

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1

Biology

Transferable Durability: Enhancing decay resistance of non-durable species with extractives from durable wood species

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Paper prepared for the 44th Annual Meeting
Stockholm, Sweden
June 16-20, 2013

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ABSTRACT

Extractive content and composition is a vital component of naturally durable woods; however, variability in extractives can limit their usefulness in the field. Two extractive-free, non-durable wood species were pressure treated with ethanol-toluene extractives from 8 durable wood species. Extracted Southern pine, Paulownia and unextracted Southern pine blocks were treated and challenged in soil bottle experiments with four common wood decay fungi. Weight loss from fungal degradation of the extractive-treated blocks was compared to untreated controls. Results indicated that in some cases, treatment with extracts from durable wood species decreased the percent weight loss from exposure to decay fungi. Chemical analysis of extractives from these durable and non-durable woods was performed using GC-MS and chemical components were compared. Several unique compounds were found in the more durable species compared to less durable species.

Keywords: natural durable wood, extractives, wood decay fungi, extractive transfer

1. INTRODUCTION

Naturally durable wood species are marketed as an alternative to treated wood, but the extractives present in these woods that impart durability vary greatly making standardization of durable species quite difficult (Morris et al. 2010). Extractives are simply the non-structural components of the wood and can be comprised of terpenoids, alkaloids, stilbenes, flavonoids, and other chemical groups. Past research has found certain extractive chemicals do have antifungal and insecticidal properties that may warrant future research (Taylor et al. 2002, Chedgy et al. 2009, Morris et al. 2010, Morrell et al. 1999, Stirling et al. 2010).

In a previous study, blocks from 8 naturally durable wood species were extracted following ASTM D1105-96, and then exposed to 6 common wood rot fungi along with unextracted blocks from the same wood species. The extracted wood blocks were significantly more susceptible to decay fungi than the unextracted blocks, and many were as susceptible as the southern yellow pine (SYP), non-durable controls (Kirker et al. in press). Some of the extracts from these naturally durable wood species were found to inhibit growth of wood rot fungi in petri plate assays (unpublished data).

Since the removal of extractives decreased durability in different wood species, the next step was to remove these extractives from durable wood species and use them to enhance the durability of wood species that are otherwise moderate to non-durable. By pooling extractives from a durable specimen, we hypothesized that the durability could be transferred and distributed more evenly into a non-durable species, such as southern pine or paulownia. This process has been previously

explored using different fractions of black locust and osage orange (Smith et al. 1989), methanol extracts of black locust, osage orange, redwood, and *Intsia bijuga* (Kamden 1994), and methanol extracts of various tropical species (Onuorah 2002) and many others (Van Acker et al. 1999, Turner and Conradie 1995, Kennedy and Powell 2000, Kennedy et al. 1995, Kennedy et al. 2000, and Powell et al. 2000). For an extraction technique, we chose the ASTM D1105-96 method because it offered relatively quick sample processing and stringent extractive removal. In the pulp and paper industry, this standard is routinely used for pre-processing of fiber stocks to remove extractives, which often hinder the pulping process or discolour the final product (Hillis 1962).

This study is part of the Research, Technology and Education portion of the **National Historic Covered Bridge Preservation** (NHCBP) Program administered by the Federal Highway Administration. The NHCBP program includes preservation, rehabilitation and restoration of covered bridges that are listed or are eligible for listing on the National Register of Historic Places; research for better means of restoring, and protecting these bridges; development of educational aids; and technology transfer to disseminate information on covered bridges in order to preserve the Nation's cultural heritage.

2. EXPERIMENTAL METHODS

2.1 Durable Wood Species

Durable wood species were obtained from various sources in North America for a concurrent study that is evaluating above and below-ground performance of naturally durable wood species in field test sites in WI and MS. The stock samples had been in conditioned storage at 26°C and 30% relative humidity (RH) for 2 years. The study contained five coniferous species (Alaskan yellow cedar *Chamaecyparis nootkanensis* [AYC], Eastern red cedar *Juniperus virginiana* [ERC], West coast juniper *Juniperus occidentalis* [WCJ], and Western red cedar *Thuja plicata* [WRC]) and four hardwood species (Black locust *Robinia pseudoacacia* [BL], Honey mesquite *Prosopis glandulosa* [HM], Paulownia *Paulownia tomentosa* [PAW], and Southern catalpa *Catalpa speciosa* [CAT]). The wood species selected in this study, with the exception of PAW and SYP, are all listed as resistant/highly resistant in the wood handbook (Clausen 2010). PAW has been reported to be resistant and we have found it to be durable to brown-rot fungi and moderately durable to white-rot fungi in our previous study (Kirker et al. in press). PAW was also added to this study because it is listed as an invasive/underutilized species in the south-eastern US in managed forests (Williams 1993).

