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Effect of Fusiform Rust (*Cronartium quercuum* f.sp. *fusiforme*) on the Composition of Loblolly Pine Lignin

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Abstract

Loblolly Pine, *Pinus taeda*, is a common southern softwood ranging from Georgia and the Carolinas to Texas. The fungus *Cronartium quercuum* f.sp. *fusiforme* is one the most destructive forest diseases in the south. This fungus infects both loblolly and slash pine (*P. elliottii* Engelm.), causing canker formation that frequently kills the infected branch. April and early May are when the pine infection cycle occurs in Georgia. The elongated swelling of the branches is the result of individual attacks on different parts of the tree. Many of the infected trees are unsuitable for product, causing millions of dollars to be lost a year because of the death of infected trees. In addition, trees with large galls on the main stem are unsuitable for many products.

We report the chemical composition of loblolly pine infected with fusiform rust. Branches were collected in Athens, Georgia, and frozen. The branches not infected by fusiform were collected as the control. Spectroscopy was used to analyze lignin. This report presents and discusses the results.

The aim of this study was to apply Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) to determine whether there were any structural modifications to the chemical composition of lignin from the fungus. Published information on fusiform rust analyzed by FTIR and NMR is limited in the literature. In this paper, we present a FTIR and NMR spectroscopic analysis of the chemical changes occurring in a softwood, loblolly pine, by fusiform rust.

Keywords: FTIR, NMR, sugar analyses, lignin, fusiform rust, loblolly pine, southern pine, invasive species

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Cover Photo: Loblolly pine infected by fusiform rust

Content

Effect of Fusiform Rust (*Cronartium quercuum* f.sp. *fusiforme*) on the Composition of Loblolly Pine Lignin

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Introduction

Loblolly pine, *Pinus taeda*, is a common southern softwood ranging from Georgia and the Carolinas to Texas. The fungus *Cronartium quercuum* f.sp. *fusiforme* is one of the most destructive forest diseases in the South. This fungus infects both loblolly and slash pine causing canker formation that frequently kills the infected branch (Brown and Coder 2009). April and early May are when the pine infection cycle occurs in Georgia. The elongated swelling of the branches is the result of individual attacks on different parts of the tree. Many of the infected trees are unsuitable for product, causing millions of dollars to be lost a year because of the death of the infected trees. In addition, trees with large galls on the main stem are unsuitable for many products.

Changes in wood chemistry resulting from fungal decay of Scots pine (*P. sylverstris* L.) have been studied directly using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR). Scots pine sapwood was exposed to brown rot, selective white rot, and nonselective white rot fungi, and the decay process was followed using FTIR (Pandey and Pitman 2003). After 12 weeks, the wood exposed to the brown rot fungus resulted in progressive increase in lignin content relative to cellulose and hemicellulose, whereas the lignin content of the wood exposed to the selective white rot decreased as decay proceeded. For the wood exposed to the nonselective white rot wood, both occurred.

The aim of this study was to apply FTIR and NMR to determine whether the pathogen caused any structural modifications to the chemical composition of lignin.

Materials and Experimental Methods

Samples

The samples selected for this study were loblolly branches affected by fusiform rust. The control was branches that grew in the same environment but were not attacked by fusiform rust. The branches were collected by Pauline Spaine of the U.S. Forest Service Southern Research Station from loblolly pines located near Athens, Georgia.

Lignin Preparation

These branches were debarked, cut, and ground into 40-mesh grain sizes. The branches (50–100 g) were soxhlet-extracted with 700 ml of acetone:water (9:1) and ethanol (95%) for 8 hours. The isolation of milled wood lignin (MWL) was slightly modified from Björkman MWL (Björkman 1956).

A portion (500 mg) of MWL was acetylated with equal portions of pyridine and acetic anhydride for 4 hours. The mixture was then co-rotary evaporated with toluene and then acetone to remove the reaction co-products. The acetylated MWLs were then dissolved in deuterated acetone for NMR analysis. Acetylation was not done for the FTIR analysis.

