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The role of extractives in naturally durable wood species☆

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ABSTRACT

There are numerous examples of wood species that naturally exhibit enhanced performance and longevity in outside exposure independent of preservative treatment. Wood extractives are largely considered to be the contributing factor when evaluating and predicting the performance of a naturally durable wood species. However, little test methodology exists that focuses on the extent of the role of extractives in wood durability. In this study, eight candidate naturally durable wood species plus a non-durable control were evaluated in laboratory soil block tests for resistance to termite attack and decay by three brown-rot and three white-rot decay fungi. Chemically extracted test blocks were compared to unextracted controls. Extracted durable species were also compared to non-durable controls. Results showed nearly all of the wood species exhibited higher weight loss due to termite or fungi when extractives were removed and extracted samples had weight losses that were comparable to the non-durable controls.

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1. Introduction

Wood extractives are the non-structural components of wood. They are typically concentrated in the heartwood and are often produced by the standing tree as defensive compounds to environmental stresses (Taylor et al., 2002). Markets for naturally durable wood expanded due to the removal of chromated copper arsenate (CCA) from the general forest products market and have been called environmentally friendly or chemical free alternatives to treated wood (Evans, 2003). However, extractive content is highly variable not only from tree to tree but also within an individual tree (Scheffer and Cowling, 1966). This fact presents an enormous challenge when attempting to standardize these materials and prescribe recommendations on predicted service life and performance and has long plagued researchers working within this area (Räberg et al., 2005; Morris et al., 2011). The study of extractives of naturally durable woods is an expansive body of literature, excellent reviews of the literature have been presented by Scheffer and Morrell (1998), Yang (2009), and Singh and Singh (2012). Using a wide array of extraction methods, extracts of naturally durable wood species have been used to inhibit a wide range of organisms from human pathogens to insects, wood decay fungi, and mold fungi. Commercially available products, such as Cedarshield® and Termilone® are now available as wood treatments, but are unproven in academic literature. Several synergistic combinations of wood extracts have also been evaluated against wood decay, such as tannin–borate combinations (Thevenon et al., 2009), condensed tannins from bark complexed with copper (Laks and McKaig, 1988), and combinations of heartwood extractives and quaternary ammonium compounds (Hwang et al., 2007). Taylor et al. (2002) suggested that micro-distribution of extractives within the wood may be more important than presence of bulk extractive in the heartwood, but also added that in-situ studies of extractive content have been difficult. The literature seems to agree that both quantity and quality of extractives have a role to play, but their relative contribution varies considerably from substrate to substrate. This study evaluated the role of extractives in durability of eight wood species by removing as much of the extractives as possible from test blocks and measuring termite feeding and decay rates compared to unextracted test blocks. This study can also be seen as a demonstration of a worst case scenario of very low extractive content; showing how decay fungi and termites may respond to durable woods of marginal quality. Extractive-free durable wood should be considered for the establishment of a baseline for comparing relative durability of different wood species.

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2. Materials and methods

2.1. Wood species

2.1.1. Durable wood species

Naturally durable wood species were obtained from various lumber producers in North America for a concurrent study evaluating above and below-ground performance of these wood species in field test sites in both Mississippi (MS) and Wisconsin (WI). Samples were in conditioned storage at 26 °C and 30% humidity for 2 years prior to testing. Naturally durable species selections were based on previous field trials with untreated southern pine [SYP] selected as the non-durable control. Aside from southern pine, the wood species chosen included four additional coniferous species (Alaskan yellow cedar *Callitropsis nootkanensis* [AYC], eastern red cedar *Juniperus virginiana* [ERC], western 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 catalpa *Catalpa* spp. [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 was added to this study because it is listed as an invasive/underutilized species in the southeastern US in managed forests (Williams, 1993).

2.2. Preparation of extractive-free wood

Ten millimeter cubes were cut from each of the 9 wood species. Blocks were numbered and conditioned for 1 week at 27 °C and 30% relative humidity (RH) before obtaining initial weights. Half of the numbered blocks were extracted following ASTM D1105-96 with minor adaptations as follows: blocks were extracted in 150 mL of a 2.32:1 mixture of 95% ethanol to toluene in soxhlet extraction apparatus, 24 blocks per soxhlet by species, at 100 °C for 6 h. Blocks were rinsed in 95% ethanol and allowed to air-dry overnight. After air-drying, blocks were extracted in soxhlet extraction apparatus for 6 h in 150 mL 95% ethanol, rinsed in 95% ethanol and allowed to dry overnight. The following day, blocks were boiled at 100 °C 3 times consecutively in 1L portions of distilled water. Blocks were rinsed with hot distilled water and allowed to dry overnight. Extracted blocks were conditioned for 1 week and weighed to determine loss due to extractive removal. Since each soxhlet apparatus could only hold 24 blocks at a time, the extraction was repeated with a second set of 24 blocks.

