International Biodeterioration & Biodegradation 75 (2012) 104–108

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

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

Biological performance of *Liquidambar orientalis* Mill. heartwood

Evren Terzi [a], S. Nami Kartal [a,*], Claudia Marcela Ibáñez [b], Coşkun Köse [a], Rachel Arango [c,1], Carol A. Clausen [c,1], Frederick Green III [c,1]

[a] Department of Forest Biology and Wood Protection Technology, Forestry Faculty, Istanbul University, Bahcekoy Sariyer, 34473 Istanbul, Turkey
[b] Unidad Académica de Gestión Tecnológica, Departamento Tecnológico, Facultad de Química, Universidad de la República, Uruguay
[c] USDA Forest Service, Forest Products Laboratory, 53725 Madison, WI, USA

ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form
25 April 2012
Accepted 25 April 2012
Available online

Keywords:
Heartwood
Extractive
Decay
Mold
Termite
Insect
Liquidambar orientalis

ABSTRACT

New approaches for wood protection based on green technologies have increased interest in using heartwood portions of certain wood species for the effects (toxic and antioxidant properties, chelate formation, hydrophobicity) of extractives located in heartwood. This study evaluated the biological performance of heartwood of *Liquidambar orientalis* Mill., trees well-known for production of balsam. Heartwood specimens were subjected to soil-block decay tests based on the American Wood Protection Association standard method using two brown-rot and two white-rot fungi. Specimens were also subjected to two different laboratory termite resistance tests. Additionally, heartwood specimens were tested for mold growth and resistance to furniture beetle larvae. Laboratory fungal decay resistance tests showed that the heartwood of the tree was not resistant against the fungi tested; however, the wood was resistant against termites and furniture beetle larvae in laboratory conditions. Mold tests revealed that the wood also showed resistance to mold growth. Complete biological resistance was not achieved in this study, suggesting that heartwood extractives do not directly correspond to resistance to wood-degrading fungi.

1. Introduction

Liquidambar orientalis Mill., a member of Hamamelidaceae family, is ecologically and economically important due to the balsam produced naturally by the species, which is used mainly in the cosmetics and pharmaceutical industries (Öztürk et al., 2008). However, *L. orientalis* wood has very limited applications, such as furniture, veneer production, ornamental and decorative goods, etc. (Bozkurt et al., 1989; Doğu et al., 2001) and very few investigations have been made on the properties of wood itself. Detailed studies are needed to evaluate the resistance of *L. orientalis* wood against wood decay and mold fungi, termites, and other insects; evaluation of its chemical properties is needed as well. Our previous study on biological performance properties of balsam from *L. orientalis* trees showed that balsam-treated pine sapwood specimens generally displayed moderate resistance to fungal decay by two white-rot fungi, based on the ASTM D2017 classification (ASTM, 2010). In an agar toxicity test, balsam at the concentration level of 0.5% inhibited all test fungi in vitro except *Trametes versicolor* and *Ceratocystis pilifera* (Kartal et al., 2012).

With regard to establishment of new plantations of the species, the wood might become important for indoor building and roofing material (Bozkurt et al., 1989). As an endemic tree species in Turkey, *L. orientalis* has been locally distributed in the southwestern coastal area of the country (Yaltırık and Efe, 2000; Sağdıç et al., 2005; Öztürk et al., 2008). Or (2007) and Yaltırık and Efe (2000) argue that *L. orientalis* forests have been seriously damaged because of habitat destruction, grazing, unsuitable balsam extraction methods that seriously harm the trees, and the continual extraction of balsam for export. In order to protect the remaining stands from overexploitation, various measures have been undertaken by the Turkish Forestry Service (Yaltırık and Efe, 2000). In recent years, the Ministry of Forestry and Water Affairs of Turkey and the Ministry of Environment and Urban Planning have started various projects regarding protection of *L. orientalis* trees, formation of new plantations, regeneration of the ecosystem in *L. orientalis* forests, and implementation of novel management plans for balsam production.

* Corresponding author. Tel.: +90 212 226 11 00; fax: +90 212 226 11 13.
E-mail address: snkartal@istanbul.edu.tr (S. Nami Kartal).

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http://dx.doi.org/10.1016/j.ibiod.2012.04.025

An attempt has been made here to present an overview of the natural durability of the wood against various biological agents. In the study, the heartwood specimens of *L. orientalis* were tested against various decay and mold fungi, termites, and insects under laboratory conditions.

