Comparative Study of Fungal Deterioration in Liquidambar *Orientalis* Mill Heartwood Extractives

*Roderquita K. Moore, Research Chemist, USDA-Forest Service, roderquitamoore@fs.fed.us
Doreen Mann, Research Technician, USDA-Forest Service, dmann@fs.fed.us
Nural Yilgor, Associate Professor, Istanbul University, yilgorn@istanbul.edu.tr
*Corresponding author

Abstract

A comparative study was done on Liquidambar Orientalis Mill heartwood extractive samples. These extractives were collected from wood decay specimens. The objective of the study was to determine the chemical composition of the extractives remaining after exposure to brown rot fungi *Tyromyces Palustris* and white rot fungi *Trametes Vericolor*. All samples were extracted according to TAPPI Standard T-204. Samples were put through two different solvent systems and characterized by analytical techniques for any changes in chemical and morphological properties. The extracted chemicals collected were control 13%, *T. versicolor* 18% and *T. palustris* 69%. The polar composition remains higher than the nonpolar composition as is usually observed in hardwoods.

Keywords: *extractives, deterioration, wood fungi, polar, nonpolar, GC-MS*

Introduction

Biodegradation by fungi will alter the chemical properties of some, if not most, of the general material that makes up tree tissue. So depending on the tree species, the tree anatomy, and the fungi group (white, brown, or soft) that a wood source is exposed to, the relative amounts of molecular structuring could change drastically.[1] It should be possible to determine structural degradation in the form of fungal decay within a tree species by comparing the extractives from a healthy wood sample along with extractives from wood samples exposed to fungi. Comparison of biodegradation from exposure to different fungi is another possibility because of the chemical alterations that are characteristically found to be associated with the fungi species.

Extractives are exudates formed by the tree through secondary metabolism after damage or foreign attack by insects or fungi. Extractives form a variety of classes of chemicals according to their origin within the tree. Resin acids are located in the resin canal, fats and waxes are in the ray parenchyma cell and phenolic extracts are in the bark and heartwood. These chemicals are important and necessary for the tree to function. For example, fats are an energy source of the wood cells and terpenoids, while resin acids and phenolics protects the tree against microbial attack.[2,3] Extractives from Liquidambar Orientalis Mill are known for anti-fungal activity and one of the chemicals identified is Benzyl benzoate which has high toxicity against insects.

In this study, Liquidambar *Orientalis* heartwood (LOH) samples were exposed to brown-rot fungi *Tyromyces Palustris* and white-rot fungi *Trametes Vericolor*.[1] Liquidambar *Orientalis* Mill is one of the most important endemic hardwoods, in Turkey. The main objective for this comparative study is to evaluate analytical and quantitative analyses of the changes in LOH extractives due to exposure to white and brown wood decay fungi.

Experimental

Sample Preparation

Researchers in Turkey sent fungi-exposed Liquidambar *Orientalis* Mill heartwood chips along with a milled offsite untreated control sample.[1] Each of the fungi-treated wood samples were Wiley milled through a 40 mesh sieve. Extraction thimbles were filled with approximately 35.0 g of milled materials. The samples were extracted using the Soxhlet extraction apparatus with a 2 cycle per hour, up to 24 hours solvent turnover rate. The initial solvent composed of 500 mls of 2:1 toluene:ethanol (T:E) mixture was followed by 95% ethanol solvent extraction. Extractives were filtered for any impurities and solvent was evaporated using a rotary evaporator. Extracts were placed in a freezer.[4]

Sample Extractions

A sample containing 60-100 mg from each solvent extractions was transferred into 5 ml vials. Polar and nonpolar partitions were created from 30 ml total of a 5:1 hexane:chloroform solution.[5] Samples were mixed thoroughly by using a vortex and sonicator. Separation was achieved by allowing the polar fraction to settle to the bottom and the nonpolar liquid fraction was decanted off and transferred into a 5 ml vial. Each fraction was dried under nitrogen, weighed, and sealed until further analysis.