Nuclear Magnetic Resonance

A Bruker 250 DPX Nuclear Magnetic Resonance Spectrometer (Bruker Corporation, Billerica, MA) was used to acquire ^{1}H, ^{13}C, Distortionless Enhancement by Polarization Transfer (DEPT) 135 and Heteronuclear Single Quantum Coherence (HSQC) spectra using standard Bruker pulse programs. The internal reference was set to the center peak of the acetone solvent 2.04/29.83 ppm.

Fourier Transform Infrared Spectroscopy

A Mattson Galaxy 5020 FT-IR system (Mattson Instruments, Inc., Madison, WI) was used to characterize KBr pelleted samples. FT-IR spectra were obtained in transmission mode by averaging 64 scans from 4000 to 450 cm^{-1} at 8 cm^{-1} resolution.

Results and Discussion

Nuclear Magnetic Resonance

Both the proton and carbon NMR spectra were acquired, along with a DEPT experiment that was run to distinguish

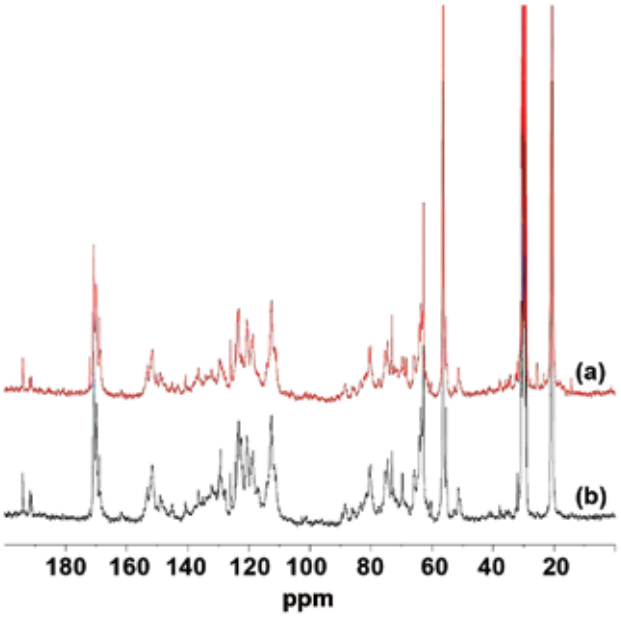

Figure 1. Full spectra of ^{13}C NMR. The red line (a) is loblolly pine infected by fusiform rust. The black line (b) is the control sample of loblolly pine.

the degree of protonation on the carbons in the ^{13}C NMR. A short range C-H correlation, HSQC, experiment was run to determine C-H correlations for signals of interest.

Carbon NMR

A comparison of the ^{13}C spectra from infected and non-infected (control) loblolly by fusiform rust shows areas of interest at approximately 170, 122, and 68 ppm (Fig. 1). The region between 173 and 169 ppm shows some differences (Fig. 2a). The signals shown in Fig. 2a are due to the carbonyl group of the acetate moieties (Shown by arrows). The primary, secondary, and phenolic hydroxyl group acetates are at 171, 170.5, and 168 ppm, respectively, in this region. The signal at 172 ppm is due to residual acetic acid from the derivitization procedure and may be disregarded.

The spectra of the carbonyl group were normalized on the gamma carbonyl signal 171 ppm. The carbonyl at the beta position 170.5 ppm intensity was the same; however, peak broadening was observed for the infected branches, indicating that neighboring groups were changing. The free hydroxyl phenolic acetates position at 169 ppm intensity increased, indicating there are more free phenolic side groups in the infected branches available for acetylation. Literature indicates that an increase in lignin and more phenolic compounds are observed in the infected sample (Rowan 1970).

Between 134 and 122 ppm were also differences (Fig 2b). Two methine carbon signals seen at 129 and 122 ppm are missing or diminished for the infected sample. This observation indicates that oxidation is occurring and is forming an aldehyde because of the fungal attack. Oxidation of this kind is observed in wood-decay-forming benzoates (Eriksson and others 1999).