2.2 Preparation of Extractive-Free Wood

Ten mm cubes were cut from 216 PAW and 450 SYP wood specimens. They were numbered and conditioned 1 week at 26°C and 30% RH before obtaining initial weights. Half of the SYP and all of the PAW blocks were extracted following ASTM D1105-96 with minor adaptations as follows: 24 blocks per wood species were extracted in 150 mL of a 1L 95% ethanol to 427 ml toluene mixture (EtOH:Tol) in soxhlet extraction apparatus at 100°C for 6 hours. Blocks were rinsed in 95% ethanol and allowed to dry overnight. The following day, blocks were again extracted in soxhlet extraction apparatus for 6 hours in 150 mL 95% ethanol and again rinsed in 95% ethanol and allowed to dry overnight. The following day, blocks were boiled at 100°C 3 times consecutively in 1 L portions of distilled water. Blocks were rinsed with hot distilled water and allowed to dry overnight. Because each soxhlet apparatus could only hold 24 blocks, the extraction was repeated with a second set of blocks. Extracted blocks were allowed to condition

for 1 week at 26°C and 30% RH, and then weighed to determine weight loss due to extractive removal.

2.3 Preparation of Extractives from durable wood species

A similar extraction process was used to obtain extractives solutions from the 8 durable wood species. Six blocks each of extracted SYP and PAW along with unextracted SYP were pressure treated following ASTM D1105 standard with the initial EtOH:Tol extraction solutions. The 150 mL of extraction solution was diluted to 300 mL in order to fully submerge all of the blocks for treatment.

2.4. Pressure Treatment

Blocks were treated in a laboratory treating vessel according to E10 (AWPA 2010). Blocks were allowed to air dry in a fume hood for 30 minutes prior to being transferred into a conditioning room to equilibrate.

2.5 Laboratory Soil block Cultures

Laboratory soil block cultures were set up according to AWPA standard E10 (AWPA 2010). Three extracted and un-extracted blocks of SYP and 3 extracted PAW blocks were challenged in duplicate soil bottles individually inoculated with 4 wood decay fungi: *Gloeophyllum trabeum* Mad 617, *Irpex lacteus* HHB-7328, *Postia placenta* Mad 698, and *Trametes versicolor* Mad 697. Southern pine and sugar maple were used as feeder strips for brown-rot and white-rot fungi, respectively. Test blocks were pre-sterilized with the propylene oxide method (E10.13.3.3) by placing groups of blocks in glass tubes separated by treatment species to prevent volatiles from crossing-over between durable and extracted wood specimens. Soil block cultures were incubated at 26°C and 70% RH for 8 weeks. After 8 weeks, blocks were removed and fungal mycelium was brushed off. Blocks were conditioned 1 week as before and final weights were obtained.

2.5 GC-MS

The initial 150 mL EtOH:Tol extraction solutions from 8 naturally durable wood species from a previous experiment were roto-vapped to dryness and resuspended in methanol to 5 mg/mL. Samples were quantified on the GC-MS against a standard curve prepared with dilutions of 7 common wood terpenes. Quantified target and non-target analytes were compiled into tables for comparisons between the species.

3. RESULTS

3.1 Extractive transferability

Pre-extraction of the study blocks was performed to enhance weight losses in order to improve detection of slight differences in durability due to treatments. In the previous study, all 6 wood decay fungal strains caused higher weight loss in PAW and SYP in extracted blocks compared with un-extracted blocks. A full set of un-extracted SYP blocks and solvent controls was also evaluated, but a limited supply of PAW blocks prohibited the evaluation of unextracted PAW. Weight gain due to treatment was variable in the extracted SYP ranging from -0.08 to 0.16%; however weight gain from treatment in all wood samples was negligible (Figure 1a). Weight gain due to treatment was also quite low in un-extracted SYP ranging from 0.18% to 0.51%, the

solvent control also gaining 0.29% (Figure 1c) The extracted PAW ranged from 0.001 to 0.009g per 10 mm cube which accounts for only a 2% (CAT on SYP) to 4% (WRC on PAW) weight gain (Figure 1b).

