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Section 1 **Biology**

Above Ground Field Evaluation and GC-MS Analysis of Naturally Durable Wood Species

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ABSTRACT

Nine wood species are being evaluated in above ground field studies in Mississippi and Wisconsin. Candidate naturally durable wood (NDW) species are being rated at yearly intervals for resistance to decay, cupping, and checking. Field ratings after 12 months exposure are presented. To date, *Paulownia tomentosa* (PAW) and southern yellow pine (SYP) are least durable and cedars are the most durable in above ground exposure. Wood samples are being taken from the deck-boards and subjected to chemical analysis using GC-MS. Fatty acids from NDW species were extracted, derivatized, and analyzed along with commercial fatty acid methyl ester (FAME) standards. With few exceptions, results indicate that FAMEs are more abundant in NDW species. However, preliminary bioassays found no inhibition of select wood decay fungi by FAMEs at naturally occurring concentrations.

Keywords: wood durability, fatty acids, extractives, GC-MS, above ground testing

1. INTRODUCTION

The allure of naturally durable wood (NDW) species has grown significantly in the last decade as consumers are increasingly conscious of chemical preservatives, especially those containing heavy metals such as chromium and arsenic. Increased regulation on potentially toxic compounds also reduces the number of effective wood preservatives on the market driving the need for alternative products. Several species of wood are considered to be naturally durable for outdoor exposure, appearing to be resistant to degradation by common wood rotting fungi. Many of these species have an excellent history of service in past studies (Scheffer and Morrell 1998), but careful attention should be paid that natural durability is not simply assumed as the mechanisms of naturally durable wood remain unknown. Natural durability has often been attributed to chemical extractives present in the wood, but these chemical compounds are often secondary metabolites produced in response to stimuli and vary considerably from one tree to the next.

Fatty acids are long chain hydrocarbons attached to a carboxylic acid. They are essential to animal diets and also have promise as fuels due to the high amounts of ATP released when metabolized. They are common in plant oils and have been extracted from plants for ages. Trees contain a diverse assortment of fatty acids, which can be both uniform and varied among tree species depending on the fatty acid. Fatty acids are often pre-cursors to secondary metabolites, such as eicosanoids (leukotrienes, prostanoids, resolvins, and jasmonates) and also serve as precursors to waxes, which naturally function to restrict and regulate water movement in plants (Dowhan, et al. 2008). An excellent review on antifungal properties of fatty acids is presented by Pohl, et al. (2011).

Antimicrobial activity has been established for several fatty acids in a range of hosts and substrates. The saturated fatty acid, Lauric acid (C18:0) has been shown to have antifungal and antibacterial properties in laboratory studies (Kabara, et al. 1972, Rihakova, et al. 2001). Walters, et al. (2004) tested linolenic, linoleic, and erucic acids against four plant pathogens (*Rhizoctonia solani*, *Pythium ultimum*, *Pyrenophora avenae* and *Crinipellis perniciosa*) and found that linoleic and linolenic acids had activity against all of the fungi tested. Fatty acids have also been evaluated as components of wood protectants; Schmidt (1984) tested C5-C16 saturated fatty acids against spores of four isolates of decay fungi: *Poria tenuis*, *Trametes hispida*, and two isolates of *Gloeophyllum trabeum*. Results indicated that C5-C7 fatty acids eliminated all spores at 1000 ppm and that C8, C9, and C10 prevented germination of all fungi. Coleman and Clausen (2008) evaluated C3-C10 fatty acids against *Penicillium chrysogenum*, *Trichoderma viride*, and *Aspergillus niger* and found that a 6% concentration of propionic (C3) and pentanoic (C5) acids were effective against test fungi, and an added lactic acid emulsifier enhanced mold suppression by roughly ten-fold.

Given the past successes using fatty acids in multi-component wood protection systems, it seems appropriate to evaluate naturally occurring levels of fatty acids in wood species that have inherent resistance to degradative microbes and determine whether these fatty acids play a role in natural durability.

The above-ground field tests involving 9 wood species exposed at test sites in Madison, WI and Saucier, MS are being visually evaluated in conjunction with chemical analyses. In this study, decking boards are being rated for overall durability under full exposure to environmental weathering. Wood samples are being taken at yearly intervals and extractive (currently fatty acid) content is being analyzed by GC-MS. This research is funded by the Federal Highway Administration under the covered bridge program in order to evaluate NDW species as effective replacement components for the United State's aging covered bridges, many of which are in disrepair.

2. EXPERIMENTAL METHODS

2.1 Wood Species

Eight wood species were selected that have been previously reported as showing degrees of natural durability. Southern yellow pine (SYP) was selected as a non-durable control. "Durable" wood species, durability ratings according to Wood Handbook (FPL 2010), and examples of common uses are listed in Table 1.

Table 1: Candidate Naturally Durable Wood species used in this study, % Extractive by weight, FPL durability rating, and examples of uses of each.