2.3. Termite soil arena choice test

Termite resistance was evaluated according to modification of the AWPA E1-09 choice test bioassay (AWPA, 2010a) using the eastern subterranean termite, *Reticulitermes flavipes* collected from Janesville, WI on the day of experiment set up. Two extracted and 2 unextracted blocks of the same species were exposed to 1 g of *R. flavipes* workers in triplicate sand arenas containing 50 g of screened, washed and heat sterilized silica sand with 9 mL deionized water in a 50 mm plastic container. After 4 weeks, percent mortality was visually approximated and blocks were reconditioned for 1 week at 27 °C and 30% humidity and final weights were obtained.

2.4. Laboratory soil block cultures

Laboratory soil block cultures were set up according to a modification of AWPA standard E10-09 (AWPA, 2010b). Three extracted and unextracted blocks were challenged in duplicate soil bottles with one of the 6 wood decay Basidiomycetes which included 3 brown rot fungi: *Gloeophyllum trabeum* (Pers.: Fr.) Murr. (Mad 617), *Postia placenta* (Fr.) Lars. & Lomb. (Mad 698), *Tyromyces palustris* (Berk. & Curt.) Murr. (TYP6137), and 3 white rot fungi: 2 strains of *Irpex lacteus* (Fr.: Fr.) Fr. (HHB7328 and Mad 517), and *Trametes versicolor* (L.: Fr.) Pil. (Mad 697). Southern yellow pine and sugar maple were used as feeder strips for brown-rot and white-rot fungi, respectively. Test blocks were pre-sterilized with propylene oxide (AWPA E10.13.3.3) in tubes separated by species to prevent volatiles from crossing-over between extracted and unextracted wood specimens. Soil block cultures were incubated at 27 °C and 70% humidity for 8 weeks to decay. After 8 weeks, blocks were removed and mycelium was brushed off. Blocks were reconditioned for 1 week as before and final weights were obtained.

2.5. Statistical analyses

The percent weight losses of the wood blocks in the fungal exposure experiment were modeled with the three fixed factors: fungal species, wood species and extracted/unextracted along with their interaction. For each fungal and wood species combination, three extracted and three unextracted blocks were exposed in a soil bottle, thus nesting occurred at this treatment level along with subsampling. Two soil bottles were replicated for each fungal and wood species combination. Since brown and white-rot fungal species appeared to be somewhat different, the statistical models were fit separately for brown and white-rot species. In addition, the termite experiment had the two fixed factors, wood species and extracted/not extracted. Three replicate arenas for each wood species, with two extracted and two unextracted blocks, were exposed to termites in each arena, thus nesting occurred at this treatment level along with subsampling. Based on models of the split-plot designs, treatment comparisons were made on the modeled percent weight losses. Percent weight loss appeared heterogeneous with variation increasing with weight loss, and this was included in the model by estimating different residual variances for the wood species. Statistical modeling was performed in SAS® Version 9.2 (Cary, NC) using mixed modeling procedures (Littell et al., 2006). Multiple comparisons were based on simulation-adjustments controlled within fungus species or termites with a family-wise error rate of 0.05, with letter assignments following the methods of Piepho (2004).

3. Results

Extraction efficiency was similar for duplicate soxhlet extractions across the nine wood species (Fig. 1). SYP, BL and AYC had similar weight loss at approximately 4%. PAW, HM and WRC had the highest percent weight loss due to extractives compared to the others and were about two times more than SYP, BL and AYC.

Subterranean termites preferred extracted wood as a food source compared to unextracted wood for every wood species except HM, where there was significant termite mortality after only a few days exposure. Weight loss differences were significant (Fig. 2) and in many unextracted samples showed no weight loss from termite feeding. Neither extracted nor unextracted HM blocks were consumed by termites and virtually all the termites expired when exposed to HM blocks. Extracted WRC, PAW, AYC, and SYP had the highest weight losses at 51, 44, 36, and 31% respectively. Overall, there was low mortality of *R. flavipes* exposed to most species except when *R. flavipes* was exposed to HM (96%) or BL (70%), while ERC had minimal mortality (6.6%) at the end of the test.

