

# Laboratory Evaluations of Durability of Southern Pine Pressure Treated With Extractives From Durable Wood Species

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## Abstract

Extracts from sawdust of four naturally durable wood species [Alaskan yellow cedar, AYC, *Cupressus nootkanensis* D. Don 1824; eastern red cedar, ERC, *Juniperus virginiana* L.; honey mesquite, HM, *Prosopis glandulosa* Torr.; and black locust, BL, *Robinia pseudoacacia* L.] were used to treat southern pine, *Pt*, *Pinus taeda* L. sapwood blocks. Extractive treated blocks were evaluated for decay resistance in standard soil bottle fungal assays challenged with brown and white rot decay fungi. Results showed that extractives did impart some improvement to decay resistance of *Pt* blocks. BL- and HM-treated *Pt* blocks were also used in choice and no-choice assays to determine feeding preference and damage by eastern subterranean termites (*Reticulitermes flavipes*) Kollar. Minimal feeding on treated blocks was seen in both choice and no-choice assays. In choice assays, there was similar mortality between HM and BL arenas; however, in no-choice assays, complete mortality was recorded for HM-treated *Pt* and high mortality was seen with BL-treated *Pt*. Subsequent dose mortality termite assays showed HM to be effective in killing *R. flavipes* at low concentrations. Both HM and BL show promise as deterrents or termiticidal protectants and will be further evaluated in field studies.

**Key words:** wood, durability, *Reticulitermes flavipes*, feeding, extractives

Naturally durable wood species are those that exhibit some degree of resistance to biodeteriorative agents, such as wood decay fungi and termites. Some durable wood species such as redwood (*Sequoia sempervirens* (D. Don) Endl.) and western red cedar (*Thuja plicata* Don ex D. Don) are commonly considered naturally durable woods because they have documented in-use performance as suitable alternatives to chemically treated wood (Scheffer and Morrell 1998). They are also readily available in sizes appropriate for dimensional lumber.

In past studies (Kirker et al. 2012, 2013), decay resistance was found for several species of wood; four of these were selected for this study. Alaskan yellow cedar (AYC, *Cupressus nootkanensis* D. Don 1824) is a softwood species that grows in the far western United States. It is considered decay-resistant (Scheffer and Morrell 1998) but is currently only used for interior woodwork, furniture, cabinets, and small boats (Wiemann 2010). In other studies, AYC wood has been found to be resistant to decay (De Groot et al. 2000, Du et al. 2011) as well as subterranean termites (Morales-Ramos and Rojas 2001, Arango et al. 2006). AYC has been of particular interest since dieback along the Alaskan coast has left thousands of

dead standing trees, which poses a serious threat for both forest fire and insect outbreaks (Hennon et al. 2007). Black locust (BL, *Robinia pseudoacacia* L.) is a very heavy, hard, and strong hardwood species with high decay resistance (Sheffer and Morrell 1998) that is grown along the Appalachian Mountains. It is currently used for mine timbers, fence posts, poles, crossies and stakes (Wiemann 2010). Extractive content of BL varies greatly within trees of different ages (Chow et al. 1996) and within the trunk (Magel et al. 1994). Durability of old growth BL has been documented for decades (Hirt 1938, Hart 1989, Taylor et al. 2002), but old growth ecosystems are sensitive to major disturbances, such as logging. Extractives of BL on cellulose pads were studied by Stellar and Labovsky (1984) to determine effects on termite resistance and mortality; they found the extractives to be moderately toxic to *Reticulitermes flavipes* (Kollar). Eastern red cedar (ERC, *Juniperus virginiana* L.) is a softwood species that is considered resistant to decay (Scheffer and Morrell 1998). It is grown throughout the Eastern United States and often used for fence posts, chests, and closet linings as well as flooring (Wiemann 2010). Heartwood of ERC has

been found to be resistant to both termites and decay fungi [Kard et al. 2007 (termites only), Eller et al. 2010]. Even included sapwood has been found to be resistant to decay and termites (Köse and Taylor 2012). However, variable extractive content can be a factor in the inherent durability of ERC (Morris et al. 2011a, b). Honey mesquite (HM, *Prosopis glandulosa* Torr.), is a hardwood species that is largely considered invasive in the plains of Texas (Brown and Archer 1989). HM has a long history of use in rangeland areas and is considered durable for use as fence posts and heavy timbers (Forbes 1895). HM overpopulation encroaches on grasslands and has been shown to have ecosystem-level impacts on above- and below-ground processes (Liaou and Boutton 2008). HM is listed as a moderately flammable wood species, and its prevalence has been shown to influence fire successional characteristics in the arid plains landscape (Streeks et al. 2005). Extracts of HM have been found to have antifungal activity based on disk bioassays against select wood decay fungi and field studies have found HM to be durable for above-ground exposure (Kirker et al. 2013). Extractives of HM have been found to contain alkylated piperidine alkaloids (Samoylenko et al. 2008) and indolizidines that exhibit antiparasitic and antimicrobial properties (Rahman et al. 2011). The main limiting factor regarding effective utilization of HM is the size of the tree.