Common	***Species***	**%EW**	**FPL rating[1]**	Examples of **Uses**
Southern Yellow Pine	*Pinus taeda*	3-7	Non-resistant	Construction
Southern Catalpa	*Catalpa bignonioides*	2-7	Resistant	Fence posts, rail ties
Black Locust	*Robinia pseudoacacia*	10	Very Resistant	Mine timbers
Paulownia	*Paulownia tomentosa*	12	Not rated	Invasive
Honey Mesquite	*Prosopis glandulosa*	18	Moderately resistant	Charcoal, smoking chips.
Eastern Red Cedar	*Juniperus virginiana*	Variable	Resistant	Fence posts
Western Juniper	*Juniperus occidentalis*	Variable	Resistant	Fence posts, furniture
Alaskan Yellow Cedar	*Callitropsis nootkatensis*	3-5	Resistant	Furniture
Western Red Cedar	*Thuja plicata*	10	Resistant	Shingles, lumber, post, and piles.
[1] Based on ratings in the Wood Handbook. (FPL-GTR-190).				

%EW- extractive content by weight.

2.2 Field Studies

Decking specimens were randomized in ten replicates on above ground racks at both field sites. The Saucier, MS field site is located in the USFS Harrison Experimental Forest and is classified as zone 5 indicating "severe" decay hazard (AWPA 2011). The Madison, WI field site is located at FPL's Valley View test site and is classified as zone 2 indicating "moderate" decay hazard (AWPA 2011). Decking specimens were installed on horizontal racks in an unobstructed location to ensure maximum exposure to weather. Decking specimens are rated yearly for decay according to AWPA E-25 standards (AWPA 2011). Measurements of checking and cupping are also being recorded at each evaluation.

2.3 Chemical Analyses

Wood samples were taken at yearly rating intervals using a paddle bit and the shavings funnelled into a centrifuge tube. Two grams of wood shavings were collected from each NDW species. Wood shavings were soxhlet extracted for fatty acids in 2:1 chloroform: methanol for 6 hours at a rate of 6-8 fluxes/hr. Extractives were dried with a rotary evaporator, resuspended in toluene, and derivatized with sodium methoxide according to methods by Christie (1989). Derivatized fatty acids were separated with the non-polar constituents in a separatory funnel and roto-vapped dry. Dry weights were used to calculate mass of fatty acids and samples were normalized to 5mg/ml total FAMEs in hexane. FAMEs were then separated by solid phase extraction using Ag-Ion columns (Supleco, St. Louis, MO, USA) into fractions based on their degree of unsaturation (#of double bonds) and collected fractions were analyzed on a Shimadzu QP-2010S GC-MS. GC-MS settings are presented in Table 2.

Table 2: GC-MS Settings for NDW species FAME analysis

Instrument	Shimadzu QP 2010-S with AOC 20 auto-injector		
Column	Restek Rxi®-5HT (30m X 0.25mm ID, 0.25µm df)		
Oven	100°C hold 4 min, ramp 4C/ min. to 240C, hold at 240° 20 min.		
Injector temp.	225°C		
Detector temp.	250°C		
Mode	split/ splitless	split	50-1
Carrier gas	helium		
Injection vol.	1 µL		

Analytical standards were purchased from Restek and fractionated using Ag-Ion columns and used to create a method and standard curve for analysis of FAME concentration at sampling intervals. Fractionated FAME reference standards are shown in Fig. 1.

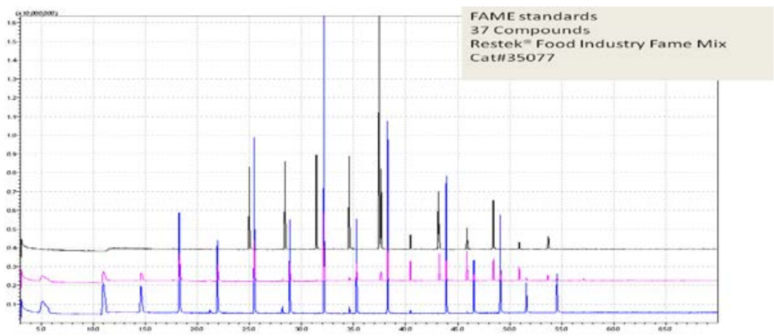

Figure 1: Stacked chromatograms of fractionated FAME standards. Frac A (bottom) contains saturated FAMEs, Frac B (middle) contains mostly mono-unsaturated FAMEs, and Frac C (top) contains mostly polyunsaturated FAMEs.

3. RESULTS AND DISCUSSION

3.1 Field test results

The decking specimens are in their second year of rating. The 2011 ratings have not been taken in MS and therefore only 2010 ratings are presented. Overall, the surfaces have greyed considerably. A comparison of freshly installed decking to 2 year weathered specimens in WI is shown in Fig. 2.

