Fusiform Rust Gall Formation and Cellulose, Lignin, and Other Wood Constituents of Loblolly Pine

S. J. Rowan

Plant Pathologist, Southeastern Forest Experiment Station, Forest Service, USDA, Athens, Georgia 30601.
Portion of Ph.D. thesis presented to the University of Georgia.
Accepted for publication 18 March 1970.

ABSTRACT

The stem wood of loblolly pine infected with *Cronartium fusiforme* was compared with that of noninfected loblolly pine. Gall wood was found to have its greatest sp gr near the point where the diam of the gall is largest (the site where the infection probably originated). This increase in sp gr is caused by an accumulation of substances extractable in neutral solvents. Holocellulose and alpha-, beta-, and gamma-cellulose (cell wall constituents) are reduced in gall wood, but lignin content is higher. Gall wood resembles conifer summerwood in lignin content and in the monosaccharide composition of holocellulose. These and other modifications caused by *C. fusiforme* infection of pine stems are discussed, along with their possible relation to gall formation. Phytopathology 60:1216-1220.

Additional key words: Pinus taeda, physiology of disease.

Although southern fusiform rust caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm. is the most serious disease of slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pines, the physiological basis of gall formation and disease development has not been studied. Several thousand papers have been published on the mechanism of gall formation in plants, but the majority have attempted to identify specific growth-regulating compounds within the affected tissue which may have stimulated the overgrowth. Although such compounds may be present in hypertrophied tissue, their presence does not prove that they have stimulated the overgrowth. Normal and abnormal growth and development result from the interaction and interrelation of many factors, and not from the activity of growth stimulants alone (13). An understanding of the changes induced by the pathogen is prerequisite to understanding the mechanism of gall formation.

This study was made for the purpose of identifying some of the modifications induced in stem tissue by the pathogen and which may be related to gall formation. The specific objective was to compare ash and dry matter content, sp gr, cellulose, lignin, and extraneous wood constituents of infected and noninfected stem tissue of loblolly pine.

MATERIALS AND METHODS.—*Determination of ash and dry matter content and sp gr.*—Samples were collected from 5- and 8-year-old loblolly pines near Macon, Georgia. The trees selected were relatively free of insect damage, approximately equal in vigor, and either free of rust or infected with girdling stem galls approximately 1 m above the ground line. Ash and dry matter content were determined on gall wood and noninfected wood from the infected trees, noninfected wood from the rust-free trees, and mature, current-year needles from the infected and the rust-free trees.

Duplicate 10-g samples (fresh wt, nearest 0.01 g) were collected from each of 10 infected and 10 rust-free trees. Wood samples representative of the stem cross section were cut into 1-cm cubes; the needle samples were cut into sections 1 cm long. All samples were placed in tared crucibles and dried to constant wt in a forced-draft oven at 65 C. Immediately after the samples were removed from the oven, the dry wt were recorded to the nearest 0.01 g. The dried samples were placed under vacuum over anhydrous magnesium perchlorate [$Mg(ClO_4)_2$], allowed to reach constant wt, and weighed to the nearest 0.01 mg. They were then placed in a muffle furnace for 12 hr at 550 C. After removal from the furnace, the samples were again placed under vacuum over $Mg(ClO_4)_2$ until they reached constant wt. The ash weights were then recorded to the nearest 0.01 mg.

Ash and dry matter content were also determined on a series of samples collected below, within, and above the gall tissue of 10 infected trees. Bolts which included areas below and above the gall were removed from each tree, and consecutive discs approximately 1 cm thick were sawed from each bolt. Duplicate 50-g samples representative of the stem cross section were cut from each consecutive disc. After removal of the rough outer bark, the wood samples, including phloem, were processed as previously described.

Specific gr measurements were made on consecutive discs, 1 cm thick, sawed from bolts removed from each of nine 8-year-old loblolly pines with girdling stem galls. The bolts included areas below and above the gall. After removal of the rough outer bark, the dry-volume sp gr of each disc was calculated as the ratio of the wt of the oven-dry disc to the wt of a volume of water equivalent to the over-all volume of the disc.

Analyses of cellulose, lignin, and extraneous constituents.—Samples of stem wood were collected from 10 rust-free and 10 stem-infected 9-year-old loblolly pines near Macon, Georgia. The gall tissue was removed from trees partially girdled ($< 75\%$) by stem galls. All wood samples were collected from areas about 1 m above the ground line. Three-g, bark-free samples from each tree were cut and ground in a laboratory pulverizing mill to pass a 40-mesh standard sieve. The wood meal from the infected trees was combined into a composite sample, as was the wood meal from the noninfected trees. From each composite, duplicate 5-g samples were set aside for determination of the percentage of dry matter, and four 2-g samples were taken for chemical analysis. The remaining wood meal was used in determining the size distribution of particles.

