Forest
Products
Laboratory

General
Technical
Report
FPL–GTR–224

In cooperation
with the

United States
Department of
Transportation

Federal
Highway
Administration

United States Department of Agriculture
Forest Service

# Evaluating Naturally Durable Wood Species for Repair and Rehabilitation of Above-Ground Components of Covered Bridges

**Grant T. Kirker**
**Carol A. Clausen**
**A.B. Blodgett**
**Stan T. Lebow**

## Abstract

More than 1,500 covered bridges remain in the United States. They are a unique part of our history; thus, replacement of bridge components is an equally important part of preserving this uncommon style of craftsmanship. The goal of this project was to evaluate seven wood species for their durability in above-ground field exposure. Chemical analysis was also conducted using gas chromatography–mass spectrometry (GS–MS) for fatty acids and terpenoids in an attempt to correlate extractive content with durability. Extracts removed from the durable wood species were also tested in laboratory bioassays to determine their biological activity against wood decay fungi and termites. This report serves as a guide for the use of these naturally durable wood species for rehabilitation of above-ground components of covered bridges and incorporates the results of field and laboratory tests into the final recommendations.



Keywords: Natural durability, above-ground testing, decking, wood decay fungi, weathering, subterranean termites.


## Contents




July 2013

Kirker, Grant T.; Clausen, Carol A.; Blodgett, A.B.; Lebow, Stan T. 2013. Evaluating naturally durable wood species for repair and rehabilitation of above-ground components of covered bridges. General Technical Report FPL-GTR-224. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 40 p.

A limited number of free copies of this publication are available to the public from the Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53726–2398. This publication is also available online at www.fpl.fs.fed.us. Laboratory publications are sent to hundreds of libraries in the United States and elsewhere.

The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin.





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.

This study is conducted under a joint agreement between the Federal Highway Administration–Turner Fairbank Highway Research Center, and the Forest Service – Forest Products Laboratory.

Federal Highway Administration Program Manager– Sheila Rimal Duwadi, P.E.

Forest Products Laboratory Program Manager– Michael A. Ritter, P.E.

# Evaluating Naturally Durable Wood Species for Repair and Rehabilitation of Above-Ground Components of Covered Bridges

**Grant T. Kirker,** Research Forest Products Technologist
**Carol A. Clausen,** Supervisory Research Microbiologist
**A.B. Blodgett,** Biological Science Technician
**Stan T. Lebow,** Research Forest Products Technologist

Forest Products Laboratory, Madison, Wisconsin

## Introduction

More than 1,500 covered bridges remain in the United States. They are a unique part of our Nation's history; thus, replacement of bridge components is an equally important part of preserving this uncommon style of craftsmanship. Covered bridges are designed for protection from the elements, but it is difficult to protect all wood components from moisture exposure. Some areas of the structures, such as weatherboarding and wood members near the ends of the bridges, are very particularly susceptible to wind-driven precipitation. Chronic wetting or wet–dry cycling can create conditions that are conducive to biodeterioration. Consequently, biodeterioration from decay and insects is frequently responsible for replacement of above-ground covered bridge components. Treatment of susceptible components with chemical wood preservatives is one way to protect them from biodeteriorating organisms. However, naturally durable domestic wood species, particularly underutilized or invasive species, could provide a more suitable alternative to chemically treated wood products for above-ground replacement components.

The original structural elements of covered bridges were not treated with preservatives. Historically, covered bridges were constructed from locally sourced old-growth stands of local wood species and wood species known to be more resistant to rot, such as white oak (*Quercus alba* L.) (Pierce and others 2005). Subsequently, covered bridges in the Eastern United States were commonly made from eastern hemlock (*Tsuga canadensis*), white pine (*Pinus strobus*), and spruce (*Picea* spp.), whereas Douglas-fir (*Pseudotsuga menziessii*) was used most often in the west, and southern covered bridges were mostly built with southern pine (Pierce and others 2005). Whereas old-growth (first-growth) southern pine (*Pinus* spp.), spruce, and Douglas-fir were commonly used and adequately durable in the 18th and 19th centuries to build covered bridges, second-growth stands of the same wood species are typically nonresistant to decay (Clausen 2010) and should not be considered for replacement components unless they are pressure treated with a wood preservative. In lieu of preservative treatment, locally sourced, naturally durable wood species should be considered for replacement components, such as floor elements and trusses, when rehabilitating a covered bridge. By design, the risk of fungal decay on interior components of a covered bridge that are not exposed to wetting is low. That risk is highly dependent on wood species, exposure to rainfall, local temperature, and ability of the component or structural design to shed moisture quickly. Decay risk can be elevated by trapping water or condensation in joints between components, around bolts, or by connectors that channel water to the interior of the bridge enclosure.

Chemical extractives have long been recognized as key features that impart natural durability in the heartwood of certain wood species (Scheffer and Cowling 1966; Taylor and others 2002). The field of naturally durable wood is a huge body of literature, but excellent reviews can be found in Taylor and others (2002), Singh and Singh (2012), and Yang (2009). The review by Taylor and others (2002) is especially useful for understanding heartwood extractives and their natural roles. Extractives from some wood species have been evaluated for use as environmentally friendly preservatives or additives to coatings (Laks and McKaig 1988; Van Acker and others 1988; Kennedy and others 2000; Smith and others 1989; Chedgy and others 2009), but little is known about the chemical makeup of many wood extractives as it relates to natural durability. Unlike extractives from a well-known durable wood such as western redcedar (*Thuja placata*), extractives from lesser-known wood species believed to be responsible for high resistance to decay and insects have not been isolated, identified, and characterized. Correlating chemical properties of extractives in laboratory tests with field performance may increase opportunities for utilizing lesser known wood species as locally sourced materials for repair and rehabilitation of covered bridge components in protected outdoor exposure. The chemical component(s) responsible for imparting durability could also provide the basis for future research on environmentally benign wood protection systems for above-ground components of covered bridges.

**Table 1. Botanical and common names for the wood species evaluated in this study and their classification as underutilized or invasive species**

| Abbreviation | Common name | Botanical name | Category |
|---|---|---|---|
| AYC | Alaska yellow cedar | *Chamaecyparis nootkatensis* | UU[a] |
| BL | Black locust | *Robinia pseudoacacia* | UU |
| CAT | Catalpa | *Catalpa* spp. | UU |
| ERC | Eastern redcedar | *Juniperus virginiana* | UU |
| HM | Honey mesquite | *Prosopis gradulosa* | IS[b] |
| PAW | Paulownia, princess-tree | *Paulownia tomentosa* | IS |
| SYP | Southern pine | *Pinus palustris* | Negative control |
| WCJ | West Coast juniper | *Juniperus occidentalis* | UU |
| WRC | Western redcedar | *Thuja plicata* | Positive control |

[a]Underutilized.
[b]Invasive species.

# Objective and Scope

Above-ground performance of treated and untreated wood has been the subject of numerous studies worldwide (Carey and Bravery 1986; Carey and others 1981; Carey 2002; Fougerousse 1976; Highley 1984, 1993; Morris and McFarling 2007; Scheffer 1971; Savory and Carey 1979; Williams and others 1995). Above-ground tests are useful for evaluating the natural durability of building components intended for applications protected from the environment (Clausen and Lindner 2011; Clausen and others 2006; Eslyn and others 1985; Highley 1995; Räberg and others 2005). A number of standardized and alternative test designs have been evaluated; e.g., post-rail, cross braces, Y-joints, simulated deck test, L-joints, and lap joints (DeGroot 1992; Hedley and others 1995; Morrell and others 1998; Morris and McFarling 2007), with L- and lap-joints most commonly used. One alternative test assembly, the simulated deck test, addresses the need for accelerated test methods to speed up the otherwise lengthy period of time necessary to acquire sufficient performance data for new preservatives (Williams and others 1995; Morris and McFarling 2007). In this study, field performance for natural durability was assessed through comparative above-ground simulated deck tests that approximate in-service conditions in Mississippi (Severe decay hazard zone 5) and Wisconsin (Moderate decay hazard zone 2). Under conditions of high decay hazard, Southern Pine typically fails in above-ground exposure in just 12 months.

Tannins, phenols, and other resin acid extracts from certain species of wood have been found to be fungitoxic and are believed to be responsible for imparting natural durability to certain wood species. The chemical composition of pine cone extracts (Micales and others 1994), pine needle extracts (Zinkel and Magee 1991), and the efficacy of pine and spruce extractives against brown-rot and white-rot fungi have been evaluated (Celimene and others 1999). Western redcedar extractives are perhaps the most studied and best understood of the highly durable wood species in the United States (Stirling and others 2007; Morris and others 2012). However, little is known about the phytochemical composition of durable underutilized or invasive wood species.

The three main objectives of this project were the following:

- To assess field performance of naturally durable wood species in accelerated above-ground field tests in high and moderate decay hazard zones.
- To assess the effectiveness of chemical extractives from those wood species in laboratory tests against decay fungi and termites.
- To characterize the chemical components of wood extractives that successfully inhibited biodeteriorating organisms for future development as “green” wood preservatives.

# Materials

## Wood Selection

Table 1 shows basic information about the species chosen for this study. Considerations for the selection of naturally durable timber species for covered bridge replacement components must also include workability and regional availability. The following excerpts on the characteristics of the wood species selected for this study are taken from Wiemann and others (2010) and Alden (1995, 1997). Distribution maps are adapted from U.S. Geological Survey tree species range maps (USGS 2012) and the United States Department of Agriculture Natural Resources Conservation Service plants database (www.plants.usda.gov/).

### Alaska Yellow Cedar

Alaska yellow cedar (*Chamaecyparis nootkatensis*) is distributed in the coastal forests from southwestern Alaska through British Columbia to northern California (Fig. 1). Alaska yellow cedar trees can attain heights of 120 ft (36.6 m) and diameters of 6 ft (1.8 m). These trees are frequently over 300- y- old to more than 700 y with a record of more than 1,040 y. The sapwood is narrow and sometimes slightly lighter than the bright yellow heartwood. The wood is moderately heavy, soft, fine-textured, straight-grained, and durable. It has moderate strength, stiffness, and hardness, shrinks little during drying, and is dimensionally stable after seasoning. Alaska yellow cedar is easily worked by

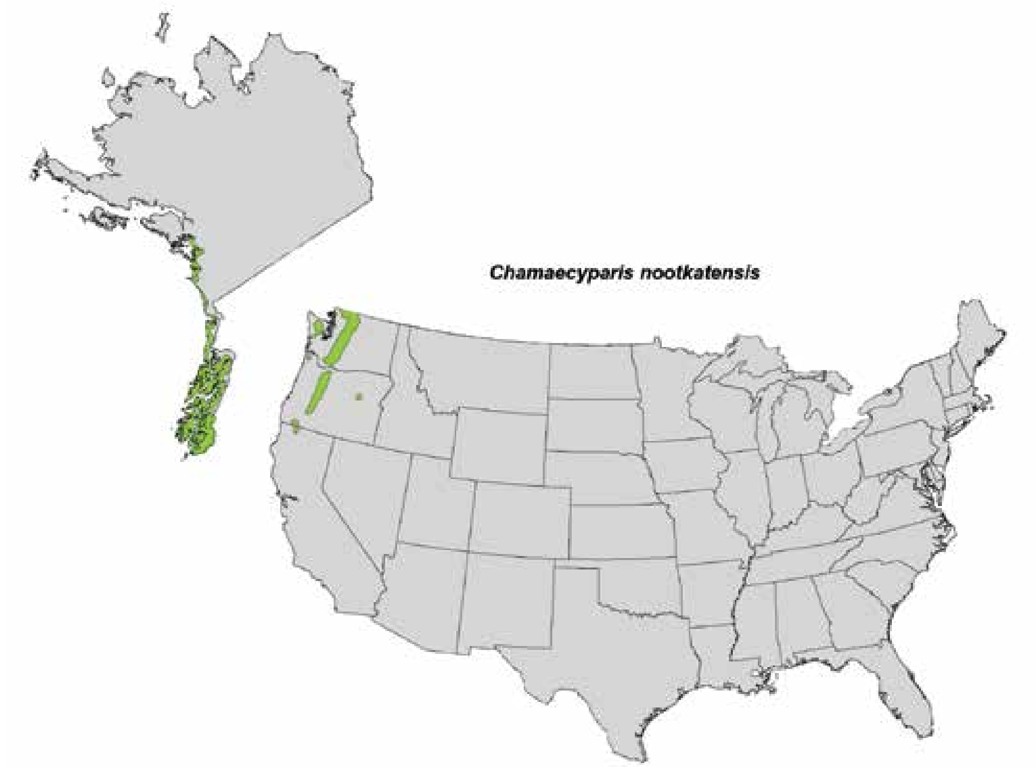


**Figure 1. Alaska yellow cedar (*Chamaecyparis nootkatensis*) distribution in the continental United States and Alaska (Little 1971).**

both hand and machine tools. The heartwood is rated as resistant to very resistant to decay. It has many commercial uses such as bedding for heavy machinery, boat building, bridge and dock decking, cabinetry, cooling towers, framing, furniture, flooring, marine piling, paneling, stadium seats, and utility poles, to name a few.

### Black Locust

Black locust (*Robinia pseudoacacia*) has been extensively naturalized in the United States and is native to the Appalachian Mountains and Ozark Mountain regions (Fig. 2). It also grows in southern Illinois and Indiana. Black locust trees can reach a height of 100 ft (30.5 m) with a diameter of 3 ft (0.9 m). The sapwood of black locust is creamy white and the heartwood varies from greenish yellow to dark brown. The wood turns reddish brown when exposed to air. It has a high density and decay resistance. It shows slight shrinkage and has high bending strength. Because black locust is one of the hardest woods in America, it is difficult to work with hand tools, but it nails well. It is rated as exceptionally resistant to heartwood decay. Common uses include fencing, furniture, and mine timbers. There are reports of dermatitis from direct skin contact with the wood.

### *Catalpa* spp.

*Catalpa* is native to the central eastern United States, but is naturalized throughout the United States and Canada (Fig. 3). There are two commercial North American *Catalpa* species and the wood characteristics are similar for both species. The sapwood is narrow and gray, while the heartwood is grayish brown, tinged with lavender. It is ring porous, straight-grained, light, and soft. It may be confused with ash. *Catalpa* dries quickly and is easy to season. It works very well with hand and machine tools, although it requires care to sand well. The heartwood of *Catalpa* is very resistant to decay, even when it is in contact with soil. It is commonly used for fence posts and rails, general construction, interior finish, picture frames, handles, cabinetry, and fuel wood.

### Eastern Redcedar

Eastern redcedar (*Juniperus virginiana*) is native to the eastern half of the United States (Fig. 4). It has the widest distribution of any other conifer in the eastern Unites States and can reach heights of 120 ft (36.6 m) and a diameter of 4 ft (1.2 m). It is known as a pioneer species as it is one of the first trees to invade disturbed areas. It grows very slowly. Eastern redcedar has thin, white sapwood, and the heartwood is red to deep reddish-brown. The sapwood may be in stripes, alternating with the heartwood. It has a fine, uniform texture and a straight grain, except where deflected by knots, making it easy to work with hand or machine tools. Eastern redcedar splits easily but holds nails well.

