Fusiform Rust Gall and Canker Formation and Phenols of Loblolly Pine

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ABSTRACT

Heartwood area and heartwood phenols increase with increasing diam, age, and sp gr in fusiform rust galls on loblolly pine. This increase occurs as water-conducting tissue decreases, indicating that this heartwood is relatively impermeable to water and may inhibit water transport. The orthodihydroxy and heartwood phenols of gall wood differ quantitatively, rather than qualitatively, from those in noninfected wood. The phenols in water-extracted *n*-butanol fractions of gall wood differ quantitatively and qualitatively from those in noninfected wood. These and other modifications caused by *Cronartium fusiforme* infection of pine stems, as well as their possible relation to gall and canker formation, are discussed. Phytopathology 60:1221-1224.

Additional key words: Pinus taeda, physiology of disease.

There are indications that phenolic compounds are involved in the formation of galls on pine stems infected by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm. Phenols have been observed to promote growth in plant tissue (20), and to accumulate in tissues wounded by parasites and other agents (12) and in cambial tissue during the period of active growth (8). A relatively large number of phenolic compounds exist in coniferous tree species (1, 6, 9, 15, 22). Pinosylvin, pinosylvin monomethyl ether (PME), pinocembrin, and pinobanksin are heartwood constituents of loblolly pine (*Pinus taeda* L.) (15). Although the accumulation of phenolic compounds is a characteristic response of plant tissue to infection (18), they may or may not have been translocated from other tissues of the plant. Certain water-soluble phenols may migrate to an infection site (18), but others are apparently formed within the infected tissue, and are not translocated (9). Substances extractable with neutral solvents accumulate in fusiform rust galls and cause an increase of wood sp gr (17). These extraneous constituents include waxes, fats, essential oils, tannins, resins, soluble saccharides (gums), soluble sugars, phenols, and proteinaceous materials (25). The sp gr is significantly greater in the area of largest gall diam (where the infection probably originated) than it is in noninfected wood. The accumulation, therefore, appears to be directly related to fungus activity, and may contain growth-regulating phenolic compounds which are involved in gall formation.

The objectives of this study were (i) to compare the phenolic constituents of infected and noninfected stem tissue of loblolly pine for differences which may be related to gall formation; and (ii) to determine if phenolic compounds are accumulated in gall tissue in direct proportion to increases of gall diam.

MATERIALS AND METHODS.—Polyphenols were extracted from galls and noninfected wood samples collected from 10 rust-free and 10 stem-infected 9-year-old loblolly pines by two methods. In the first method, wood samples (100 g fresh wt), representative of the stem cross section, were extracted for 24 hr in two changes of cold methanol at −15 C after triturating in cold methanol. The extract was filtered, concd on a rotary film evaporator at 38 C, and brought to 100 ml with methanol. In the second extraction method, 20 g of wood tissue, ground to pass a 40-mesh standard sieve, were extracted in boiling water and partitioned into *n*-butanol (6). The *n*-butanol fraction was concd on a rotary film evaporator at 50 C and brought to 10 ml with *n*-butanol (these two methods were used because the former extracted water-insoluble phenols not isolated by the latter method, and because this methanolic fraction was more representative of the total phenolic composition). Many interfering substances, including tannins, were eliminated by extraction in hot water; this elimination improved chromatographic separation of the phenols.

Extracts were applied to Whatman No. 1 or 3 MM paper and chromatographed at 20-24 C either with benzene:ligroin:methanol:water (upper phase; 60:50:1:50) or in two dimensions with (i) 2% acetic acid and (ii) *n*-butanol:acetic acid:water (BAW 4:1:5) solvents (all solvent ratios are by volume). Descending chromatography was employed after equilibrating for 4 hr. The developed chromatograms were examined under ultraviolet light for visible spots. The spray reagents used for locating phenols were bis-diazotized benzidine (14), Arnow reagent (11), diazotized sulphanilic acid (19), and Pauly's reagent (19). The Folin-Ciocalteu (21) and the Arnow reagents (11) were used to estimate the amount of phenols in the methanol extracts.