Extractive content is an important consideration when evaluating naturally durable wood species and quite often imparts much of the durability to a given species. When extractives are removed with a stringent method for preparing extractive free wood (ASTM D1105-96), the majority of these durable wood species lose most or all of their durability in soil bottle and termite assays (Kirker et al. 2012). Extractive content depends on a wide range of factors and can vary greatly from tree to tree and even within the tree (Taylor et al. 2002). This variation is a hurdle in the standardization of naturally durable wood species (Morris et al. 2011b). One approach to overcome this hurdle is to select wood species that exhibit resistance to biodeterioration and then transfer the nonstructural constituents (extractives) to a nondurable, more millable wood species. This eliminates the necessity for naturally durable wood to be of millable size and structure, as well as uniform extractive content. The objective of this study was to evaluate the use of extractives from naturally durable wood species for treatment of a nondurable wood species to improve biological resistance to selected wood decay fungi and eastern subterranean termites (*R. flavipes*).

## Materials and Methods

### Nondurable Sample Material

Southern pine (*Pt*, *Pinus taeda* L.) was selected for the study owing to its common use, availability, and size. *Pt* is widely used in interior and exterior grade lumber and represents the greatest proportion of the North American lumber market. The name “southern pine” actually represents a species group, but *P. taeda* is the species most commonly sold in the Midwestern United States and was used throughout this study. It is considered nondurable (Scheffer and Morrell 1998) but exhibits excellent material properties (workability, nail holding, dimensional stability, etc.). Currently, the southern pine group accounts for the majority of residential lumber sales in the United States and represents 85% of all preservative-treated wood sold in the United States (Wiemann 2010).

### Durable Species Material and Extractive Removal

Naturally durable wood species (AYC, BL, ERC, and HM) were obtained from various suppliers in North America. Sawdust was

prepared from each species by milling wood blocks to pass a 40 mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Extractives were removed from sawdust of each individual durable species using a modified protocol from ASTM D1105-96 “Standard Test Method for Preparation of Extractive-Free Wood” (ASTM 2014). Twelve grams of sawdust was Soxhlet-extracted in 900 ml of a 2:1 mixture of ethanol:toluene for 6 h, followed by a second 6-h extraction with another 12 g of sawdust, to obtain a concentrated treatment solution. A 100-ml aliquot was rotary evaporated and weighed to determine approximate extractive content for each wood type. Extractive content for fungal assay treatment solutions were approximately 9, 8, 8, and 11 mg/ml for AYC, BL, ERC, and HM, respectively, and for termite assays were approximately 12 mg/ml for BL and 7 mg/ml for HM.

### Treatment Process

Experimental *Pt* blocks (20-mm cubes for fungal assays and 25 by 25 by 6-mm ( $t \times r \times l$ ) blocks for termite assays) were conditioned to constant weight at 27°C and 30% RH and weighed. Blocks were then pressure-treated using a vacuum desiccator as described in the AWP A E-10-12 standard (AWPA 2014) with the solvent extractions (AYC, BL, ERC, and HM, respectively) along with solvent-only (2:1 ethanol: toluene mixture) controls for 30 min at 29 PSI followed by 30-min soak. Blocks were air-dried overnight, conditioned to constant weight at 27°C and 30% RH, and weighed to obtain approximate retention of extractive solutions. Treatment retentions for the fungal assays were approximately 9, 11, 8, and 14 mg/g of wood for AYC, BL, ERC, and HM, respectively, and for termite assays were approximately 10 mg/g of wood for BL and 12 mg/g of wood for HM.

### Fungal Soil Bottle Bioassays

Soil fungal bioassays were conducted according to AWP A E10-12 (AWPA 2014). Assays were conducted with two brown rot fungi (*Postia placenta* (Fr.) M.J. Larson and Lombard (Mad-698-R) and *Gloeophyllum trabeum* (Pers.) Murrill (Mad-617-R)) and two white rot fungi (*Trametes versicolor* (L. Lloyd) (Mad-697) and *Irpex lacteus* Fr. (Fr.) (HHB-7328-Sp)). De-ionized water and soil were added to glass jars, followed by a 40 by 30 by 3-mm ( $t \times r \times l$ ) feeder strip of *Pt* (red maple, rm, *Acer rubrum* L., was used for white rot fungal feeder strips) and autoclaved 45 min at 121°C to sterilize. Feeder strips were inoculated with two 5-mm plugs of fungal mycelium from the leading edge of 2-week-old cultures grown on malt extract agar plus 0.1% yeast extract (MEA), and then grown until the feeder strip was covered with mycelium, approximately 3 weeks. Two 20-mm cubes were placed into each bottle and replicated with four bottles for each treatment and fungal isolate, except AYC, which had three bottles for each. Bottles were placed in a 27°C and 70% RH growth chamber for 12 wk, then blocks were removed and mycelia lightly brushed off. Blocks were then oven-dried overnight to kill the remaining fungal tissue and placed in a 27°C and 30% RH conditioning chamber until constant weight was achieved and weights were then recorded. Percent weight loss of blocks were calculated and reported. A couple of *P. placenta* (Mad-698-R) replicates unexpectedly experienced no growth and were excluded.