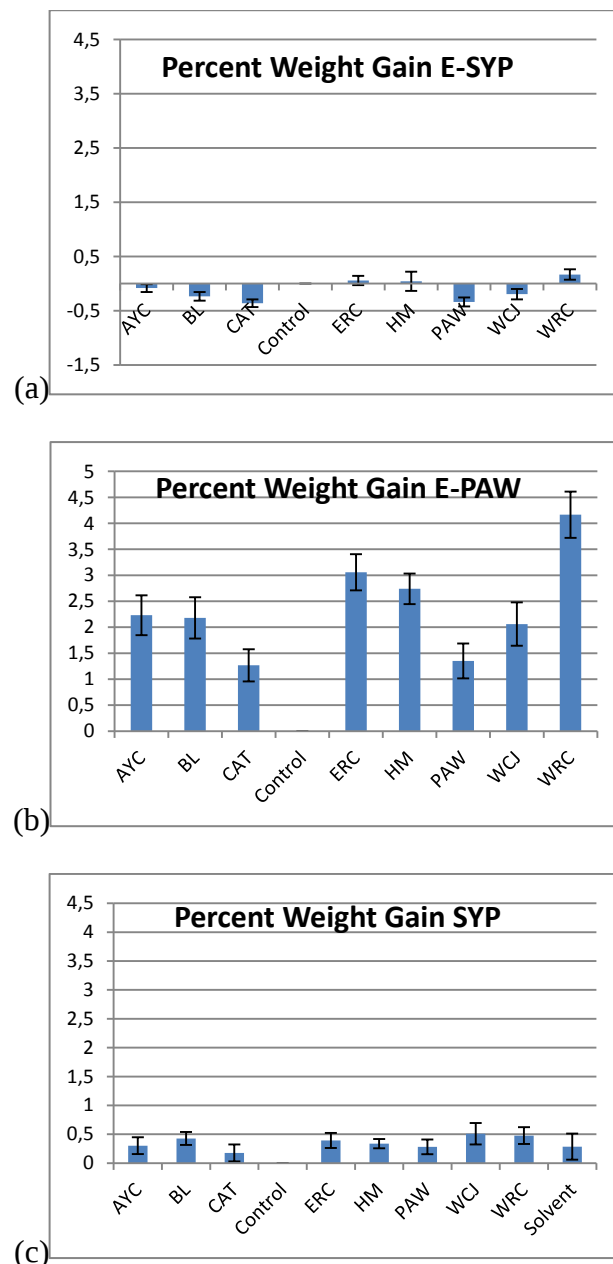


Figure 1: Percent weight gain of (a) pre-extracted SYP, (b) pre-extracted PAW, and (c) un-extracted SYP due to treatment with durable wood extractives. Y-axis represents percent weight gain due to treatment.

3.2 Soil bottle results

3.2.1 PAW-White Rot Fungi

Extracted PAW control blocks had an average weight loss of 28.7% when decayed by *T. versicolor* (Mad 697) (Figure 2a). AYC treated PAW had the lowest mean weight loss (9.4%), followed by PAW treated (14.1%) and BL treated (15.7%), all three had around half the weight loss of the control. All other treatments (WCI, HM, CAT, and ERC) were lower than the control except WRC (28.9%). *Irpex lacteus* (HHB-7328) was more effective at decaying the PAW test blocks than *T. versicolor* (Figure 2b). Extracted PAW controls had an average weight loss of

44.5% when decayed by *I. lacteus* (HHB-7328). ERC and HM were the most effective treatments at decreasing weight loss (35.3% and 39%, respectively). All other treatments had mean weight losses nearly equal to or greater than the control.

