Lignin Analysis of Resistant and Susceptible Aspen Trees Wounded and Inoculated with *Hypoxylon mammatum*, the Causal Agent of *Hypoxylon* Canker

B. Bucciarelli, C.P. Vance and H.G. Jung

Introduction

Aspen (*Populus tremuloides* Michx.), the predominant forest tree in the Great Lakes States, is widely grown for use in the paper and construction industries. *Hypoxylon mammatum* (Wahl.) Mill, the causal agent of Hypoxylon canker, is the most important pathogen of aspen. Unrestricted infection to the main bole of the tree results in loss of wood quality and increases the risk of tree breakage. Studies of adult trees show tremendous clonal variation towards susceptibility to *Hypoxylon* infection. The goal of this research was to identify characteristics that contribute to disease resistance or susceptibility. Previous studies have shown that both resistant and susceptible genotypes develop a lignified zone along the wound margin in response to either wounding only or wounding plus inoculation with the pathogen. The focus of this study was to determine if differences exist in lignin concentration and/or in the monomeric composition of the response lignin deposited by these two genotypes.

Materials And Methods

Green internodal stem tissue of greenhouse grown aspen was either wounded only or wounded plus inoculated with mycelium of *Hypoxylon mammatum*. Stem tissue (3 to 4 mm diameter) was sampled at 24-, 48-, 72- and 96-h after treatment. Lignin content of treated internodes and nonwounded controls was measured using the Klason lignin method. This method measures the lignin residue after the tissue is hydrolyzed in concentrated sulfuric acid. Pyrolysis-GC-MS was used to qualitatively analyze the lignin composition of stem tissue 96 h after treatment and in nonwounded controls.

Results

Klason lignin concentration of stem internodes was greater in the resistant aspen genotype in nonwounded controls (Fig. 1). After wounding only, both genotypes exhibited rapid increases in lignin concentration; however, the resistant genotype continued to consistently contain more lignin than the susceptible genotype. After wounding plus inoculation, both genotypes again rapidly deposited lignin, but in this treatment the susceptible aspen genotype had similar lignin concentrations to the resistant genotype at 24-, 48- and 72-h after inoculation. By 96-h after inoculation, the susceptible genotype actually contained more lignin than the resistant aspen.

Pyrograms from pyrolysis-GC-MS analysis showed that the syringyl-to-guaiacyl (S/G) ratio of monolignols for nonwounded controls was statistically similar for both resistant and susceptible genotypes (Table 1), suggesting that the initial syringyl and guaiacyl content of lignin for both genotypes are proportionately the same. Subsequently, in response to wounding only and wounding plus inoculation, there was a decrease in the S/G ratio for both genotypes (Table 1). The decrease, however, was more pronounced for the susceptible than for the resistant genotype.

Analysis of the pyrograms indicated the presence of more hydroxyphenyl-type lignin in wounded only and wounded plus inoculated stem tissue from both genotypes than seen in nonwounded controls. This observation is based on the appearance of *t*-4-propenylphenol after wounding (Table 1). Additionally, the susceptible genotype contained more 4-vinylphenol after wounding only and wounding plus inoculation than in its nonwounded control or any of the resistant genotype samples. 4-Vinylphenol can arise during pyrolysis from hydroxyphenyl lignin and, also, from *p*-coumaric acid. Whether the increased 4-vinylphenol observed in the susceptible genotype due to wounding is from hydroxyphenyl lignin or *p*-coumaric acid is unknown.

Conclusion

The results indicate that lignin concentration of aspen increases in response to *H. mammatum* infection, but the susceptible and resistant genotypes are similar in the magnitude of this lignification response. However, the two genotypes display differences in the composition of the lignin polymer deposited. Both genotypes deposit a lignin lower in syringyl units in response to wounding, but additionally the susceptible genotype response lignin is enriched in hydroxyphenyl lignin and, perhaps, *p*-coumaric acid.

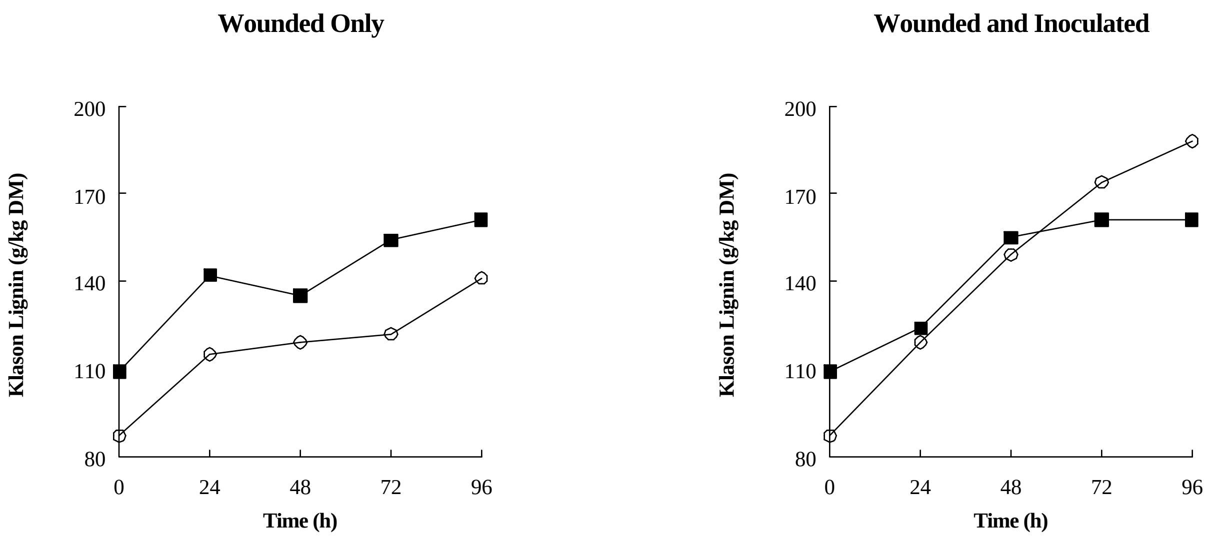

Figure 1. Klason lignin concentration of green internodal stem tissue of putative resistant (■) and susceptible (O) aspen that was wounded only or wounded and inoculated with Hypoxylon mammatum.

Table 1. Lignin composition of green internodal tissue from putative resistant and susceptible aspen 96-h after wounding only or wounding and inoculation with *Hypoxylon mammatum*.

Genotype-treatment	S/G Ratio	*t*-4-Propenylphenol	4-Vinylphenol
	- - - - - - - standardized to guaiacol - - - - - - -		
Susceptible-nonwounded	1.23	0	1.44
Susceptible-wounded	0.86	0.27	3.43
Susceptible-wounded & inoculated	0.93	0.22	3.18
Resistant-nonwounded	1.10	0.26	1.21
Resistant-wounded	0.93	0.19	1.50
Resistant-wounded & inoculated	0.99	0.19	1.50