All six wood degrading fungal strains caused higher percent weight loss in extracted blocks compared to unextracted blocks except *G. trabeum* on PAW (Fig. 3) and *I. lacteus* on HM (Fig. 4) which

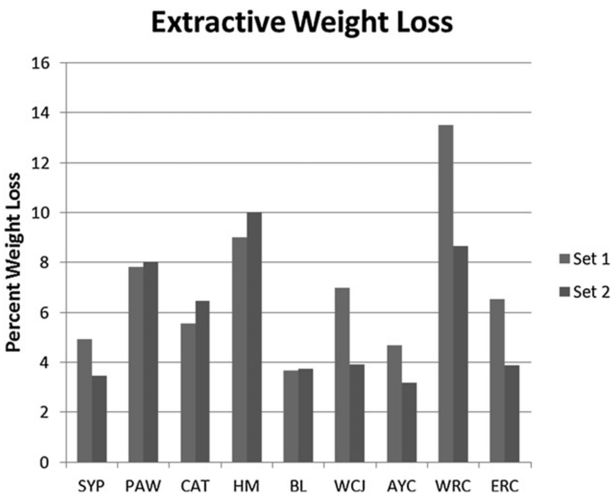

Fig. 1. Percent weight loss of sample blocks for each naturally durable wood species in duplicate extractions using ASTM D-1105 method for preparation of extractive free wood.

were equivalent. The weight loss differences between extracted and unextracted wood blocks were statistically significant for *T. palustris* on BL, CAT, ERC, HM, and WRC, *G. trabeum* on AYC, BL, CAT, ERC, WCJ, and WRC, and *P. placenta* on AYC, BL, CAT, ERC, and WRC., Very low weight losses were noted for the two *I. lacteus* strains overall. HHB 7328 had significant differences only between extracted and unextracted ERC, while Mad 517 had significant weight loss differences on CAT. The final white-rot fungus, *T. versicolor* had higher weight losses overall and there were significant differences between extracted and unextracted BL and CAT wood species.

Brown-rot fungi produced higher weight loss on SYP controls (Fig. 3) than all white-rot test fungi (Fig. 4). *P. placenta* had the highest weight loss at 61% followed by *G. trabeum* at 46% and *T. palustris* at 31%. White-rot fungi *I. lacteus* and *T. versicolor* caused low weight losses in SYP, around 10–15%. *T. versicolor* caused marginally to significantly greater weight loss on extracted PAW

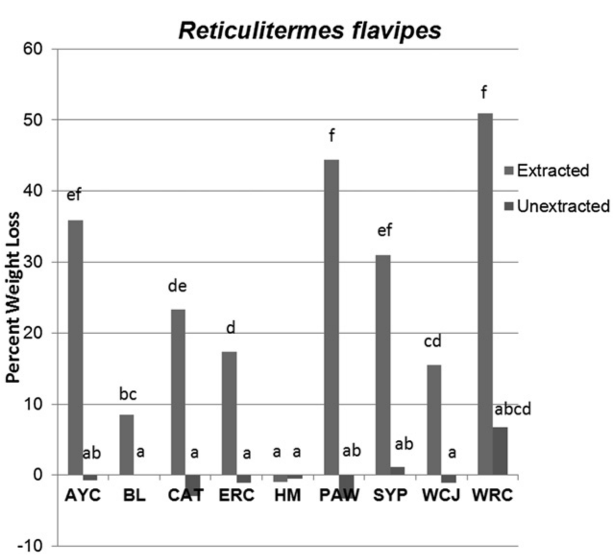

Fig. 2. Mean percent weight loss (PWL) of extracted and unextracted wood blocks of 9 wood species due to termite attack in a modified E1-09 termite choice bioassay. Letters above columns indicate statistical groupings, differ significantly at 0.05 (a = least PWL, f = highest PWL).