Results and Discussion

Klason lignin and sugars were analyzed from the T:E extracted samples (Table 1). Total yield and total carbohydrates between the control and the *T. versicolor* samples are similar. White rot fungi are known to degrade cellulose, hemicellulose, and lignin.[1] So, it is not clear why the unmodified control would contain amounts that are similar to *T. versicolor*. Compared to the control T. versicolor percent differences were 4.5% for Klason lignin and 3.2% for Glucan. The different values between polysaccharides do run along a general trend for the fungi groups. Previous research has shown that, unlike white rots, brown rots are not likely to digest lignin while they are decomposing the polysaccharides.[1] This is observed by the glucan decrease difference by 21.2% and an increase difference in the Klason lignin by 41.5%. The increase in Klason lignin could be indication of lignin composition changing during the exposure. The percent of Klason Lignin is greater for *T. palustris* than compared to the *T. versicolor*. It is known that brown rot fungi have greater effect on the chemical properties than white rot fungi. These two analytical trends are congruent with how two groups of fungi behave within the wood tissue compared to untreated wood samples.

Table 1. Klason lignin and sugar analysis of extractives free LOH wood specimen

Sample	**Klason Lignin**	**Arabanan**	**Galactan**	**Glucan**	**Xylan**	**Mannan**	**Total Carb.**	**Total Yield**
Control	20.4%	0.54%	0.54%	42.2%	16.3%	2.7%	62.2%	86.0%
T. palustris	28.9%	0.00%	0.00%	33.3%	13.9%	1.2%	48.4%	80.9%
T. versicolor	19.5%	0.69%	0.69%	44.9%	18.0%	2.8%	63.1%	85.7%

The percentages presented in figure 1 for polar fractions are compared to each other and the same for nonpolar fractions. This allows for comparison of samples to be related to the control. The chemical compositions extracted during the fractionation are known for removing nonpolar chemicals like fatty acids, waxes, and sterols.[5] The polar chemicals are typically identified as phenols and low carbohydrates.

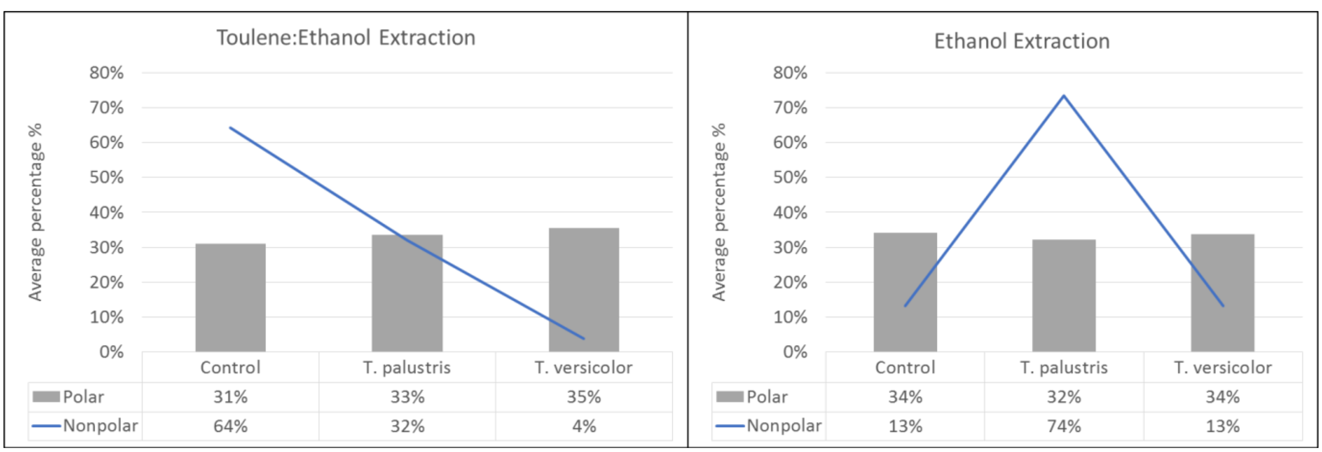

Figure 1. LOH average percentages of nonpolar and polar fractionations.