2. Materials and methods

2.1. Wood specimens

Two trees were obtained for experimentation: one *L. orientalis* Mill. tree, obtained from Mugla, Turkey, and a *Pinus slyvestris* L. (Scots pine) tree, harvested from Bolu, Turkey. Since decay resistance can vary according to the position in the heartwood from which an item of wood is taken (the upper versus the lower trunk), heartwood portions from the trees used in the study were cut from a disk (20 cm thick) obtained at breast height (1.3 m above ground level) of each tree. Test specimens were cut from the center area of the heartwood discs since durability can change from the heartwood boundary toward the pith. The specimens chosen were free of knots or any visible concentration of resins, and showed no visible evidence of infection by mold, stain, or wood-destroying fungi.

Prior to decay and termite resistance tests, wood specimens were extracted with either cold water (48 h), hot water (48 h), hot water (48 h)/ethanol (48 h), or ethanol/cyclohexane (1:2 v/v) (6 h) in accordance with the Technical Association of the Pulp and Paper Industry standards TAPPI T 207 cm-99 and TAPPI T 204 cm-97 (TAPPI, 1999). Unextracted *L. orientalis* heartwood served as a control. For termite resistance tests unextracted *L. orientalis* heartwood and ethanol-extracted (48 h) Scots pine specimens were tested. Following extractions, all test specimens were conditioned at 27 °C and 70% relative humidity (RH) for 4 wk.

2.2. Decay resistance tests

Heartwood specimens of *L. orientalis* (19 by 19 by 19 mm) were tested against two brown-rot and one white-rot fungi in soil block tests based on the ASTM D2017-05 standard method with some modifications (ASTM, 2010). In the tests, southern pine feeders were inoculated with the brown-rot fungi *Tyromyces (Fomitopsis) palustris* (Berkeley et Curtis) Murrill (FFPRI 0507) and *Gloeophylum trabeum* (Pers:Fries) Murrill, MAD 617, and maple feeders were inoculated with the white-rot fungus *T. versicolor* (L.: Fr.) Quel. MAD 697. Bottles were incubated at 27 °C and 70% RH for 3 wk until the fungus completely colonized each feeder. Following the conditioning period, wood specimens were steam-sterilized and placed on actively growing feeders in soil bottle cultures. Soil bottles were incubated at 27 °C, 70% RH for 12 wk. Following incubation, surface mycelium was brushed from each specimen before the specimens were oven-dried at 40 °C for 24 h, and reconditioned at 27 °C and 70% RH to a constant weight. Average percentage mass loss was calculated for each specimen.

2.3. Mold resistance tests

Heartwood specimens of *L. orientalis* (7 mm tangential by 20 mm radial by 7 cm long) were evaluated for resistance to mold fungi according to a modification of ASTM D4445-10 (ASTM, 2010). Three mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476, were grown and maintained on 2% malt agar (Difco, Detroit, MI, USA) at 27 °C, and 80% RH. All fungi were obtained from the USDA Forest Service Forest Products Laboratory, Madison, WI, USA. A mixed spore suspension of the three test fungi was prepared by washing the surface of individual 2-wk-old petri plate cultures with 10–15 ml of sterile DI water. Washings were combined in a spray bottle and diluted to approximately 100 ml with DI water to yield approximately 3×10^7 spores ml^{-1}. The spray bottle was adjusted to deliver 1 ml of inoculum per spray. Wood specimens were sprayed with 1 ml of mixed mold spore suspension and incubated at 27 °C and 80% RH for 12 wk. Even though the standard test required a 4-wk incubation, we extended the test duration to 12 wk to reach complete coverage in control Scots pine specimens. Following incubation, specimens were visually rated on a scale of 0–5, with 0 indicating the specimen is completely free of mold growth and 5 indicating the specimen was completely covered with mold growth (0: no growth, 1: 20% coverage with mold fungi, 2: 40%, 3: 60%, 4: 80%, 5: 100%).

2.4. Termite resistance-ASTM D3345-08 test method

A no-choice termite resistance test with *Reticulitermes flavipes* Kollar (Eastern subterranean termites) was performed using heartwood specimens of *L. orientalis* (25 by 25 by 5 mm). Termites were collected from Janesville, WI, USA. One specimen was placed in the bottom of an acrylic cylindrical container (90 mm diameter and 60 mm height) with 1 g of *R. flavipes* and moist sand. The containers were maintained at 27 °C and 85% RH for 4 wk based on the ASTM D3345-08 standard method (ASTM, 2010). Tests were periodically checked for moisture and mortality. At the end of the test, wood specimens were oven-dried, reconditioned at 27 °C and 70% RH, and reweighed to calculate mass losses.