Figure 3: Composite image showing NDW decking specimens at installation (top) compared to NDW specimens after 24 months above ground field exposure (bottom). (HM=honey mesquite, AYC=alaska yellow cedar, BL=black locust, PAW=paulownia, WCJ=west coast juniper, CAT=southern catalpa, ERC=eastern red cedar, SYP=southern yellow pine (control), and WRC= western red cedar.)

Some decay has initiated in the Madison non-durable controls (SYP). Fungal colonization was recorded after 12 months exposure on the underside of several SYP decking specimens. Fungal colonization almost always occurred underneath the board where the board joins with the rack and forms a natural catch point for moisture. Mean decay ratings for deck-boards in WI and MS for the 2010 rating cycle are presented in Fig.4.

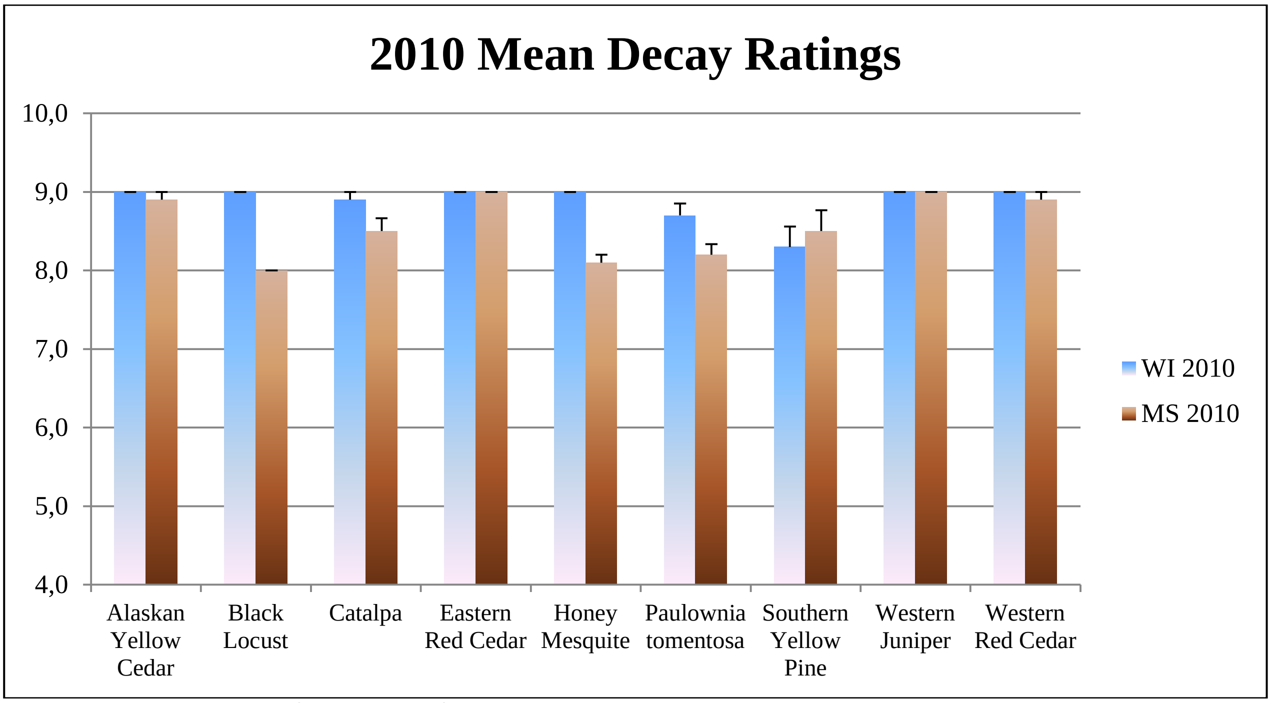

Figure 4: Mean Decay Ratings of Deck-boards after 12 months above ground exposure in WI and MS.

The durable softwoods (AYC, ERC, WRC, and WCJ) all appear to be performing quite well in the field study with mean ratings of 9.0 in WI and slightly less than 9.0 in MS. AYC does appear to have some surface blemish that is most likely caused by wasps (see lower AYC, Fig. 3). The wasps peel the surface of the wood for nests and food and this leaves a characteristic mark on the surface. Black Locust and Honey Mesquite are performing well in WI (9.0), but are showing signs of degradation in MS (~8.0).

Figure 5: Cross checking of juvenile wood in Southern Catalpa after 12 months of weathering in WI.

Catalpa is performing well in WI (9.0), but not as well in MS (~8.5). Catalpa is showing moderate cupping and quite a bit of checking (Fig. 5). Catalpa is also showing signs of cross checking on the surface that appears to be juvenile wood causing defects on the decking surface.

Paulownia is not holding up well in this test, the ratings suggest that it is equivalent to Honey Mesquite, but the surface of the decking is severely checked (Fig 6). In-ground tests of *Paulownia* have almost all failed in WI after one year (unpublished data).