The wood is moderately low in strength and stiffness, but high in shock resistance. It has good dimensional stability and shrinks little during drying. The heartwood is highly

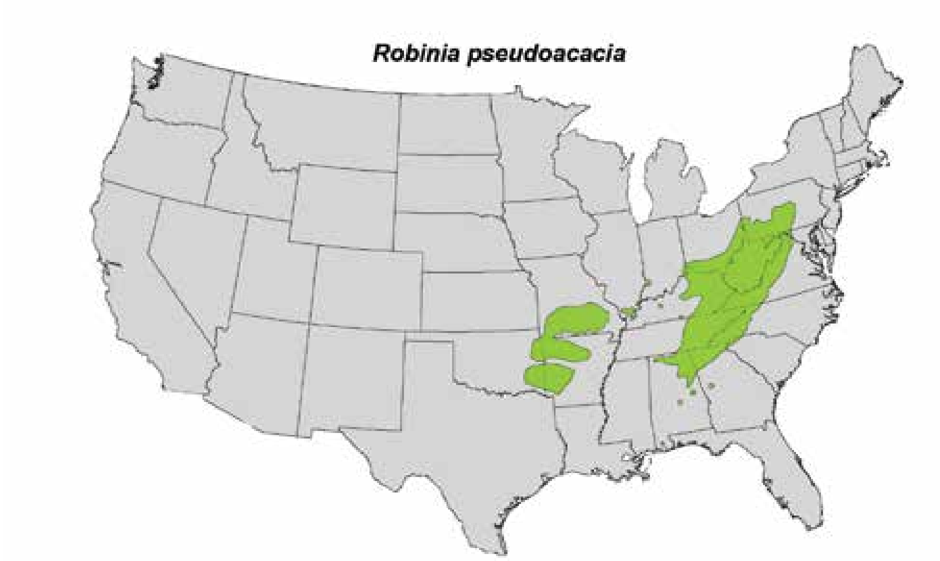


**Figure 2. Black locust (*Robinia pseudoacacia*) distribution in the United States (Little 1971).**

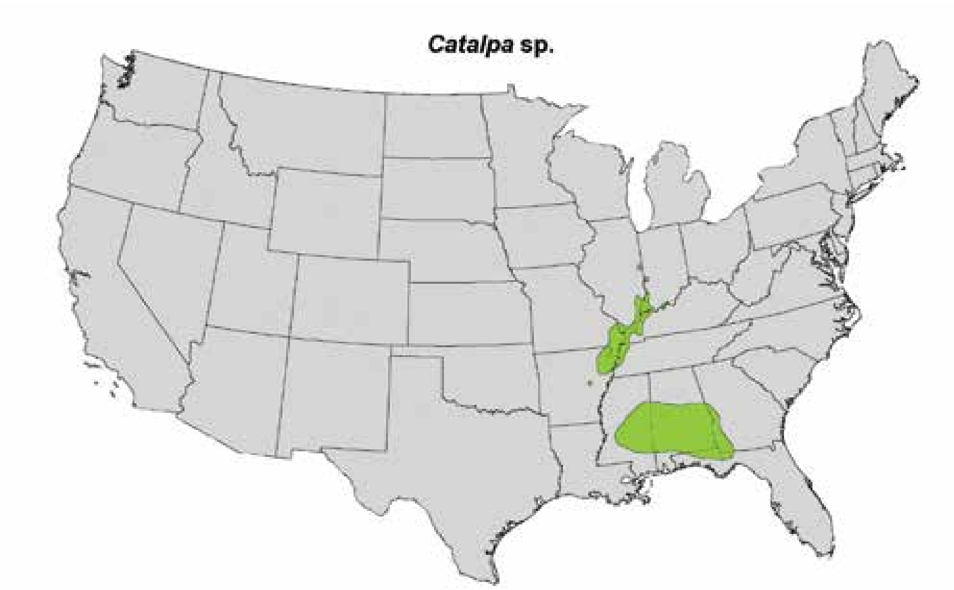


**Figure 3. Distribution of *Catalpa* spp. in the United States (Little 1971, 1977).**

resistant to decay and insects. It is commonly used for fence posts, chests, closet linings, pencils, carvings, pet bedding, furniture, flooring, small boats, and household items. It may cause dermatitis and respiratory problems.

## Honey Mesquite

Honey mesquite (*Prosopis glandulosa*) is one of three North American species of mesquite. Its native range is shown in Figure 5. It reaches heights of 40 ft (12.2 m), with diameters varying from 10 in. (25.4 cm) to 4 ft (1.2 m). The sapwood is a lemon yellow, while the heartwood is deep reddish brown. The wood is dense, close-grained, very hard and heavy, but somewhat brittle. It is exceedingly resistant to heartwood decay, with thin sapwood. It contains high concentrations of tannins and has been reported to cause dermatitis. Honey mesquite is used for buildings, cabinetry, posts, charcoal, fuel, railway crossties, and paving blocks.

## Princess-tree

Princess-tree (*Paulownia tomentosa)* is a popular ornamental and widely recognized as an invasive species and specifically as a noxious weed in some states (Fig. 6). The growth rate is rapid and trees reach their maximum height of 60 ft (18.3 m) and 2 ft (0.6 m) in diameter. They are used for fuel, lumber, and naval stores.

## Western Juniper

*Juniperus occidentalis* is native to the mountains of the Pacific Coast region (Fig. 7). It reaches heights of 35 ft

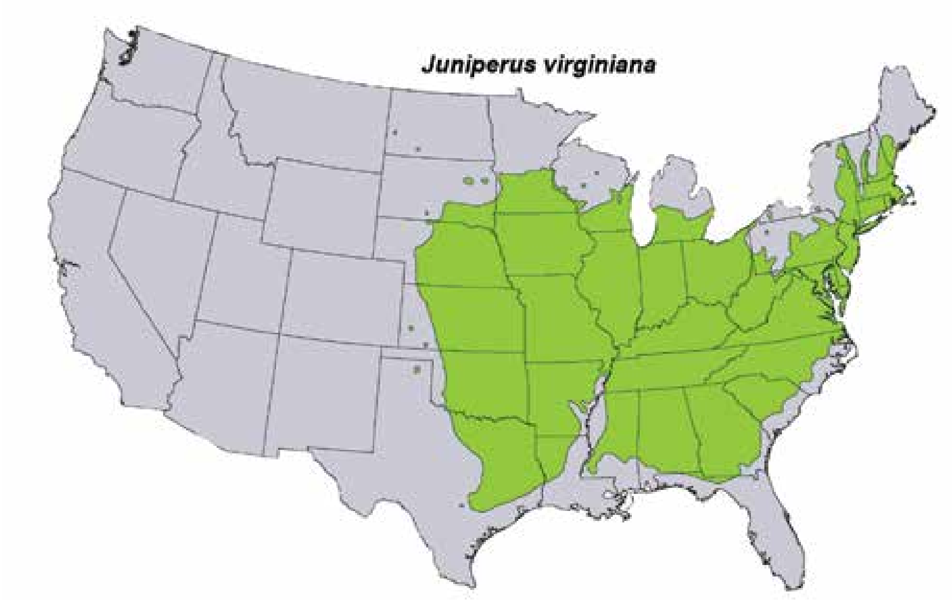


**Figure 4. Eastern redcedar (*Juniperus virginiana*) distribution in the United States (Little 1971).**


**Figure 5. Honey mesquite (*Prosopis glandulosa*) distribution in the United States.**

(10.7 m) with exceptional trees reaching heights of 87 ft (26.5 m), with a diameter of more than 13 ft (4 m). Older trees may live for 1,000 years. The heartwood of western juniper is light red to reddish-brown. It is durable, fragrant, close-grained, moderately dense, lightweight, relatively brittle, and splits easily. The heartwood is reported to have good natural durability. It is easy to work and finish. It is used for fence posts, fuel wood, novelties and pencil wood. Western juniper may cause dermatitis and respiratory problems, so appropriate protection should be used when handling this wood.

## Controls

### Western Redcedar

Western redcedar (*Thuja plicata*) was selected as the positive control in this study. It is lightweight, moderately soft, subject to compression, splinters easily, and is low in strength. These characteristics make it undesirable for replacement components in covered bridges compared with other naturally durable wood species. However, western redcedar is renowned for being very resistant to decay, and volumes of published literature have characterized the

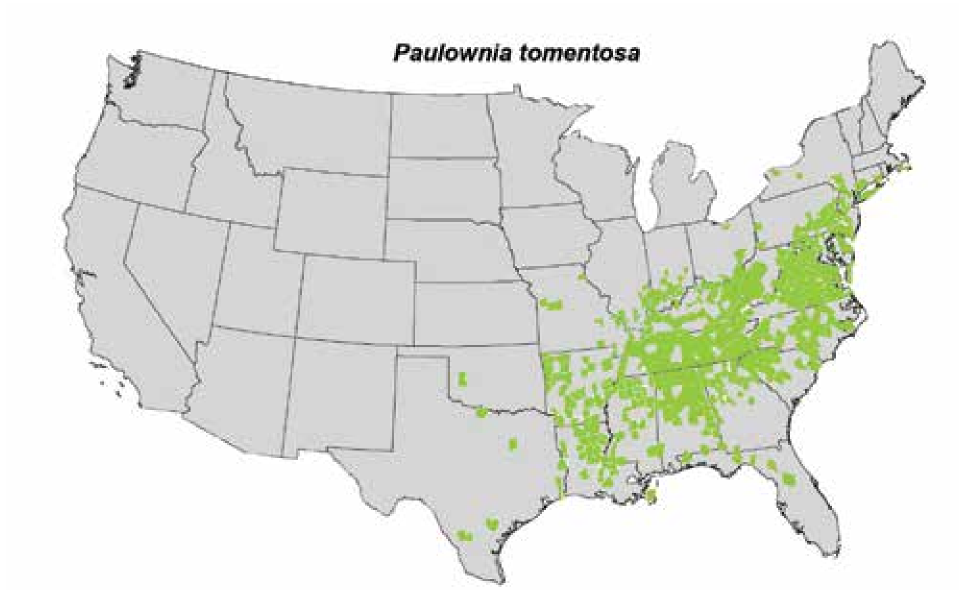


**Figure 6. Princess-tree (*Paulownia tomentosa)* distribution in the United States (NRCS 2012).**

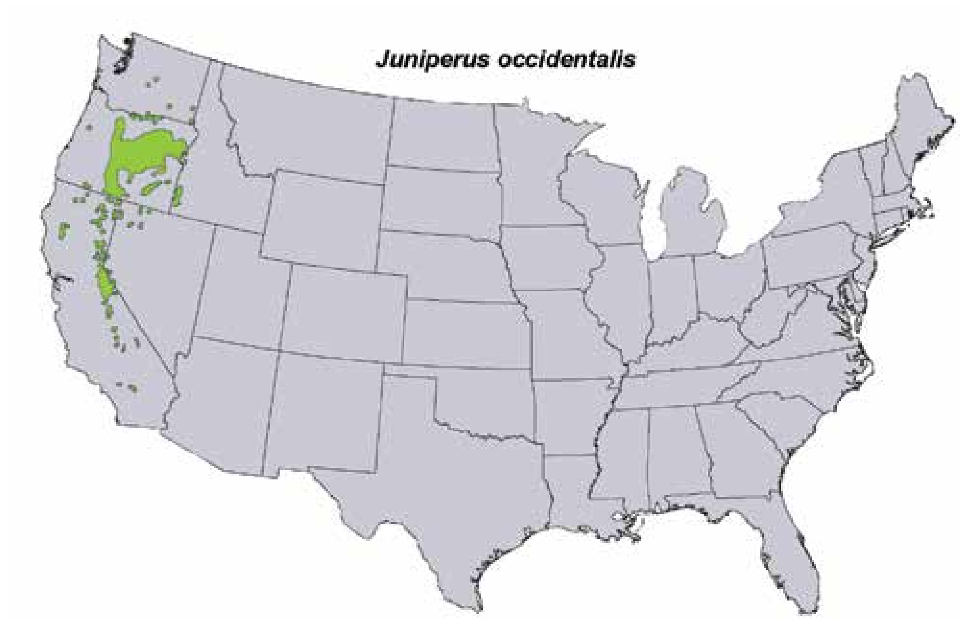


**Figure 7. Western juniper (*Juniperus occidentalis*) distribution in the United States (Little 1971).**

extractives responsible for its natural durability making it a model comparative wood species.

### Southern Pine

Longleaf pine (*Pinus palustris*), designated as SYP, was selected as the negative control in this study. The sapwood is usually wide in second-growth stands and is not resistant to decay. Southern Pine is a term used for a group of pines that includes longleaf pine, shortleaf pine, loblolly pine, and slash pine and has numerous other common names. The southern pines are native to southeastern United States. Because the otherwise nondurable sapwood is quite permeable to preservative treatments, Southern Pine makes up 75% of the treated wood market in the United States.

# Research Methods

## Field Testing

Above-ground durability was assessed at the Forest Products Laboratory exposure site near Madison, Wisconsin, (decay hazard zone 2) and in the Harrison National Forest in Gulfport, Mississippi, (decay hazard zone 5) using a modification of the E25-08 field test decking method (AWPA 2011a). Five untreated decking specimens (5.1 by 15.2 by 45.7 cm long) or (2-in. by 6-in. nominal by 18-in. long) cut

**Table 2. Rating system for above-ground deck test**

| Scale | Condition of specimen |
|---|---|
| 10 | Sound |
| 9.5 | Trace/Suspicion of decay |
| 9 | Slight attack |
| 8 | Moderate attack |
| 7 | Moderate /Severe attack |
| 6 | Severe attack |
| 4 | Very severe attack |
| 0 | Failure |

from each wood species were fastened to a simulated deck structure 91.4 cm (36 in.) above the ground at each test site. The exposure platform was designed so that the front edge of each specimen hung over the platform and the back edge of each specimen was flush with the platform and abutted a pine feeder (5 cm by 15 cm (2-in. by 6-in.) nominal) positioned at a 90° angle to the edge of the test specimen. Each year for 3 y, the specimens were visually evaluated for decay, insect attack, dimensional stability (cupping and checking), and overall appearance. Specimens were rated according to E25-08 (AWPA 2011a) with additional measurements for cupping and checking recorded during each evaluation (Table 2).

### Chemical Extraction and Characterization

Wood samples were Wiley-milled to 40 mesh size, extracted using various solvents (details follow) using a soxhlet apparatus, concentrated on a rotary evaporator and re-suspended in hexane or methanol (depending on target analytes) for analysis. The chemical analysis focused on two major groups of chemical compounds: (1) fatty acids and (2) terpenoids. Fatty acids were chosen because previous research indicated the presence of antifungal and antibacterial properties at high concentration, and we hypothesized that the more durable species might contain and retain higher concentrations of certain fatty acids. Terpenoids were an obvious second choice; they have been implicitly linked to the durability of western redcedar (Morris and Sterlling 2012) and have demonstrated antifungal properties (Abad and others 2007). The goal of the fatty acid analysis was to observe the changes in concentration as the samples weathered.

### Gas Chromatography–Mass Spectrometry (GS–MS) Analysis

#### Fatty Acids

Fatty acids were extracted in 2:1 chloroform: methanol, derivitized using methanolic hydrochloric acid, and quantified using custom c7-c26 fatty acid methyl ester (FAME) standards (Restek) (Fig. 8). Standards and samples were fractionated using solid phase extraction (SPE) columns to separate saturated, monounsaturated, and polyunsaturated fatty acids. Fatty acid concentrations were quantified for years 1–2 and compared with unexposed samples. GS–MS conditions are presented in Table 3.

#### Terpenoids

Terpenoids were the second target in the analysis of extractives of these naturally durable wood species. Terpenoids are a large class of chemicals that are widely distributed in many different plant species. Terpenoids are chemically modified terpenes and are also sometimes referred to as isoprenoids. The name terpene refers to turpentine, which is the most famous of the compounds derived from conifers and frequently used as a solvent and to make other chemicals. Pure terpenoids were selected for analysis and used to create a standard curve for quantitation and an analytical method was produced that fully separated the peaks of interest (Fig. 9). Ethanol: toluene extractives from the preparation of extractive-free wood (See Chemical Analyses section) were analyzed using the terpenoid method and quantified. Additional terpenoids not included in the standard set were identified using the GS–MS spectral library.

## Data Analysis

GS–MS data were compiled into tables to compare fatty acid and terpenoid composition across species and time intervals. Fatty acid profiles were compared from unexposed and exposed samples to determine depletion of fatty acid methyl esters (FAME)s over time. FAME composition was also compared between durable species in an attempt to correlate initial FAME composition with field durability. Terpenoid composition of quantified targets was compared between species for unexposed samples and additional terpenoids not in the standard curve were identified using the GS–MS library and tabulated.

## Laboratory Bioassays

### Disk Assay Inhibition Test (Fatty Acids)

Mycelial inoculum was prepared by aseptically inoculating 100 ml of modified Bailey's minimal medium in a 250-ml Erlenmeyer flask with an agar plug from the margin of active fungal growth in a Petri dish culture of four wood decay fungi (two white-rot fungi: *Irpex lacteus* (HHB 7823) and *Trametes versicolor* (Mad 697), and two brown-rot fungi: *Tyromyces palustris* (TYR 6137) and *Gloeophyllum trabeum* (Mad 617). Stationary cultures were incubated at 27 °C for 3 wk until a mat of mycelial growth had covered the surface of the flask. The mycelial mat was aseptically placed in a sterile blender cup with 100-ml sterile deionized (DI) water and blended briefly to create a mycelial suspension of the individual test fungi. Petri dishes of 2% malt extract agar were inoculated with 1 mL of mycelia suspension from individual test fungi. Extractives obtained from naturally durable wood species using ethanol: toluene (See preparation of extractive-free wood (Chemical analyses section)) was used to treat sterile Whatman disks. 100 µL of each ethanol: toluene fraction was added to Whatman cellulose discs and allowed to dry. Plates were incubated at 27 °C and inhibition of fungal growth was recorded at 1, 3, and 7 days.