2.5. Termite resistance-JIS K 1571 test method

Heartwood specimens of *L. orientalis* (20 by 20 by 10 mm) were exposed to the subterranean termites *Coptotermes formosanus* Shiraki according to the JIS K 1571 standard method (JIS, 2004). An acrylic cylinder (80 mm diameter, 60 mm height) whose lower end was sealed with a 5-mm-thick hard plaster (GC New Plastone, Dental Stone, GC Dental Industrial Corp., Tokyo, Japan) was used as a container. A test specimen was placed at the center of the plaster bottom of the test container. A total of 150 worker termites collected from a laboratory colony of the Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Japan, were introduced into each test container together with 15 termite soldiers. The assembled containers were set on damp cotton pads to supply water to the specimens and kept at 28 °C and >85% RH in darkness for 3 wk. The mass losses of the specimens due to termite attack were calculated based on the differences in the initial and final oven-dry (60 °C, 3 days) weights of the specimens after cleaning off the debris from the termite attack.

2.6. Resistance to Anobium punctatum larvae

The assay was carried out based on the BS EN 48:2005 standard (BSI, 2005). Heartwood specimens of *L. orientalis* (50 by 25 by15 mm) were conditioned at 20 °C and 65% RH for 2 wk before testing. Sapwood specimens of *Pinus taeda* (pine) and *Quercus* sp. (oak) served as controls.

The larvae of *A. punctatum* were obtained from the Unidad Académica de Gestión Tecnológica Departamento Tecnológico, Facultad de Química, Universidad de la República, Uruguay. They were kept in storage blocks in the culture chamber for two months until needed. The mean mass of the larvae used was approximately 3.5 mg. Insect specimens selected were in good condition and were not damaged or infested with mites.

Wood specimens were kept in the conditioning chamber for 2 wk before the holes to house the larvae were drilled. Six cylindrical holes approximately 5 mm deep were drilled in the two

Table 1
Solubility of sapwood and heartwood of *L. orientalis*.

Properties	Sapwood %	Heartwood %
Ethanol solubility	0.68	1.98
Hot water solubility	4.82	5.64
Ethanol/toluene solubility	2.46	2.73

transverse cross sections of each test specimen with a pattern of holes in two lines of three 10 mm from the large faces of the test specimen; the distance between the holes within a row was 15 mm, and the distance between the two rows was 10 mm.

The selected larvae were inserted into the 12 holes of each specimen. The entrances of the holes were sealed by means of a glass plate and fixed with an adhesive tape. The test specimens were kept in the culturing chamber at 20 °C and 80% RH placed horizontally on their cross-section face. After the first 2 wk of the test, dead larvae or larvae that had not started boring were replaced, and at that time, the glass plate was removed and the transverse cross section was sealed with paraffin wax. All specimens were kept in the culture chamber for 16 wk. The results were considered valid when at least 70% of the larvae retrieved from the control test specimens were alive, the larvae showed a satisfactory level of activity, and live larvae were present in all control test specimens. Results were expressed as the number of larvae retrieved from test specimens separating dead and moribund larvae from living larvae along with mortality rates.

2.7. Statistical analysis

Statistical analysis was conducted using the SPSS program in conjunction with analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to test statistical significance at the $\alpha = 0.05$ level.

3. Results

Table 1 gives solubilities and ash content of heartwood and sapwood of *L. orientalis* by various solvents. Higher solubilites were obtained by hot water extraction in comparison with ethanol only and ethanol/toluene extractions. In general, the ethanol/toluene extractable content of wood consists of waxes, fats, resins, oils, and tannins. The cold-water extraction removes part of the extraneous components, such as inorganic compounds, tannins, gums, sugars, and components imparting color to the heartwood, while the hot-water procedure removes starches in addition to the compounds removed by cold-water extraction.

Heartwood specimens of *L. orientalis* were susceptible to fungal decay by the two brown-rot fungi and one white-rot fungus (Table 2). According to the ASTM classification (Table 2 footnote), heartwood specimens of *L. orientalis* were "non-resistant" against the brown-rot fungus, *T. palustris*; however, the specimens were moderately resistant against the brown-rot fungus *G. trabeum* and the white-rot fungus *T. versicolor* based on the average mass losses in the specimens. The extraction method had a mixed effect on the mass losses.

Termite tests results based on the JIS K 1571 test method with *C. formosanus* are given in Table 3. Mass losses in the unextracted specimens after termite attack for 3 wk were less than those in extracted specimens, suggesting that extractions removed some compounds that are toxic to the termites. Low termite mortalities indicate that heartwood compounds might be non-toxic repellant. Termites caused higher mass losses in Scots pine specimens when compared to heartwood specimens from *L. orientalis*. Termite mortalities were also lower in comparison with *L. orientalis*. Mass losses in the heartwood specimens of *L. orientalis* exposed to *R. flavipes* termites based on the ASTM D3345-08 test method were considerably higher than those in the specimens in the JIS K 1571 test method using *C. formosanus* termites (Table 4). However, the two termite species caused identical mass losses in unextracted Scots pine specimens.

