

## Fusiform Rust Gall Formation and Cytokinin 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.

Mention of commercial products does not constitute endorsement by the USDA over other similar products. Accepted for publication 18 March 1970.

### ABSTRACT

Cytokinin activity was 10 times greater in fusiform rust gall tissue than in noninfected tissue of loblolly pine. This increase of cytokinin activity in gall tissue suggests that cytokininlike compounds

play a significant role in gall formation. *Phytopathology* 60:1225-1226.

*Additional key words:* *Pinus taeda*, *Cronartium fusiforme*, physiology of disease.

Gall formation caused by *Cronartium fusiforme* Hedg. & Hunt ex Cumm. may result from a complex interaction of fungus activities and host growth, including competition for food or substrate material and the increased synthesis of certain growth-stimulating compounds (5, 6). The growth-promoting cytokinins control or influence a number of plant-growth phenomena (2). Although the accumulation of cytokinins in the tissue of fusiform rust galls has not been previously reported, many of the phenomena controlled or influenced by cytokinins are also involved in the development of fusiform rust galls. In this study, cytokinin activity was measured in concd extracts of stem tissue from loblolly pine (*Pinus taeda* L.). Trees infected with fusiform rust and noninfected trees were sampled.

Cytokinins were extracted by a modification of a previously described method (8). Samples of stem wood were collected from approximately 50-75 rust-free and 50-75 infected 1-year-old loblolly pine seedlings actively growing in a greenhouse. The stem tissue from infected trees was combined into a composite (50-g) sample, as was the stem tissue from noninfected trees. Each sample was triturated in a Waring Blender for 15 min in 500 ml of distilled-deionized water. The aqueous homogenate was adjusted to 10% perchloric acid and triturated an additional 15 min. The homogenate was then filtered through a glass-wool plug, and the residue was washed with two 100-ml portions of 10% perchloric acid. After it was adjusted to pH 3.0, 7.0, and 8.0, successively, with 2 N sodium hydroxide, the filtrate was extracted at each pH with three 100-ml portions of chloroform. The combined chloroform (900 ml) fraction of each sample was brought to dryness under vacuum in a flash evaporator at 40 C, and 1 ml of 0.2% Tween 80 (polyoxyethylene sorbitan monooleate) was added.

Cytokinin activity was measured by a bioassay based upon the retention of chlorophyll in detached first leaves of oat (*Avena sativa* L. 'Victory') seedlings (8). Five  $\mu$ liters each of the extract, of 1:10 and 1:100 dilutions, were applied to the center of each of five leaves. Acetone extracts were prepared from the "green islands" after 4 days' incubation in the dark at 20 C. The OD of the extracts was read at 665 m $\mu$ . Cytokinin concn were determined by the use of a standard curve prepared with kinetin at log-molar concentrations from  $10^{-8}$  to  $10^{-4}$  M. The

bioassay was repeated 5 times on each extract, and the entire study was repeated.

Cytokinin activity was 10 times greater in gall tissue than in noninfected tissue (Table 1). The extract from 50 g of gall tissue was equivalent to a  $10^{-4}$ -M solution of kinetin, and dilutions of 1:10 and 1:100 were equivalent to  $10^{-5}$ - and  $10^{-6}$ -M solutions of kinetin. The extract from noninfected tissue and its dilutions were equivalent to  $10^{-5}$ -,  $10^{-6}$ -, and  $10^{-7}$ -M solutions of kinetin. From these equivalents it was estimated that infected tissue contained 0.4  $\mu$ g of cytokininlike substances/g of tissue (kinetin equivalents) and that noninfected tissue contained 0.04  $\mu$ g/g of tissue. Although the chlorophyll-retention bioassay used will detect activity in a variety of substances, the linear response and steepness of slope obtained with kinetin