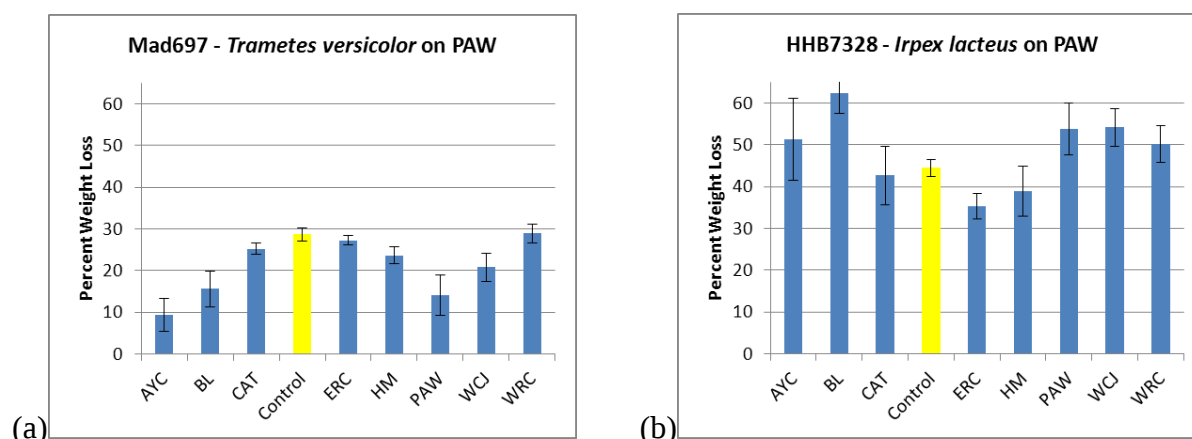


Figure 2: Percent weight loss of extracted *P. tomentosa* (PAW) blocks treated with EtOH:Tol extracts of eight durable wood species challenged with 2 white-rot fungi (a) *T. versicolor* (Mad 697) and (b) *I. lacteus* (HHB-7328). Controls are extracted, untreated *P. tomentosa*.

3.2.2 PAW-Brown Rot Fungi

Extracted PAW control blocks had a mean weight loss of 37.3% when decayed by *P. placenta* (Mad 698) (Figure 3a). CAT treated PAW had the lowest mean weight loss with 26.8%, followed by BL (30.4%), PAW (32.5%) and AYC (34.8%). All other treatments (ERC, HM, WCI and WRC) had mean weight losses equal to or greater than the control. When decayed by *G. trabeum* (Mad 617), extracted PAW controls had a mean weight loss of 39.4% (Figure 3b). PAW treated with PAW extracts had the lowest mean percent weight loss (28.1%) followed by WRC at 33.2%. All other treatments had mean weight losses nearly equal to or greater than the control.

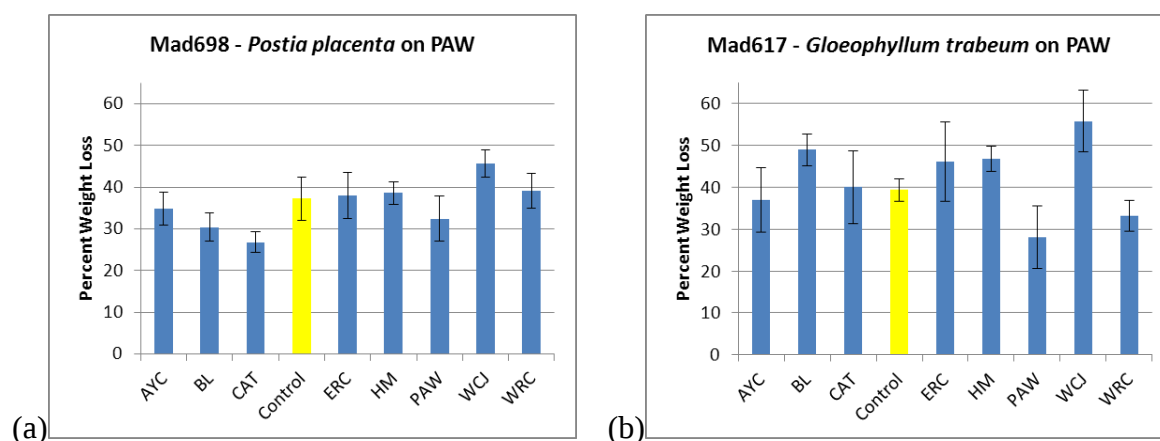


Figure 3: Percent weight loss of extracted *P. tomentosa* (PAW) blocks treated with EtOH:Tol extracts of eight durable wood species challenged with 2 brown-rot fungi (a) *P. placenta* (Mad 698) and (b) *G. trabeum* (Mad 617). Controls are extracted, untreated *P. tomentosa*.