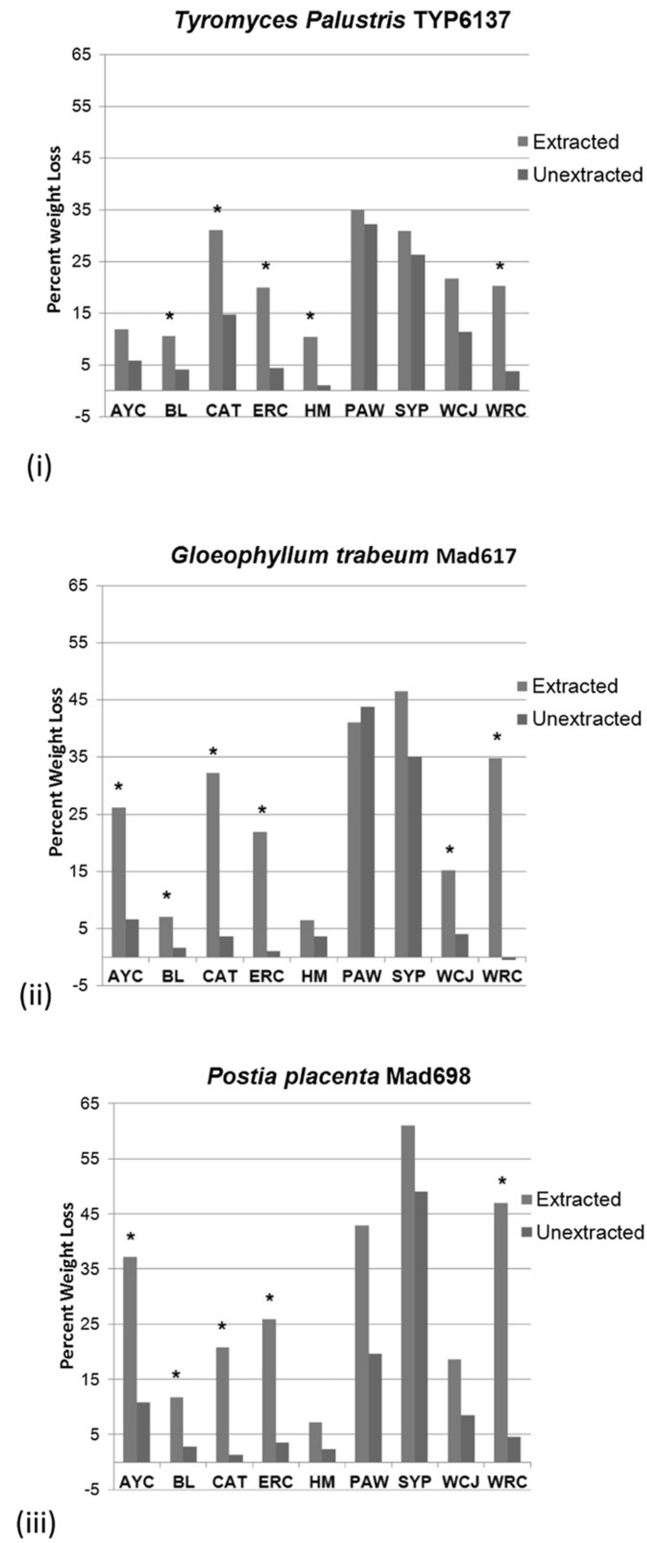

Fig. 3. Mean percent weight loss for extracted and unextracted blocks of 9 wood species exposed to (i) *Tyromyces palustris* (TYP 6137), (ii) *Gloeophyllum trabeum* (Mad 617), and (iii) *Postia placenta* (Mad 698) for 8 weeks in an E–10 soil bottle test. Asterisks indicate where extracted mean weight loss for a wood species was significantly different at the 0.05 significance level from the corresponding unextracted mean weight loss.

