

Inhibition of *Poria weirii* and *Fomes annosus* by Linoleic Acid

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Abstract. The potassium salt of linoleic acid, a long-chain fatty acid, inhibited growth of *Poria weirii* and *Fomes annosus* *in vitro* when incorporated into synthetic liquid media at concentrations of 0.1 mg/ml or higher. Increasing concentrations (up to 4.0 g/ml) resulted in significantly increasing inhibition in both fungi. Inhibitory effects were particularly striking for *Poria weirii*: growth was negligible at concentrations of 1.0 mg/ml or higher. Resistance of *Alnus rubra* to infection by *P. weirii* is likely due in part to alder's ability to produce linoleic acid. **Forest Sci. 16:329-330.**

Additional key words. Biological control, *Alnus rubra*.

PORIA WEIRII Murr. is a serious root pathogen of western North American conifers. Red alder (*Alnus rubra* Bong.) is resistant to infection (Wallis 1968), and its presence as pure stands or in mixture with conifers has been associated with reduced longevity of *P. weirii* in forest soil (Nelson 1968).

No feasible mechanical or chemical control method for reducing severity of established infections of *P. weirii* is in prospect. Further, considerations of environmental impact lead us to seek nonchemical methods of pest control whenever possible. Thus, the suggestion that alder acts as a biological control agent against *P. weirii*, and perhaps other pathogens, merits high priority research attention.

The specific mechanisms of alder's antifungal activity are important in development of concepts in biological control of root diseases in forests. Red alder produces numerous phenolic compounds, many of which inhibit *P. weirii* in laboratory experiments (Li *et al.* 1969). In defatting soil extracts preparatory to chromatographic determination of phenolic content, we discovered that soil from under pure alder or alder-conifer mixtures has a far higher lipid content than soil from under pure conifers. Only one lipid has been isolated from red alder to date, linoleic acid (Kurth and Becker 1953), an unsaturated 18-carbon fatty acid. Linoleic acid inhibits *Fusarium nivale* (Fr.) Ces. (Honkanen and Virtanen 1960), and numerous other fatty acids have antifungal properties (e.g., see Kitajima and Kawamura 1931, Baechler 1939, Wyss *et al.* 1945). The following experiment was conducted as a start in evaluating the effects of alder-produced fatty acids on *P. weirii*. *Fomes annosus* (Fr.) Cooke was included as another destructive root pathogen in western forests.

Methods

Linoleic acid (95-97 percent K & K Laboratories, Inc., Plainview, N.Y.) was converted to its potassium salt with KOH in absolute ethanol. The salt was precipitated, washed with anhydrous, peroxide-free ether, and dried in a vacuum desiccator. The dry salt was dissolved at a concentration of 0.1 percent in Trione's (1964) medium, modified by substitution of 10 g glucose for 20 g sucrose, and of Na_2SO_4 for $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ and KCl for CaCl_2 . The resulting medium was adjusted to pH 5.5 with 0.1 N HCl and sterilized by filtration through ultrafine-porosity fritted discs. Aliquots were then added to similar but linoleic acid-lacking medium to produce final concentrations of the linoleic acid salt of 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 4.0 mg/ml. Seven ml of each solution were aseptically poured into eight 15 × 150 mm test tubes.

Shake cultures of *P. weirii* (isolate T-124) and *F. annosus* were meanwhile grown on Trione's medium for 20 and 24 days, respectively. The mycelia were harvested and washed three times and homogenized in sterile distilled water. One-tenth ml of mycelial suspension (0.175 mg of mycelium) of each fungus was added to each of four tubes of each concentration of linoleic acid salt. Cultures of *F. annosus* and *P. weirii* were incubated at 20°C for 17 and 22 days, respectively. Final weight of mycelium in each tube was determined by the microchemical method of Lu *et al.* (1959).

Data were subjected to a polynomial regression analysis.