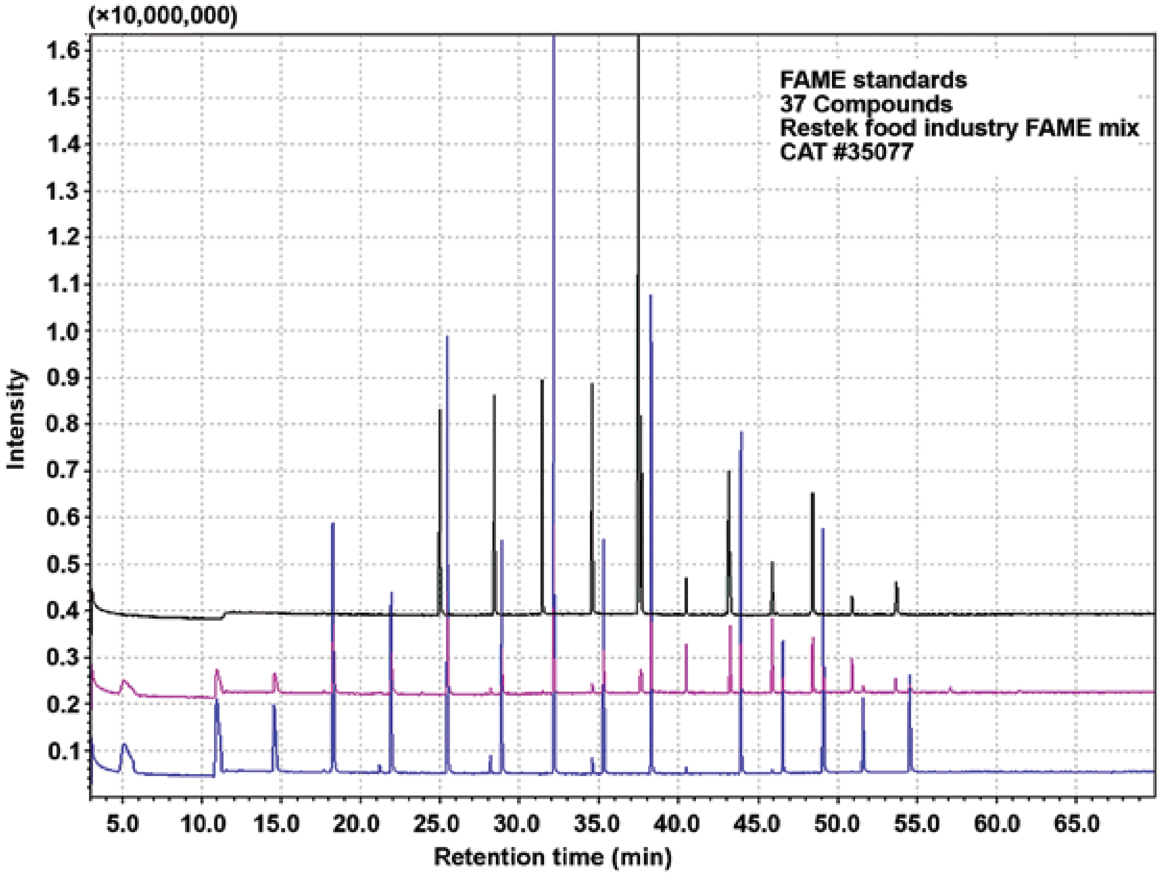


**Figure 8. Fatty acid methyl esters (FAMES) used for quantification of durable wood species. Standards were fractionated to separate saturated FAMES (no double bonds), monosaturated (1 double bond), and polyunsaturated (>1 double bond) FAMES.**

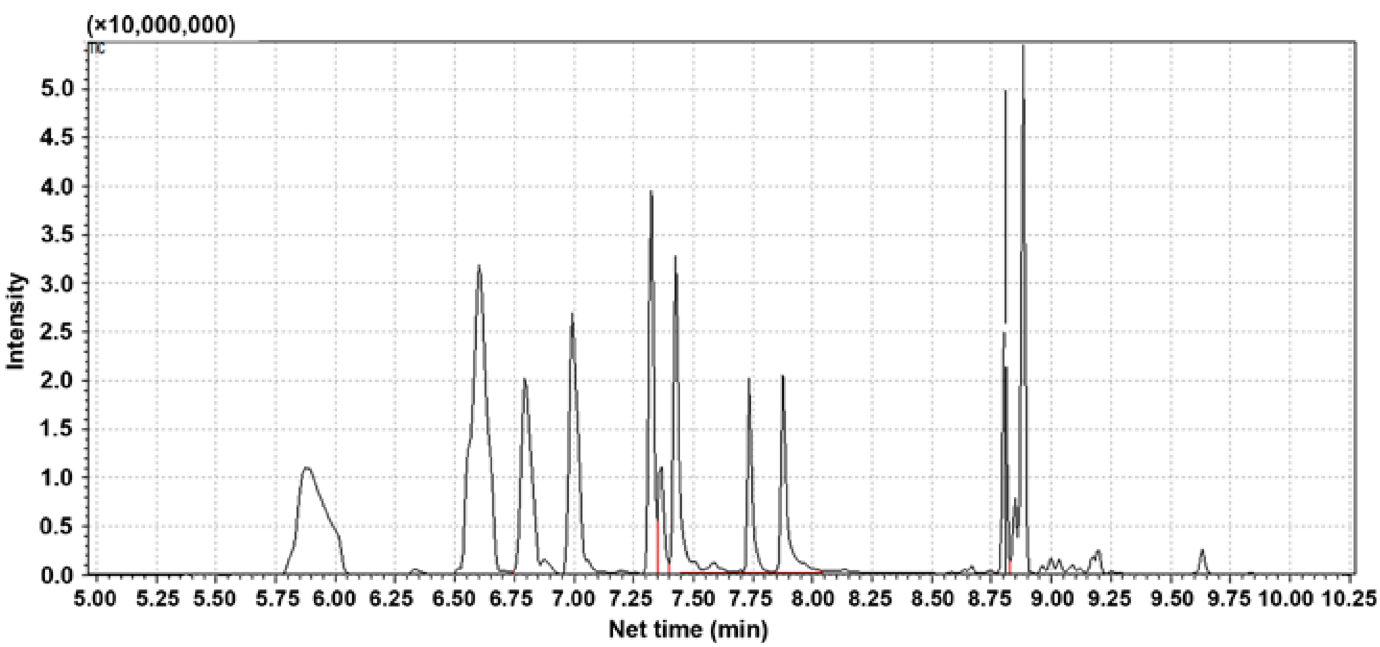


**Figure 9. New targets for terpenoid analyses. 1. Alpha-Pinene, 2. Limonene, 3. Gamma.-Terpinene, 4. Terpinolene, 5. Citronellal, 6. 1-Nonanol, 7. Geraniol, 8. Cedrene 9. Thujopsene.**

### Disk Assay Inhibition Test (EtOH:Tol Extractives)

Extractives were removed from test blocks of the nine naturally durable wood (NDW) species using an ethanol: toluene mix according to ASTM standard D1105 (ASTM 2012). The removed extractives were added to sterile assay disks and aseptically placed on agar plates covered with mycelia of four wood decay fungi (two white-rot fungi: *Tyromyces palustris* and *Trametes versicolor*, and two brown-rot fungi: *Irpex lacteus* and *Gloeophyllum trabeum*). Inhibition of fungal growth was recorded at 1, 3, and 7 days.

### Preparation of Extractive-Free Wood

Ten millimeter cubes were cut from each species of wood. They were numbered and conditioned 1 wk 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 1:0.43 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. Blocks were again rinsed in 95% ethanol and allowed to dry overnight. The following day, blocks were boiled at 100 °C three times consecutively in 1-L portions of distilled water. Blocks were rinsed with hot distilled water and allowed to dry overnight. Blocks were allowed to condition for 1 wk then weighed to determine loss caused by extractive removal. Because each soxhlet apparatus could only hold 24 blocks, the extraction was repeated with a second set of 24 blocks.

### Soil Bottle Experiments (Extracted vs. Unextracted Naturally Durable Samples)

#### Termite Soil Bottle Choice Test

Termite soil bottle choice tests were conducted with one extracted and one unextracted block (10 mm) exposed to 1-g termites in triplicate jars following a modification of AWPA E1-07 choice test bioassay (AWPA 2007). Weighed conditioned blocks were added to plastic cups containing 50 g of sterile washed and sifted sand plus 9 ml of deionized water. After 4 wk, the percentage of termite mortality was visually approximated. Blocks were removed, then conditioned for 1 wk at 26 °C and 30% humidity and final weights were obtained.

#### Laboratory Soil Block Cultures

Laboratory soil block cultures were set up according to a modification of AWPA standard E10 (AWPA 2010). Three extracted and un-extracted blocks were challenged in duplicate soil bottles with one of six wood-decay Basidiomycetes, which included three brown-rot fungi: *G. trabeum* (Pers.: Fr.) Murr. (Mad 617), *Postia placenta* (Fr.) Lars. & Lomb. (Mad 698), *T. palustris* (Berk. & Curt.) Murr. (TYP 6137), and three white-rot fungi: 2 strains of *I. lacteus* (Fr.: Fr.) Fr. (HHB7328 and Mad 517), and *T. versicolor* (L.: Fr.) Pil. (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 propylene oxide (AWPA E10.13.3.3) in glass tubes separated by species and extraction process to prevent volatiles from crossing over between extracted and nonextracted wood specimens. Soil block cultures were placed in an incubation room at 27 °C and 70% humidity for 8 wk to decay. After 8 wk, blocks were removed and mycelium was brushed off. Blocks were conditioned 1 wk before final weights were obtained.

## Results and Discussion

### Field Tests

Over the 3-y study, above-ground decking samples became bleached in appearance because of normal UV exposure, more heavily in Mississippi than Wisconsin (Fig. 10). Some fungal growth characteristic of incipient decay was noted as early as 12 months in Wisconsin (Fig. 11). Similar patterns of weathering were seen in Wisconsin and Mississippi. SYP was moderately attacked by decay fungi (Fig. 12) and showed the highest degree of cupping and checking (See Figs. 13, 14). Paulownia (PAW) was slightly attacked and showed moderate cupping and checking. The entire cedar group (western redcedar (WRC), eastern redcedar (ERC), West Coast juniper (WJN), and AYC) showed no signs of deterioration and low levels of cupping and checking. The durable hardwoods, honey mesquite (HM) and black locust (BL), showed very little deterioration or cupping, but BL did have moderate checking on the wood surface. CAT was slightly attacked in Mississippi, but remained sound in Wisconsin. CAT also exhibited moderate cupping, being more pronounced in Mississippi than in Wisconsin.

### Chemical Extractives Characterization

The phytochemical composition of the wood species selected for field tests in this study were chemically extracted and both extractives and the extractive-free wood were individually evaluated for resistance to decay fungi and termites. Extractives were analyzed by gas chromatography–mass spectroscopy (GS–MS). Components were characterized on the basis of their GS–MS spectra compared with corresponding known spectra in the GS–MS library. Two classes of compounds were singled out for this study: (1) fatty acids, analyzed as FAMES and terpenoids. Both classes of chemical have documented fungicidal properties and are thought to play a protective role in natural durability.

#### Fatty Acid Methyl Ester (FAME) Analysis

For quantification of common fatty acids, the Restek Food Industry FAME mix was used and targets were quantified and compared with unexposed samples (Fig. 15). Initial


**Figure 10. Visual comparison of decking specimens at installation (top row) and after 3 y above-ground exposure at the Valley View test site in Madison, Wisconsin (bottom row). Note the checking of SYP and BL.**

**Figure 11. After 12 months outdoor exposure in Wisconsin (Decay hazard zone 2) the underside of the Southern Pine controls with fruiting bodies present and stringy bleached undersides, indicating colonization by white-rot fungi along with black mold.**

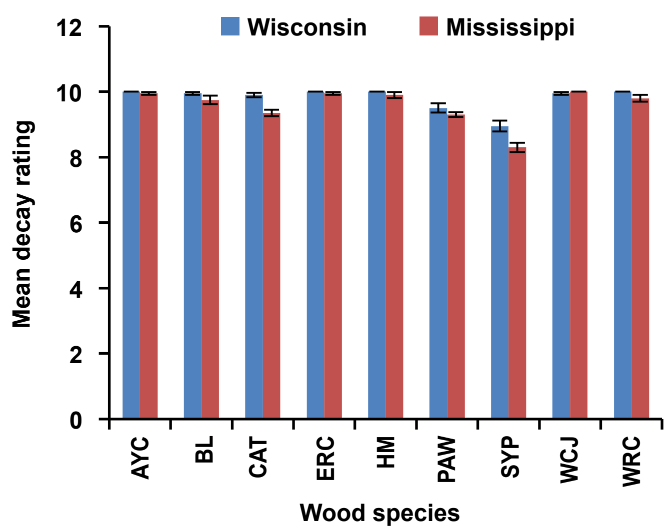


**Figure 12. Mean decay ratings of each nondurable wood species after 3-y field exposure in Wisconsin and Mississippi.**

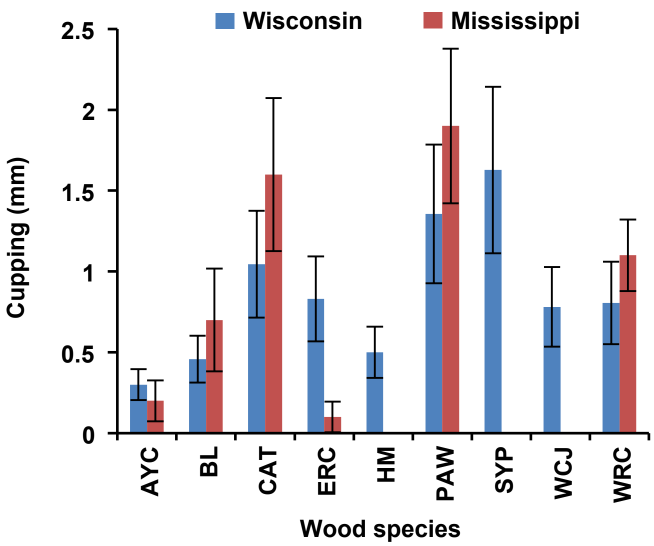


**Figure 13. Mean cupping in millimeters from level of each wood species after 3-y field exposure in Wisconsin and Mississippi.**

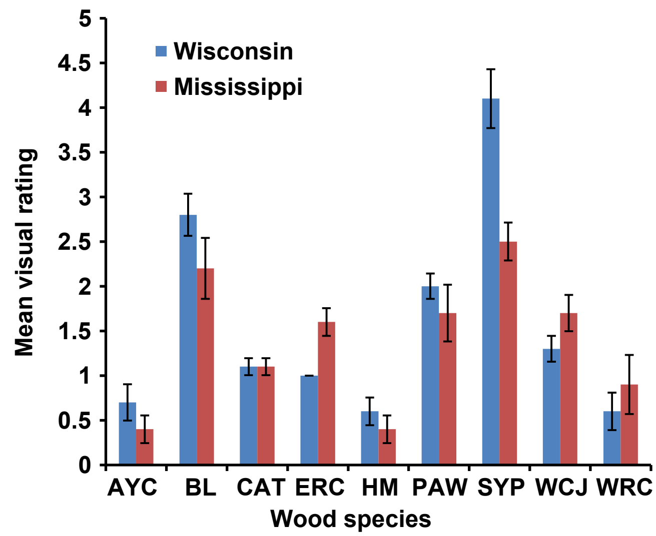


**Figure 14. Mean visual ratings of degree of checking on a scale of 1–5 by wood species after 3-y field exposure in Wisconsin and Mississippi.**