The resistance of *L. orientalis* heartwood against the larvae of *A. punctatum* is shown in Table 5. The results were considered valid because 100% of the larvae were alive at the end of the test in pine control specimens. In oak control specimens 76% of the larvae were alive due to different hemicelluloses content when compared to pine wood since the hemicelluloses are apparently the main component of larval nutrition in conifers (Serdjukova and Toskina, 1996). The mortality rates of the larvae in *L. orientalis* heartwood specimens were higher compared to those in control specimens due to heartwood components being toxic to the larvae. The larvae showed no growth or activity in *L. orientalis* heartwood test specimens. Also, larval tunnels toward the surface were absent, and there was a disruption of regular boring, probably due to larval feeding patterns.

Mold resistance tests revealed that heartwood specimens of *L. orientalis* were resistant against the mold fungi when compared to control Scots pine specimens. The Scots pine specimens were almost completely covered by the mold fungi after a 12-wk-exposure period; however, heartwood specimens of *L. orientalis* showed mold growth of around 20% based on the visual rating system used in the study (Fig. 1).

4. Discussion

Heartwood specimens of *L. orientalis* were not resistant against the wood-decaying fungi tested even though the wood has

Table 2
Mass losses (%) in the specimens exposed to test fungi for 12 weeks.

Wood specimens	Fungi					
	T. palustris		*G. trabeum*		*T. versicolor*	
	Average	Std Dev	Average	Std Dev	Average	Std Dev
L. orientalis heartwood-unextracted	50.66B	5.56	40.2C	8.93	37.08B	9.03
L. orientalis heartwood-cold water/48 h-extracted	49.65B	5.14	38.25D	3.72	40.59A	5.5
L. orientalis heartwood-hot water/48 h-extracted	49.92B	7.65	41.04C	7.43	38.35B	6.19
L. orientalis heartwood-hot water-48 h/ethanol-48 h-extracted	48.89B	6.55	42.53C	8.45	34.05BC	8.29
L. orientalis heartwood-ethanol/cyclohexane-extracted-6 h-extracted	49.82B	4.56	37.26D	2.83	36.72B	8.02
Scots pine sapwood-ethanol-48 h-extracted	57.23A	3.45	61.46A	6.06	42.28A	4.28
Scots pine sapwood-unextracted	51.11B	3.02	56.06B	2.36	40.95A	3.91

Each value is average of nine specimens for each fungus and each wood specimen group. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test ($p < 0.05$). ASTM D 2017 classification based on mass losses in wood specimens: 0–10%: highly resistant; 11–24%: resistant; 25–44: moderately resistant; 45 or above: slightly resistant or non-resistant.

Table 3
Resistance of *L. orientalis* heartwood to *C. formosanus*.

Wood specimens	Mass loss in the specimens (%)		Mass loss in the specimens (g)		Wood consumption rate (ug/termite/day)		Termite mortality (%)
	Average (%)	Std Dev	Average	Std Dev	Average	Std Dev	Average
L. orientalis heartwood-unextracted	9.14D	6.01	0.24	0.15	68.88D	44.63	14.55
L. orientalis heartwood-cold water/48h-extracted	15.40B	1.38	0.40	0.05	115.34B	13.98	10.91
L. orientalis heartwood-hot water/48h-extracted	15.49B	3.87	0.40	0.09	114.05B	26.85	9.09
L. orientalis heartwood-hot water-48h/ethanol-48h-extracted	15.26B	0.96	0.39	0.02	112.07B	6.08	11.52
L. orientalis heartwood-ethanol/cyclohexane-extracted-6h-extracted	12.68C	0.41	0.33	0.00	95.72C	1.17	7.27
Scots pine sapwood-ethanol-48h-extracted	29.45A	2.09	0.76	0.08	219.92A	12.56	2.42
Scots pine sapwood-unextracted	23.67A	3.21	0.62	0.04	178.86A	13.45	3.03

Each value is average of five specimens for wood specimen group. Values in parentheses are standard deviations. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test ($p < 0.05$).

Table 4
Resistance of *L. orientalis* heartwood to *R. flavipes*.