The cross-sectional areas of heartwood, vascular tissue, and total area inside bark were estimated on trees varying from 2 to 9 years old. Infected trees were selected to include trees of apparent high vigor with green foliage and trees of apparent low vigor with chlorotic or dying foliage. Selected stems from three rust-free and 11 infected loblolly pines were cut and immediately immersed in (i) a 0.5% (v/v) aqueous solution of acid fuchsin or in (ii) a 5.0% solution of green India ink (v/v) and 5.0% fast green (w/v) in 10% ethanol (23). The first 5-7.5 cm of stem were cut off below the surface of the dye solution. After the dye solution had moved about 18 cm above the gall, the stems were removed and cut into

consecutive 1.2-cm cross-sectional discs, and the dyed areas on lower surfaces of the discs were immediately outlined with ink. Upper surfaces of the discs were treated alternately with bis-diazotized benzidine (14) and the Arnow reagent (11). The former reagent forms a red-brown deposit on contact with phenolic compounds, while the latter reacts specifically with orthodihydroxy phenols to form a brown-green deposit. The distribution of such phenolic compounds was estimated by outlining the intensely colored sectors with ink and measuring their area.

A planimeter was used on each disc to measure the total area inside bark, the dye-conducting area on lower surfaces, and the phenolic area on upper surfaces. The data were plotted in line graphs, and the area under each curve was measured with the planimeter. Thus, the cross-sectional areas of wood, vascular tissue (dye-conducting), and heartwood were determined at 1.2-cm intervals. The total vascular and heartwood areas in each gall were calculated as the percentage of total area inside bark.

Results.—Among the 10 infected and 10 noninfected trees sampled, concn of orthodihydroxy phenols were higher within infected tissue (152 mg/100 g tissue) than in noninfected tissue (132 mg/100 g), and concn of Folin-Ciocalteu reactant phenols were also higher within gall tissue (1230 mg/100 g) than in noninfected tissue (870 mg/100 g). Gall tissue contains approximately 15 times the normal concn of heartwood phenols (Fig. 1). The area and shape of each spot in Fig. 1 are drawn to scale from chromatograms run with volumes of extract equivalent to 40 mg of infected and 600 mg of noninfected tissue on a fresh-wt basis. Authentic samples of pinosylvin, pinocembrin, and pinosylvin monomethyl ether (PME) were chromatographed with the extracts from galls and noninfected tissue. The R_F values and color reactions of compounds 1, 3, and 5 (Fig. 1) and those of the known compounds are similar, indicating that they are pinosylvin, pinocembrin, and PME, respectively. The R_F value and color reactions of compound 2 compare with published data (15) and indicate it is probably pinobanksin. The remaining two unidentified compounds absorbed ultraviolet light, became light red following treatment with the benzidine reagent, and probably are flavanones or stilbenes (15). The approximately 15-fold increase of these heartwood phenols is a quantitative rather than a qualitative difference between gall and noninfected tissue.

A large number of compounds were observed in water-extracted *n*-butanol fractions of infected and noninfected wood (Table 1), indicating quantitative and qualitative differences between the two. A total of 57 compounds was noted on chromatograms from infected tissue and 54 from noninfected tissue. R_F values and color reactions of 41 compounds on infected and noninfected tissue chromatograms were similar, and these compounds were classified as normal constituents of galls and noninfected tissue. The R_F values and color reactions of the remaining 29 unidentified compounds are shown in Table 1. The increased concn of orthodihydroxy phenols in galls apparently