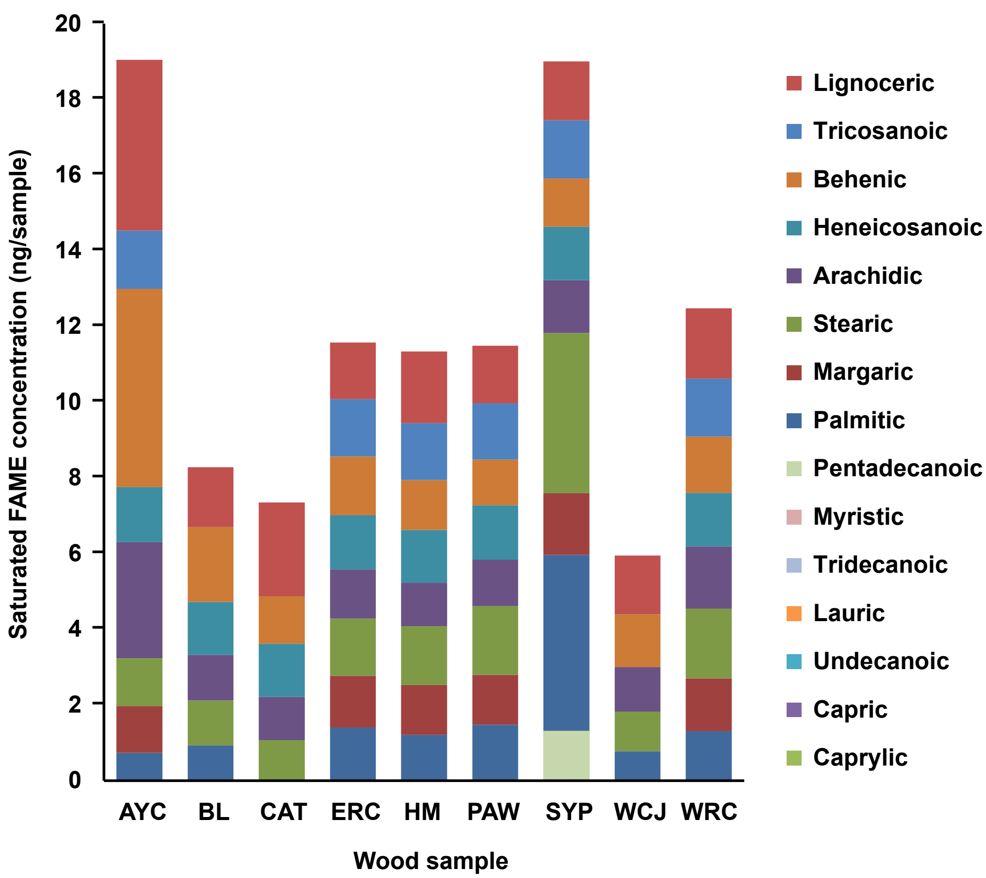


**Figure 15. Concentration of saturated FAMEs in unexposed wood samples.**

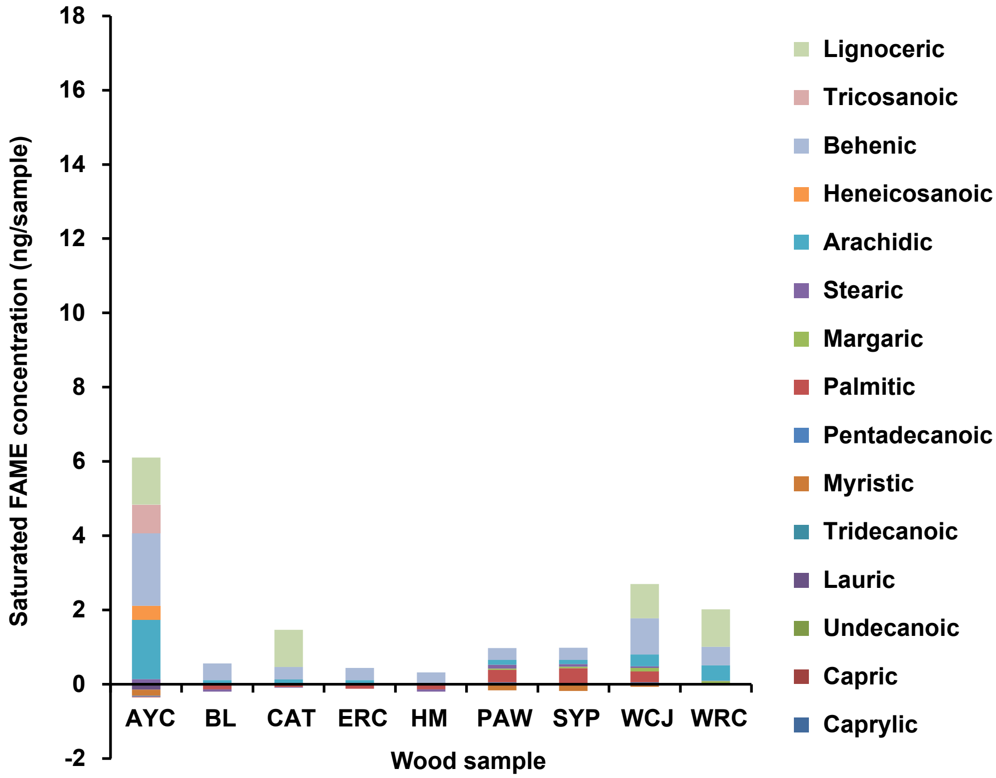


**Figure 16. Concentration of saturated FAMEs (fatty acid mothyl esters) after 2-y field exposure in Wisconsin.**

fatty acid analysis found highest amounts of target fatty acids in AYC and SYP (both 19 ng/mg wood). Lowest amount was in WCJ (5.5 ng/mg wood). Unexposed SYP had the highest diversity of FAMEs (9). The largest amount of any one fatty acid was behenic acid found in AYC (5.7 ng/mg wood).

After 2-y exposure at both test sites, the amounts of fatty acids diminished significantly, particularly SYP and ERC (Figs. 16, 17). Saturated fatty acids were markedly low in the 2-y Wisconsin samples (Fig.16). Unsaturated fatty acids (cis-10-heptanoic, oleic, cis 11eicosanoic, 13-docosenoic, and nervonic acids) were still detectable in several of the species samples after 12 months of outdoor exposure. The unsaturated fatty acids varied considerably among the different wood species. For a more complete explanation of this portion of the study, refer to Appendix I for the complete results of the FAME analysis.

### Analysis of Terpenoids

Common terpenoids were selected as standards and used to create a standard curve for analysis. Concentrations of the targeted compounds in unexposed samples are shown in Figure 18. Western redcedar had the highest diversity of terpenoids (5) and the hardwood species, not surprisingly, contained no terpenoids, as they are typically asociated with conifers. Additional terpenes not included in the standard curve are shown in Table 4. Beta sitosterol was the most widely isolated compound, present in all but SYP, WRC, and ERC. It is likely also present in those species and may not have been detectable in those samples because of coelution or masking by other compounds. Several unique compounds were found in AYC (trans-shisool, cubenol, tau-muurolol, alpha-cadinol, coniferyl aldehyde) and WRC (isopugelol acetate, flavone, and nortrachelogenin). Terpenoid analysis was carried out on unexposed samples only, but future analyses will look at compounds over time in weathered samples.

## Laboratory Decay Resistance

### Disk Assays

Derivatized FAME fractions were used in a disk assay inhibition test to evaluate possible antifungal activities of saturated and unsaturated FAMEs against four wood decay fungi. No inhibition of any of the test fungi was found using the FAME fractions. Based on these results, fatty acids do not seem to have biological activity at the concentrations we have found in the NDW species.

In a second experiment, extractives that were removed from test blocks of the nine NDW species using an ethanol: toluene mix according to ASTM standard D1105-96 were used in a similar disk assay. The removed extractives were added to sterile assay disks and aseptically placed on agar plates covered with mycelia of four wood decay fungi (two

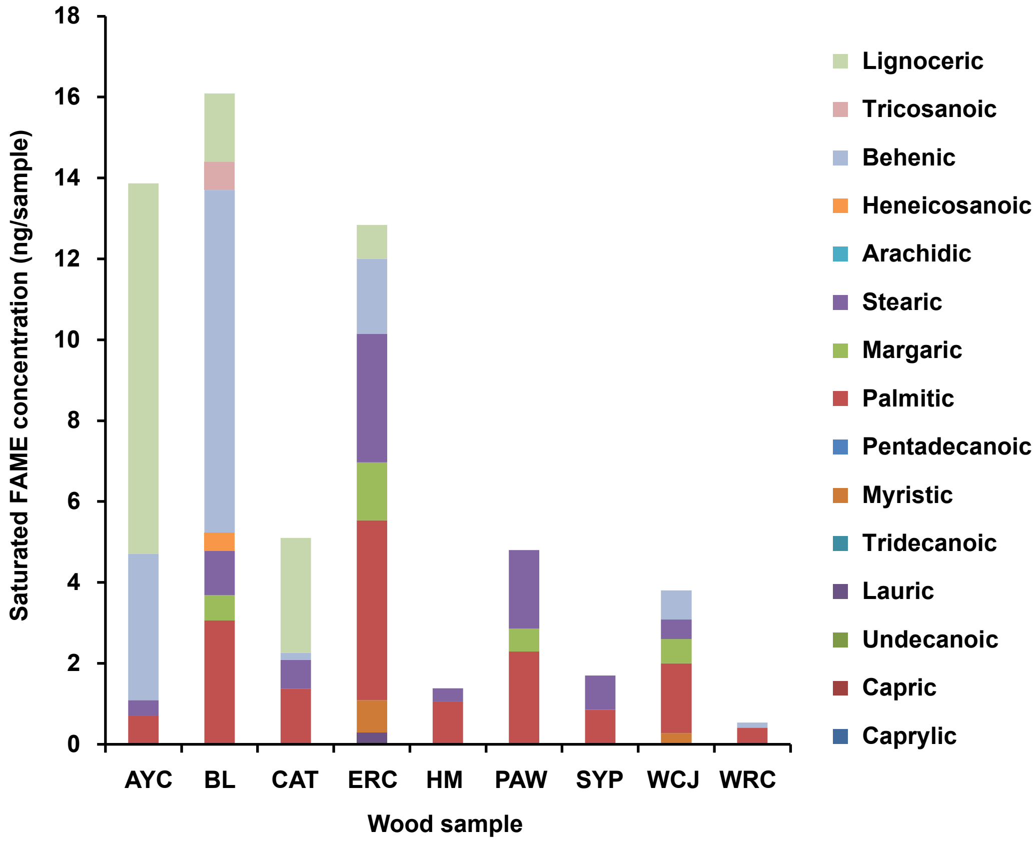


**Figure 17. Concentration of saturated FAMEs after 2-y field exposure in Mississippi.**

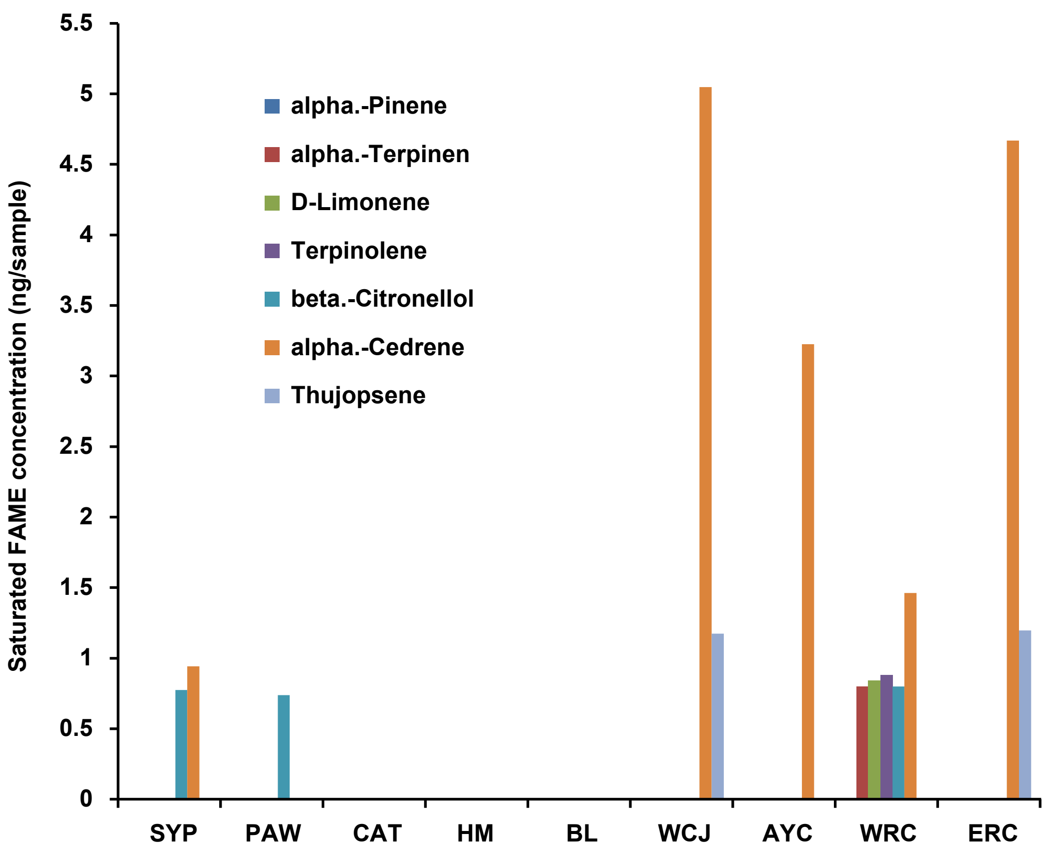


**Figure 18. Quantities of standard terpenoids in EtOH. Tol extracts of unexposed naturally durable wood species. Note high number of compounds present in WRC, which exhibited inhibitory activity against 4 test fungi in disk assays.**

**Table 4. Additional identified compounds found in NDW WtOH: Tol extractions. WRC and AYC had several unique compounds listed beneath the table columns**

| Identified chemical compounds in Etoh: Tolune extraction | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CMPD | SYP | PAW | CAT | HM | BL | WCJ | AYC | WRC | ERC |
| (-)-Myrtenol | | Y | | | | Y | Y | Y | |
| .beta.-Sitosterol | | Y | Y | Y | Y | Y | Y | Y | |
| .gamma.-Sitosterol | | | | | | | Y | Y | Y |
| alpha-cedren | Y | | | | | Y | Y | | Y |
| Andrographolide | | | | Y | | | | | |
| Aromadendrene | | | | | | Y | Y | | Y |
| Campesterol | | Y | | | | | | | |
| Ferruginol | | Y | | 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 | | | Trans-Shisool | | Isopulegol acetate | | | | |
| | | | Cubenol | | Flavone, 5,7-dihydroxy-8- methoxy- | | | | |
| | | | .tau.-Muurolol | | (-)Nortrachelogenin | | | | |
| | | | .alpha.-Cadinol | | | | | | |
| | | | Coniferyl aldehyde, methy ether | | | | | | |

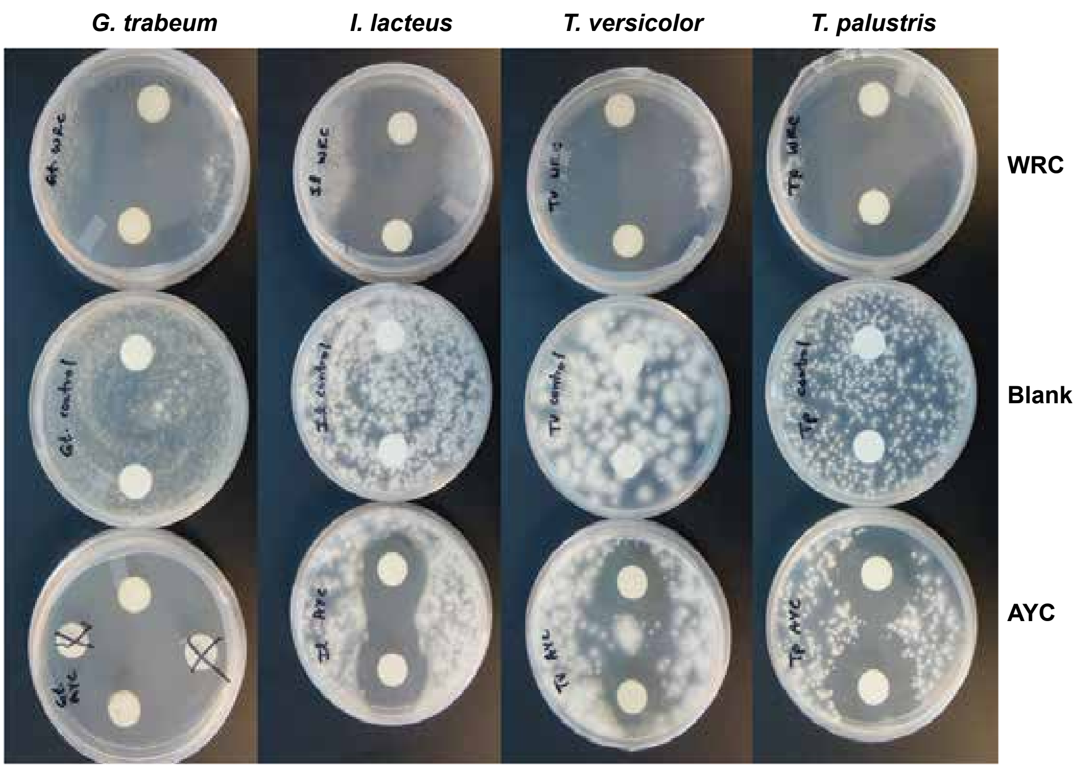


**Figure 19. Disk assay experiment evaluating ethanol.toluene extractives from WRC and AYC against *Gloeophyllum trabeum* (Gt), *Irpex lacteus* (Il), *Trametes versicolor* (Tv), and *Tyromyces palustris* (TP). Inhibition (zones of clearing) of each fungus was apparent after 7-d exposure compared with solvent-only controls (blank).**

white-rot fungi, *Irpex lacteus* and *Trametes versicolor*, and two brown-rot fungi, *Tyromyces palustris* and *Gloeophyllum trabeum*). Extracts of WRC and AYC showed direct antifungal activity against all four fungi tested (Fig. 19).