The proton NMR observed peaks at 5.4 and 5.1 ppm (see arrows) in the control proton spectrum (Fig. 3). From 2D NMR, the two peaks seen at 68.8 and 68.3 ppm in the ^{13}C NMR correlates, respectively, to the two proton peaks. From the DEPT experiment, the peaks observed represented CH groups. HSQC showed the proton was low field. This observation correlated with the diminished peaks observed in the aromatic region at 129 and 122 ppm.

Fourier Transform Infrared Spectroscopy

FTIR spectra of infected and control loblolly pine are shown in Figures 4–7. The areas of interest are 2900 and 1640 cm^{-1} (See arrows). A strong hydrogen bonded (O-H) stretching absorption is seen at 3400 cm^{-1} and a prominent C-H stretching absorption around 2900 cm^{-1}. In addition, there are many peaks in the fingerprint region between 1800 and 600 cm^{-1}. It has been shown that the guaiacyl component (softwood lignin) absorbs near 1268 and 1230 cm^{-1} (Pandey 1999; Pandey and Pitman 2003).

One area of difference occurs at around 1650–1600 cm^{-1}; unfortunately, this is also an area where overlapping with moisture occurs. Several spectra were run to eliminate other possibilities for the difference (Fig. 5). Both samples as well as an extra sample of loblolly pine were oven dried (Fig. 6). The peak at 1640 cm^{-1} remained for the infected sample.

Another area of difference occurs at 2900 cm^{-1}. This is the area where C-H stretching arises. The small new shoulder that forms on the infected loblolly pine is not seen on either the control or the loblolly sample. This could be a result of a new CH bond that has been formed (Fig. 7). Because of the shoulder at 2900, this could result in the modification at 1640 where we see broadening of the band of infected sample. Quinones are known intermediates in modification of lignin by fungi. The shoulder at 2900 cm^{-1} and the peak broadening at 1640 cm^{-1} could be due to this degradation of lignin by the fungi (Eriksson and others 1999; Heitner and others 2010).

Klason Lignin

The percentage difference in Klason lignin (Hatfield and others 1994) was calculated from the control and the infected loblolly pine (Table 1). The Klason lignin percentage increased for the infected sample. This percentage increase correlates with modifications in the chemical composition changes in the lignin that were observed in the NMR spectra. The percentages for the mannan, glucan, and xylan and total carbohydrates decreased significantly for the infected loblolly pine. The infected sample acid soluble

