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Chapter 5

Effects of Lauricidin on *Fomes annosus* and *Phellinus weirii*

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ABSTRACT

Fomes annosus (Fr.) Karast. and *Phellinus (Poria) weirii* (Murr.) Gilbertson are destructive root rot fungi in coniferous forests of western North America. We tested response of these forest pathogens to three concentrations (5, 10, and 100 ppm) of Lauricidin (monolaurin) in Trione's nutrient solution at 24 C in the dark. Growth of both fungi was significantly reduced by Lauricidin at 100-ppm concentration. Addition of 5 ppm EDTA had no significant effects on either pathogen. Protecting stump surfaces from colonization by *F. annosus* is advocated in some forest operations to reduce incidence of this disease. Colonization of western hemlock (*Tsuga heterophylla*) stem sections by spores of *F. annosus* was significantly reduced when disks were first treated with Lauricidin at 1.3 mg/cm^2 or in combination with EDTA at 0.3 or 1.3 mg/cm^2. *F. annosus* did not colonize the wood surfaces that had been treated with Lauricidin at 6.5 mg/cm^2 with or without EDTA.

INTRODUCTION

Fomes annosus (Fr.) Karst. causes a destructive root rot of conifers in temperate zones around the world. Spread of the disease in managed forests often occurs when airborne basidiospores colonize freshly cut stump surfaces during thinning operations. The fungus then grows through the stump and its roots and infects other trees at points of root graft or contact (1). The primary means of control is the application of chemicals or spores of antagonistic fungi to stump surfaces to prevent colonization. Dry borax, 10% zinc chloride, or 20% ammonium sulphamate are effective (2). Borax, however, fails to control *F. annosus* during periods of high precipitation, and ammonium sulphamate and zinc chloride are relatively costly and toxic to man. *Peniophora gigantea* (Fr.) Massee is a vigorous competitor and has been used successfully as a stump protectant on pines in Europe and southeastern United States. It does not, however, satisfactorily inhibit the growth of *F. annosus* on hemlock stumps in western North America (2).

Phellinus (Poria) weirii (Murr.) Gilb. (3), a destructive root rot in conifer forests in western North America, is characterized by its slow spread, long survival, and wide host range. It spreads along live roots from tree to tree much like *F. annosus*. The role of its basidiospores, however, is not known (4). No quick and easy control options are available.

It has been known for many years that certain fatty acids possess antifungal properties (5,6). The systematic investigation of Kiesel (7) in the Pasteur Institute revealed that fatty acids containing from 1-6 carbon atoms inhibited germination of spores, formation of mycelium, and production of conidia in *Aspergillus niger* van Tiegham. The toxicity increased as the chain length increased up to 11 carbons. Kitajima and Kawamura (8),

working with *P. vaporaria* (Pers.) Fr. and *Paxillus panuoides* Fr., essentially confirmed Kiesel's findings. They did find, however, that toxicity increased as carbon chains increased in length up to 12 atoms, and that unsaturated fatty acids were more toxic than the corresponding saturated acids; branched-chain acids were less potent than the corresponding straight-chain acids.

The studies of Baechler (9) on wood-destroying fungi, of Hoffman et al. (10) on molds, and of Rigler and Greathouse (11) on *Phymatotrichum omnivorum* (Shear) Duggar essentially substantiated results of earlier workers. Since then, several scientists have reported fungicidal activities of fatty acids: linoleic acid on *F. annosus, P. weirii,* and *Fusarium nivale* Cesati (12); 13-methyltetradecanoic acid of *Myxococcus xanthus* Beebe origin on spore germination of *F. roseum* (13); and an ornithin-containing lipid isolated from *Gluconobacter cerinus* Asai on *A. niger* (14).

The mechanisms of antifungal actions of lipids are not well understood. It was postulated that lipids are involved in a change in permeability of the cell wall or interference with cellular metabolism either by complexing critical nutrients or by allowing the diffusion of essential metabolites (15).

The ability to utilize fatty acids as sole carbon source, however, is not unique to hymenomycetous fungi. Mukherjee (16) reported the utilization of butyric acid by *A. niger*. Steric acid and oleic acid were utilized by *Mucor mucedo* (L.) Fres. (17). Both long- and short-chain fatty acids were used for growth and energy by *Spicaria violaceae* Abbott (18,19) and *Cunninghamella thaxter* (20-22).