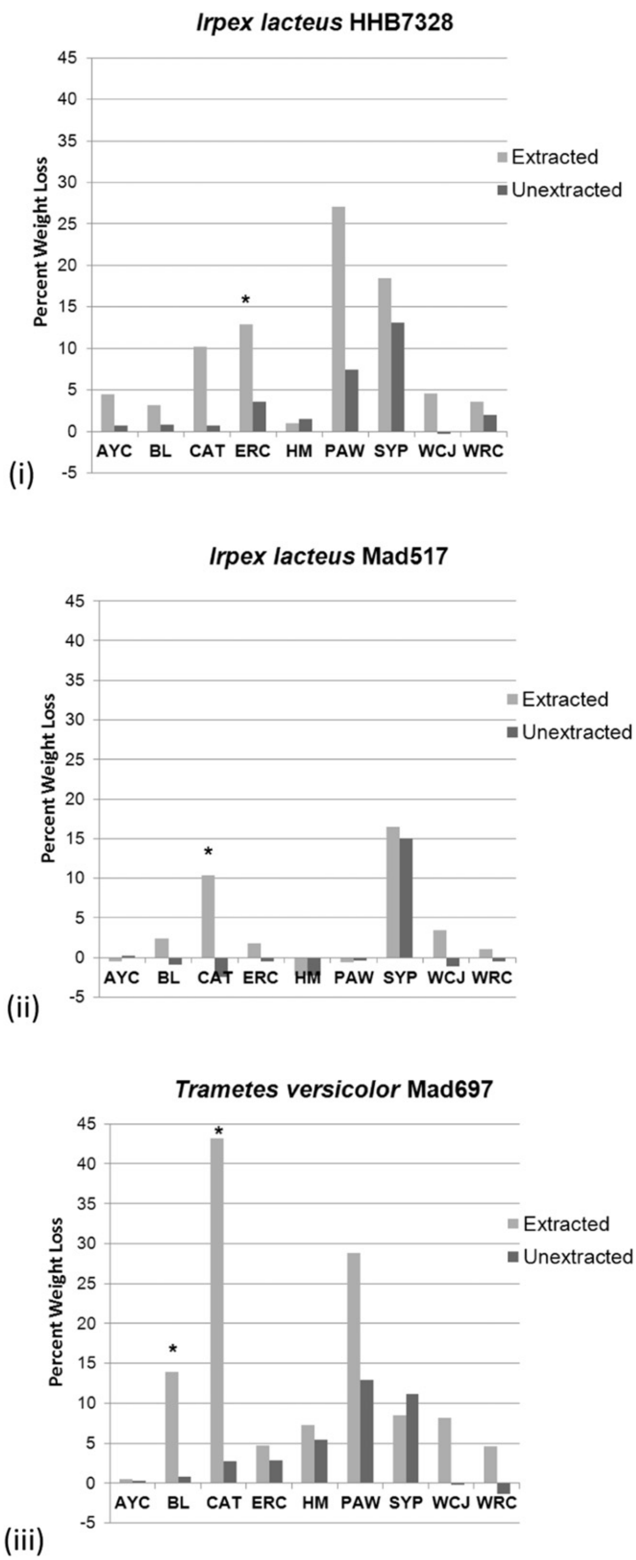

Fig. 4. Mean percent weight loss for extracted and unextracted blocks of 9 wood species exposed to (i)*Irpex lacteus* (HHB 7328), (ii) *Irpex lacteus* (Mad 517), and (iii) *Trametes versicolor* (Mad 697) for 8 weeks in an E−10 soil bottle test. Asterisks indicate where extracted mean weight loss for a wood species was significantly different at the 0.05 significance level from the corresponding unextracted mean weight loss for the wood species.

and CAT, 29% and 43% respectively (Fig. 4). HM and BL had very low weight loss at around 10% or lower across all fungi and these losses were significantly lower than SYP for every isolate except *T. versicolor*. Western juniper (WCJ) had slightly higher weight loss (~20%) and was significantly lower than SYP exposed to *G. trabeum*, *P. placenta*, and *I. lacteus* strain Mad 517.

Those woods commonly marketed as cedars, AYC, WRC and ERC, were quite durable as unextracted wood blocks (≤10%), but became susceptible to brown-rot fungi after they were extracted. Unextracted WRC samples had less than 5% weight loss for both brown-rot and white-rot fungi, but suffered a 5–10 fold decrease in durability when they were extracted and exposed to brown-rot fungi. Similarly, all unextracted ERC had less than 5% weight loss, but exhibited a 5-fold decrease in durability when extracted and exposed to brown-rot fungi. Unextracted CAT was also quite durable against fungal decay (≤15%) but when extracted, CAT exhibited a 2–20-fold decrease in durability. With the exception of *T. versicolor* and *P. placenta*, CAT exhibited significantly lower percent weight losses than SYP in the unextracted group, but performed similarly to SYP after extraction. Extracted CAT exposed to *T. versicolor* had significantly higher weight loss than SYP; while CAT extracted and exposed to *P. placenta* had significantly lower weight loss than SYP. *P. tomentosa* (PAW), in general, was not very durable and when extracted and exposed to *I. lacteus* (HHB 7328) and *T. versicolor* appeared to become even more susceptible than SYP, although the variability in PAW exposures precluded statistically significant differences in weight loss.