3.2.3 SYP-White Rot Fungi

Weight loss of *T. versicolor* (Mad 697) exposed SYP was low overall compared the other fungi, with control extracted (14.9%) and un-extracted (12.4%) SYP (Figure 4a). The lowest weight loss on extracted SYP was treatment with BL (7.7%) followed by WRC (10%), CAT (11.6%), ERC (11.7%) and WCJ (12%). Weight losses for AYC, HM and PAW ranged between 13-15%. The lowest weight loss on un-extracted SYP was treatment with BL extractive (8.8%), however all other treatments were similar to the control (12.4%). Weight loss for SYP exposed to *I. lacteus* (HHB-7328) was also relatively low overall with control extracted SYP at 24.1% and un-extracted at 18.4% (Figure 4b). The lowest weight loss on extracted SYP was in blocks treated with HM extractive (16.9%) followed by WCJ, AYC, and CAT around 18%. The lowest weight loss on un-extracted SYP was in blocks treated with WRC extractive (15%) and AYC extractive (15.2%), followed by HM extractive treatment (16.8%).

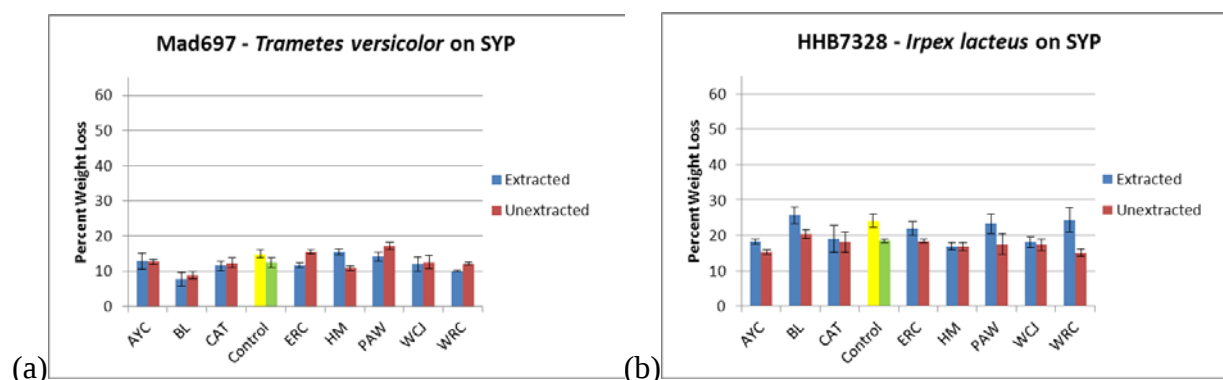


Figure 4: Percent weight loss of extracted southern yellow pine (SYP) blocks treated with EtOH:Tol extractives of eight durable wood species challenged with 2 white-rot fungi (a) *T. versicolor* (Mad 697) and (b) *I. lacteus* (HHB-7328). Controls are extracted, untreated SYP.

3.2.4 SYP-Brown Rot Fungi

Weight losses for brown rot fungi on SYP were much higher (~30%-55%) for controls (Figure 5). Extracted SYP exposed to *P. placenta* (Mad 698) was 54.9% (Figure 5a). All of the extracted SYP treatments had mean weight losses greater than 50%, however HM and WRC were lower than controls at 51.5% and 51.6%, respectively. Un-extracted control SYP weight loss was about 10% lower than extracted at 43.2%. HM and WRC were again the lowest at 38.1% and 38.6% followed by AYC (41%) and ERC (41.7%). Weight loss for control extracted SYP exposed to *G. trabeum* (Mad 617) was 41.3% (Figure 5b). The lowest weight loss was seen in the AYC treated blocks at 37.8%. All the other treatments had 40-50% weight loss; nearly equal to or greater than the control. Mean weight loss for the un-extracted control was 28.6%, again around 10% lower than the extracted control. The treatment with the lowest weight loss again was AYC (26.4%) and the other treatments were nearly equal to or greater than controls.