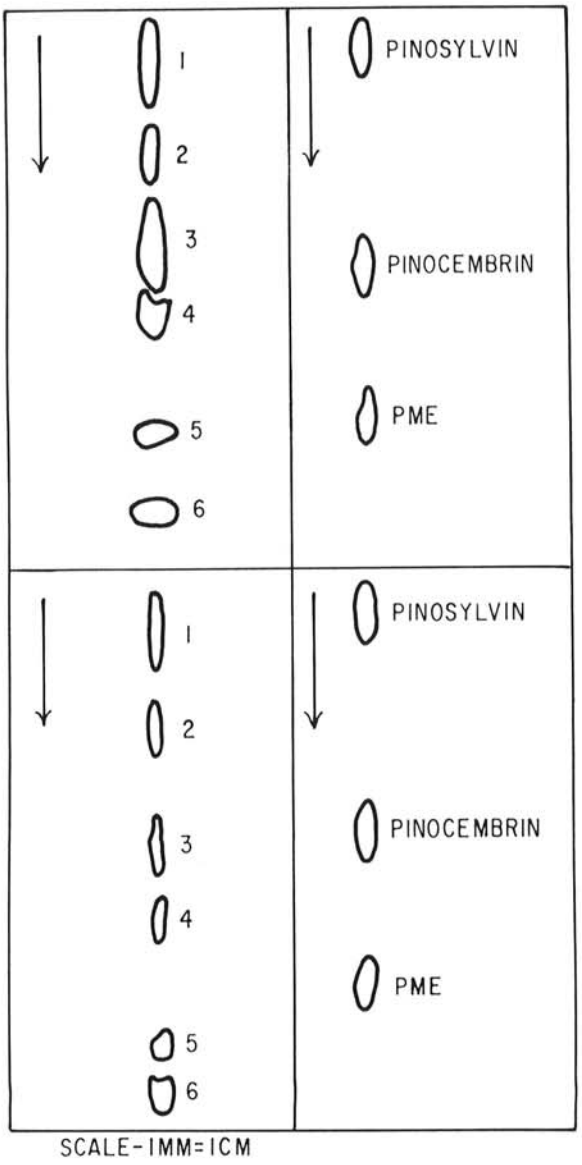

Fig. 1. Cochromatographic illustration of known pine phenols and the probable identity and comparative concn of methanol-extracted phenolic compounds in infected (upper left) and noninfected (lower left) pine stem tissue.

is a quantitative rather than a qualitative difference between gall and noninfected tissue, because none of the 29 compounds reacted with the Arnow reagent. The appearance of 16 compounds on infected tissue chromatograms and the disappearance of 13 compounds from noninfected tissue chromatograms indicate a significant modification of phenol metabolism in gall tissue. Compounds 9, 12, 13, 14, 15, and 16 from infected tissue and compound 20 from noninfected tissue did not react with any of the detecting reagents. Their failure to react may indicate that these compounds are nonphenolic in nature. Gall tissue, therefore, contains at least 10 phenols not found in noninfected tissue. An additional 12 phenols not found in gall tissue were

TABLE 1. R_F values and color reactions of compounds on chromatograms of the water-extracted *n*-butanol fraction of infected and noninfected tissue

Compound no.	R_F AcW[a]	R_F BAW[a]	Ultraviolet light[b]	Color reactions/reagent[b] Pauly's	Diazotized sulphanilic acid	Bis-diazotized benzidine
Infected						
1	.07	.83		B		R
2	.22	.77			B	
3	.27	.66			B	
4	.41	.93			B	
5	.47	.57	A		B	R
6	.60	.74		B		
7	.75	.70			B	
8	.86	.83	A			R
9	.86	.21	A			
10	.94	.64		B		
11	.95	.63		B		
12	.90	.29	A			
13	.93	.21	A			
14	.94	.15	A			
15	.90	.14	A			
16	.97	.07	A			
Noninfected						
17	.15	.70			B	
18	.18	.32			B	
19	.18	.14			B	
20	.27	.91	A			
21	.35	.91	A	B		
22	.38	.91	A	B		
23	.32	.16			B	
24	.42	.18			B	
25	.45	.18			B	
26	.48	.13			B	
27	.64	.28			B	
28	.83	.80			B	
29	.94	.39		B		

[a] Descending chromatography in acetic acid:water (AcW, 1:4, v/v); 2nd dimension in *n*-butanol:acetic acid:water (BAW, 4:1:5, v/v).
[b] A = absorbing; B = brown; R = red.

found in noninfected tissue. Tests were not made for the presence of amino acids, sugars, or other types of compounds.

Vascular tissue (tissue allowing passage of stain) increases in area as the area inside bark increases and is maximal in the area of largest stem diam. The amount of heartwood area also increases with increasing area inside bark (Fig. 2). But in infected stems with chlorotic or dying foliage (Fig. 2-C), the heartwood area is maximal and the vascular tissue area is minimal near the point of largest gall diam. In the stem with dying foliage (Fig. 2-D), the stain did not move beyond the seventh section, indicating that the heartwood occupying the entire area inside bark of section 10 through 15 is relatively impermeable to water and inhibits water transport. Among the three noninfected stems and 11 fusiform rust galls sampled, the total amount of heartwood increased directly as the amount of vascular tissue decreased. Therefore, the accumulation of heartwood in gall tissue probably inhibits water transport when the accumulation extends into the physiologically active transport stream.