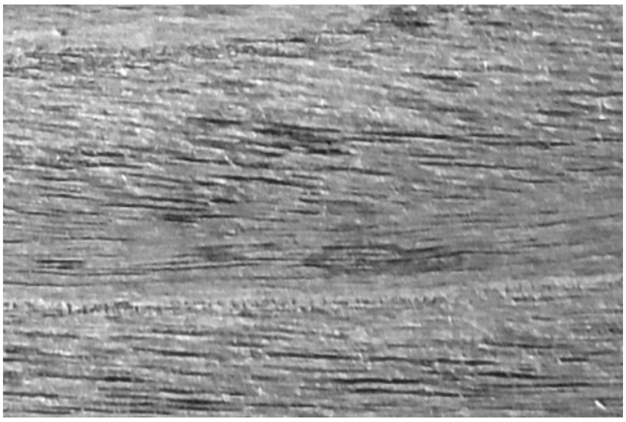

Figure 6: Severe checking on decking specimens of *Paulownia* after 12 months field exposure in WI.

Southern yellow pine is considered the non-durable control in this test and is performing precisely at that level (8.3 (MS), and 8.5 (WI)). Underneath the decking specimen, decay has already initiated at the catch point between the rack and the deck boards. Incipient white rot as well as severe mould growth was noted on SYP after only 12 months of exposure in WI (Fig 7.).

Figure 7: Incipient decay by white rot fungi noted on the underside of SYP decking specimens after 12 months field exposure in WI.

3.2 Results of Chemical analyses

Initial fatty acid composition extracted from the naturally durable wood species is shown in Fig. 8 as a percent weight. From initial to 2 year exposure in WI, there was a significant decrease in saturated fatty acids across all of the species, however, mono- and poly-unsaturated FAMEs held steady from year 1-2 (data not shown).

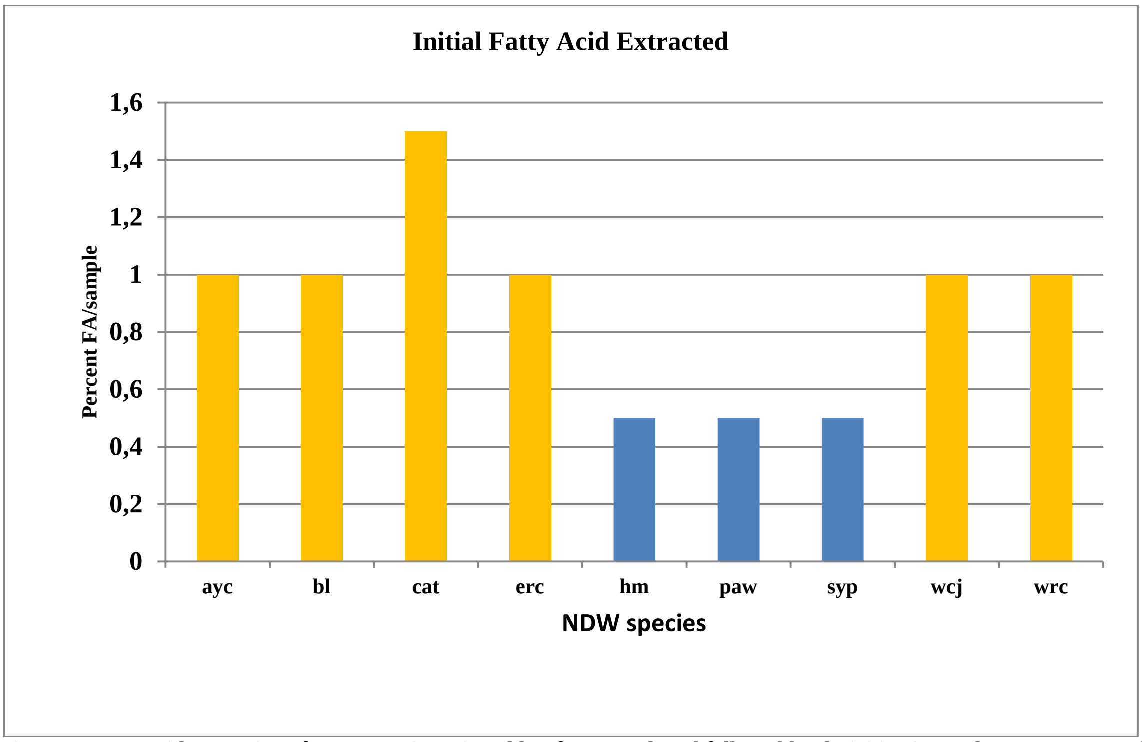

Figure 8: Fatty acid extraction of NDW species using chloroform: methanol followed by derivitization. Solvent was removed by rotary-evaporation and samples weighed to determine % FA by weight of wood for each species.

The initial FA extraction yielded 0.5-1.5% fatty acid per unit weight (g). PAW and SYP had lower amounts (0.5%) while the "more durable" species yielded closer to 1% fatty acid. CAT had the highest recovery at 1.5% per unit weight. HM, however, was lower than the other "durable" species, with 0.5% FA. Overall, it appears that the more "durable" species contain more fatty acids.