The procedures used in preparing samples of wood meal are similar to those outlined in the TAPPI Standard T 11m-59 (22) and modified by Cowling (2). Within a 20-min span, wood samples were collected, ground, separated into subsamples, and placed in a freezer at −20 C. In each subsample, the particle sizes were determined by weighing the meal that was retained by standard sieves with meshes of 40, 50, 60, 80, 100, and 200. The stacked sieves were agitated by hand until complete separation was obtained. The distribution of particle sizes was expressed in terms of percentages by wt of the total subsample.

Extraneous constituents were separated from cell-wall material by subjecting the samples of wood meal to successive extractions with ethanol:benzene (1:2, v/v), 95% ethanol, and hot water (21). The wt of the total extractives was obtained by determining the difference between the wt of the original and that of the extracted, moisture-free sample. This difference was expressed as a percentage of the original wt.

The amount of soluble carbohydrate in the extractives was determined by the anthrone method (11) and expressed in glucose equivalents as a percentage of the original wt of the moisture-free sample. The extractive liquids were combined and concd in a flash evaporator at 50 C. The aqueous residue was then slurried at 50 C with analytical Celite and filtered. The filtrate was deionized by passage through 10-cm resin columns (14). The neutral fraction containing the sugars was collected after the filtrate had passed through the columns and was then brought to volume with distilled-deionized water. The neutral fraction was analyzed for carbohydrate content. The results were compared to a glucose standard curve, and calculations were made on the basis of the average reading of five subsamples from each duplicate extract. This neutral fraction is referred to as the soluble carbohydrate fraction to distinguish it from the insoluble holocellulose fraction.

Holocellulose was prepared from the extractive-free samples (2, 19), and the lignin content obtained by determining the difference between the moisture-free wt of the holocellulose and of the extractive-free wood. This difference was expressed as a percentage of the original moisture-free wt (20). Two holocellulose samples were subsequently fractionated into alpha-, beta-, and gamma-cellulose, and two samples were hydrolyzed into constituent monosaccharides (2, 19, 20). The monosaccharides were resolved by descending paper chromatography after the hydrolysates had been passed through a column of Amberlite IR-45 resin to remove the acid (15). An aliquot was applied to Whatman No. 1 paper and allowed to equilibrate 4 hr; the chromatogram was then run for 48 hr in *n*-butanol: acetic acid:water (4:1:5, v/v). From each chromatogram, three strips located at the two margins and the center were used cochromatographically to mark the zones of individual sugars in the extract. The three strips were cut from the chromatogram and treated with *P*-anisidine hydrochloride reagent (17), and the chromatogram was reassembled. Areas of the chromatograms corresponding to the zones of the sugars galactose, glucose, mannose, arabinose, and xylose were cut from the paper, and each sugar was eluted into warm, distilled-deionized water. The amount of sugar was then estimated by the anthrone method; standard curves of each sugar were used for the calculations. The actual amounts present in the samples were calculated from the relative proportions in the hydrolyzate; the holocellulose content served as a basis of calculation. The results are expressed as the percentage of the wt of the original moisture-free sample.

RESULTS.—Gall wood contains more dry matter than does noninfected wood removed from either infected or rust-free trees (Table 1). Foliage of either infected or noninfected trees did not differ in dry matter or ash content. There were no significant differences in ash content among any of the wood samples (Table 1).

Among the nine trees sampled, the average sp gr of gall wood (0.52) did not differ from that of noninfected wood above (0.46) or below the galls (0.48). But sp gr apparently increased directly as the amount of infected tissue in each consecutive stem section increased, and was significantly greater near the point where the diam of the gall was largest than in noninfected tissue above or below the gall (Fig. 1). Noninfected tissue is visibly distinguishable from infected tissue, because hyphae of the parasite are always contiguous to abnormal host tissue (7). The point of maximum sp gr corresponded to the approximate point where the diam of the gall was largest and to the approximate locus of the original infection.

