaniel, C.A., Carter, F.L., 1988. Termiticidal activities in the heartwood, bark/sapwood and leaves of *Juniperus* species from the United States. Biochem. Systemat. Ecol. 16, 453–456.
Alavijeh, E.S., Habibpour, B., Moharramipour, S., Rasekh, A., 2014. Bioactivity of *Eucalyptus camaldulensis* essential oil against *Microcerotermes diversus* (isoptera: termitidae). J. Crop Prod. 3, 1–11.
American Wood Protection Association, 2012. Standard Method of Testing Wood

Preservatives by Laboratory Soil Block Cultures. E10–12. Annual Book of AWPA Standards, Birmingham, AL, USA, pp. 327–335.
American Wood Protection Association, 2016. Laboratory Method for Evaluating the Termite Resistance of Wood-Based Materials: Choice and No-choice Tests. E1-16. AWPA Standards, Birmingham, AL, USA, pp. 379–384.
Brocco, V.F., Paes, J.B., daCosta, L.G., Brazolin, S., Arantes, M.D.C., 2017. Potential of teak heartwood extracts as a natural wood preservative. J. Clean. Prod. 142, 2093–2099.
Cheng, S.-S., Chang, H.-T., Wu, C.-L., Chang, S.-T., 2007. Anti-termitic activities of essential oils from coniferous trees against Coptotermes formosanus. Bioresour. Technol. 98, 456–459.
Eller, F.J., Clausen, C.A., Green, F., Taylor, S.L., 2010. Critical fluid extraction of *Juniperus virginiana* L. and bioactivity of extracts against subterranean termites and wood-rot fungi. Ind. Crop. Prod. 32, 481–485.
Eller, F.J., Vander Meer, R.K., Behle, R.W., Flor-Weiler, L.B., Palmquist, D.E., 2014. Bioactivity of cedarwood oil and cedrol against arthropod pests. Environ. Entomol. 43, 762–766.
Eller, F.J., King, J.W., 2000. Supercritical carbon dioxide extraction of cedarwood oil: a study of extraction parameters and oil characteristics. Phytochem. Anal. 11, 226–231.
Eller, F.J., Taylor, S.L., 2004. Pressurized fluids for extraction of cedarwood oil from Juniperus virginiana. J. Agric. Food Chem. 52, 2335–2338.
Evans, P.D., Wingate-Hill, R., Cunningham, R.B., 2009. Wax and oil emulsion additives: how effective are they at improving the performance of preservative-treated wood? For. Prod. J. 59, 66–70.
Fanta, G.F., Felker, F.C., Hay, W.T., Selling, G.W., 2017. Increased water resistance of paper treated with amylose-fatty ammonium salt inclusion complexes. Ind. Crop. Prod. 105, 231–237.
Fanta, G.F., Felker, F.C., Selling, G.W., 2016a. Films prepared from poly(vinyl alcohol) and amylose-fatty acid salt inclusion complexes with increased surface hydrophobicity and high elongation. Starch Staerke 68, 874–884.
Fanta, G.F., Felker, F.C., Selling, G.W., Hay, W.T., Biswas, A., 2016b. Poly (vinyl alcohol) composite films with high percent elongation prepared from amylose-fatty ammonium salt inclusion complexes. J. Appl. Polym. Sci. 133.
Fanta, G.F., Kenar, J.A., Felker, F.C., 2013. Preparation and properties of amylose complexes prepared from hexadecylamine and its hydrochloride salt. Carbohydr. Polym. 98, 555–561.
Hassan, B., Mankowski, M.E., Kirker, G., Ahmed, S., 2017. Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species. Int. Biodeterior. Biodegrad. 123, 27–36.
Hay, W.T., Behle, R.W., Fanta, G.F., Felker, F.C., Peterson, S.C., Selling, G.W., 2017a. Effect of spray drying on the properties of amylose-hexadecylammonium chloride inclusion complexes. Carbohydr. Polym. 157, 1050–1056.