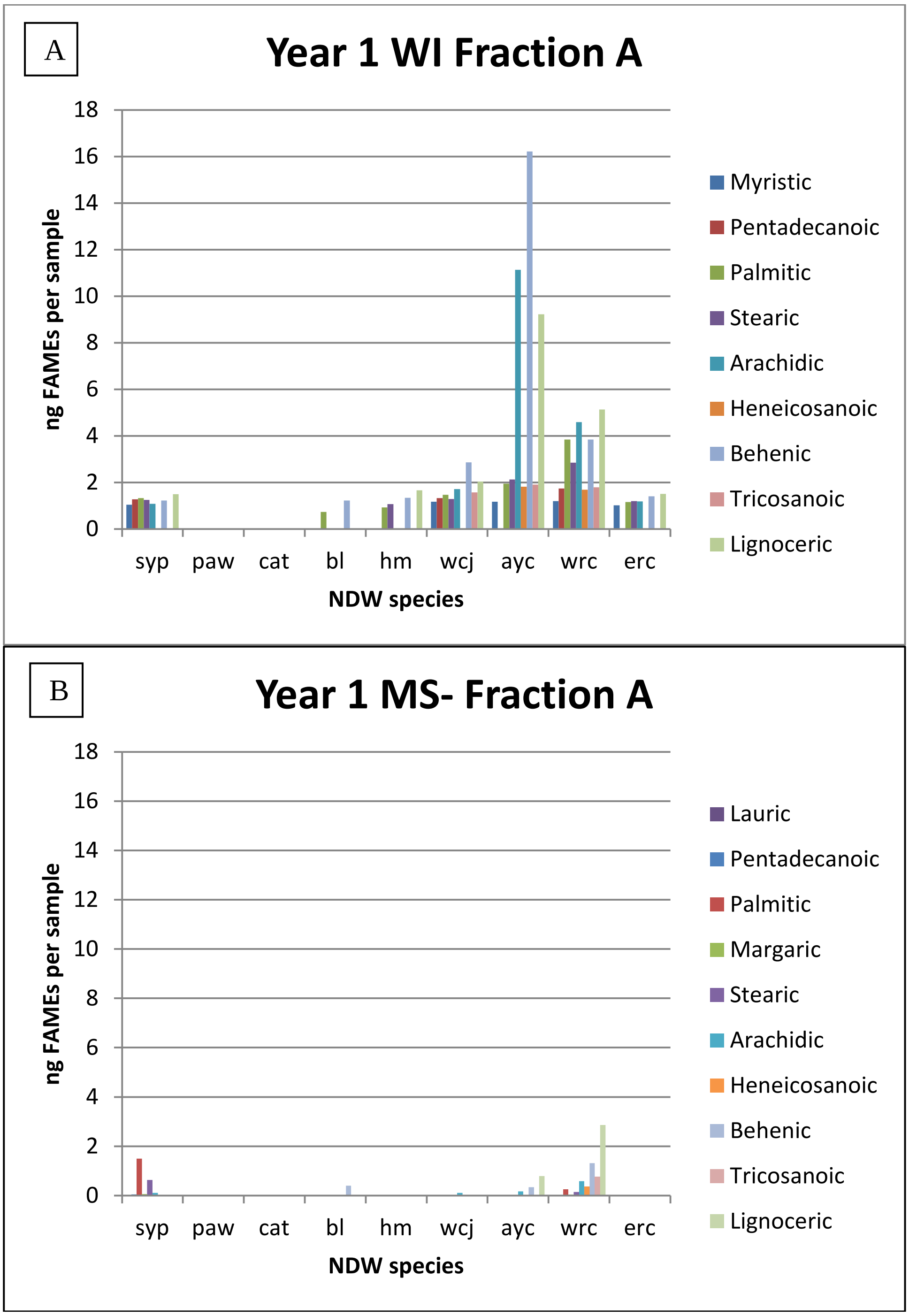

Figure 9: Identified saturated FAMEs in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample.

Fraction A contains saturated FAMEs (no double bonds), trans monoenes, and conjugated linoleic acids. In WI, AYC contained the highest single FAME (behenic,16 ng) and had the highest diversity of saturated FAMEs (7) . PAW and CAT had no saturated FAMEs detected. Overall, saturated FAMEs were ~11times lower in samples from MS compared to WI. Lignoceric acid in WRC was the highest saturated FAME in MS (2.86 ng) and WRC had the highest diversity of FAMEs of the MS samples (7).