More particles of wood meal from noninfected trees than from infected trees were retained by the 200-mesh sieve (Table 2). This greater percentage of fine particles probably resulted from the fact that a greater

TABLE 1. Average % ash and dry matter in stems and foliage collected from *Cronartium fusiforme*-infected and noninfected loblolly pines

Type of tissue	Avg % dry matter[a]	Avg % ash[a]
Gall wood	67.3[b]	0.6400[b]
Noninfected wood from infected trees	53.5	0.5149
Wood from non-infected trees	42.7	0.4879
Foliage from infected trees	41.2[b]	1.5234[b]
Foliage from non-infected trees	41.4	1.3355
Gall wood	70.6[c]	0.5775[c]
Wood above gall	57.6	0.4396
Wood below gall	57.6	0.4345

[a] Figures not enclosed by the same bracket are significantly different at the 1% level (Duncan's multiple range test).

[b] Duplicate 10-g samples were collected from each of 10 infected and 10 noninfected trees.

[c] Duplicate 50-g samples representative of the stem cross section were collected from each consecutive cm of stem wood above, within, and below the galls on each of 10 infected trees.

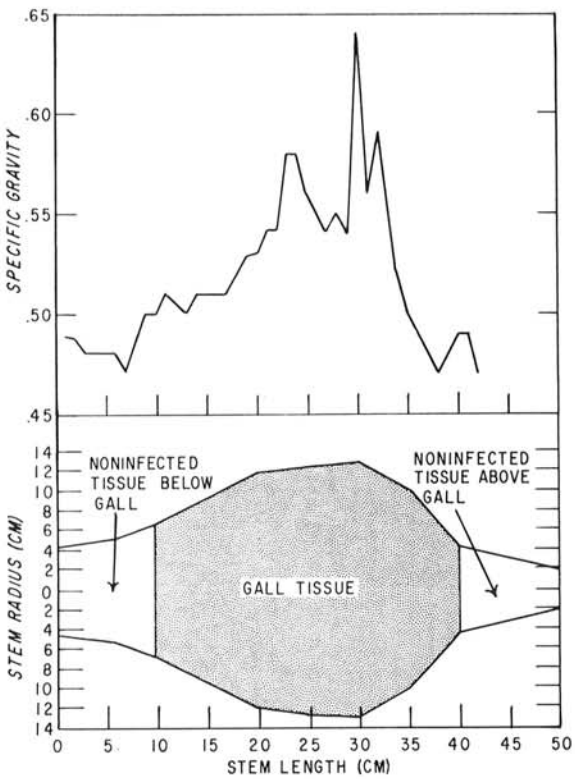

Fig. 1. The relationship of gall morphology to sp gr of consecutive cross sections of an infected loblolly pine stem. A sp gr greater than .55 is significantly different (1% level) from that in noninfected wood.

proportion of the noninfected wood required regrinding. During the grinding of infected wood, particles which would pass a 40-mesh screen were produced at a relatively rapid speed.

The increase of dry matter and sp gr in gall tissue results from a threefold increase in substances extracted with neutral solvents (Table 3). These extraneous constituents include waxes, fats, essential oils, tannins, resins, soluble saccharides (gums), soluble sugars, phenols, and proteinaceous materials (24). There was no significant difference between the content of soluble carbohydrates in galls and that in noninfected wood (Table 3). The amount of holocellulose in gall wood was considerably less than that in noninfected wood. This decrease, which did not change when expressed as a percentage of extractive-free wood, indicates a significant reduction of cell-wall synthesis in infected tissue. The lack of a significant difference between the lignin content of galls and normal wood is misleading (Table 3). When expressed as the percentage of extractive-free wood, the lignin content of gall wood was significantly higher than that of noninfected wood (33.1% versus 23.8%).

TABLE 3. Chemical composition of *Cronartium fusiforme*-infected and noninfected wood from loblolly pine stems[a]

Chemical composition	Infected wood		Noninfected wood	
	%	%	%	%
Total extractives	32.9	32.6	9.0**[b]	8.7**
Soluble carbohydrates	5.1	5.1	4.4	4.4
Holocellulose	44.9	45.4	69.3**	69.7**
Total carbohydrates	50.0	50.5	73.7**	74.1**
Alpha-cellulose	25.8		40.7**	
Hemi-cellulose	19.1		28.6**	
Beta-cellulose	5.6		8.4	
Gamma-cellulose	13.5		20.2**	
Glucan		35.7		54.8**
Galactan		1.5		2.3
Mannan		5.6		7.9**
Arabinan		0.5		0.9
Xylan		2.1		3.8
Lignin	22.2	22.0	21.7	21.6

[a] The data represent the average of duplicate samples and are expressed as percentages of the wt of the moisture-free samples.

[b] ** = significant differences between the infected and the noninfected samples at the 1% confidence level (LSD).