## Laboratory Termite Resistance

The results of the choice termite bioassays showed higher wood consumption in the extracted blocks compared with unextracted blocks (Fig. 20) in all wood species except for

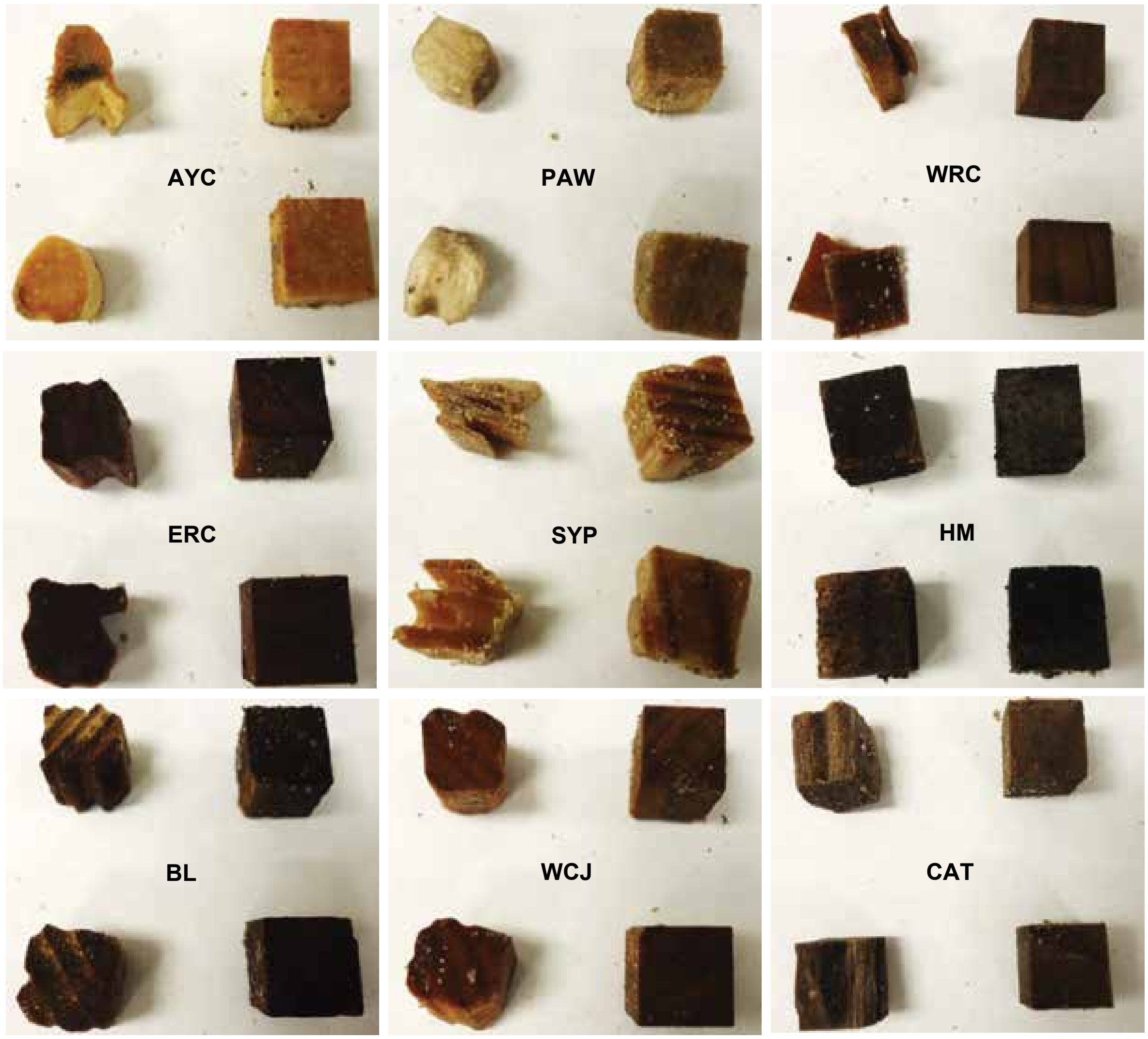


**Figure 20. Visual comparisons of extracted and unextracted naturally durable woods exposed to eastern subterranean termites (*Reticulitermes flavipes* Kollar) in a choice bioassay. Blocks on the right side of each image are unextracted controls, and blocks on the left have extractives removed by solvent extraction. Virtually no feeding was seen in HM (center) because of high mortality in the soil jars.**

honey mesquite, which had high mortality in the soil jars presumably because of insecticidal toxins present in the unextracted blocks. WRC-, PAW-, AYC-, and SYP-extracted blocks had the highest mass loss because of consumption (over 30% weight loss), whereas BL extracted wood had the lowest weight loss (under 10%) (Fig. 21).

## Laboratory Soil Block Assays

The results of this study stress the importance of extractive content when evaluating natural durability of wood. Blocks from the naturally durable wood species were extracted following ASTM D1105-96, to the point where virtually all extractives were removed, then exposed to six common wood rot fungi along with unextracted blocks. The extracted wood blocks were significantly more susceptible to decay fungi than the unextracted blocks, and many became as susceptible as the SYP nondurable controls. Differences in fungal colonization of the blocks could be seen by 4 wk in the soil bottles (Figs. 22, 23). Overall, brown-rot fungi were more efficient at degrading blocks than white-rot fungi, causing 30% to 60% weight loss compared with 10% to 15% weight loss, respectively (Figs. 24–26). Many durable wood species, AYC, ERC, WRC, CAT, and BL, became significantly less durable when extractives were removed. In both the termite and fungal block assays, BL and HM remained quite durable even after extractives were removed.

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

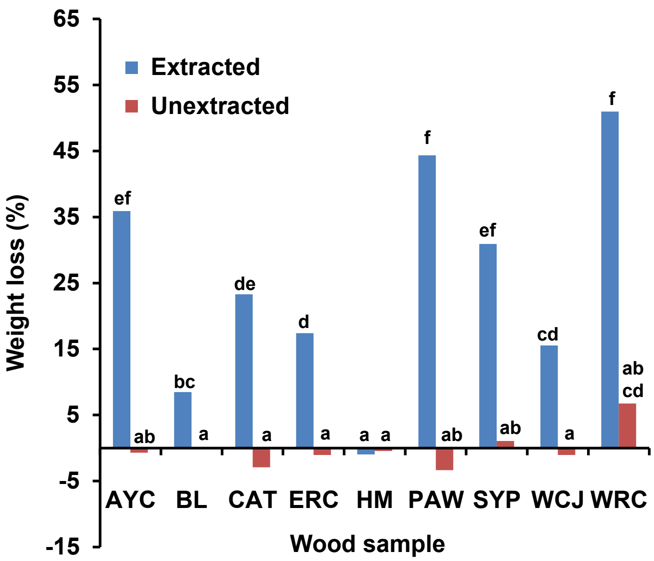


**Figure 21. Percentage of weight loss (PWL) of wood blocks because of termite attack of nine wood species in a modified E7 termite choice bioassay. Letters above columns indicate statistical groupings (a is least PWL, f is highest PWL). Termites readily consumed all species when extractives were removed with the exception of honey mesquite (HM), which caused high mortality (96%).**

(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 durable to moderately durable. *The Wood Handbook* (Clausen 2010) (table 14-1) classifies all of these durable wood species 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 against white-rot fungi and not durable (class 5) to brown-rot fungi.

Of particular interest in the brown-rotted blocks were the patterns noted on unextracted Alaska yellow cedar. There was an area of "cubical rot" on the underside of the block that was in direct contact with the feeder strip, suggesting that the fungus was actively attempting to colonize the block but was unsuccessful. The presence of fruiting bodies is indicative of the fungal response to a lack of nutrients from encountering an unsuitable substrate (Rayner and Boddy 1988).

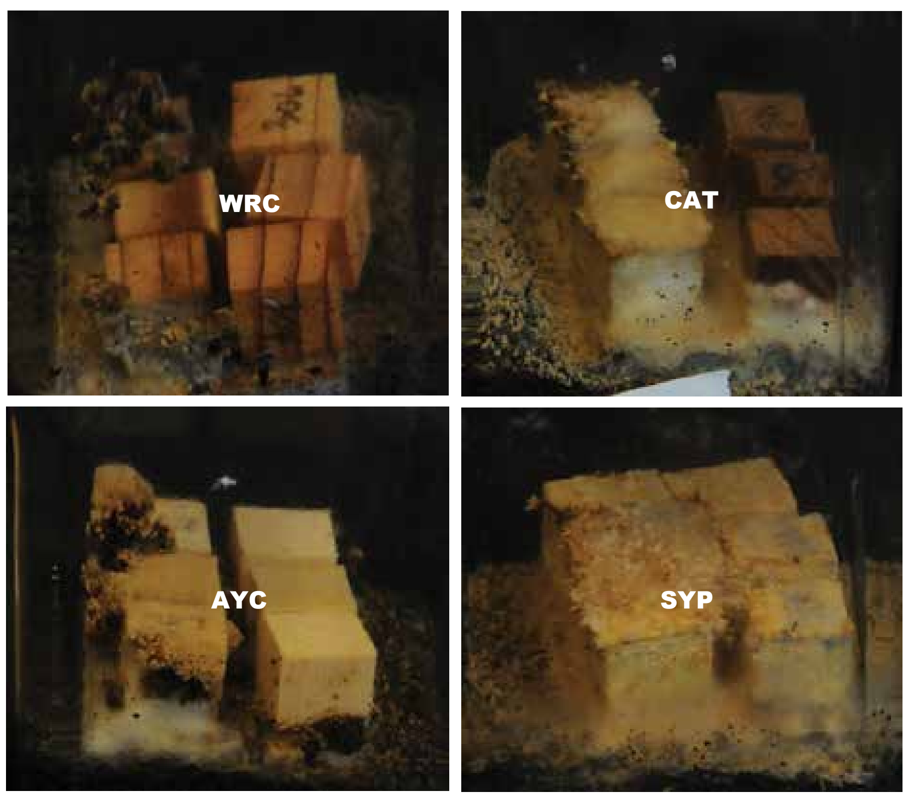


**Figure 22. Soil blocks exposed to *G. trabeum* at 8 wk. Blocks on the left side of each image have extractives removed and right side are unextracted.**

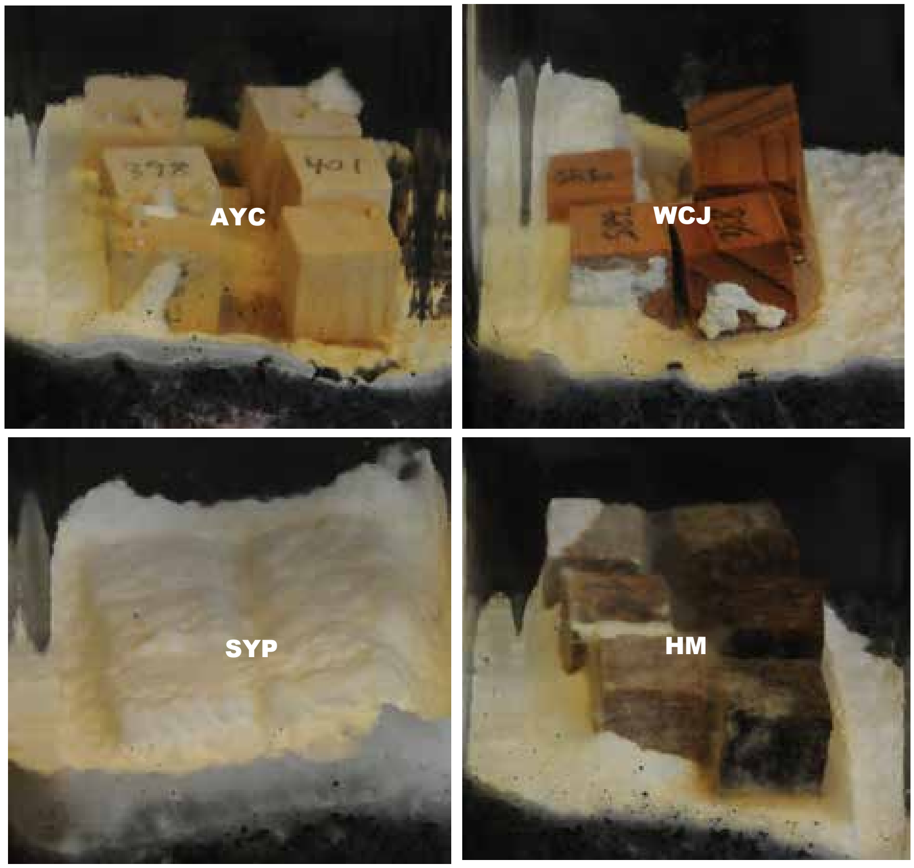


**Figure 23. Soil blocks exposed to *I. lacteus* at 8 wk. Blocks on the left side of each image have extractives removed and right side are unextracted.**

**Figure 24. Unextracted blocks of AYC after 8 wk of soil bottle exposure to *G. trabeum*. Note localized patterns of brown-rot only on the underside of block that abutted the feeder strip.**

## Summary and Discussion

### Field Tests

The results of the field test indicate that with the exception of PAW and SYP all of the “durable” wood species are sufficiently decay resistant for use in above-ground conditions. All of the softwood NDW species (ERC, WRC, AYC, and WCJ) exhibited excellent performance in our tests. Of the hardwood NDW species, HM and BL performed exceptionally; CAT was slightly less durable and was also prone to cupping. Black locust had moderate checking at both field test sites, which does diminish the overall appearance of the wood and would be more of a problem underfoot for deck replacement than when used in vertical orientation, such as cladding. The visual observations of these decking specimens do indicate that bleaching is inevitable for uncoated lumber in this particular situation and a surface protection (i.e., stains or clear polyurethane) would minimize the bleaching that is no doubt caused by UV exposure. Clear coatings would also slow leaching of extractives from the wood and prolong the useful service life of the above-ground components. As demonstrated by our laboratory

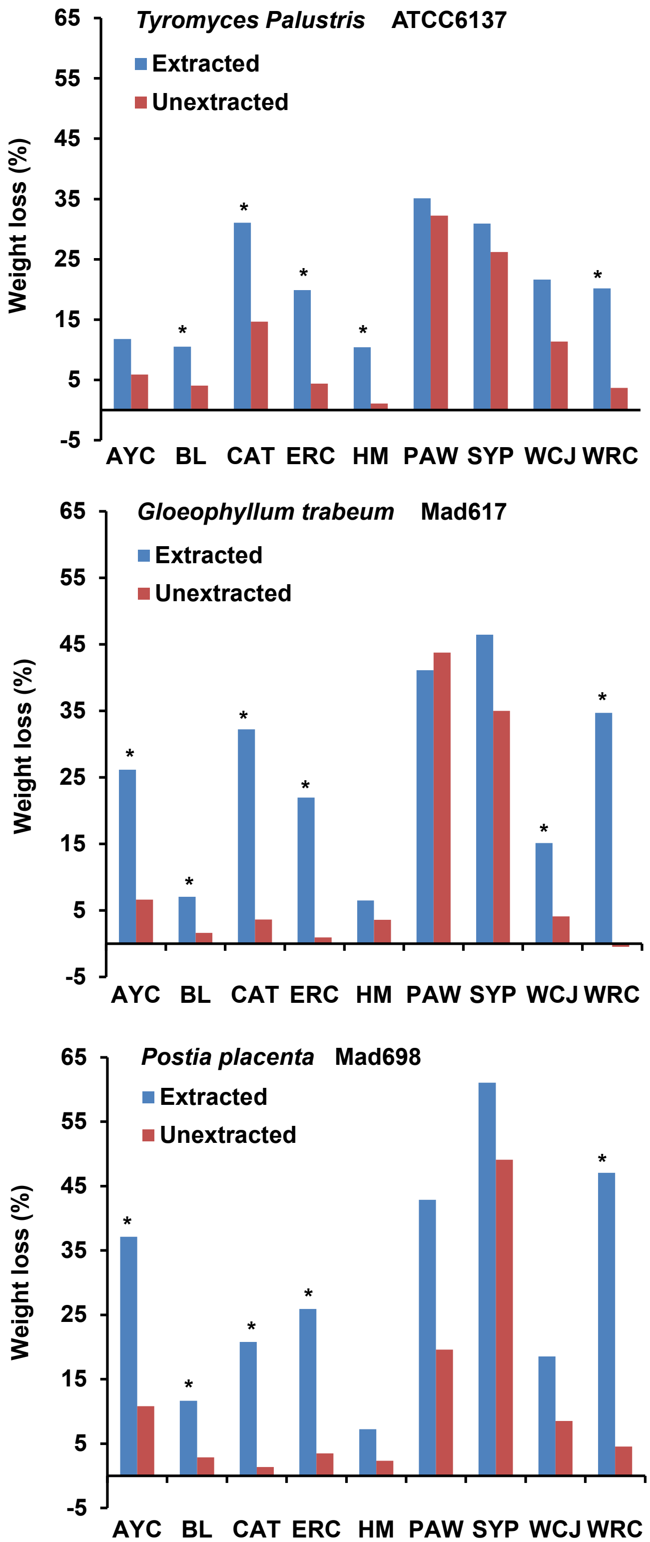


Figure 25. Mean percentage of weight loss for extracted and unextracted blocks of nine wood species exposed to (i) *Tyromyces palustris* (TYP 6137), (ii) *Gloeophyllum trabeum* (Mad 617), and (iii) *Postia placenta* (Mad 698) for 8 wk in an E-10 soil bottle test. Asterisks indicate where extracted mean weight loss for a wood species was significantly different from the corresponding unextracted mean weight loss.