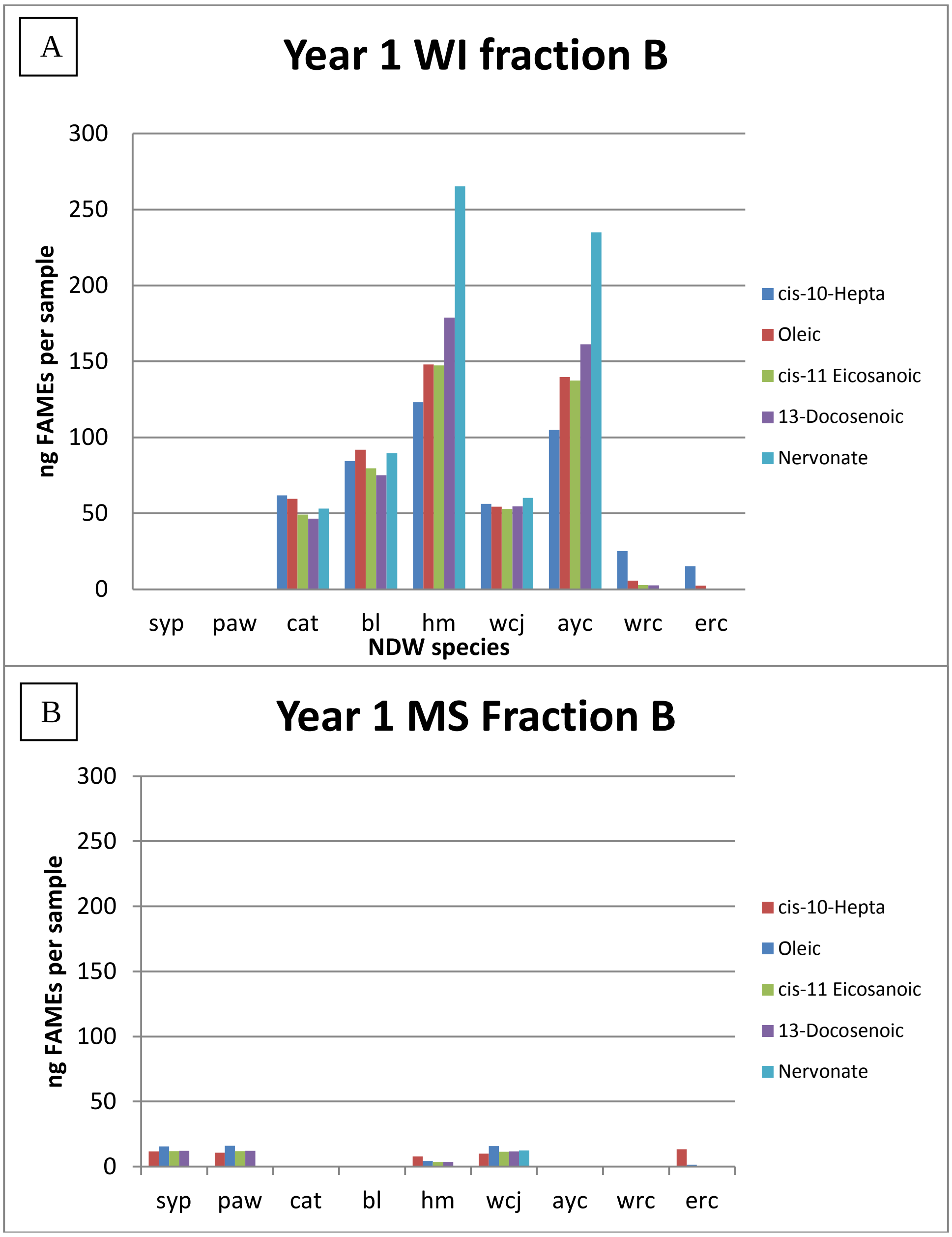

Figure 10: Identified mono-unsaturated FAMEs in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample

Fraction B contains cis monoenes (single bonds), and trans/trans dienes (double bonds). In WI, HM contained the single highest FAME in fraction B (nervonate, 265 ng), followed by AYC (nervonate, 234 ng). HM and AYC also contained the highest amounts of 13-docosenoic acid (178 and 161 ng, respectively), Oleic (147 and 139 ng), 11-Eicosanoic (147 and 137 ng), and cis-10 heptanoic acid (123 and 104 ng).

In MS, WCJ had the highest diversity of single bonded FAMEs (5). CAT, BL, AYC, and WRC all had no single bonded FAMEs detected. FAMEs were ~13 times lower in MS compared to WI after 1 year of exposure.