Wood specimens	Mass loss (g)	Mass loss (%)	Consumption rate (ug/termite/day)
L. orientalis heartwood-unextracted	0.40 (0.05)C	16.04 (2.27)C	47.65 (9.03)C
Scots pine sapwood-ethanol-48 h-extracted	0.84 (0.11)A	43.71 (13.53)A	100.00 (21.03)A
Scots pine sapwood-unextracted	0.52 (0.11)B	25.87 (5.41)B	61.93 (11.34)B

Each value is average of five specimens for wood specimen group. Values in parentheses are standard deviations. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test ($p < 0.05$).

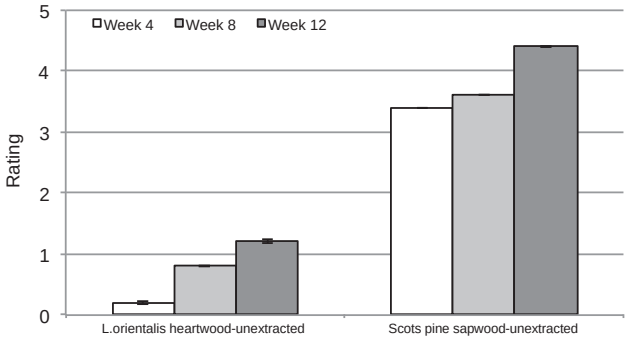

Fig. 1. Mold resistance test results. (Specimens were visually rated on a scale of 0–5, with 0 indicating the specimen is completely free of mold growth and 5 indicating complete coverage. 0: no growth, 1: 20% coverage with mold fungi, 2: 40%, 3: 60%, 4: 80%, 5: 100%. Each value is the average of ten specimens).

a reddish brown color and it has been theorized that the color of heartwood represents the presence/absence, characteristics, and concentrations of extractives in the wood (Gierlinger et al., 2004). Scheffer and Morrell (1998) also classified the heartwood of a similar wood species, *Liquidambar styraciflua* (sweetgum), as non-resistant or perishable based on laboratory tests. The heartwoods of some wood species have varying degrees of natural decay resistance, which is greatly affected by differences in the protective qualities of the wood extractives. However, a review by Taylor et al. (2002) states that extractive content in heartwood does not necessarily correspond to natural durability, and decay resistance is sometimes weakly correlated with variations in the heartwood compounds. In this study, hot water, ethanol, and ethanol/toluene extractions showed that the heartwood of *L. orientalis* contained some amount of extractives that were not effective against the test fungi based on the decay resistance tests. Based on the review study by Taylor et al. (2002), even though some wood species such as *Larix* spp. contain high concentrations of extractives, the wood is not resistant to decay. In this study, termite and larvae resistance tests revealed that the heartwood of *L. orientalis* was more resistant against insects than fungi.

Table 5
Resistance of *L. orientalis* heartwood to *A. punctatum* larvae.

Specimen #	Number of larvae retrieved	Number of dead or moribund larvae	Number of live larvae	Mortality rate (%)
#1	12	12	0	100
#2	11	10	1	91
#3	11	11	0	100
#4	12	12	0	100
#5	12	12	0	100
#6	11	11	0	100
Total	69	68	1	
Mean	11.50	11.33	0.17	98.5

Control test specimens	Pine	Oak	Pine	Oak	Pine	Oak	Pine	Oak
#1	12	11	0	8	12	3	0	73
#2	12	11	0	8	12	3	0	73
#3	11	11	0	9	11	2	0	82
Total	68		25		43			
Mean	11.67	11	0	8.33	11.67	2.67	0	76

5. Conclusion

Several wood species may have specific components in their heartwoods that are effective against a number of wood-degrading organisms. In this study, even though the heartwood of *L. orientalis* contained some amount of extractives proved by water and solvent extractions, the wood was not resistant to fungal decay. However, mass losses in the specimens exposed to termites were considerably lower than those in control sapwood specimens prepared from pine species. The heartwood was also resistant to the larvae of *A. punctatum* in laboratory conditions when compared to pine and oak controls. Even though the heartwood was easily colonized by the wood-degrading fungi, the wood was resistant against the mold fungi. Further studies are in progress to determine chemical properties and identify heartwood extractives of *L. orientalis* to correlate durability properties of the wood with the extractives.

Acknowledgements

The authors acknowledge financial support by the Coordination Unit for Scientific Research Projects, Istanbul University, Turkey (Project No: 4436). We also thank Lourdes Silva y Ana Ibarra, Carlos Mantero, and Mario Rabinovich of Unidad Académica de Gestión Tecnológica Departamento Tecnológico, Facultad de Química, Universidad de la República, Uruguay, for their assistance and support for insect resistance tests.

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