This study was initiated to determine the effect of Lauricidin (monolaurin), an antibacterial lipid, on growth of *F. annosus* and *P. weirii* in vitro, and to determine if it could be used to prevent colonization of wood sections by *F. annosus*. Lauricidin is nontoxic to man (5,6), has been approved by the U.S. Food and Drug Administration as a food additive, and is rather inexpensive.

MATERIALS AND METHODS

The experiments on growth of *F. annosus* and *P. weirii* were conducted with 250-ml Erlenmeyer flasks containing 50 ml of Trione's (23) synthetic nutrient solution, modified by substituting 10 g glucose for 20 g sucrose.

Stock solutions of Lauricidin (Med-Chem Laboratories, brand of monolaurin with monoester greater than 90%) in water were prepared and added to the nutrient solutions to give 5-, 10-, and 100-ppm concentrations. The solutions were then adjusted to pH 5.4 with sterile 0.3 N NaOH. Nutrient solutions containing EDTA (ethylenediaminetetraacetic acid disodium salt) at 5, 10, and 100 ppm, or EDTA plus Lauricidin were similarly prepared. Addition of EDTA increases the solubility of monolaurin and permeability of fungal cells to monolaurin.

A plug of inoculum 4 mm in diameter, taken from the edge of week-old colonies of *F. annosus* or *P. weirii* grown on malt agar plates, was introduced into each flask. Flasks were then incubated at 24 C for 21 days for *F. annosus* and 30 days for the slower growing *P. weirii* isolate. After harvest, mycelia were dried for 48 hours at 85 C and weighed. Final pH of the medium was determined for each flask. There were 5 replicates for each treatment. The effects of Lauricidin, EDTA, Lauricidin-EDTA and the control were compared by orthogonal contrasts.

For wood colonization experiments, stem disks, 7-7.5 cm in diameter and about 2.5 cm in length, were cut from 11 to 13-year-old living western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) and immediately brought to the laboratory where bark was removed. Surfaces of the disks were sterilized for 1 hr with ultraviolet light (254 nm). One flat surface of each disk was dipped in melted paraffin and the paraffin-treated end was placed downward on the bottom half of a sterile 50x90-mm glass petri dish. The opposite surface was coated by painting with Lauricidin alone at 0.3 mg, 1.3 mg, and 6.5 mg/cm^2,

TABLE I

Growth of *Fomes annosus* in a Nutrient Medium Containing Lauricidin, EDTA, or Lauricidin plus EDTA[ab]

	Mycelium growth and final pH[c]							
	Concentration							
	Control		5 ppm		10 ppm		100 ppm	
Compound	mg	pH	mg	pH	mg	pH	mg	pH
Control	162	(4.8)						
Lauricidin			103	(5.2)	118	(5.1)	82	(5.2)
EDTA			119	(5.1)	120	(5.1)	32	(4.9)
Lauricidin + EDTA			123	(5.2)	20	(5.1)	4	(4.9)

[a]Growth was measured in mg of dry weight after 21 days.

[b]EDTA = ethylenediaminetetraacetic acid disodium salt.

[c]Average of 5 replicates; initial pH 5.4.

or Lauricidin at these concentrations plus EDTA at 0.3 mg and 1.3 mg/cm^2. Untreated disks and those inoculated with spores and treated with EDTA only or sterile distilled water were used as controls.

One day after the treatment, the nonparaffin coated cut surface of each disk was inoculated with an $8x10^2$/ml spore suspension of *F. annosus* in water. Ten ml of sterile distilled water were poured into each petri dish to maintain a high relative humidity. A lid, which fit well but did not prevent gas exchange, was placed on each petri dish. There were 10 replicates for each treatment.

Disks were incubated at 24 C and examined for the presence of mycelium and the *Oedocephalum* spore stage of *F. annosus*. Those disks that showed no signs of *F. annosus* were split; 4 chips were taken from the split surface of one of the resulting halves with a pair of chiesel forceps, then transferred to a selective medium devised by Kuhlman and Hendrix (24) for the detection of *F. annosus*.

RESULTS AND DISCUSSION

Growth in Culture

Analysis of variance indicated that growth of *F. annosus* and *P. weirii* in the nutrient medium was significantly reduced by Lauricidin, EDTA, or Lauricidin-EDTA combinations; the growth was significantly less in 100 ppm than in the other two concentrations (Table I, Table II). At 10 ppm and 100 ppm, the growth of both fungi in the medium containing Lauricidin-EDTA combinations was significantly less than that in the medium containing Lauricidin or EDTA alone. Growth was least with Lauricidin-EDTA at 100-ppm level. At 100 ppm, Lauricidin alone was as effective as Lauricidin-EDTA in reducing the growth of *P. weirii* (Table II). The means of final pH values of the media for both fungi were not much different among treatments. The final pH of the medium in which *P. weirii* was grown, however, was generally lower than that for *F. annosus*.