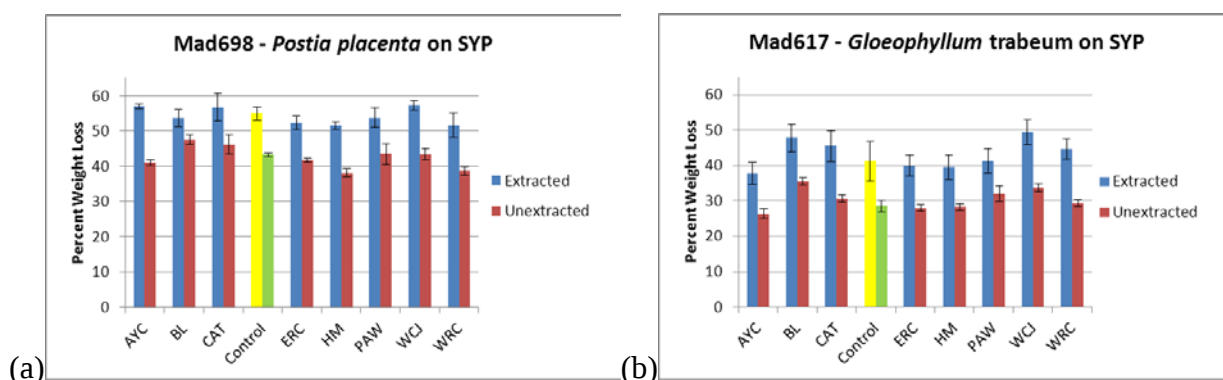


Figure 5: Percent weight loss of extracted and unextracted southern yellow pine (SYP) blocks pressure treated with EtOH:Tol extractives of 8 durable wood species and challenged with 2 brown rot fungi (a) *P. placenta* (Mad 698) and (b) *G. trabeum* (Mad 617). Controls are extracted, untreated SYP.

3.3 Chemical analysis of ethanol:toluene fractions

Chemical analysis was conducted using selected terpenes to generate a standard curve, in order to quantify known terpenes in our naturally durable wood extracts (Figure 6). Alpha-cedrene was common and plentiful in the cedar and Juniper extracts. WRC extracts revealed some unique compounds including alpha-terpinene, d-limonene, terpinolene and beta-citronellol. Additional non-target analytes found in the EtOH:Tol fractions are summarized by species in Table 1. The greatest variety of compounds was discovered in AYC with several unique compounds listed below (see footnote Table 1). The other cedars and WCJ also showed a variety of terpenes as well as sterols. Only two compounds, beta-sitosterol and stigmasterol, stand out in HM or BL as being unique from the SYP control.

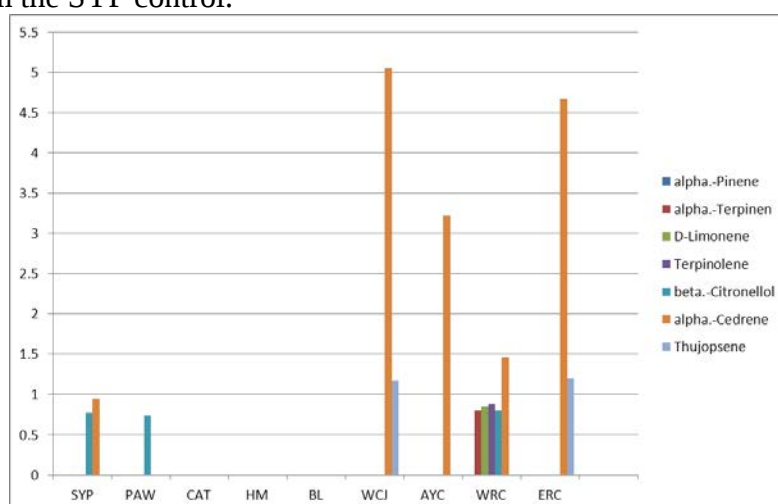


Figure 6: Quantification of target terpenoid analytes from EtOH:Tol fractions of naturally durable wood species. Concentrations are represented in ug/ml.

Table 1: Non-target analytes found in EtOH:Tol fractions of the 9 naturally durable wood species.