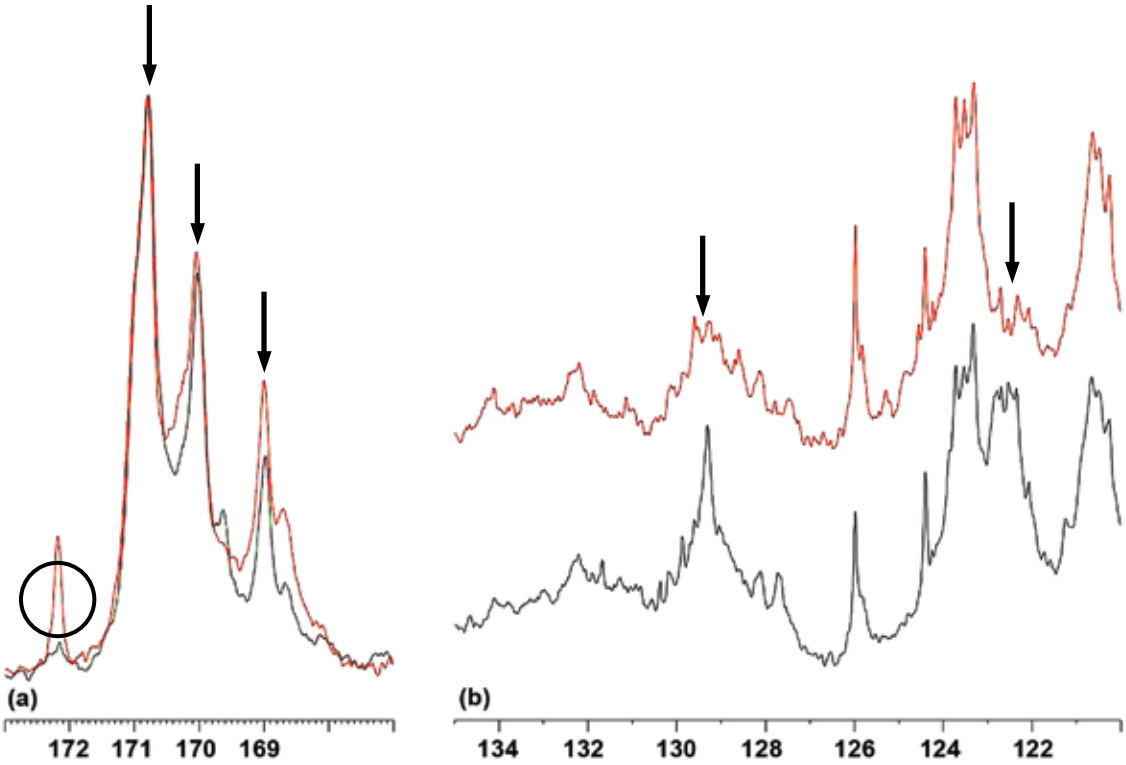

Figure 2. (a) The red line shows the ^{13}C NMR between 173 and 167 ppm of loblolly pine infected by fusiform rust and the black line is the loblolly pine control. (b) The red line shows ^{13}C NMR between 135 ppm and 120 ppm of loblolly pine infected by fusiform rust, and the black line shows the loblolly pine control.

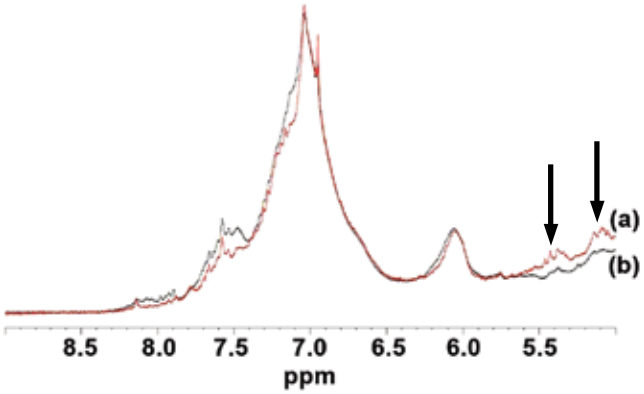

Figure 3. Partial expansion of ^{1}H NMR spectrum. The red line (a) shows loblolly pine infected by fusiform rust. The black line (b) shows the control sample of loblolly pine.

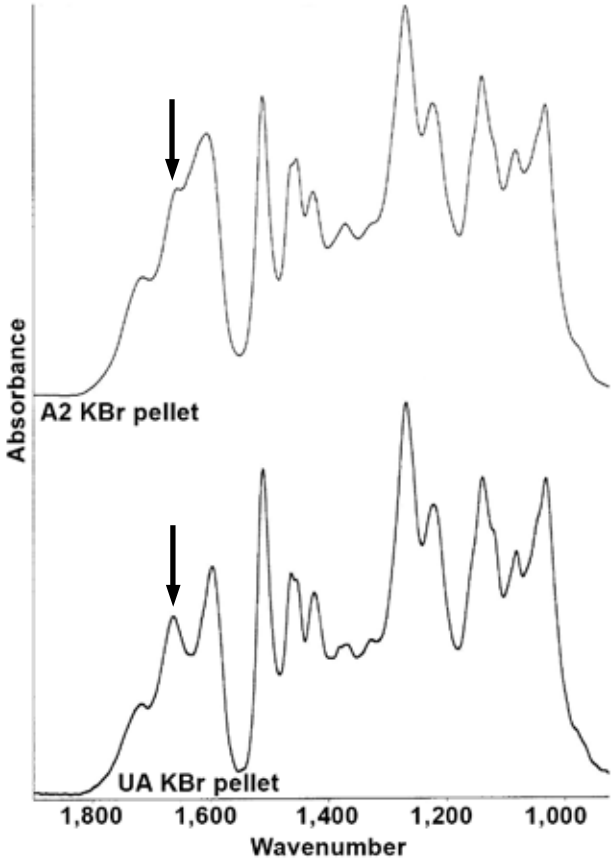

Figure 4. Full FTIR spectra of (a) infected (A2) and (b) control (UA) loblolly pine.