Glucans and mannans, the principal constituents of the holocellulose fractions, were reduced in gall wood (Table 3). Infected and noninfected wood did not differ significantly in glucan content or in galactan content when these were expressed as percentages of the holocellulose fraction. However, when so expressed, mannan content was higher in gall wood than in noninfected wood (12.3% versus 11.3%), whereas arabinan (1.1% versus 1.3%) and xylan (4.7% versus 5.5%) contents were lower in gall wood than in noninfected wood.

DISCUSSION.—Southern fusiform rust galls on pine stems arise from hypertrophy and hyperplasia of the cortex, phloem, and xylem (6, 7). The reduction in the length of tracheid cells (hypotrophy) in gall tissue (6, 7) suggests that galls also arise from either

TABLE 2. Distribution of particle sizes in wood meal from 10 *Cronartium fusiforme*-infected and 10 noninfected loblolly pines[a]

Wood sample	Mesh size of particles							Probability of a larger value of Chi-square
	0-40	40-50	50-60	60-80	80-100	100-200	200+	
				%				
Noninfected	0.00	6.24	1.97	55.97	13.50	21.99	0.35	
Infected	0.00	4.22	0.17	67.91	22.70	4.59	0.41	<.001

[a] The data represent the average of triplicate 4-g samples and are expressed as percentages of the wt of each wood sample.

hyper- or hypo-plasia of the xylem. The annual growth of hyphae only into the cambial regions and the presence of intra-cellular haustoria in parenchyma cells of the rays, cortex, and phloem, where food is normally most abundant (7), suggest that fungus activity is related to host food reserves. The presence of materials that stain darker than normal in phloem, cortex, and ray cells of galls may indicate an increase of food reserves (6, 7) or an accumulation of waste products. An accumulation of dry matter has been reported in a number of diseases as a result of the transport of substrates to infection sites from surrounding tissue (16). Such an accumulation of dry matter occurs in fusiform rust galls. It appears to be directly related to fungus activity, because the sp gr of galls is highest in the area of largest gall diam (where the infection probably originated). The fact that wood collected above or below the gall did not differ significantly in dry matter content or sp gr indicates that the accumulation in gall tissue does not cause a significant reduction in dry matter in surrounding, noninfected tissue relatively distant from the gall. But a slight reduction of wood sp gr at points above and below the gall occurs, and this reduction may indicate a migration of substrates to the infection site from tissue nearest the gall. The accumulation of dry matter apparently is not related to an increase of dry matter in foliage. It may, however, be related to an increase in the amount of crown foliage, because infected trees often have more foliage.

The increase of dry matter and sp gr in gall wood is caused by an accumulation of substances extractable in neutral solvents, and is not caused by an increase in cell wall material. Reduced cell wall synthesis in infected tissue causes a reduction of alpha-, beta-, gamma-, and holocellulose as well as a reduction in the ratio of dry matter per unit wt of extractive-free wood. This reduction of cell wall synthesis should cause an increase of soluble carbohydrates if the sugars are not utilized in other metabolic pathways. The amount of soluble carbohydrates in infected tissue, however, does not differ significantly from that in noninfected tissue; soluble carbohydrates apparently are utilized in the synthesis of other substances and may be utilized by the fungus (16). Fungal hyphae contribute to the wt of dry matter, but the higher number of resin ducts in gall wood (7) suggests that the resin content of gall wood is higher and that it adds significantly to the wt of dry matter accumulated in gall tissue.

The growth of the fungus, particularly during pycnial and aecial sporulation, and the hypertrophy and hyperplasia in gall tissue suggest that the fungus and the host have an increased requirement, and competition, for food materials. The utilization of host carbohydrates by rusts and other fungi reduces host reserves concurrent with the formation of fruiting structures by the pathogens (3, 16). Harper (5) reported that the cell walls of summerwood fail to thicken normally if starvation is induced by repeated or late-season defoliation. Similar failures have been obtained by experimental defoliation (10), and after defoliation by insects and other agents (8, 9, 18).