### Termite Bioassays

Termites (*R. flavipes*) were obtained from cardboard baits in Janesville, WI, USA, on 26-VI-2014 (used in choice assays) and 14-VIII-2014 (used in no-choice assays), stored in a 27°C and 90% RH chamber and added to assays within one week. Termite choice and

no-choice assays were conducted according to AWP A E1-13 (AWPA 2014). HM and BL were selected based on performance in preliminary experiments (unpublished). Termites were added to the bioassay arenas as indicated by the standard 1 d prior to the addition of test blocks. For the choice assay, one block each of treated, untreated, and solvent control was included in an arena with 20 replicate arenas. For no-choice assays, only one block was included per termite arena with 10 replicate arenas. One hundred fifty grams of sand with 30 ml sterile water was used for larger 110 by 110 by 35-mm-square choice arenas, whereas 50 g sand with 9 ml water was used for smaller 54-mm-round  $\times$  36-mm-high no-choice arenas. Additionally, 1.4 g of termites ( $\sim$ 500) was added to choice arenas and 1 g of termites ( $\sim$ 300) for no-choice arenas. Both experiments were placed in a 27°C and 90% relative humidity chamber for 4 weeks when blocks were removed and visually rated according to the standard. The termites were separated from sand as follows: the contents of each arena were individually poured out into a pan, and then flooded with water while mixing. The live termites floated to the top and were poured out into another pan. The flooding and pouring was repeated twice and then the water with the termites was poured through a screen that captured the termites and allowed the water and sand to pass through. The screen was blotted with paper towel from the bottom to remove as much water as possible; the termites were then tapped out into weighing boats. Termites were allowed to dry for approximately 30 min and then weighed. Percent mortality was calculated from the original termite weight. Blocks were scraped free of debris, visually rated for soundness according to the standard, and then placed in a 27°C and 30% RH chamber for one week before weight was recorded.

#### Dosage-Mortality Bioassays

Owing to high mortality of termites in the earlier HM choice and no-choice assays, a dosage mortality assay was conducted to determine the median effective dose and 90% effective dose of the extractives to the termites. Five serial 1:2 dilutions were made of treatment solutions with solvent 2:1 ethanol: toluene; these dilutions were used to treat test blocks. No-choice arenas were set up as previously described with 1 g termites in 10 replicate arenas. Termite separation and analyses were conducted as previously described.

#### Statistical Methods for Fungal Bioassays

The fungal bioassays were carried out at separate times for each naturally durable species treatment. That is, a full two-factor experiment, with factors of wood block type (untreated control, solvent-treated control, and extract-treated) and fungal exposure type (Mad-698-R, Mad-617-R, Mad-697, and HHB-7328-Sp), based on a completely randomized design was deployed for each naturally durable species. The experimental unit was a bottle, which contained two blocks of the same type of wood. For each experiment, models of percent weight loss owing to fungal decay were fit to a two-way analysis of variance in SAS v9.4<sup>®</sup> (PROC GLIMMIX, SAS Institute 2012) and main effect or simple hypotheses were investigated, using Tukey adjustments for multiple mean comparisons.

#### Statistical Methods for Termite Bioassays

*Termite Choice Bioassays.* The termite choice experiment was analyzed as an incomplete nested experiment, as an arena was designated as a BL arena or as an HM arena—each arena included the designated treated (HM – 7 mg/ml or BL – 12 mg/ml) southern pine block, an untreated block, and a solvent-treated control block. There were 20 replicate BL arenas and 20 replicate HM arenas each

exposed to termites (1.4 g per arena). Beginning block weight, final block weight, percent weight loss, and E1 rating were recorded for each block. A final live termite weight per arena was also recorded. Percent weight losses for the test blocks were compared between each BL- or HM-treated group and the associated untreated and solvent controls; comparisons between controls in the two types of arenas were also made to understand possible differences in choice feeding behavior. To accommodate the correlations within each of the two arena types and the heterogeneous variance that occurred across treatment groups, the statistical model included separate unstructured covariance matrices for the two arena types. The model was fit in SAS v9.4<sup>®</sup> (PROC GLIMMIX, SAS Institute 2012) and particular hypotheses were tested via contrast statements.