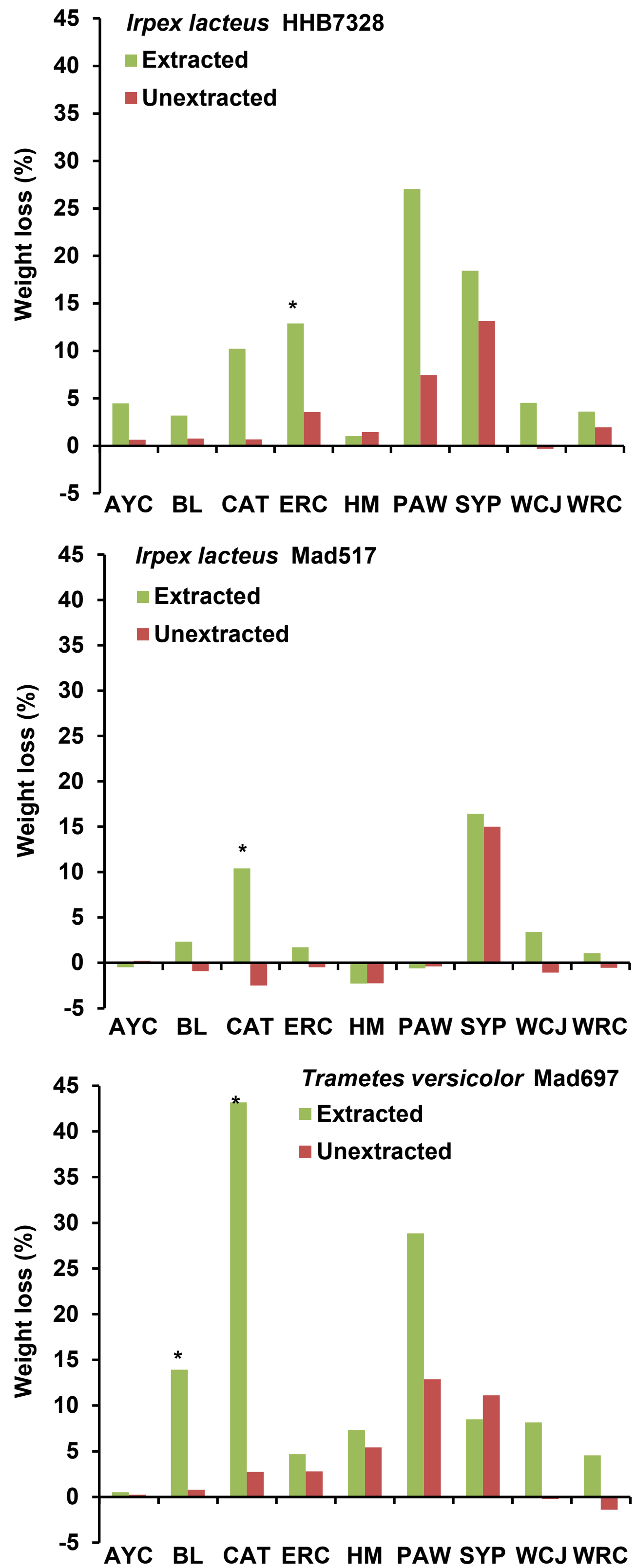


Figure 26. Mean percentage weight loss for extracted and unextracted blocks of nine wood species exposed to (i) *Irpex lacteus* (HHB 7328), (ii) *Irpex lacteus* (Mad 517), and (iii) *Trametes versicolor* (Mad 697) for 8 wk in an E-10 soil bottle test. Asterisks indicate where extracted mean weight loss for a wood species was significantly different from the corresponding unextracted mean weight loss for the wood species.

tests, extractive content is a critical component of natural durability, and diminished extractive content diminishes the durability of the wood.

### Chemical Characterization

Overall, the chemical analyses conducted on these wood species netted a significant amount of information. Our initial GS–MS analysis found upwards of 150 compounds in each sample, thus the reasoning behind narrowing our focus to fatty acids and later terpenoids. Because fatty acids were severely diminished in the wood samples after 2-y exposure and exhibited no biological activity in our laboratory disk bioassays, we conclude that fatty acids do not play a direct protective role in naturally durable wood species. Terpenoids look more promising as having a protective role in natural durability; two of the species having the highest amount and diversity of our targeted terpenoids were classified as durable (AYC and WRC). For these same two species, antifungal activity was determined for EtOH: Tol extracts against several wood decay fungi (See Fig. 19). For the softwood species, terpenoids seem to be a major contributing factor to durability, but the durable hardwoods likely contain additional compounds, such as flavonoids, polyphenols, and alkaloids. Terpenoid-rich resins (Van Acker and others 1999; Kennedy and others 2000) have been used in the literature to treat nondurable sapwoods, so it is possible to use terpenoids as wood protectants.

### Laboratory Bioassays

The termite-resistance assays indicate the importance of extractive content in naturally durable wood species especially emphasizing the maintenance of high extractive content. Once the extractives were removed, two of the most durable species (AYC and WRC) were consumed by termites to a higher degree than the extracted nondurable control (SYP). Similarly, once extractives were removed, NDWs showed significantly higher weight loss when challenged by brown-rot fungi. Many of the wood species chosen for these studies were softwoods, which are typically not preferred by white-rot fungi. However, the general pattern was observed and was more prominent in hardwoods when exposed to white-rot fungi. Overall, most wood species were rendered considerably less durable when extractives were removed, but some of the naturally durable wood species remained durable to moderately durable after extraction. It is possible that with honey mesquite and black locust this is due to the inability to fully extract the durable components, either because of insolubility or the presence of physical barriers such as densely packed cells, resins, or waxes. (For a more complete discussion of the soil bottle assays, refer to Appendix II, page 35.)

## Conclusions and Recommendations

The overall findings of this study indicate that several of the species evaluated have benefits for use in above-ground replacement components for covered bridges.

- Alaska yellow cedar exhibited good performance in the above-ground test. It was slightly more prone to discoloration in the field, but showed excellent resistance to decay and was not prone to cupping or checking. Extractives from Alaska yellow cedar showed some direct antifungal activity against several of our test fungi in laboratory bioassays. Laboratory soil bottle tests indicated Alaska yellow cedar is durable to decay fungi and termites, but durability is severely diminished once extractives are removed. It is always important to source lumber with sufficient heartwood content and protect wood from weathering and leaching with a sealant.
- Black locust exhibited similar durability in the field with quite a bit more checking. Black locust also performed similarly in laboratory assays and it maintained more termite and fungal resistance after extraction. Black locust appears to be difficult to extract possibly because of the wood structure, which could also be contributing to inhibition of degradation by termites and fungi and makes it a very good choice for replacement repairs.
- Catalpa has shown good durability in the field, more so in Wisconsin than Mississippi, but with quite a bit of cupping and some checking. It showed very good resistance to termites and fungi, but upon leaching, durability was severely diminished. Catalpa could perform well in the field with the benefit of weather protectants.
- Eastern redcedar has exhibited good performance in the above-ground decking study. The wood is durable but shows some cupping and cracking. Extractive content is, however, an important factor to consider when selecting cedar as a naturally durable wood. Our tests concluded that eastern redcedar also becomes quite susceptible to fungal and termite attack once extractives are removed. As with the other cedars evaluated in this study, it is important to source lumber with sufficient heartwood content and protect wood from weathering and leaching with a sealant.
- Honey mesquite exhibited excellent durability in the field, with low cupping and checking. Both with and without extractives present, Honey mesquite showed excellent fungal resistance in laboratory bioassays and caused high mortality in termites. Microscopic examinations showed plugged vessels in honey mesquite test blocks, combined with the tight cellular arrangement; its structure could inhibit extraction or even inhibit accessibility by fungi. The scarcity of large dimensions of honey mesquite may limit its usefulness for covered bridge components. A better understanding of the gums and waxes in honey mesquite as well as other chemical components will help us better understand its excellent decay resistance.
- *Paulownia tomentosa* cannot be recommended based on the results of this study. Above ground, the wood was susceptible to decay, cupping, and checking and performed

only slightly better than Southern Pine controls. Laboratory tests indicated very little resistance to test decay fungi and once extracted, it performed worse than Southern Pine against several decay fungi and termites. Additional in-ground tests using *P. tomentosa* all failed within a year and faster than Southern Pine controls.

- Western juniper exhibited good performance in the field tests with some checking by year 3. In laboratory bioassays, Western juniper showed excellent durability to termites and fungi and even maintained some durability after stringent extraction making it another excellent choice.
- Western redcedar also exhibited good performance in the field tests with some cupping by year 3. Extractives from Western redcedar showed some direct antifungal activity against several of our test fungi in laboratory bioassays. Western redcedar does diminish in durability once extractives are removed, so the same recommendations would be made for western redcedar as the other cedars. Always make sure that the material is mostly heartwood to ensure highest possible extractive content. Sealants may also improve the durability of western redcedar as it would slow leaching of extractives caused by weathering.

## References


Abad, M.J.; Ansuategui, M.; Bermejo, P. 2007. Active antifungal substances from natural sources. ARKIVOC (vii): 116–145. ISSN 1424–6376.

Alden, H.A. 1995. Hardwoods of North America. Gen. Tech. Rep. FPL–GTR–83. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 136 p.

Alden, H.A. 1997. Softwoods of North America. Gen. Tech. Rep. FPL–GTR–102. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 151 p.

ASTM. 2012. D1105-96. Standard test method for preparation of extractive-free wood. West Conshocken, PA: American Society for Testing Materials International. pp. 147–148.

AWPA. 2010a. Standard method for laboratory evaluation to determine resistance to subterranean termites. Standard E1-09. Book of Standards. Birmingham, AL: American Wood-Preservers' Association. pp. 351–359.

AWPA. 2010b. Standard Method of Testing Wood Preservatives by Laboratory Soil-block Cultures. Standard E10-09. Book of Standards. Birmingham, AL: American Wood-Preservers' Association. pp. 383–392.

AWPA. 2011a. Standard field test for evaluation of wood preservatives to be used above ground (UBC3): Decking method. E25-08. In: Annual book of AWPA Standards. Birmingham, AL: American Wood Protection Association. pp. 448–450.

AWPA. 2011b. Standard method of testing wood preservatives by laboratory soil-block cultures. E10–11. In: Annual book of AWPA Standards. Birmingham, AL: American Wood Protection Association. pp. 387–396.

AWPA. 2011c. Standard method for laboratory evaluation to determine resistance to subterranean termites E1–09. In: Annual book of AWPA Standards, Birmingham, AL: American Wood Protection Association. pp. 354–362.

Carey, J.K; D.F. Purslow; Savory, J.G. 1981. Proposed method for out-of-ground contact trials of exterior joinery protection systems. IRG/WP/2157. Stockholm, Sweden: International Research Group on Wood Preservation. 15 p.

Carey, J.K. 2002. L-joint trials Part 3: Relative performance of a range of preservative products. IRG/WP/30292. Stockholm, Sweden: International Research Group on Wood Preservation. 15 p.

Carey, J.K.; Bravery, A.F. 1986. Co-operative research project on L-joint testing. Progress report to March 1986. IRG/WP/2272. Stockholm, Sweden: International Research Group on Wood Preservation. 16 p.

Celimene, C.C.; Micales, J.A.; Ferge, L.; Young, R.A. 1999. Efficacy of pinosylvins against white-rot and brown-rot fungi. Holzforschung, 53(5): 491–497.

Chedgy, R.J.; Lim, Y.W.; Bruille, C. 2009. Effects of leaching on fungal growth and decay of western redcedar. Canadian Journal of Microbiology, 55: 578–586.

Clausen, C.A. 2010. Wood Handbook, Chapter 14: Biodeterioration of wood. Gen. Tech. Rep. FPL–GTR–190. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory: 14-1–14-16.

Clausen, C.A.; Lindner, D.L. 2011. Shading aboveground L-joint and lap-joint tests: comparison of white pine and sugar maple test assemblies. Forest Products Journal, 61(3): 265–269.

Clausen. C.A., T.L. Highley, Lindner, D.L. 2006. Early detection and progression of decay in L-joints and lap-joints in a moderate decay hazard zone. Forest Products Journal, 56(11/12):100–106.

DeGroot, R.C. 1992. Test assemblies for monitoring decay in wood exposed above-ground. International Biodeterioration and Biodegradation, 29:151–175.

EN 350e1. 1994. Durability of wood and wood-based products-natural durability of solid wood. British Standards Association. p. 12.

Eslyn. W.E.; Highley, T.L.; Lombard, F.F. 1985. Longevity of untreated wood in use above-ground. Forest Products Journal, 35(5):28–35.

Fougerousse. M. 1976. Wood preservatives field tests out of ground contact. Brief survey of principles and methodology. IRG/WP/269. Stockholm, Sweden: International Research Group on Wood Preservation. 34 p.

Hedley, M.; Page, D.; Foster, J.; Patterson, B. 1995. Above-ground field tests undertaken in New Zealand. IRG/WP 95-20063. Stockholm, Sweden: International Research Group on Wood Protection. 6 p.

Highley, T.L. 1984. In-place treatments for waterborne preservatives for control of decay in hardwoods and softwoods above-ground. Material und Organismen, 19(2):95–104.

Highley, T.L. 1995. Comparative durability of untreated wood in use above ground. International Biodeterioration and Biodegradation, 409–419.

Highley, T.L. 1993. Above-ground performance of surface-treated hardwoods and softwoods. Wood Protection, 2(2):61–66.

Kennedy, M.J.; Stephens, .M.; Powe, M.A. 2000. Natural durability transfer from sawmill residues of white cypress (*Caitris glaucophylia*). Part 3: full penetration of the refractory sapwood of white cypress.

Laks, P.E.; McKaig, P.A. 1988. Flavanoid biocides: wood preservatives based on condensed tannins. Holzforschung, 42(5): 299–306.

Little, E.L., Jr. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146. 9 p. 200 maps.

Micales, J.A., Han, J.S. Davis, J.L. Young, R.A. 1994. Chemical compositions and fungitoxic activities of pine cone extracts. In: Llewellyn, G.C., Dashek, W.V., and O'Rear, C.E., eds. Biodeterioration research 4: mycotoxins, wood decay, plant stress, biocorrosion, and general biodeterioration: Proceedings of the 4th Meeting of the Pan American Biodeterioration Society; 1991 August 20-25; New York: Plenum Press. pp. 317–332.

Morrell, J.J.; Miller, D.J.; Lebow, S.T. 1998. Above-ground performance of preservative-treated Western wood species. In Proceedomgs of the 94th Annual Meeting of the American Wood-Preservers' Association, May 17–19, 1998, Scottsdale, AZ. pp. 249–253.

Morris, P.I.; McFarling, S. 2007. Accelerated above-ground testing of wood preservatives. IRG/WP 07-20358. Stockholm, Sweden: International Research Group on Wood Protection,. 8 p.

Morris, P.I.; Stirling, R. 2012. Western red cedar extractives associated with durability in ground contact. Wood Science and Technology, 46: 991–1002.

Pierce, P.C.; Brungraber, R.L.; Lichtenstein, A.; Sabol, S.; Morrell, J.J.; Lebow, S.T. 2005. In: Covered Bridge Manual. Publication # FHWA-HRT-04-098. McLean, VA: Federal Highway Administration, Research, Development and Technology. 346 p.

Räberg, U.; Edlund, M.L.; Terziev, N.; Land, C.J. 2005. Testing and evaluation of natural durability of wood in above ground conditions in Europe-an overview. Journal of Wood Science, 51:429–440.

Savory, J.G.; Carey, J.K. 1979. Decay in external framed joinery in the United Kingdom. Journal of the Institute of Wood Science, 8(3):176–180.

Scheffer, T.C. 1971. A climate index for estimating potential for decay in wood structures above-ground. Forest Products Journal, 21(10):25–31.

Scheffer, T.C., Cowling, E.B. 1966. Natural resistance of wood to microbial deterioration. Annual Review of Phytopathology, 4:147–168.