Application of the benzidine and Arnow reagents to surfaces of stem cross sections indicates that the highest concn of orthodihydroxy and benzidine-reactant phenols are in the heartwood of gall tissue. Infected stem tissue other than heartwood contains higher concn of these phenols than does contiguous noninfected tissue.

DISCUSSION.—Quantitative and qualitative differences were observed in the phenolic constituents of infected and noninfected tissue. The approximately 3-fold increase in material extracted in neutral solvents, the increase of sp gr (17), and the accumulation of heartwood in fusiform rust galls appear to be interrelated. The increase of lignin in gall wood (17) may be due to lignification of heartwood. Pinosylvin, PME, pinocembrin, pinobanksin, two unknown heartwood phenols, orthodihydroxy, and certain water-soluble phenols are accumulated in gall tissue and apparently are synthesized at a rate proportional to increases in gall diam. Apparently, gall formation is directly related to fungus activity; the reduction of cell-wall holocellulose, the increase of lignin, the increase of wood sp gr (17), and the increase of heartwood and phenolic compounds are most pronounced near the area of largest gall diam and the probable locus of the original infection. The point of largest gall diam is the most probable site of

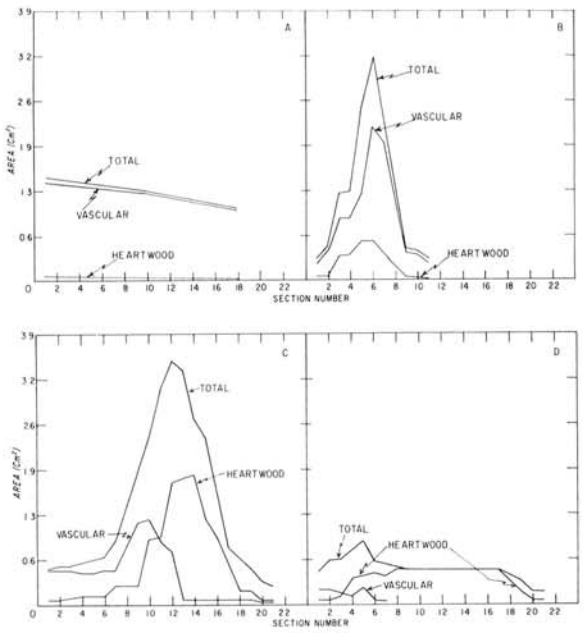

Fig. 2. Comparative amounts of heartwood, vascular tissue, and total area inside bark in consecutive sections of **A)** a rust-free and **B)** an infected pine stem of apparently high vigor and green foliage and of two infected pine stems of apparent low vigor and **C)** chlorotic or **D)** dying foliage.

the original infection, because gall development is related to the pattern of tissue invasion by the fungus (10) and is a host response to activities of the fungus (13, 16, 17, 18).

Indole acetic acid (4) and a number of phenolic compounds (20) possess growth-promoting activities and may promote hypertrophy and hyperplasia in fusiform rust galls. In the presence of polyphenolase and orthodihydroxy phenols, tryptophan is converted to indole acetic acid (5). The increased amount of these phenols in gall tissue suggests that they promote auxin synthesis and are involved in gall formation.

Canker formation may be related to an accumulation of heartwood which is formed in living sapwood by a change in metabolism of dying cells (12). The dead heartwood in a branch-origin infection would form a continuous area to the pith or natural heartwood area of the main stem. If the area of heartwood in such a branch-origin infection were large, a correspondingly large branch scar would be formed on the main stem when the branch died and was pruned. The stimulated heartwood formation in the infected stem would increase the area of heartwood scar tissue, resulting in an enlarging zone of dead tissue corresponding to the flat or necrotic area of the canker. The stimulation of heartwood formation by fungi and insect attacks (2, 3, 7, 12, 24) and infection of the exposed heartwood by wood-rotting fungi (2, 24) would augment canker development, reduce the mechanical strength of gall wood, increase the susceptibility of trees to drought, and increase mortality of infected trees.

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