*Termite No-Choice Bioassays.* The no-choice termite assay included the same four types of blocks as the choice assay: BL (12 mg/ml)-treated *Pt*, HM (7 mg/ml)-treated *Pt*, untreated control, and solvent-treated control. For each type of block, there were 10 individual arenas with a single block ( $n=40$ ) each exposed to termites (1.0 g per arena). Beginning block weight, final block weight, percent weight loss, E1 rating, and final live termite weight were recorded for each block. Percent block weight losses were compared based on a one-way analysis of variance with four treatment groups, but assuming unequal variances for each of the four groups (Levene's test for homogeneity,  $F_{3,36}=46.52$ ,  $P<0.0001$ ). The model was fit in SAS v9.4<sup>®</sup> (PROC GLIMMIX, SAS Institute 2012) with separate residual variances for each block type, and particular hypotheses were tested via contrast statements.

*Dosage-Mortality Bioassays.* Owing to the high termite mortality using HM-treated blocks, a second no-choice experiment was conducted to determine the nature of the dose–response relationship between HM concentration and percent weight loss and termite mortality. With this experiment, sets of replicate *Pt* blocks were treated at HM concentrations of 0.4375, 0.875, 1.75, and 3.5 mg/ml or solvent controls ( $n=10$ , except for 0.4375 mg/ml, which had  $n=11$ ). An additional set of 10 untreated control blocks was also included. Each block was individually exposed to 1 g of termites per arena as previously described. An initial mixed-effect model of percent weight loss combined the controls, solvents, and HM (7 mg/ml) blocks from the first no-choice experiment with the controls, solvents, and HM (0.4375, 0.875, 1.75, and 3.5 mg/ml) blocks from the dose experiment and included a random effect to capture the additional variation that could be contributed to conducting the experiments at different times (covtest marginal  $P=0.0940$ ). As with the initial no-choice experiment, the residual variance within each of the groups was heterogeneous and separate variances were fit for each treatment group. Mean comparisons are given both with unadjusted and adjusted  $P$ -values (based on the simulation method). Additional models were fit to determine dosages that can effectively achieve certain levels of termite mortality.

## Results

#### Fungal Bioassays

AYC showed some improvement in durability compared with untreated and solvent controls (wood type  $F_{2,23}=3.79$ ,  $P=0.0377$ , Fig. 1). Differences were detected between the weight losses owing to brown rot and those owing to white rot (fungus type  $F_{3,23}=33.69$ ,  $P<0.0001$ ), while significant interactions between wood type and fungus were not detected ( $F_{6,23}=0.12$ ,  $P=0.9927$ ).

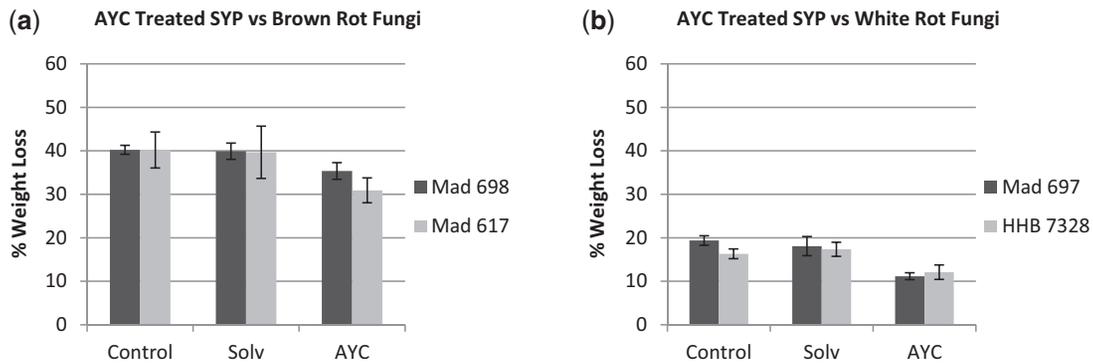


Fig. 1. Percent weight loss of southern pine blocks treated with Alaskan yellow cedar, AYC, extracts exposed to both: (a) brown and (b) white rot fungi in an AWPA E-10 soil block test showing some improvement to durability compared with untreated and solvent controls.

Mean weight loss was similar for both brown rot strains (Mad-698-R and Mad-617-R) in the untreated control and solvent controls (~40%), and similar for the white rot strains (Mad-697 and HHB-7328-SP.), but lower than the brown rot strains, between 16 and 20%. Weight loss was 5% lower (vs Mad-698-R) and 9% lower (vs Mad-617-R) than controls for *Pt* when treated with AYC extracts. When challenged with two strains of white rot fungi (Mad-697 and HHB-7328-SP.), AYC-treated *Pt* weight losses were 8% (vs 697) and 4% (vs HHB-7328-SP.) lower than controls. When combined, the AYC-treated *Pt* weight losses averaged 6.7% lower than the controls (adjusted  $P = 0.0551$ ). Weight losses were typically lower for white rot fungi because softwoods are not their typical host species.

BL showed some improvement in durability compared with untreated (adjusted  $P = 0.0323$ , Fig. 2), although there was no statistical difference from the solvent control (adjusted  $P = 0.8738$ ). Mean percent weight loss was approximately 40% for both brown rot strains (Mad-698-R and Mad-617-R) in the untreated controls and solvent controls, and there were minor differences in percent weight loss for *Pt* treated with BL extracts. When challenged with two strains of white rot fungi (Mad-697 and HHB-7328-SP.), untreated and solvent controls showed approximately 20% weight loss, with BL treatment showing no difference with Mad-697, but a 5.5% decrease in percent weight loss with HHB-7328-SP (however, this was not statistically significant).