Singh, T.; Singh, A.P. 2012. A review of natural products as wood protectant. Wood Science Technology, 46:851–870.

Smith, A.L.; Campbell, C.L.; Walker, D.B.; Hanover, J.W. 1989. Extracts from black locust as wood preservatives: extraction of decay resistance from black locust heartwood. Holzforschung, 43(5):293–296.

Stirling, R.; Daniels, C.R.; Clark, J.E.; Morris, P.I. 2007. Methods for determining the role of extractives in the natural durability of western red cedar. International Research Group on Wood Protection. Doc No. IRG-WP 07-20356.

Taylor, A.M.; Gartner, B.L.; Morrell, J.J. 2002. Heartwood formation and natural durability–a review. Wood and Fiber Science, 34 (4): 587–611.

USGS. 2012. Digital representations of tree species range maps from *Atlas of United States Trees* by Elbert L. Little, Jr. (and other publications). Reston, VA: United States Geological Survey. http://esp.cr.usgs.gov/data/atlas/little/

Van Acker, J.; Nurmi, A.; Gray, S.; Militz, H.; Hill, C.; Kokko, H.; Rapp, A. 1988. Decay resistance of resin treated wood. International Research Group on Wood Protection. Doc. No. IRG/WP 99-30206.

Wiemann, M.C. 2010. Chapter 2: Characteristics and availability of commercially important woods. In: Forest Products Laboratory. Wood handbook—wood as an engineering material. R.J. Ross, ed. Gen. Tech. Rep. FPL–GTR–190. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI. 508 p.

Williams, G.R.; Fox, R.; Drysdale, J.A. 1995. A note on testing the efficacy of wood preservatives above-ground. IRG/WP 95-20078. Stockholm, Sweden: International Research Group on Wood Protection. 5 p.

Yang, D.Q. 2009. Potential utilization of plant and fungal extracts for wood protection. Forest Products Journal, 54: 37–39.

Zinkel, D.F.; T.V., Magee. 1991. Resin acids of *Pinus ponderosa* needles. Phytochemistry, 30(3): 845–848.

# Appendix I—Above-Ground Field Evaluation and GC-MS Analysis of Naturally Durable Wood Species

Proceedings IRG Annual Meeting (ISSN 2000-8953)


**IRG/WP 12-10764**

**THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION**

**Section 1** **Biology**

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

Kirker, G.T., Blodgett, A.B., Lebow, S.T., and C.A. Clausen
USDA-FS, Forest Products Laboratory
One Gifford Pinchot Drive
53726-2398, Madison, WI, USA

Paper prepared for the 43rd Annual Meeting
Kuala Lumpur, Malaysia
6-10 May 2012



**IRG SECRETARIAT**
**Box 5609**
**SE-114 86 Stockholm**
**Sweden**
**www.irg-wp.org**

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

Kirker, G.T., Blodgett, A.B., Lebow, S.T., and C.A. Clausen

USDA-FS FPL, One Gifford Pinchot Drive, Madison, WI 53726

gkirker@fs.fed.us, abblodgett@fs.fed.us, slebow@fs.fed.us, cclausen@fs.fed.us

## 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.

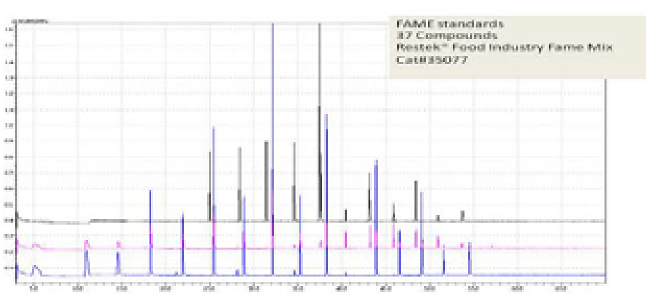


**Figure 1: Stacked chromatograms of fractionated FAME standards. Frac A (bottom) contains saturated FAMEs, Frac B (middle) contains mostly mono-unsaturated FAMEs, and Frac C (top) contains mostly polyunsaturated FAMEs.**

## 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.

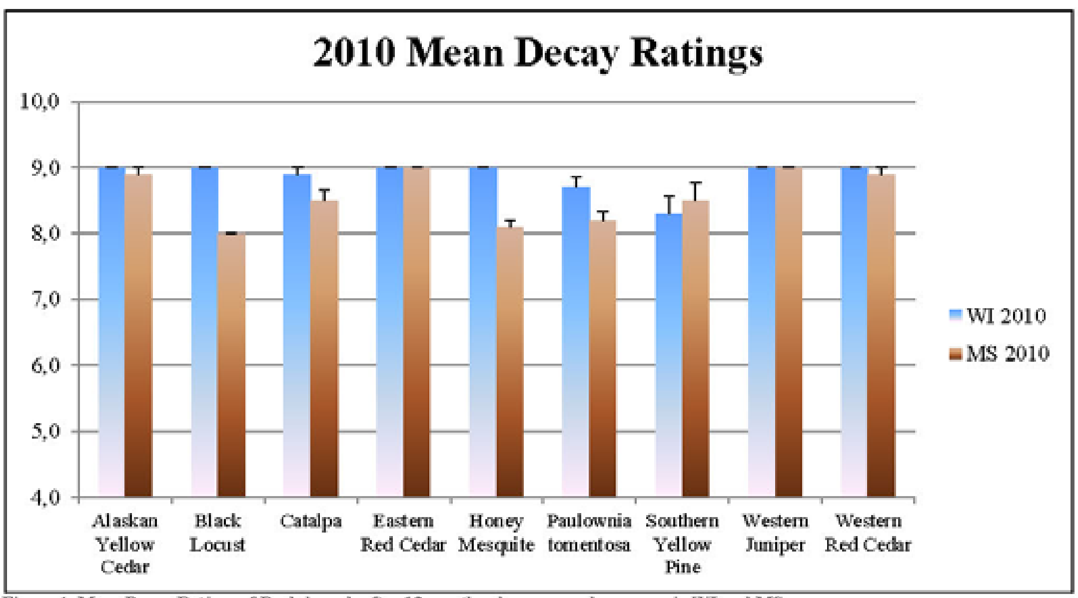


**Figure 4:** Mean Decay Ratings of Deck-boards after 12 months above ground exposure in WI and MS.

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).

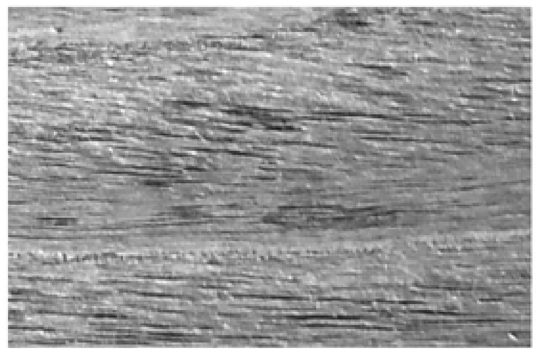

**Figure 6: Severe checking on decking specimens of *Paulownia* after 12 months field exposure in WI.**

**Figure 7: Incipient decay by white rot fungi noted on the underside of SYP decking specimens 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.).

### 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).

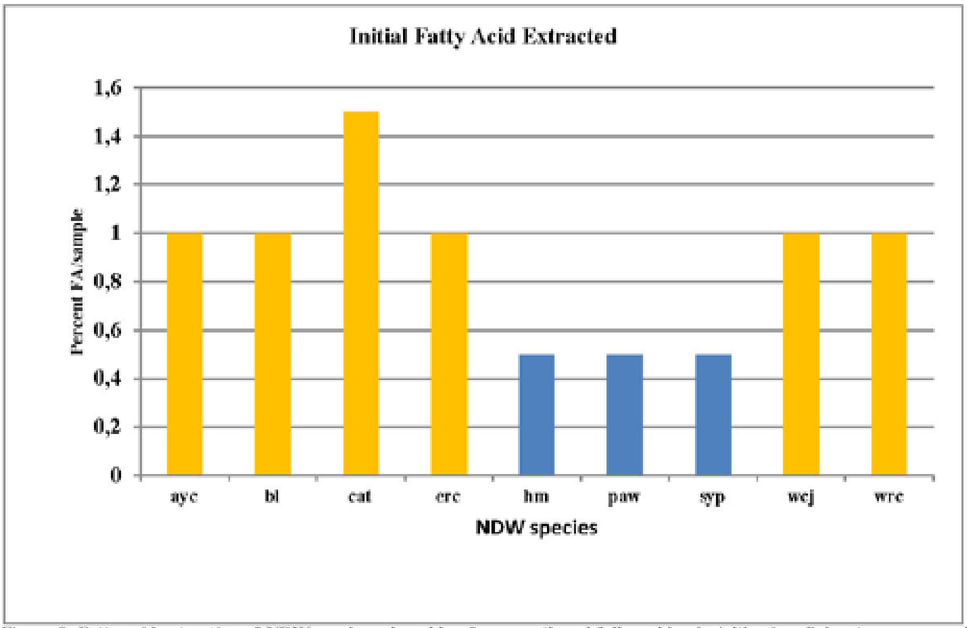


Figure 8: Fatty acid extraction of NDW species using chloroform: methanol followed by derivitization. Solvent was removed by rotary-evaporation and samples weighed to determine % FA by weight of wood for each species.

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.

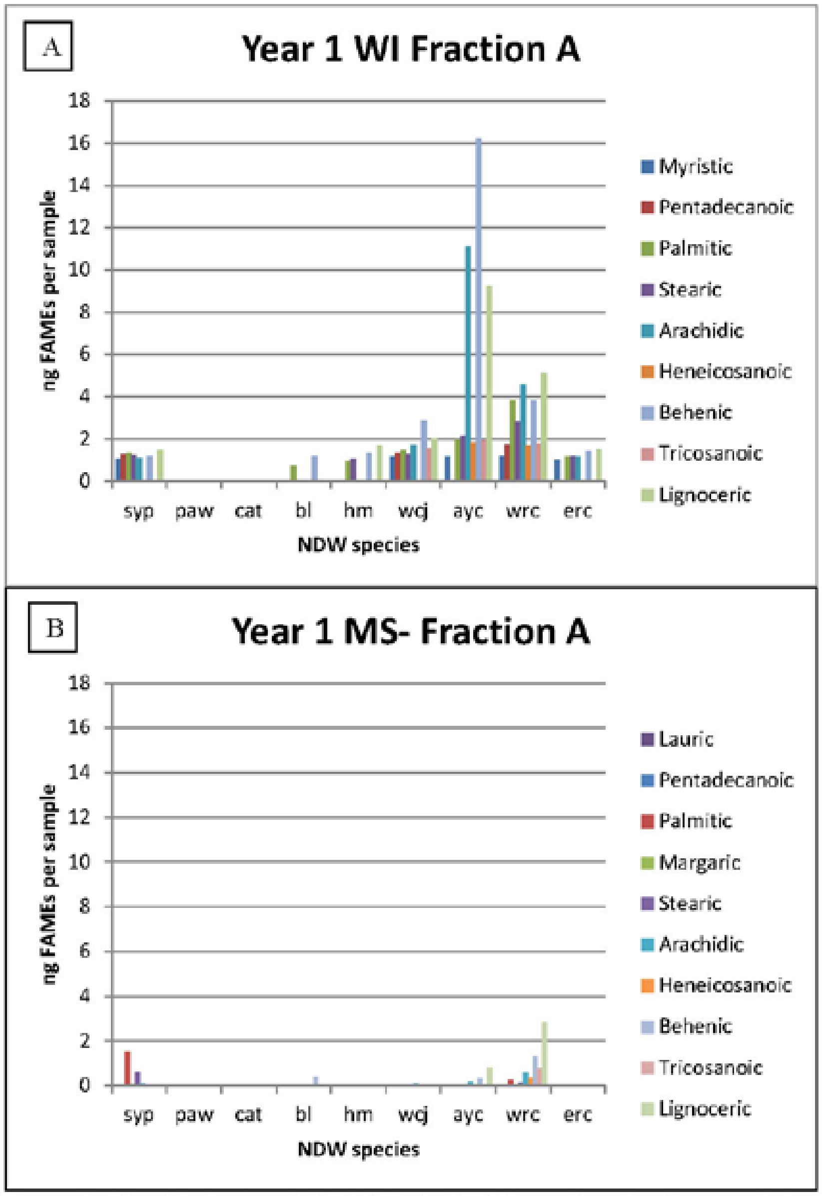


Figure 9: Identified saturated FAMEs in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample.

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).

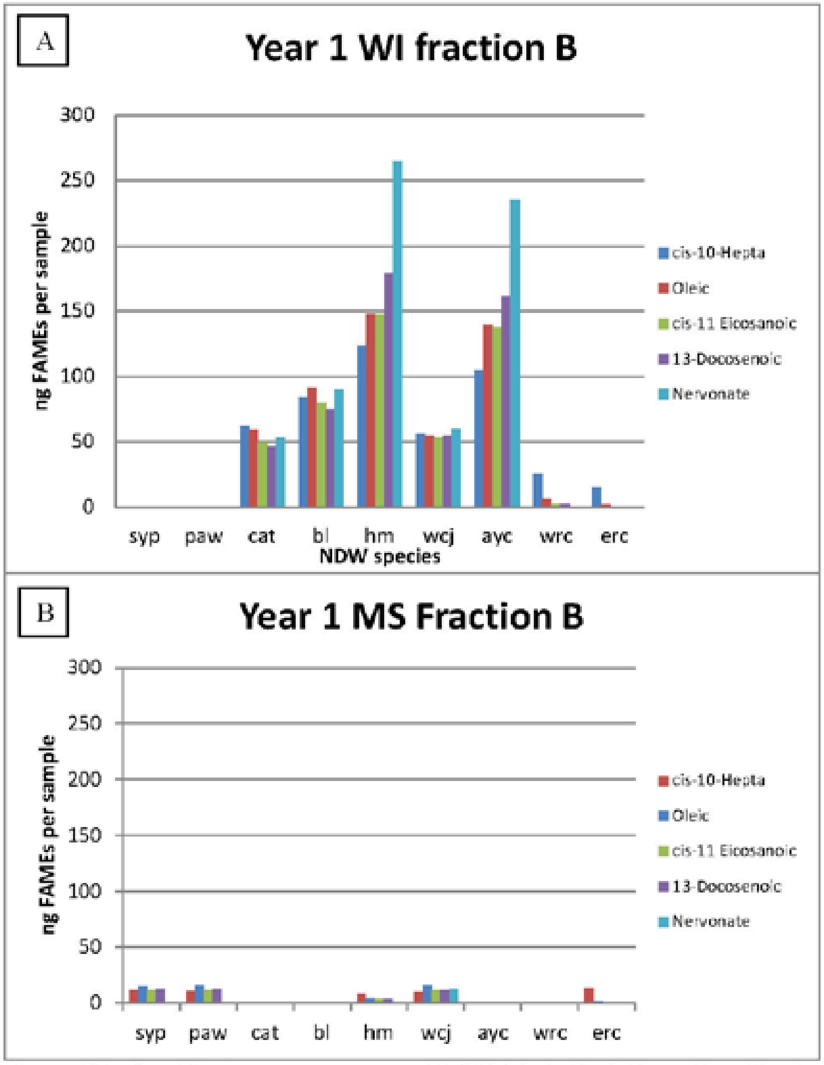


Figure 10: Identified mono-unsaturated FAMEs in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample

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.