Colonization of Stem Disks

Three days after inoculation, mycelium and the *Oedocephalum* stage of *F. annosus* were observed on the untreated surfaces of stem sections; those treated with EDTA, Lauricidin (0.3 mg/cm^2), or a combination of these two did not show signs of *F. annosus* until 1 week after inoculation. After 21 days, fungal colonization of these disks did not detectably differ from that of untreated disks. Few clusters of mycelia or conidio-

TABLE II

Growth of *Phellinus weirii* in a Nutrient Medium Containing Lauricidin, EDTA, or Lauricidin plus EDTA[ab]

	Mycelium growth and final pH[c]							
	Concentration							
	Control		5 ppm		10 ppm		100 ppm	
Compound	mg	pH	mg	pH	mg	pH	mg	pH
Control	142	(4.4)						
Lauricidin			73	(4.3)	75	(4.3)	6	(5.0)
EDTA			128	(4.3)	107	(4.5)	23	(4.8)
Lauricidin + EDTA			70	(4.5)	46	(4.5)	2	(5.3)

[a]Growth was measured in mg of dry weight after 30 days.

[b]EDTA = ethylenediaminetetraacetic acid disodium salt.

[c]Average of 5 replicates; initial pH 5.4.

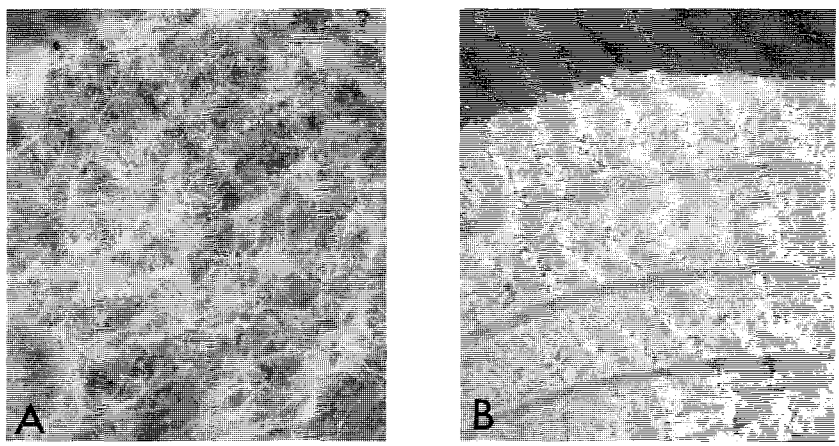

FIG. 1. Wood disks of *Tsuga heterophylla* where exposed to spores of *Fomes annosus* for 2 weeks; (A) Control and (B) 1% Lauricidin plus 1% EDTA.

phores were observed on the disks that had been treated with Lauricidin alone at 1.3 mg/cm^2 or in combination with EDTA (Fig. 1).

F. annosus did not become established on wood surfaces coated with Lauricidin at 6.5 mg/cm^2 with or without EDTA, nor did wood chips from split disks initiate development of *F. annosus* colonies on the selective medium. These results indicate that at this concentration, Lauricidin alone or in combination with EDTA could prevent *F. annosus* from colonizing wood.

Effects of lipids on *F. annosus* and *P. weirii* have been reported previously. Puritch and Etheridge (25) reported that capric acid at 0.1% was able to inhibit both fungi, and wood disks of western hemlock treated with 1% solution of capric acid prevented the mycelial growth of *F. annosus*. Li et al. (3) also reported that linoleic acid, present in *P. weirii*-resistant red alder (*Alnus rubra* Bong.) inhibited both pathogens in vitro at 0.1% or higher concentrations. The results of this study indicate that Lauricidin, a monoglyceride, is more effective than the other two lipids in suppressing the growth of both pathogens in vitro. But its relative effectiveness toward *F. annosus* in vivo, in comparison with capric and linoleic acids, is difficult to evaluate, because the concentrations of capric or linoleic

acid on wood disks were not given. Nevertheless, Lauricidin would be advantageous over capric or linoleic acid because of its low cost and stability.

Probably owing to its anionic surfactant properties, Lauricidin, once applied, can hold firmly onto the wood surfaces—an advantage for its use on stump surfaces during periods of high precipitation. Its effectiveness under field conditions is now being investigated.

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