The percentage of polar composition are similar in wood rot fungi and control. The same is observed for the ethanol extraction. The T:E extraction percent change between the brown-rot and white rot fungi are 6% and 13% higher than the control respectively. The ethanol extraction extractives percent change were the virturally the same for control, *T. versicolor* and *T. palustris*. This information can imply the phenols and other chemical concentrations may or may not change but the same polarity of chemicals remains the same during exposure to the wood rot fungi. This does not give specific names of chemicals but it does characterize the class of chemicals for identification. The nonpolar fractions however, are different. The extractives percent change for the control chemical composition is 50% and 94% decrease in the T:E extraction. This implies the chemical composition of the nonpolar content decreases when LOH is exposed to wood rot fungi.

The nonpolar ethanol extraction shows no change for *T. versicolor* and the control extracts which is also observed in the polar fraction. However, the increase in LOH extractives exposed to *T. palustris* is 61. The average percentages of polar and nonpolar extractives from the exposure of T. palustris is 93% and 7% respectively. (not shown) The chemical composition is significantly high in *T. palustris* than the control extractives. So, further analyses will be done on the chemical composition of the extractives exposed to T. palustris.

Figure 2 is a sum of the total percentage of extractives from both extractions. The extractives collected from the LOH exposed to *T. palustris*, *T. versicolor* and the control were 69%, 18% and 13% respectively. Brown-rot fungi exposed to wood is known for increasing and changing the chemical composition of the wood. Knowing the chemical characteristics and known individual chemicals that caused this increase can give more information on the class of chemicals being formed or increased.

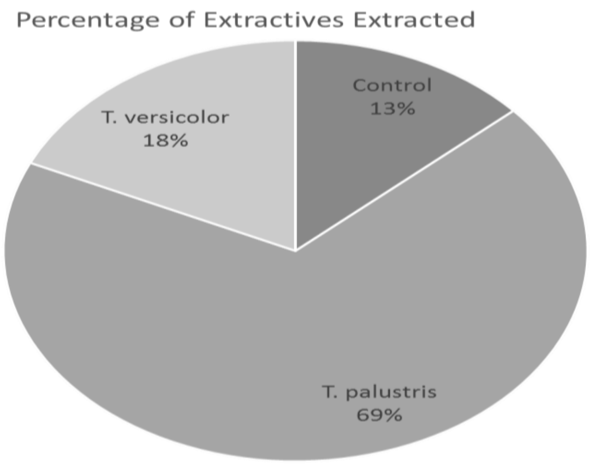

Figure 2. The percent distribution of the extractives collected from *T. versicolor*, *T. palustris* and control of LOH wood samples.

Conclusions

This study investigated Liquidambar *Orientalis* Mill heartwood extractives chemicals polarity and weight changes when expose to fungi. The LOH extractives collected under the fungi condition did change significantly. However further investigations will determine whether these changes are within the extractives or whether other chemicals are forming due to the wood's decomposition. The goal of future work is to identify and characterize the chemicals resulting from fungi exposure. In the present study:

- The sugar analysis of the LOH *T. versicolor* exposure showed the total yield and total carbohydrates between the control and the *T. versicolor* samples are similar and not much change occurred.
- The polar fraction of T:E extraction had similar percentages which implied the phenols and other chemical concentrations may or may not have changed.
- The percentage changes in T:E extraction nonpolar fractions implies the chemical composition decreased when LOH is exposed to the wood rot fungi.
- Further analyses on chemical composition in the nonpolar fraction is significantly higher in *T. palustris* than the control so identifying the chemicals will be next step.

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