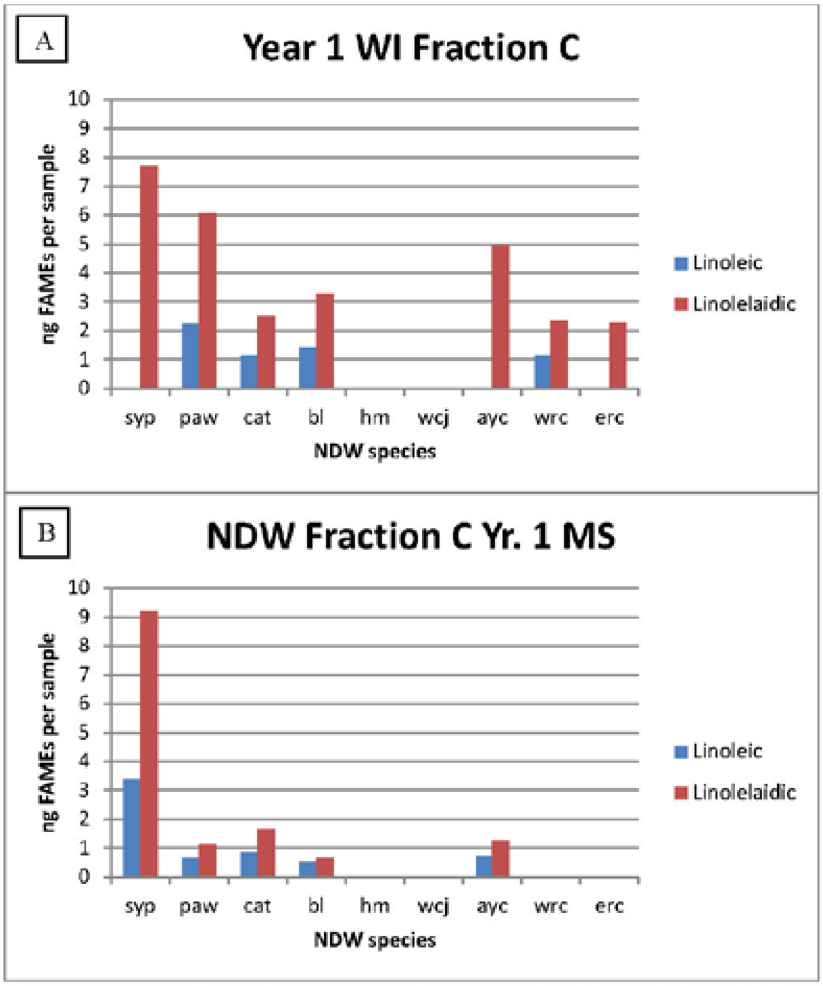


Figure 11: Identified poly-unsaturated FAMEs, linoleic and linolelaidic acids, in NDW species after 1 year of above ground exposure in (A) WI and (B) MS. Concentrations of identified FAMEs are in ng/sample.

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

This research is funded through the United States Federal Highway Administration's Covered Timber Bridge Research Program.

## 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.

# Appendix II—The Role of Extractives in Naturally Durable Wood Species

International Biodeterioration & Biodegradation 82 (2013) 53–58

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

## The role of extractives in naturally durable wood species☆

G.T. Kirker*, A.B. Blodgett, R.A. Arango, P.K. Lebow, C.A. Clausen

USDA-FS Forest Products Laboratory One Gifford Pinchot Drive, Madison, WI 53726-2398, USA

ARTICLE INFO

*Article history:*
Received 5 February 2013
Received in revised form
6 March 2013
Accepted 8 March 2013
Available online

*Keywords:*
Natural durability
Wood extractives
Decay fungi
Brown-rot
White-rot
Termite

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.

Published by Elsevier Ltd.

### 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 (Råberg 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.

☆ The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service. The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin. This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain and not subject to copyright.

* Corresponding author. Tel.: +1 608 231 9256; fax: +1 608 231 9592.
*E-mail addresses:* gkirker@fs.fed.us, gkirker@gmail.com (G.T. Kirker).

0964-8305/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.ibiod.2013.03.007

## 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* [WC]], 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

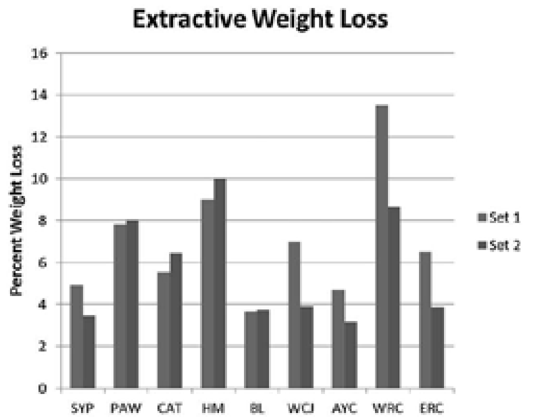


**Fig. 1.** Percent weight loss of sample blocks for each naturally durable wood species in duplicate extractions using ASTM D-1105 method for preparation of extractive free wood.

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

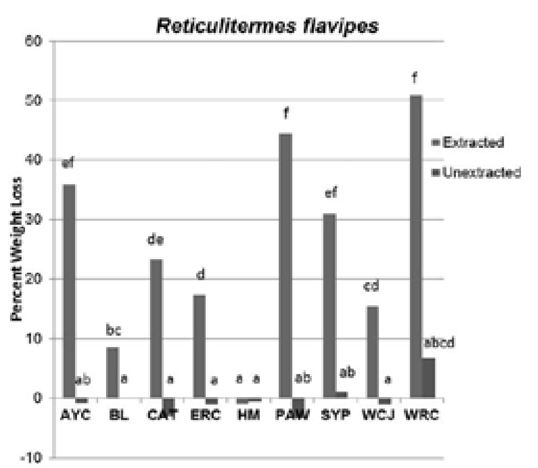


**Fig. 2.** Mean percent weight loss (PWL) of extracted and unextracted wood blocks of 9 wood species due to termite attack in a modified E1-09 termite choice bioassay. Letters above columns indicate statistical groupings, differ significantly at 0.05 (a = least PWL, f = highest PWL).

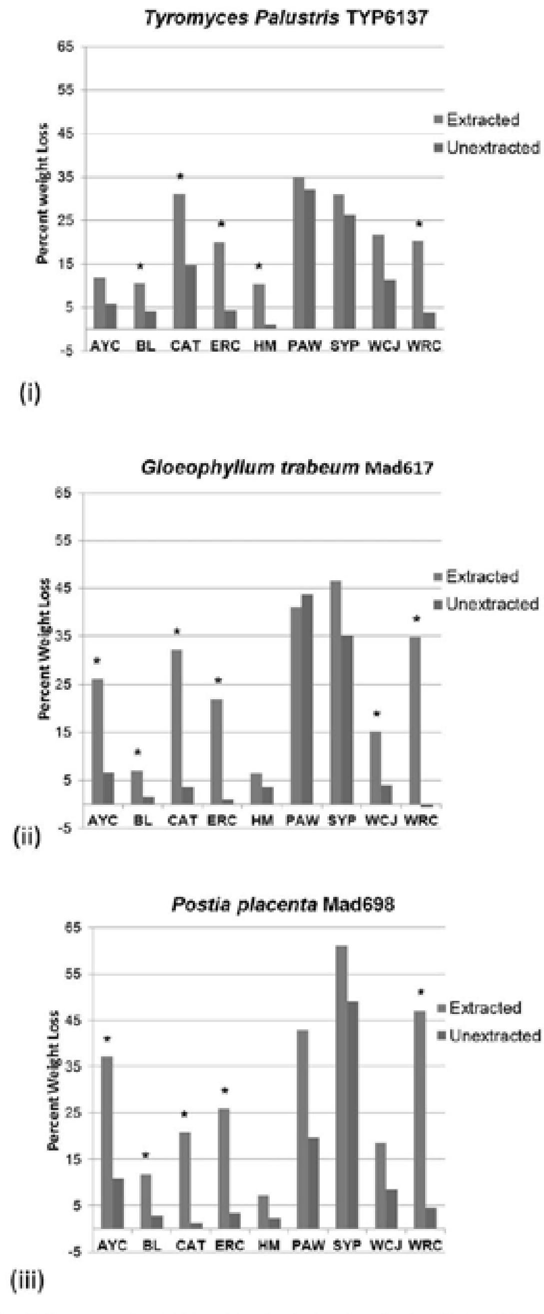


**Fig. 3.** Mean percent weight loss for extracted and unextracted blocks of 9 wood species exposed to (i) *Tyromyces palustris* (TYP 6137), (ii) *Gloeophyllum trabeum* (Mad 617), and (iii) *Postia placenta* (Mad 698) for 8 weeks in an E-10 soil bottle test. Asterisks indicate where extracted mean weight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

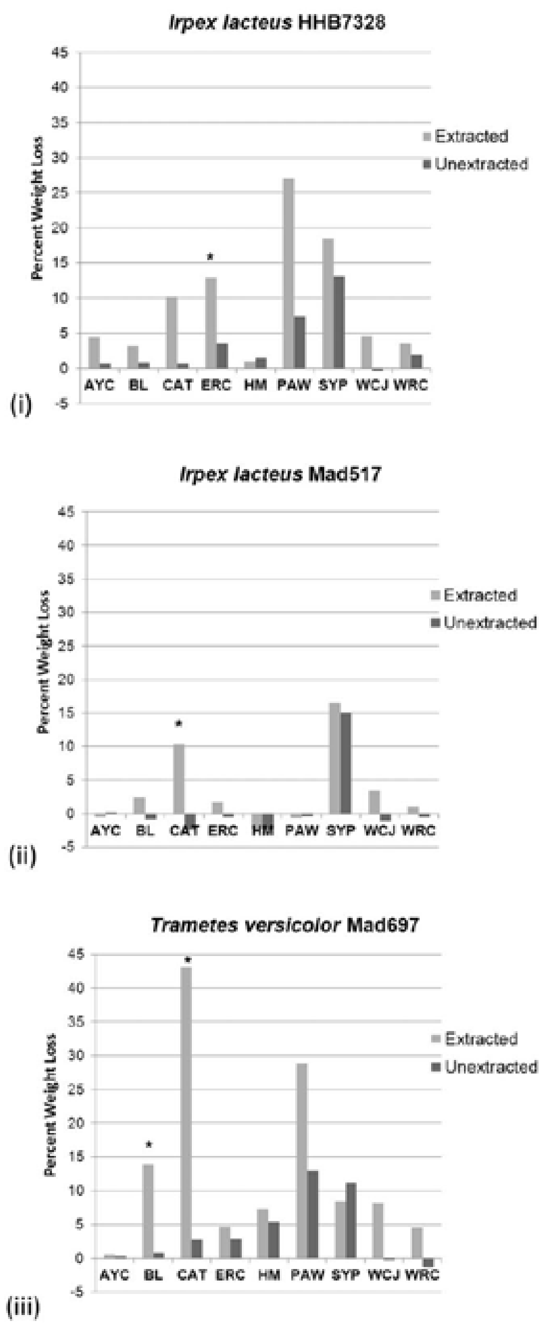


**Fig. 4.** Mean percent weight loss for extracted and unextracted blocks of 9 wood species exposed to (i)*Irpex lacteus* (HHB 7328), (ii) *Irpex lacteus* (Mad 517), and (iii) *Trametes versicolor* (Mad 697) for 8 weeks in an E–10 soil bottle test. Asterisks indicate where extracted mean weight loss 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, epicatalponol, 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 juliflora*, 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 *Coriolus 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.

## Acknowledgments

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.

## References

D1105-96 American Society for Testing Materials (ASTM), 2012. Standard Test Method for Preparation of Extractive-Free Wood. ASTM International, West Conshocken, PA, pp. 147–148.

American Wood-Preservers' Association (AWPA), 2010a. Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites. Standard E1-09. Book of Standards. American Wood-Preservers' Association, Birmingham, AL, pp. 351–359.

American Wood-Preservers' Association (AWPA), 2010b. Standard Method of Testing Wood Preservatives by Laboratory Soil-block Cultures. Standard E10-09. Book of Standards. American Wood-Preservers' Association, Birmingham, AL, pp. 383–392.

Chedgy, R.J., Lim, Y.W., Bruille, C., 2009. Effects of leaching on fungal growth and decay of Western Redcedar. Canadian Journal of Microbiology 55, 578–586.

Clausen, C.A. 2010. Chapter 14: biodeterioration of wood. In. Forest Products Laboratory. Wood Handbook-Wood as an Engineering Material. R.J. Ross [Ed.], General Technical Report FPL-GTR 190. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI: 508 p.

Cushnie, T.P., Lamb, A.J., 2005. Antimcrobial activity of flavonoids. International Journal of Antimicrobial Agents 26, 343–356.

EN 350–1, 1994. Durability of Wood and Wood-Based Products-Natural Durability of Solid Wood. British Standards Association, p. 12.

Evans, P., 2003. Emerging technologies in wood protection. Forest Products Journal 53 (1), 14–22.

Hwang, W.J., Kartal, S.N., Yoshimura, T., Imamura, Y., 2007. Synergistic effect of heartwood extractives and quaternary ammonium compounds on termite resistance of treated wood. Pest Management Science 63, 90–95.

Jun-Qing, C., et al., 1983. Studies on the wood properties of the genus Paulwonia I, II, and III. Scienta Silvae Sinicae 19, 57–63, 19: 153–167, 19: 284–291.

Laks, P.E., McKaig, P.A., 1988. Flavanoid biocides: wood preservatives based on condensed tannins. Holzforschung 42, 299–306.

Latorraca, J.V.F., Dunisch, O., Koch, G., 2011. Chemical composition and natural durability of juvenile and mature heartwood of Robinia pseudoacacia L. Anais da Academia Brasileira de Ciencias 83, 1059–1083.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS® for Mixed Models, Second Edition. SAS Institute, Inc, Cary, NC, p. 814.

MacDaniel, C.A., 1982. Major antitermitic components of the heartwood of southern Catalpa. Journal of Chemical Ecology 18, 359–369.

Mori, A., Nichino, C., Enoki, N., Tawata, S., 1987. Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphyllococcus aureus*. Phytochemistry 26, 2231–2234.

Morrell, J.J., 2011. Resistance of selected wood-based materials to fungal and termite attack in non-soil contact exposures. Forest Products Journal 61, 685–687.

Morrell, J.J., Miller, D.J., Schneider, P.F., 1999. Service Life of Treated and Untreated Fence Posts: 1996 Post Farm Report. Research Contribution 26. Forest Research Laboratory, Oregon State University, p. 24.

Morris, P.I., Stirling, R., 2012. Western red cedar extractives associated with durability in ground contact. Wood Science Technology 46, 991–1002.

Morris, P., Laks, P., Lebow, S., 2011. Standardization of naturally durable wood species. In: Proceeding of the American Wood Protection Association. American Wood Protection Association, Birmingham, AL.

Olson, J.R., Carpenter, S.B., 1985. Specific gravity, fiber content, and extractive content of young Paulownia. Wood and Fiber Science 17, 428–438.

Piepho, H.P., 2004. An algorithm for a letter – based representation of all-Pairwise comparisons. Journal of Computational and Graphical Statistics 13, 456–466.

Räberg, U., Edlund, M.L., Terziev, N., Land, C.J., 2005. Testing and evaluation of natural durability of wood in above ground conditions in Europe-an overview. Journal of Wood Science 51, 429–440.

Samoylenko, V., Ashfaq, M.K., Jacob, M.R., Tekwani, B.L., Khan, S.I., Manly, S.P., Joghi, V.C., Walker, L.A., Muhammad, I., 2009. Indolizidine, Antiinfective and Antiparasitic Compounds from *Prosopis glandulosa* var. *glandulosa*. Journal of Natural Products 72, 92–98.

Scheffer, T.C., Cowling, E.B., 1966. Natural resistance of wood to microbial deterioration. Annual Review of Phytopathology 4, 147–168.

Scheffer, T.C., Morrell, J.J., 1998. Natural Durabilty of Wood: A Worldwide Checklist of Species. Research Contribution 22. Forest Research Laboratory, Oregon State University, p. 58.

Singh, T., Singh, A.P., 2012. A review of natural products as wood protectant. Wood Science Technology 46, 851–870.

Sirmah, P., Iaych, K., Poaty, B., Dumarcay, S., Gerardin, P., 2009. Effect of Extractives on Durability of Prosopis Juliflora Heartwood. International Research Group on Wood Protection. Doc. No. IRG-WP 09–30518.

Smith, A.L., Campbell, C.L., Walker, D.B., Hanover, J.W., 1989. Extracts from black locust as wood preservatives: extraction of decay resistance from black locust heartwood. Holzforschung 43, 293–296.

Stirling, R., 2010. Residual extractives in Western Red Cedar Shakes and Shingles after long-term field testing. Forest Products Journal 60, 353–356.

Stirling, R., Daniels, C.R., Clark, J.E., Morris, P.I., 2007. Methods for Determining the Role of Extractives in the Natural Durability of Western Red Cedar. International Research Group on Wood Protection. Doc No. IRG-WP 07–20356.

Taylor, A.M., Gartner, B.L., Morrell, J.J., 2002. Heartwood Formation and natural durability – a review. Wood and Fiber Science 34 (4), 587–611.

Thevenon, M.F., Tondi, G., Pizzi, A., 2009. High performance tannin resin-boron wood preservatives for outdoor end-uses. European Journal of Wood and Products 67, 89–93.

Weldon, D., 1986. Exceptional physical properties of Texas mesquite. Forest Ecology Management 16, 149–153.

Wiemann, M.C., 2010. Chapter 2: characteristics and availability of commercially important woods. Forest Products Laboratory. In: Ross, R.J. (Ed.), Wood Handbook-Wood as an Engineering Material. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison WI, p. 508. General Technical Report FPL-GTR 190.

Williams, C.E., 1993. The exotic empress tree, *Paulownia tomentosa*: an invasive pest of forests. Natural Areas Journal 13, 221–222.

Yang, D.Q., 2009. Potential utilization of plant and fungal extracts for wood protection. Forest Product Journal 54, 37–39.