Gall wood resembles summerwood in that each has a high lignin content and a similar monosaccharide composition of holocellulose. Meier (12) reported that in *Pinus sylvestris,* summerwood contains more mannan and less arabinan and xylan than does springwood. The lack of alteration of galactan content in infected wood indicates that gall wood also differs from compression wood, which contains considerably more galactan than does normal wood (12). The reported reduction of summerwood in fusiform rust galls (6) is not substantiated by these results, but the competition for food within galls may cause a depletion of host reserves and a failure of normal thickening of the cell walls in summerwood. The internal competition for food between fungus and host may also cause the reduction in the size of tracheid cells (1). The length and volume of tracheids of normal pine stems are correlated with the length and volume of tracheid initials (4), and the reduction in length of gall tracheids might indicate a reduction in length of the tracheid initials or a failure of the growth of gall tracheids to equal that of the tracheids in noninfected wood. Wood tracheids make up as much as 90% of the wood volume in pine (24), and their reduction in length in fusiform rust galls is probably correlated to the observed reduction of holocellulose.

Hypertrophy and hyperplasia in fusiform rust may be caused by the synthesis of certain growth-stimulating compounds. However, the utilization of food by host and fungus may result in the hypertrophy of host cells if the increased (dual) utilization reduces transport of nutrients away from infected cells and increases the nutrients supplied to these cells (1, 4, 23).

LITERATURE CITED

1. Bannan, M. W. 1956. Some aspects of the elongation of fusiform cambial cells in *Thuja occidentalis* L. Can. J. Bot. 34:175-196.
2. Cowling, E. B. 1961. Comparative biochemistry of the decay of sweet gum sapwood by white-rot and brown-rot fungi. USDA Bull. 1258. 79 p.
3. Daly, J. M., A. A. Bell, & L. R. Krupka. 1961. Respiratory changes during development of rust diseases. Phytopathology 51:461-471.
4. Esau, K. 1960. Plant Anatomy. John Wiley & Sons, N. Y. 735 p.
5. Harper, A. G. 1913. Defoliation: its effect upon the growth and structure of the wood of *Larix*. Ann. Bot. 27:621-642.
6. Jackson, L. W. R., & J. N. Parker. 1958. Anatomy of fusiform rust galls on loblolly pine. Phytopathology 48:637-640.
7. Jewell, F. F., R. P. True, & S. L. Mallett. 1962. Histology of *Cronartium fusiforme* in slash pine seedlings. Phytopathology 52:850-858.
8. Jost, L. 1891. Über Dickenwachstum und Jahresringbildung. Bot. Ztg. 49:485-495, 501-510, 525-531, 541-547, 557-563, 573-579, 589-596, 605-611, 625-630.
9. Kny, L. 1882. Über das Dickenwachstum des Holzkörpers in seiner Abhängigkeit von äussern Einflüssen. Parey, Berlin. 136 p.
10. Kühns, R. 1910. Die Verdoppelung des Jahresringes durch Künstliche Entlaubung. Biblioth. Bot. 15(Heft 70). 53 p.
11. Levitt, J. 1954. Investigations of the cytoplasmic particulates and proteins of potato tubers. II. Nitrogen, phosphorus, and carbohydrate contents. Physiol. Plantarum 7:117-123.

12. MEIER, H. 1964. General chemistry of cell walls and distribution of the chemical constituents across the walls, p. 137-151. *In* M. H. Zimmerman [ed.] The formation of wood in forest trees. Academic Press, N. Y.
13. ROMBERGER, J. A. 1963. Meristems, growth, and development in woody plants. USDA Tech. Bull. 1293. 214 p.
14. ROMBERGER, J. A., & G. NORTON. 1961. Changing respiratory pathways in potato tuber slices. Plant Physiol. 36:20-29.
15. SAEMAN, J. F., W. E. MOORE, R. L. MITCHELL, & M. A. MILLETT. 1954. Techniques for the determinations of pulp constituents by quantitative paper chromatography. TAPPI 37:336-343.
16. SHAW, M. 1963. The physiology and host-parasite relations of the rusts. Annu. Rev. Phytopathol. 1:259-294.
17. SMITH, I. 1960. Chromatographic and electrophoretic techniques. I. Intersci. Pub., Inc. N. Y. 617 p.
18. STUDHALTER, R. A. 1955. Tree growth. I. Some historical chapters. Bot. Rev. 21:1-72.
19. TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY. 1954. Holocellulose in wood. TAPPI Standard T 9m-54, 2 p.
20. TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY. 1954. Lignin in wood. TAPPI Standard T 13m-54, 2 p.
21. TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY. 1959. Preparation of extractive-free wood. TAPPI Standard T 12m-59, 1 p.
22. TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY. 1959. Sampling and preparing wood for analysis. TAPPI Standard T 11m-59, 2 p.
23. WARDLAW, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.
24. WISE, L. E., & E. C. JAHN. 1952. Wood chemistry. Rheinhold Pub. Corp., N. Y. 1343 p.