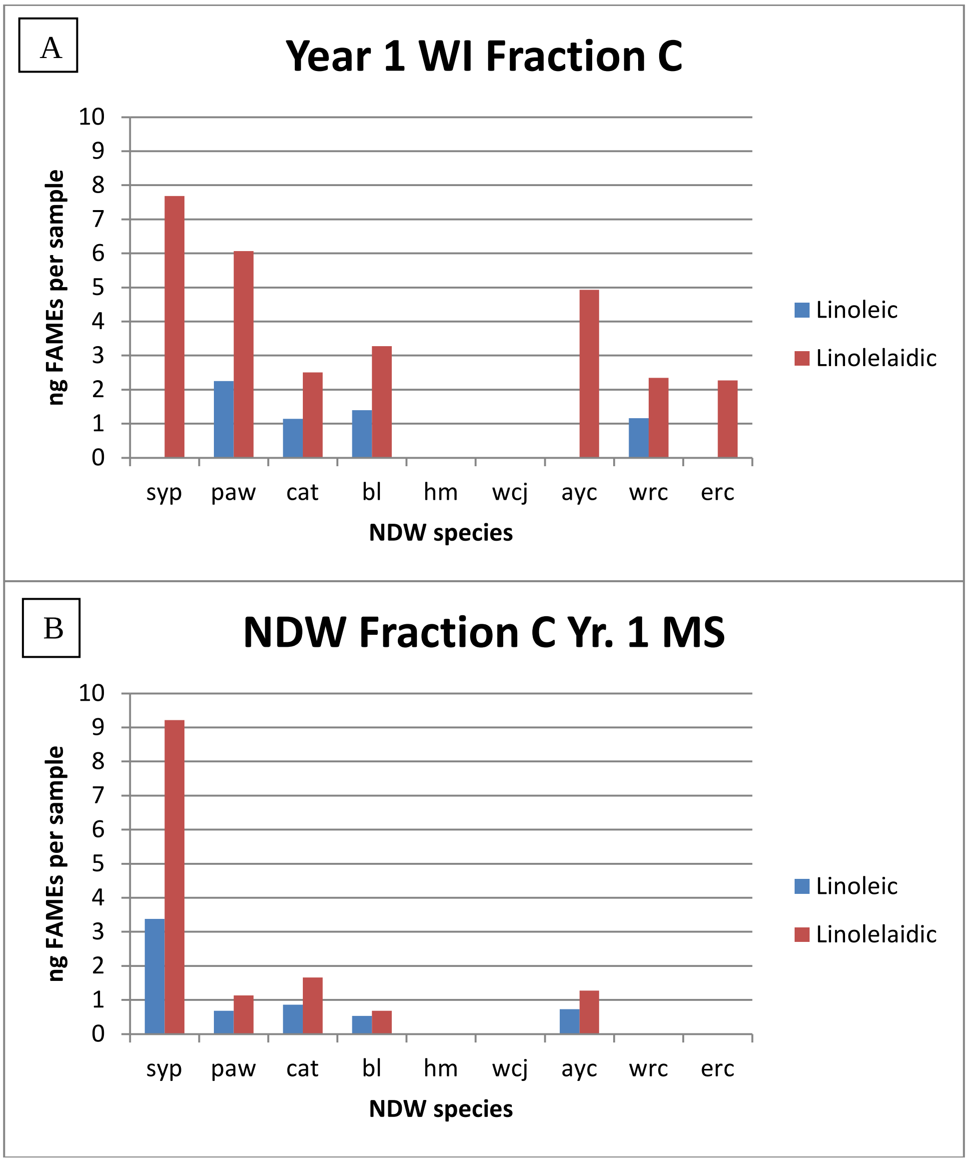

Figure 11: Identified poly-unsaturated FAMEs, linoleic and linolelaidic acids, in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample.

Fraction C contains dienes and trienes (triple bonds). Linoleic and linolelaidic acids were the only FAMEs identified. In WI, PAW had the highest amount of linoleic (2.25 ng), while SYP had the highest amount of linolelaidic acid (7.69 ng). PAW had 6.6 ng of linolelaidic acid. In MS, SYP had the highest levels (9.22 ng linolelaidic, 3.38 ng linoleic). All others had less than 2 ng of both linoleic and linolelaidic acids. Identified FAMEs were ~2 times lower in MS compared to WI.

It should be noted that leaching of FAMEs was evident in this study when MS is compared to WI. Total amounts of FAMEs were always lower from the MS samples, but the difference becomes less with increasing unsaturation of the FAMEs.

3.3 Fungal Inhibition Assay

In order to test the hypothesis of direct biological activity against decay fungi, an inhibition assay was conducted. FAME fractions (saturated, mono-, and polyunsaturated) from the year one WI samples along with solvent controls were added to sterile filter paper disks and allowed to dry. Disks were placed on agar plates spread with mycelium of three decay fungi (*G. trabeum*, *T.*

versicolor, and *P. placenta*). No inhibition of these fungi was seen in this assay. It was concluded that these FAMEs at this concentration do not have direct antifungal activity against these decay fungi.

4. CONCLUSIONS

4.1 Field Evaluations

Field evaluations will continue on these specimens for the next 4 years. Based on observations thus far, *Paulownia* is non-durable for above ground applications. *Paulownia* has been suggested to have anti-termitic properties, but it is not generally considered resistant to decay. In-ground studies of *Paulownia* in WI have all failed within 12 months, showing great susceptibility to white rot fungi (unpublished data). *Paulownia* has also been evaluated at FPL against *Reticulitermes flavipes*, and it was found to be susceptible to termite attack (*Rachel Arango, Personal communication*). ERC, WRC, WCJ, and AYC are all performing well as anticipated. CAT, HM, and BL seem to be performing slightly better in WI compared to MS, but not as well as the cedars.