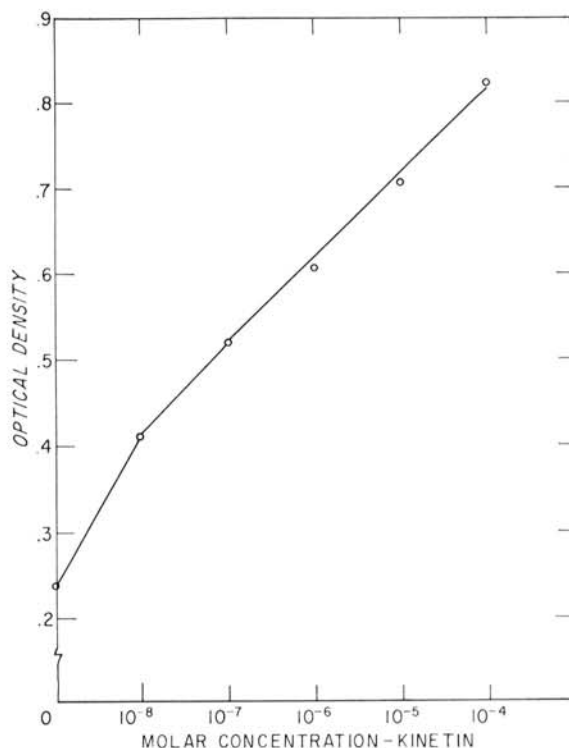


Fig. 1. Standard sensitivity curve of oat-leaf kinetin bioassay. The optical density is proportional to the amount of chlorophyll in acetone extracts of the treated zone.

TABLE 1. Comparative cytokinin activity in extracts of *Cronartium fusiforme*-infected and noninfected loblolly pine stem tissue as measured by OD

Extract dilution	OD (665 m $\mu$ )	
	Infected tissue	Noninfected tissue
1:1	0.84	0.69
1:10	0.70	0.59
1:100	0.62	0.50

(Fig. 1) is approximately the same as reported by Thimann & Sachs (8). Their data indicate that this degree of response is related to the detection of cytokinin activity in extracts.

The phenomenon of gall formation has been attributed to an excess production of auxin or certain other growth-regulating compounds (1, 8). Although a particular growth substance may be involved in the production of plant over-growth when its synthesis is increased within the affected tissue, there is increasing evidence that growth substances (auxins, cytokinins, gibberellins) and other metabolites interact to affect normal and abnormal growth and development (1, 2, 7).

The formation of fusiform rust galls does not appear to be due to cytokinin effects alone, since the length of tracheid cells and the synthesis of cell walls are reduced in gall tissue (3, 4, 5). But cytokinin affects cell division and elongation, apical dominance,

regulation of phloem transport, and mobilization of metabolites (2). The disturbance of these phenomena in pine infected with *C. fusiforme* and the presence of 0.4  $\mu$ g of cytokininlike substances (kinetin equivalents) in gall tissue suggest that one or more of these compounds plays a significant role in the development of fusiform rust galls.

#### LITERATURE CITED

1. FUCHS, Y., & M. LIEBERMAN. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production and their interaction in growth of seedlings. *Plant Physiol.* 43:2029-2036.
2. HELGESON, J. P. 1968. The cytokinins. *Science* 161: 974-981.
3. JACKSON, L. W. R., & J. N. PARKER. 1958. Anatomy of fusiform rust galls on loblolly pine. *Phytopathology* 48:637-640.
4. JEWELL, F. F., R. P. TRUE, & S. L. MALLETT. 1962. Histology of *Cronartium fusiforme* in slash pine seedlings. *Phytopathology* 52:850-858.
5. ROWAN, S. J. 1970. Fusiform rust gall formation and cellulose, lignin, and other wood constituents of loblolly pine. *Phytopathology* 60:1216-1220.
6. ROWAN, S. J. 1970. Fusiform rust gall and canker formation and phenols of loblolly pine. *Phytopathology* 60:1221-1224.
7. SHAW, M. 1963. The physiology and host-parasite relations of the rusts. *Annu. Rev. Phytopathol.* 1:259-294.
8. THIMANN, K. V., & T. SACHS. 1966. The role of cytokinins in the "fasciation" disease caused by *Corynebacterium fascians*. *Amer. J. Bot.* 53:731-739.