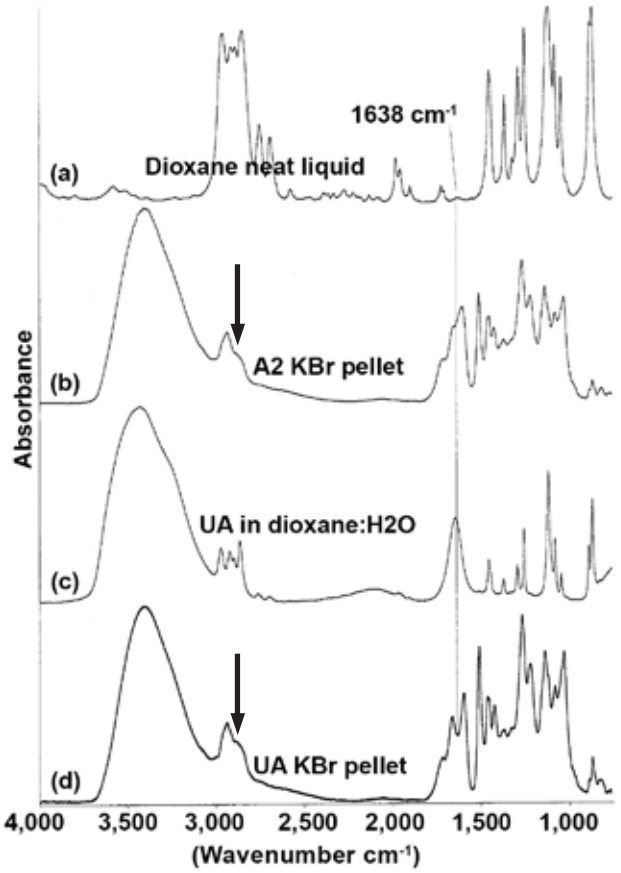

Figure 5. Full FTIR spectra of (a) dioxane, (b) infected (A2) loblolly pine, (c) control (UA) sample of loblolly pine in dioxane: H_2O and (d) control (UA) loblolly pine.

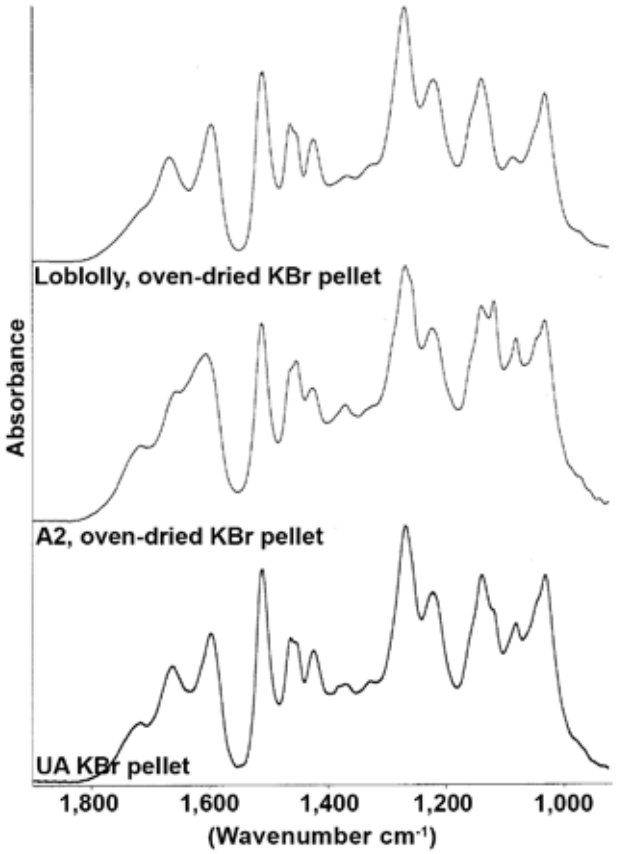

Figure 6. Partial FTIR spectra of oven-dried (a) loblolly pine, (b) infected (A2) loblolly pine, (c) control (UA) loblolly pine.

percentage increased, leading us to conclude that low molecular weight acids were formed during the fusiform rust attack.