4.2 Chemical analyses

Wood samples will continue to be taken at yearly intervals for chemical analysis. The focus of these chemical analyses will likely shift to other chemical compounds, as it seems FAMEs are a poor choice due to their lack of permanence and limited biological activity against decay fungi. Other classes of compounds (terpenoids, phenolics, tannins, sterols) are currently being explored. Due to the great taxonomic diversity of these species, narrowing the focus to discreet classes of compounds has proven to be challenging. Based on our initial observations, terpenoids analysis would be well suited for the coniferous species in this study, but terpenoids are absent or lacking in the catalpa, honey mesquite, and black locust, all of which are still “durable”. Black locust has flavonoids that have been shown to have antifungal properties (Shain 1977), and honey mesquite contains stilbenes and hydrolyzed tannins that may contribute to its longevity (Hillis 1962). Southern catalpa heartwood contains sesquiterpine alcohols, ketones, and phthalides that have anti-termitic activity (McDaniels 1992) and antifungal properties (McGray and McDonough 1954). Each group of chemical compounds mentioned has its own associated solvent affinities, extraction procedure, and analytical parameters. The goal of this project is to track the degradation of these chemical compounds and correlate loss of extractive components to how the specimens weather. Inversely, remaining extractives could be used as biological markers to assess the condition of the wood based on chemical composition.

Our goal in this study was to gain insight into what chemicals may be contributing to natural durability. Fatty acids were selected initially due to their cited biocidal activity against decay fungi, or at least a co-biocidal effect due to their interactions with cell membranes (Pohl, et al. 2011). Previous work done at FPL has focused on fatty acid chemistry and their efficacy as mould inhibitors and decay prevention (Coleman and Clausen 2008), but these are typically C3-C8 range (propionic, butyric, caprylic, octanoic acids). The majority of fatty acids evaluated in this study fall in the C10-C21 range. It should be noted that fatty acids represent a huge class of chemical compounds, and lack of activity by this select group in this study does not rule out their biological relevance during the decay process. Continued analyses of chemical compounds contained within these wood species may provide a better understanding of what confers natural durability.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

American Wood Protection Association (2011). AWPA Book of Standards. Birmingham, AL.

Arango, Rachel (2012). Personal Communication. 2/8/2012.

Christie, W.W. (1989): Gas chromatography and lipids. 4th Ed. The Oily Press Ltd.

Coleman, R.D., and C.A. Clausen (2008): Multifactorial antimicrobial wood protectants. IRG WP Document IRG/WP 08-30484. Paper prepared for the IRG Americas Regional Meeting, Play Flamingo, Guanacaste, Costa Rica.

Dowhan, W. and M. Bogdanov (2008): Functional roles of lipids in membranes. In: *Biochemistry of Lipids, Lipoproteins and Membranes* (5th Edition), eds. D.E. Vance and J.E. Vance, Elsevier Science. pp. 1-37.

Hillis, W.E. (1962): *Wood extractives and their significance to pulp and paper industry*. Academic Press. New York . pp. 59-131.

Kabara, J.J., Sweiczkowski, D.M., Conly, A.J., and J.P. Truant (1972): Fatty Acids and derivatives as antimicrobial agents. *Antimicrobial Agents and Chemotherapy*. 2(1): 23-28.

McDaniels, C.A. (1992): Major anti-termitic compounds of the heartwood of southern catalpa. J. of Chemical. Ecology.: 18(3): 359-369.

McGray, R.J. and E.S. McDonough (1954): Antimycotic effects of an extract of catalpa. Mycologia, 46(4): 463-469.

Pohl, C.H., Koch, L.F., and V.S. Thibaine. (2011): Antifungal free fatty acids. In: *Science against microbial pathogens: communicating current research and technological advances.* ed A. Méndez-Vilas (Ed.). Formatex 2011.

Rihakova, Z.,. Plocková, M., · Milada · Filip, V., and J. Smidkral (2001): Antifungal activity of lauric acid derivatives against *Aspergillus niger*. European Food Research and Technology 213: 488–490.

Scheffer, T.C. and J.J. Morrell. (1998): Natural durability of wood: a worldwide checklist of species. Forest Research Laboratory, Oregon State University. Research contribution 22, 528p.

Schmidt, E.L. (1984): Influence of aliphatic fatty acids on spore germination of wood decay fungi. IRG WP Document IRG/WP/2224. Paper prepared for the 15th Annual Meeting, Sweden.

Shain, L. (1977): The Effects of extractives from black locust heartwood on *Fomes rimosus* and other decay fungi. Proceedings of the American Phytopathological Society. 3: 216.

USDA Forest Products Laboratory (2010): Wood Handbook, Wood as an Engineering Material. FPL-GTR-190. Madison, WI. 508pp.

Walters, D., Raynor, Mitchell, Walker, and K. Walker (2004): Antifungal activities of four fatty acids against Plant Pathogenic fungi. *Mycopathologia* 157: 87–90.