ERC also showed some improvement in durability to one of the fungal strains compared with untreated and solvent controls (Fig. 3). Mean percent weight loss was similar for untreated and solvent control for Mad-698-R (~40%) and showed a nonsignificant 4% decrease in weight loss when treated with ERC extractives. There was more variability for Mad-617-R controls with a difference of 10% between the untreated and solvent controls (adjusted  $P = 0.0005$ ), but treatment with ERC extractives showed a 10% decrease from the untreated control (adjusted  $P = 0.0030$ ). When challenged with two strains of white rot fungi, controls were again much lower than brown rot fungi (16–22%), and ERC-treated *Pt* had weight losses of 21% (Mad-697) and 17% (HHB-7328-SP.), with Mad-697 actually producing more weight loss on ERC-treated *Pt* than the solvent control and HHB-7328-SP only 3% lower than untreated and solvent controls; in this case, other than differences between the white rot and brown rot fungi, these differences were not statistically significant.

HM showed little improvement in durability compared with untreated and solvent controls, with only differences between brown rot and white rot fungi being statistically significant (fungus type

$F_{3,32} = 41.28$ ,  $P < 0.0001$ , Fig. 4). Mean weight loss was around 40% to 50% for brown rot strains (Mad-698-R and Mad-617-R) in the untreated and solvent controls. There was a slight nonstatistical increase in weight loss exposed to Mad-698-R on HM-treated *Pt* (44%) and a slight nonstatistical decrease in weight loss exposed to Mad-617-R on HM-treated *Pt* (42%). When challenged with two strains of white rot fungi (Mad-697 and HHB-7328-SP.), again there was lower percent weight loss overall at 24 and 19%, respectively, than when exposed to the brown rot fungi. There was no decrease in weight loss owing to HM treatment exposed to HHB-7328-SP. and only a nondetectable 2% decrease in weight loss when exposed to Mad-697.

#### Termite Bioassays

**Choice Bioassays.** Mortality in BL and HM choice arenas averaged 49 and 55% based on termite weights, respectively; BL treatment caused 100% mortality in 3 of 20 arenas, while HM caused 100% mortality in 2 of 20 arenas (not shown). Average E1 rating of controls in both assays was approximately six, while BL and HM treated blocks were rated nine and eight, respectively, showing little to no feeding on either (Fig. 5). Similarly, there was an average of 12% weight loss in the untreated and solvent controls in both assays and only 2.3 and 4.9% in BL- and HM-treated blocks, respectively, and these were significantly ( $P < 0.0001$ ) different from their controls (Fig. 6).

**No-Choice Bioassays.** In the no-choice assays, the higher degree of mortality was seen in HM (100%) compared with BL (75%) (Fig. 7); however, BL-treated blocks did cause 100% mortality in 6 of 10 arenas. E1 ratings of the no-choice assays showed no feeding on the HM-treated blocks and an average rating of six for the BL-treated blocks, while most control blocks, both untreated and solvent, failed with an average zero rating (Fig. 7a). Average percent weight loss was approximately 40% for both untreated and solvent controls, while BL treated was at 14% and HM only 1% (Fig. 7b). Combined the untreated and solvent-treated control blocks lost significantly more weight than either the BL-treated ( $P < 0.0008$ ) or the HM-treated ( $P < 0.0001$ ), as tested with a single-degree-of-freedom hypothesis test.

Considering that E1 ratings are ordinal measures and that live termite weights appear to be a zero-inflated response (if all the termites die in an arena, it has a live termite weight of zero), relationships between responses were measured with a nonparametric measure of association, Spearman's rank correlation coefficient.

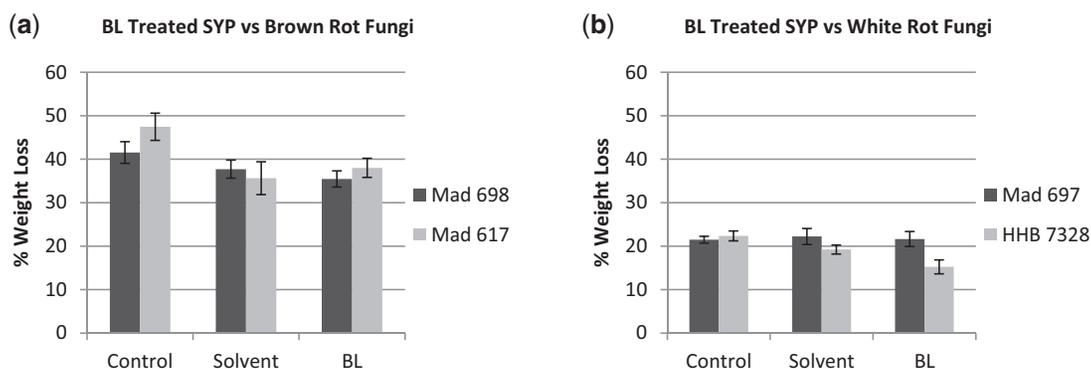


Fig. 2. Percent weight loss of southern pine blocks treated with black locust, BL, extracts exposed to both: (a) brown and (b) white rot fungi in an AWPA E-10 soil block test showing marginal improvements to durability compared with untreated and solvent controls.