Conclusions

FTIR and NMR spectroscopy were used to examine changes in the lignin chemical composition after fusiform rust fungal attack on loblolly pine. Changes were observed in the spectra for the infected loblolly pine lignin. NMR spectra showed changes in the carbonyl groups. These changes indicated there are more ß-O-4 linkages forming that could result in higher molecular weight because of the attack. NMR results supported the observation from the FTIR that modification of the lignin is occurring. Klason lignin percentage increased from the fungal attack. The acid soluble from the infected lignin increased, which could indicate that low molecular weight compounds are formed. Sugar analysis percentage differences were similar to what is found in literature (Rowan 1970), even though fusiform rust is not a wood-decaying fungi glucan, and xylan decreased because of fungal attack. The chemical composition of lignin was investigated, and from the study lignin chemical composition is being modified because of the fusiform rust attack in loblolly pine. Future investigation will include chromatography. This analytical method will be added to further investigate what type of changes and degradation are occurring because of this fungal attack.

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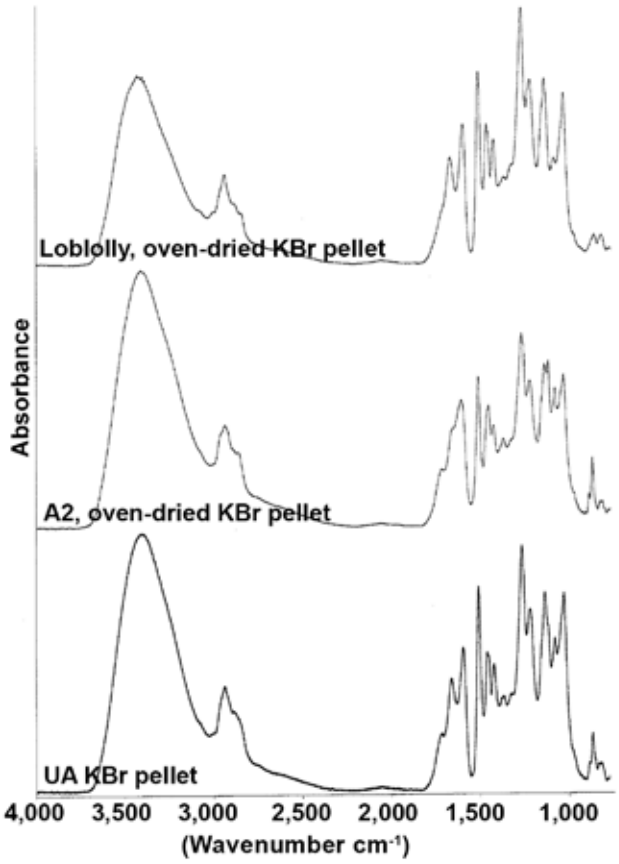

Figure 7. Full FTIR spectra of oven-dried (a) loblolly pine, (b) infected (A2) loblolly pine, (c) control (UA) loblolly pine.

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Table 1. Klason lignin percentage difference of infected and control loblolly pine including sugars found in lignin

Sample	Control (UA) (%)	Infected (A2) (%)	Difference (%)	Higher percentage
AI ash	0.00	0.20	0.20	A2
AS lignin	0.80	1.10	0.30	A2
K. lignin	33.70	38.40	4.70	A2
Arabinan	1.24	1.99	0.75	A2
Galactan	4.83	5.16	0.33	A2
Rhamnan	ND	0.67	0.67	A2
Glucan	33.00	26.74	6.26	UA
Xylan	9.52	5.92	3.60	UA
Mannan	7.62	7.53	0.09	UA
Total carb	56.21	48.01	8.20	UA
Total yield	90.70	87.70	3.00	UA