Hay, W.T., Byars, J.A., Fanta, G.F., Selling, G.W., 2017b. Rheological characterization of solutions and thin films made from amylose-hexadecylammonium chloride inclusion complexes and polyvinyl alcohol. Carbohydr. Polym. 161, 140–148.
Hyvönen, A., Piltonen, P., Niinimäki, J., 2006. Tall oil/water–emulsions as water repellents for Scots pine sapwood. European J. Wood Wood Prod. 64, 68–73.
Kadir, R., Ali, N.M., Soit, Z., Khamaruddin, Z., 2014. Anti-termitic potential of heartwood and bark extract and chemical compounds isolated from *Madhuca utilis* Ridl. H. J. Lam and *Neobalanocarpus heimii* King P. S. Ashton. Holzforschung 68, 713–720.
Kard, B., Hiziroglu, S., Payton, M.E., 2007. Resistance of eastern redcedar panels to damage by subterranean termites (Isoptera: Rhinotermitidae). For. Prod. J. 57, 74–79.
Kartal, S.N., Shinoda, K., Imamura, Y., 2005. Laboratory evaluation of boron-containing quaternary ammonia compound, didecyl dimethyl ammonium tetrafluoroborate (DBF) for inhibition of mold and stain fungi. Holz als Roh-und Werkstoff 63, 73–77.
Klem, R.E., Brogly, D.A., 1981. Method for selecting the optimum starch binder preparation system. Pulp Pap. 55, 98–103.
Konemann, C.E., Kard, B.M., Payton, M.E., 2014. Palatability of field-collected eastern redcedar, *Juniperus virginiana* L., components to subterranean termites (isoptera: Rhinotermitidae). J. Kans. Entomol. Soc. 87, 269–279.
Mishra, T., Pal, M., Rai, A.K.D., Tewari, S.K., 2017. Termiticidal Activity of Punica granatum fruit rind fractions and its compounds against Microcerotermes beesoni. Ind. Crop. Prod. 107, 320–325.
Mun, S.P., Prewitt, L., 2011. Antifungal activity of organic extracts from Juniperus virginiana heartwood against wood decay fungi. For. Prod. J. 61, 443–449.
Rowell, R.M., Banks, W.B., 1985. Water Repellency and Dimensional Stability of Wood. USDA General Technical Report, FPL-50. pp. 1–24.
Santana, A.L.B.D., Maranhão, C.A., Santos, J.C., Cunha, F.M., Conceição, G.M., Bieber, L.W., Nascimento, M.S., 2010. Antitermitic activity of extractives from three Brazilian hardwoods against *Nasutitermes corniger*. Inter. Biodeter. Biodegrad. 64, 7–12.
Scheffer, T.C., 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4, 147–168.
Schmidt, T.L., Leatherberry, E.C., 1995. The expansion of eastern red cedar in the lower Midwest. NJAF (North. J. Appl. For.) 12, 180–183.
Tumen, I., Eller, F.J., Clausen, C.A., Teel, J.A., 2013. Antifungal activity of heartwood extracts from three *Juniperus* species. Bioresources 8, 12–20.
Wahyudi, W., Ohtani, Y., Ichiura, H., 2012. Significant feeding deterrent of berberine from tali kuning (*Tinospora dissitiflora* diels) against two subterranean termites *Coptotermes formosanus* shiraki and *Reticulitermes speratus* kolbe. Wood Res. J. 3, 18–22.
Wang, J., Li, J., Li, S., Freitag, C., Morrell, J.J., 2011. Activities of *Cunninghamia lanceolata* heartwood extractives. Bioresearch (Ujjain) 6, 606–614.
Watanabe, Y., Mihara, R., Mitsunaga, T.T., Yoshimura, T., 2005a. Termite repellent sesquiterpenoids from *Callitris glaucophylla* heartwood. J. Wood Sci. 51, 514–519.
Watanabe, Y., Mitsunaga, T., Yoshimura, T., 2005b. Investigating Antitermitic Compounds from Australian White Cypress Heartwood (*Callitris glaucophylla*Thompson et Johnson) Against *Coptotermes formosanus* Shiraki. J. Essent. Oil Res. 17, 346–350.