Durability indices were calculated for both extracted and unextracted species in this study according to EN 350-1 (1994) using SYP as the reference specimen. Wood species were averaged separately across brown and white-rot fungi to calculate the indices. For brown-rot fungi, unextracted BL, HM, ERC, and WRC were classified as very durable (class 1) while AYC, CAT, and WCJ were classified as durable (class 2). Even with extractives removed, BL, HM, and WCJ remained moderately durable (class 3) to durable. For white-rot fungi, unextracted AYC, BL, CAT, HM, WCJ, and WRC were all classified as very durable while ERC was only durable. AYC was still very durable after extractive removal, and HM, WRC, BL, ERC, and WCJ were still durable to moderately durable. The wood handbook (Table 14-1) classifies all of these durables as resistant, except for BL, which is classified as very resistant. PAW is not listed in the table, however, we found it to be moderately durable to white-rot fungi and not durable (class 5) to brown-rot fungi.

4. Discussion

The results for the soil bottle assays were consistent with our observations of in-ground field performance at the WI test site (unpublished data). At 24 months of in-ground exposure, the most durable in order were ERC > WCJ > BL > HM > WRC > AYC while the least durable were PAW < SYP < CAT.

PAW was not durable in this study when exposed to either termites or fungi. PAW generally performed no differently than SYP and often became less durable than SYP after extraction and exposure to wood decay fungi. Termites readily consumed extracted PAW to a slightly higher degree than SYP, though not significant. In ground field stakes of PAW have all failed within two years exposure in WI (unpublished data). Our findings indicate that *P. tomentosa* is not naturally durable for ground exposure, although above ground exposure tests are still on-going. Jun-Qing et al. also indicated that PAW is only slightly resistant to decay (1983) and Arango has found it un-resistant to termites (unpublished data). Olson and Carpenter (1985) reported extractive content of *P. tomentosa* averaged 13% and that much of that could be due to soluble sugars from the hemicelluloses in the sapwood, as extractive concentration was greater in sapwood than heartwood. Catalpa performed well in soil bottles when all extractives remained, but when extracted was non-durable. These observations correlated with our field experiments, where CAT was susceptible to decay fungi in soil contact, likely due to leaching of extractives into the soil. CAT also exhibited similar behavior in the termite test; with no weight loss in the unextracted and 23% weight loss after specimens were extracted. MacDaniel (1982) studied anti-termitic compounds from CAT and found catalponol, epi-catalponol, catalpanone, and catalpalactone to be the active compounds. No-choice tests using catalpalactone resulted in 99% termite mortality with no termite feeding on the test blocks (MacDaniel, 1982). Our termite bio-assay results showed decreased feeding in the arenas with unextracted and extracted blocks, but little mortality, possibly due to the choice of another more suitable food source.

R. flavipes preferred extracted cedars in choice tests, and extracted AYC and WRC both had higher weight losses due to *R. flavipes* feeding compared to extracted SYP. Results obtained from the soil block study indicated that the cedars were significantly less durable to decay fungi, particularly brown-rot fungi, once extractives were removed. Chedgy et al. (2009) studied the effects of leaching and fungal growth of WRC and found that leached WRC contained 80% fewer extractives than un-leached and that maintaining extractives was essential for preventing decay of WRC. We noted direct antifungal activity for WRC and AYC in disk assays, however after 7 days the fungi began to re-grow over the zones of inhibition (unpublished data). Stirling et al. (2007) has developed a micro-bioassay method to rapidly evaluate individual components of western red cedar extracts and determine their role as either biocidal, radical scavenger, or metal chelators and found that thujaplacins and B-thujaplcinol were directly toxic to decay fungi and were all excellent metal chelators as well as plicatic acid. Plicatic acid and B-thujaplacinol was also found to be excellent radical scavengers. According to Stirling (2010) and Morris and Stirling (2012), it appears that some of the protective compounds in the cedars remain under typical field exposure (UV, rain), but are removed through solvent exposure, which presents additional avenues for chemical discovery. Leachability of cedar extractives presents a major obstacle in their utility in ground exposure, but our field stake evaluations are still on-going and cedar remains relatively sound compared to controls (unpublished data).