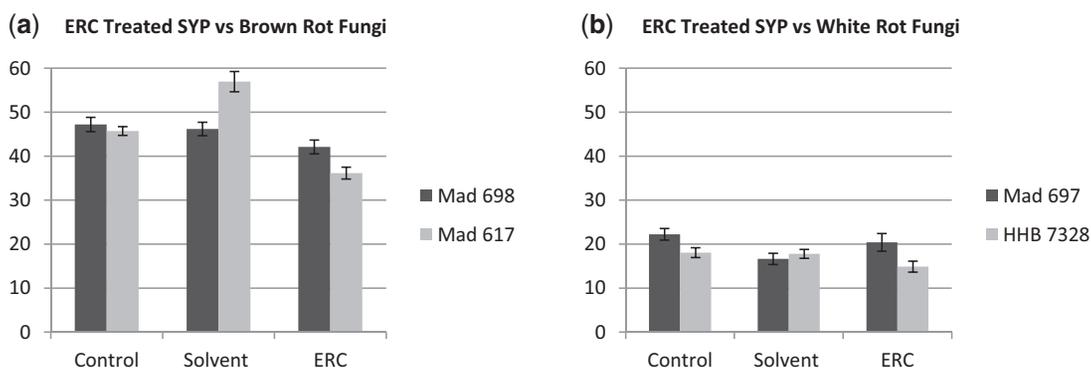


Fig. 3. Percent weight loss of southern pine blocks treated with eastern red cedar, ERC, extracts exposed to both: (a) brown and (b) white rot fungi in an AWPA E-10 soil block test showing marginal improvements to durability compared with untreated and solvent controls.

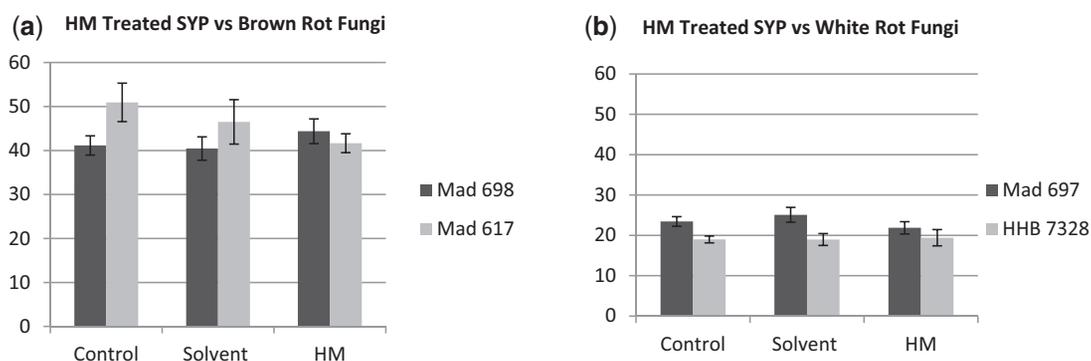


Fig. 4. Percent weight loss of southern pine blocks treated with honey mesquite, HM, extracts exposed to both: (a) brown and (b) white rot fungi in an AWPA E-10 soil block test showing minor improvements to durability compared with untreated and solvent controls.

Termite mortality was defined as the beginning weight (1 g) minus the ending live weight (g) times 100. Aggregating across treatment groups, it appears that mortality was positively associated with E1 visual ratings (Spearman's correlation coefficient = 0.84,  $P < 0.0001$ ) but negatively associated with percent weight loss (Spearman's  $r = -0.86$ ,  $P < 0.0001$ ).

**Dosage-Mortality Bioassays.** In the dosage-dependent bioassays, all but the final serial dilution were effective at reducing mass loss owing to termite feeding. E1 rating and mortality steadily improved as concentration increased and mass loss steadily decreased with increasing concentration (Fig. 8a).

An initial mixed-effect model of percent weight loss combined the controls, solvents, and HM-7 mg/ml blocks from the first no-choice experiment with the controls, solvents, HM-0.4375, 0.875, 1.75, and 3.5 mg/ml blocks from the dose experiment and included a random effect to capture the additional variation that could be contributed to conducting the experiments at different times (a test that additional variation equaled zero had a marginal  $P = 0.0940$ ). The least square means and the comparisons show the significant decline in percent weight loss associated with increased concentration of HM (Fig. 8b).