Identified Chemical Compounds in EtOH:Toluene Extraction									
CMPD	SYP	PAW	CAT	HM	BL	WCJ	AYC	WRC	ERC
(-)-Myrtenol		Y				Y	Y	Y	
.beta.-Sitosterol		Y	Y	Y	Y	Y	Y		
.gamma.-Sitosterol								Y	Y
alpha-cedrene	Y						Y	Y	Y
Andrographolide						Y	Y		Y
Aromadendrene						Y	Y		Y
Campesterol		Y		Y					
Ferruginol		Y				Y		Y	Y
Longipinocarveol, trans-						Y	Y		Y
Phenanthrene						Y	Y	Y	Y
Stigmasterol				Y	Y				
Thujopsene						Y			Y
Thymene		Y	Y						
Vanillin	Y	Y	Y				Y		
*Unique Compounds *									
AYC-trans-Shisool, Cubenol, tau-Muuroolol, alpha-Cadinol, Coniferyl aldehyde									
WRC-Isopulegol acetate, Flavone, 5,7-dihydroxy8-methoxy, Cubenol, (-)-Northrachelogenin									

4 DISCUSSION

This series of soil bottle tests indicated that a notable quantity of effective extractives can be removed from naturally durable wood species and transferred to non-durable species. In this study the extracts from 24 blocks were pressure treated into 76 blocks in a treatment solution that was diluted 2X. It is possible that a more concentrated treatment solution would provide increased protection of non-durable wood blocks.

With the variability of wood species along with fungal species there was no clear “best” naturally durable wood extract for treatment. However, AYC and WRC generally performed well against the brown-rot fungi selected for this study, while BL and ERC performed well against white-rot fungi. Other treatments showed modest improvement in decay inhibition for one or more test fungus, but were not conclusively effective against either the brown- or white-rot fungi used in this study.

Chemical analyses indicated multiple chemicals that could be contributing to the durability factors observed in this study. Particularly for the cedars and WCJ, there seems to be a great diversity of chemicals that could individually or in combination be contributing to the overall durability of these wood species. The discovery of alpha cedrene in the cedar group was not at all surprising, and together with thujopsene, make up a major component of cedar wood oil. The biotoxicity of cedar oil has been widely studied, however our GC-MS analysis only found thujopsene in WCJ and ERC, but it should be present in WRC and AYC (Chedghey et al. 2009). The other terpenes found in WRC are very common in many plants, but numerous studies have found antifungal properties of essential oils containing these compounds, albeit in different relative concentrations. Limonene is a cyclic terpene commonly associated with citrus and has a broad range of uses ranging from food additives to natural insecticides. Alpha terpinene and gamma terpinene (also referred to as terpinolene) are also cyclic terpenes and commonly isolated from plants. *Melaleuca alterniflora* (tea tree) oil is rich in terpinolene and has reported antifungal properties. Yang and Clausen (2007) evaluated essential oils from seven sources, which included both geranium (beta-citronellol) and tea tree oils (terpinol), against both mold and decay fungi and found that geranium oil was effective at controlling mold, but tea tree oil was only 80% effective. Their results also found thyme and geranium oil to be effective at controlling *Trichoderma viride*, *Penicillium chrysogenum*, and *Aspergillus niger*.

5. CONCLUSION

The results of this study show slightly increased durability in some of the non-durable wood blocks treated with extractives from durable wood species, indicating that durability can potentially be transferred from durable to non-durable wood species. Because the extractives were obtained from a small number of blocks and diluted to obtain sufficient treatment solution in this study, we conclude that the concentration of the extractives should be increased for future studies. One of the most comprehensive studies of this nature to date would be that of Powell et al. 2000, which used sawmill residues of cypress heartwood to treat non-durable white cypress sapwood. In this study, the concentrations for establishing fungal toxicity ranged for 0.2% to 20% extractive (by volume) and our efforts are falling considerably short of this mark. Future efforts will attempt to increase extractive concentrations into a more appropriate range. Pre-extraction of the test blocks was done to increase overall weight losses in order to improve detection of slight changes in durability. In future experiments involving more concentrated extractives, pre-extraction of test blocks should not be necessary; moreover, in a large scale lumber application this would not be practical. Several unique compounds were identified from the chemical analyses of some of our more durable species (AYC and WRC) and warrants further research to elucidate their specific role. We will continue our efforts to increase the extractive concentrations and conduct further studies in transferrable durability which would provide economic advantage to under-utilized or invasive wood species.

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