Western juniper (WCJ) was overall moderately to very durable throughout this test and became slightly less durable when extractives were removed. Extracted WCJ was preferred by *R. flavipes* in our choice tests. Junipers, which would include western juniper, are all classified as resistant according to the Wood Handbook (Clausen, 2010), which would make them equivalent to catalpa, eastern and western red cedar, or honey mesquite. WCJ fence posts were reported to have lasted 56 years in ground at the Oregon post farm (Morrell et al., 1999) and above ground tests in Hilo, HI showed WCJ heartwood to be durable to both termites and decay, but presence of heartwood had no effect on durability of adjacent sapwood (Morrell, 2011).

The performances of honey mesquite in this entire series of tests indicated that it maintained excellent durability even after stringent extraction protocols. Honey mesquite appeared to be quite toxic to *R. flavipes* in our choice test arenas, and should be studied further. Various chemical components of HM have been found to exhibit anti-infective and anti-parasitic properties and are also being evaluated as natural treatments against antibiotic resistant strains of bacteria (Samoylenko et al., 2009). SEM micrographs from a sampling of blocks from this assay (unpublished) reveal a very dense, resinous structure that may restrict access or limit movement of fungal hyphae within the wood or inhibit full extraction and potentially the leachability of this wood species. The structure of honey mesquite may simply prevent hyphal movement and colonization, or efficient extractive removal, but further study will be required. Honey mesquite has received some attention as a naturally durable species, but the size of the trees is a limiting factor in production of straight grained wood of larger dimensions (Weldon, 1986). Mesquitol, a flavonoid, has been isolated from *Prosopis julipiflora*, and has been found to have antioxidant properties, but has not been identified in Honey Mesquite (Sirmah et al., 2009). Honey mesquite is also considered invasive in the southwest, so effective utilization of this durable species would reduce burdens on grassland managers.

Black locust exhibited similar durability properties to honey mesquite, but still exhibited some weight loss (~10%) when extractives were removed. Historically, black locust was used as fence post material prior to pressure-treated pine, and also used in ship building (Wiemann, 2010). In ground black locust fence posts remained sound for 53 years in tests at Oregon State (Morrell et al., 1999). The most studied compound to date from BL is dihydrorobinetin, a flavonoid which accounts for about 4% of the wood (Scheffer and Cowling, 1966). It has exhibited anti-oxidant properties (Cushnie and Lamb, 2005) and is antibacterial (Mori et al., 1987), but its role in preventing fungal decay is so far unstudied. Smith et al. (1989) increased durability of aspen wafers through pressure treatment with different solvent fractions of black locust extractives against attack by *G. trabeum*. Latorraca et al. (2011) found lower durability of juvenile heartwood to *Coniophora puteana* and *Corilous versicolor* due to lower concentrations of phenolic compounds and flavonoids than in mature heartwood. Additional research into the durability of above and in-ground deterioration of black locust as it relates to extractive content would provide useful information.

5. Conclusions

Our durability indices were consistent with the literature on all of these naturally durable species. *P. tomentosa* has not had much study, but we found it to be durable to brown rot and moderately durable to white rot. Our study found HM and WRC to also be very durable in addition to BL, which was the only species listed as very resistant in the Wood Handbook (Clausen, 2010). The durability indices of the extracted blocks may offer a "worst case" scenario for predicting performance of durable woods containing less than ideal amounts of extractive to sufficiently inhibit decay fungi. The overall results of these tests indicate that extractive content is primarily responsible for durability; however, percent extractive content was not directly correlated with durability. Therefore, it is probable that individual components of extractives confer durability rather than bulk presence of extractive, as concluded in various other studies. However, the issue of micro-distribution of heartwood extractives shouldn't be ignored, *in situ* studies of extractives and where they are localized within the heartwood and sapwood and how they impact fungal colonization are crucial in able to properly understand their proper function. This study does indicate that additional factors may also confer resistance to decay as in the case of black locust and honey mesquite which retained resistance to decay after extraction. Physical barriers may provide additional protection against fungal colonization or perhaps our stringent extraction failed to remove some of the less extractable chemicals present in some of the hardwood species (Scheffer and Cowling, 1966). The presence of these barriers may have also prevented full removal of extractives, which could explain the high mortality of *R. flavipes* and possibly prevent leaching in field exposure. More directed studies of honey mesquite and black locust may prove useful in discovery of potential compounds or concepts for wood protection.

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