A concentration-dependent logistic model was fit to the termite mortality data following the methods of Agresti (2013), setting the

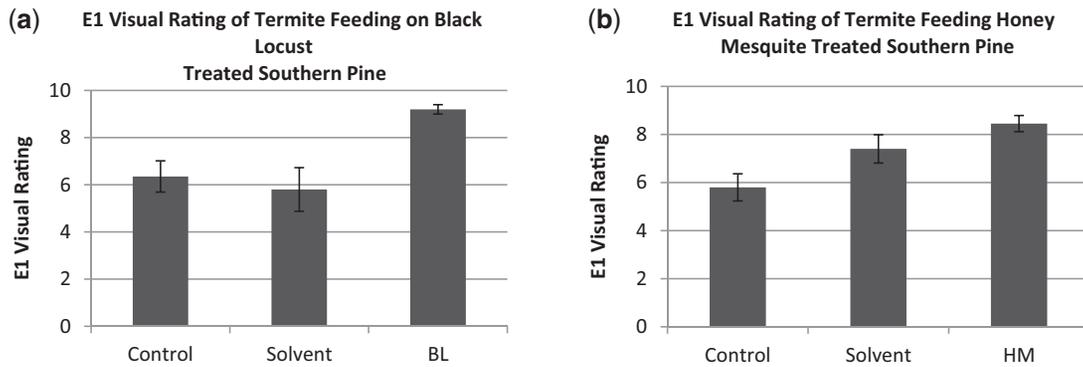


Fig. 5. Comparisons of E1 visual ratings (0 = failure-10 = sound) for termite damage of southern pine blocks treated with (a) black locust, BL, and (b) honey mesquite, HM, both compared with untreated and solvent controls. Both treatments improved ratings of treated pine after the 4-week test.

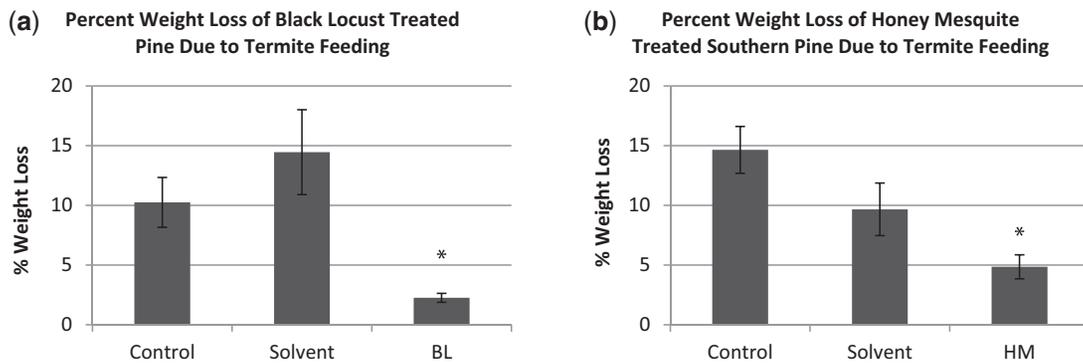


Fig. 6. Percent weight loss of (a) southern pine treated with 12 mg/ml solution of black locust, BL, extracts compared with untreated and solvent controls and (b) southern pine treated with 7 mg/ml solution of honey mesquite, HM, extracts compared with untreated and solvent controls. Weight loss was significantly ( $P < 0.0001$ ) reduced in both treatments after the 4-week test compared with controls.

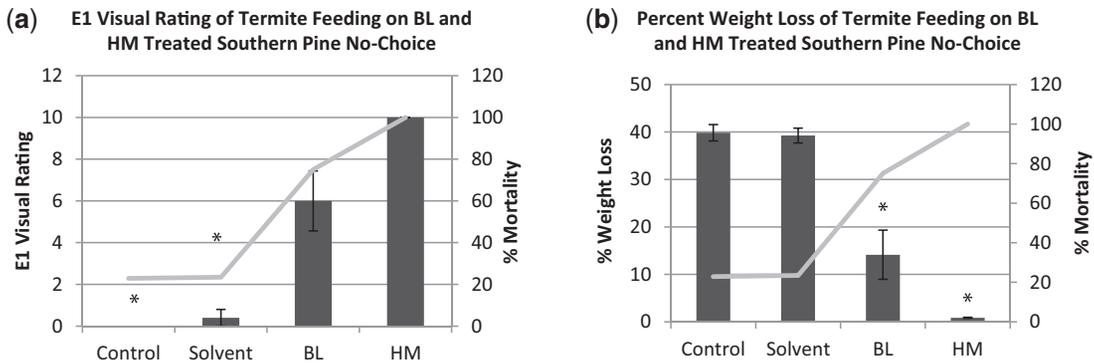
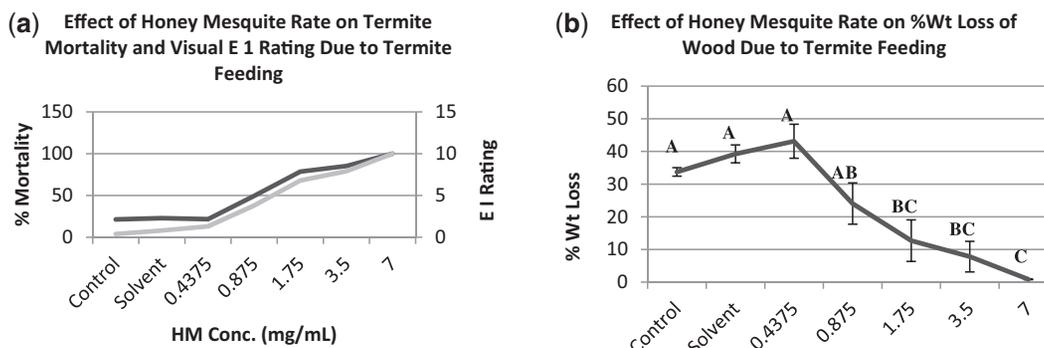