Results and Discussion

Poria weights decreased linearly with the concentration of linoleic acid up to the 1.0 mg/ml concentration of acid, and then dropped abruptly to a level which corresponded roughly to the initial weight of *Poria* in the tubes. There was no evidence to indicate a curvilinear relationship between weight and concentration (up to 1.0 mg/ml). The negative linear relationship was

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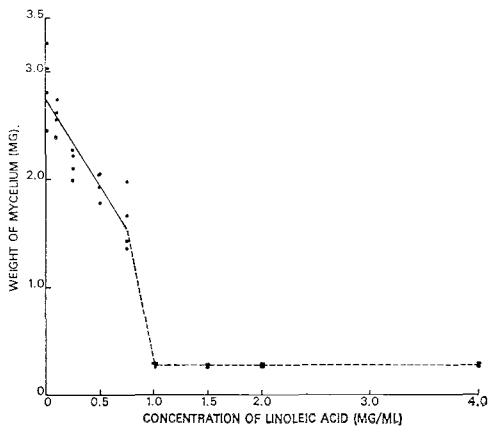


FIGURE 1. Weight of *Poria weirii* mycelia grown for 22 days on media with different concentrations of linoleic acid. Solid line represents computed, highly significant linear regression; broken line, uncomputed line drawn through mean values.

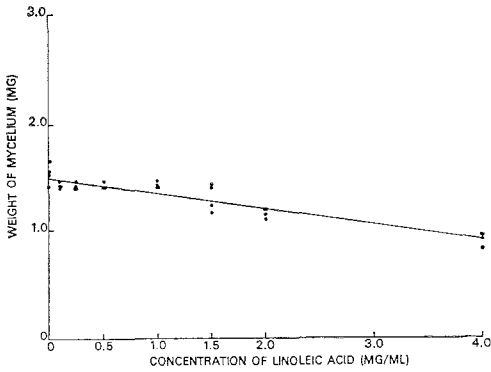


FIGURE 2. Weight of *Fomes annosus* mycelia grown for 17 days on media with different concentrations of linoleic acid (linear relationship highly significant).

highly significant and was expressed as mg of *P. weirii* = $2.75 - 1.603$ (mg/ml linoleic acid salt) (Fig. 1). In other words, through the 0.75 mg/ml concentration, the 22-day growth of *P. weirii* was less than that of the control by a factor of 1.6 for each mg of linoleic acid salt in the solution. Growth became negligible when the concentration was increased from 0.75 to 1.0 mg/ml and higher.

F. annosus grew moderately well at all concentrations of linoleic acid tested. However, increased inhibition occurred with each increase in concentration of linoleic acid salt (Fig. 2), as expressed by this highly significant negative linear regression:

Mg of *F. annosus* = $1.496 - 0.1494$ (mg/ml linoleic acid salt).

These results suggest that linoleic acid is an important factor in the resistance of red alder to infection by *Poria weirii* (Wallis 1968) and may play a role in reducing longevity of this fungus in soil under alder. A similarly strong inhibition of *Fomes annosus* in nature seems less likely on the basis of our data.

Literature Cited

- BAECHLER, R. H. 1939. Toxicity of normal aliphatic alcohols, acids, and sodium salts. Amer Wood Pres Ass Proc 35:364-372.
- HONKANEN, E., and A. I. VIRTANEN. 1960. Unsaturated fatty acids in rye plants. Suomen Kem B 33:171.
- KITAJIMA, K., and J. KAWAMURA. 1931. Antiseptic action of higher fatty acids against wood-attacking fungi. Imp Forest Exp Sta Bull Tokyo 31:108-113.
- KURTH, E. F., and E. L. BECKER. 1953. The chemical nature of the extractives from red alder. Tappi 36:461-466.
- LI, C. Y., K. C. LU, W. B. BOLLEN, and J. M. TRAPPE. 1969. Effect of phenolic and other compounds on growth of *Poria weirii* in vitro. Microbios 3:305-311.
- LU, K. C., J. E. DAWSON, and M. ALEXANDER. 1959. A microchemical method for detecting antifungal substances. Arch Mikrobiol 33:182-185.
- NELSON, E. E. 1968. Survival of *Poria weirii* in conifer, alder, and mixed conifer-alder stands. USDA Forest Serv Res Note PNW-83, 5 pp. Pacif Northwest Forest Range Exp Sta, Portland, Ore.
- TRIONE, E. J. 1964. Isolation and *in vitro* culture of the wheat bunt fungi *Tilletia caries* and *T. controversa*. Phytopathology 54:592-595.
- WALLIS, G. 1968. Resistance of *Alnus rubra* to infection by the root rot fungus *Poria weirii*. In J. M. Trappe, J. F. Franklin, R. F. Tarrant, and G. M. Hansen (eds.), Biology of Alder, p. 195. USDA Forest Serv Pacif Northwest Forest Range Exp Sta, Portland, Ore.
- WYSS, O., B. J. LUDWIG, and R. R. JOINER. 1945. The fungistatic and fungicidal action of fatty acids and related compounds. Arch Biochem 7:415-425.