Fig. 7. Honey mesquite, HM, and black locust, BL, treatment caused significant differences in (a) E1 visual ratings and (b) percent weight loss compared with untreated and solvent controls in the no-choice bioassays. Percent mortality is plotted as a line on the secondary axis on both graphs, which was 75% for BL and 100% for HM. BL and HM weight losses were significantly different from the untreated and solvent controls at  $P < 0.0008$  and  $P < 0.0001$ , respectively. Mortality (black line) is positively associated with E1 visual ratings and negatively associated with percent weight loss.

response to zero if zero live termite weight, one if positive live termite weight, and resulting in parameter estimates of 0.58 ( $SE = 0.3872$ ) for the intercept and  $-2.2874$  ( $SE = 0.4681$ ) for the slope. This model gives the estimated treatment solution concentration that will cause 50% of the no-choice arenas to have a zero live termite weight, i.e., median effective dose, of 1.29 mg/ml with a 95% confidence interval of (0.92, 1.90) (from SAS<sup>®</sup> PROC PROBIT, SAS Institute 2012). The 90% effective dose is estimated as 3.37 mg/ml with a 95% confidence interval (2.21, 7.67).

## Discussion

Pressure treatment with extractives was only marginally effective against wood decay fungi in all of our assays. This is most likely owing to the stringency of the soil bottle assay. Designed to challenge chemicals for protectant abilities, the test is conducted in the most favorable conditions for the fungus. It is unlikely to mimic protective abilities in the field or any degree of repellency. BL and HM extractive treatment, however, showed drastic improvement in the durability of *Pt* to *R. flavipes*. Three choice arenas showed very low



**Fig. 8.** Graphs depicting: (a) percent mortality plotted over concentrations for honey mesquite, HM-treated southern pine (E I ordinal ratings listed on the secondary axis) and (b) effects of rate (concentration in mg/ml) of honey mesquite on percent weight loss of wood. Least squares means showing significance in percent weight loss are indicated by letters above plot points ( $\pm$  1SE, means with a common letter are not significantly different at a 5% level, adjusted for multiple comparisons).

feeding on BL and HM compared with untreated and solvent controls, with only 2–5% weight loss of treated blocks. Both sets of arenas showed higher average mortality around 50%, which explains the lower feeding overall with only 12% weight loss for untreated and solvent controls. Complete mortality was recorded in no-choice bioassays with HM-treated blocks and 75% mortality with BL-treated blocks. This was higher than the choice arenas presumably owing to the lack of an alternate untreated food source. All controls in the no-choice were fed upon until failure, except for one solvent control, which was rated a four (very severe). BL-treated blocks were, on average, severely fed upon, while HM-treated blocks were uneaten, probably owing to high mortality. Similarly, the weight loss data showed that controls had severe weight loss, BL-treated blocks had moderate weight loss, and HM blocks remained unchanged.

Owing to this high mortality experienced in the HM no-choice bioassay, a follow-up experiment was conducted in attempt to establish a dose curve for the HM extractives. Subsequent logistic modeling of the data yielded a median effective concentration of 1.27 mg/ml and a 90% effective dose of 3.37 mg/ml. In the native range of HM, the desert termite *Gnathotermes tubiformans* (Isoptera:Termitidae) is reported to avoid HM woody debris (Johnson and Whitford 1975) based on field survey observations. The results of these laboratory assays show that transferring durability from a durable host to a nondurable surrogate does, in fact, improve termite resistance in the case of HM and BL, but higher concentrations may be required to control wood decaying fungi. Two potential downsides to the use of HM as a wood protectant are that it is listed as a severe human allergen (Killian and McMichael 2004), but respiratory and dermal reactions are not uncommon with more durable wood species—the sap of Osage orange (*Maclura pomifera* (Raf.) Schneid) being an excellent example (Nelson et al. 2007). Second, tannin/polyphenol rich extractives are often found to increase corrosion of fasteners (Zelinka et al. 2011). The next step in our approach is to pressure-treat full-sized specimens and install them in test sites in both moderate and high hazard decay zones in North America.

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