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# Effect of Lauricidin and Ethylenediaminetetraacetic Acid on Growth of Nine Hymenomycetous Fungi

C. Y. Li and Paul E. Aho



## Abstract

Growth of nine wood-decaying basidiomycetes was measured on media containing 10, 100, and 1,000 parts per million (p/m) Lauricidin with or without 0.1 percent ethylenediaminetetraacetic acid (EDTA). EDTA alone significantly reduced the growth of all fungi tested. Lauricidin at 1,000 p/m significantly retarded the growth of all fungi except two: *Ganoderma applanatum* and *Armillariella mellea*. The addition of EDTA to 1,000 p/m Lauricidin completely inhibited *Echinodontium tinctorium, Fomitopsis officinalis*, and *Perenniporia subacida*. These compounds show promise as constituents of tree-wound dressings.

Keywords: Decay fungi (wood), lipids, fatty acids, wounds, tree injury.

## Introduction

Lauricidin[1/] is the trade name of monolaurin with more than 90-percent monoester attached to the first glycerol hydroxyl group. This compound has shown remarkably high activity against oral streptococci and actinomycetes and has been incorporated into products used to prevent dental caries in humans (Kabara and others 1978). In vitro growth of *Heterobasidion annosum* (Fr.) Bref. and *Phellinus weirii* (Murr.) Gilb. was inhibited by Lauricidin (Li and Kabara 1978). A preliminary field test indicated that Lauricidin applied on stump surfaces of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) could prevent colonization by *H. annosum* (Nelson and Li 1980).

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1/ Lauricidin is a compound available from Med-Chem Laboratories, Monroe, Michigan. Trade names are included for information only and do not imply endorsement by the U. S. Department of Agriculture.

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C. Y. LI is microbiologist and PAUL E. AHO is research plant pathologist, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon 97331.

Intensive forest management usually requires repeated stand entries with logging equipment. Aho and others (1983) have shown that up to 50 percent or more of the residual (crop) trees were injured during thinning in young true fir stands in northern California. About 14 percent of the board-foot volume of the wounded trees was lost to decay after only 13 years. Wounds on trees in recreation and urban areas may lead to extensive decay and tree failure, resulting in property damage and injury or death to people.

Recent studies have shown that commonly used wound dressings do not prevent invasion by bacteria and fungi that cause discoloration and decay (Shigo and Wilson 1977). Shigo and Wilson (1971) suggested that an effective dressing should protect wounds not only from invasion by decay fungi, but also from the bacteria and nondecay fungi that are often the pioneer invaders of exposed wood and contribute to discoloration and decay. Because Lauricidin is relatively inexpensive, is not toxic to animals and higher plants, and inhibits both bacteria and fungi, it is a potential component of tree-wound dressings. We tested the inhibitory effects of Lauricidin alone and combined with EDTA in vitro on nine hymenomycetous fungi. EDTA was added because it increases the solubility of Lauricidin and the permeability of microbial cells to Lauricidin (Shibasaki and Kato 1978).

## Materials and Methods

The fungi used in this experiment were *Armillariella mellea* (Fr.) Karst., *Coniophora puteana* (Fr.) Karst., *Echinodontium tinctorium* E. and E., *Fomitopsis pinicola* (Fr.) Karst., F. *officinalis* (Vill. ex Fr.) Bond. et Sing., *Ganoderma applanatum* (Pers. ex Wallr.) Pat., G. *tsugae* Murr., *Phellinus pini* (Fr.) Pilát, and *Perenniporia subacida* (Pk.) Donk. Cultures of these fungi were provided by the Center for Mycology Research, Forest Products Laboratory, Madison, Wisconsin. Cultures had been maintained on malt or potato dextrose agar since 1968. Inocula of the fungi obtained were from colonies grown on malt agar in petri plates at 25 °C until they reached 25 to 30 mm in diameter.

Lauricidin was added to malt agar and malt agar containing 0.1 percent EDTA to give concentrations of 10, 100, and 1,000 p/m. Controls were malt agar and malt agar containing 0.1 percent EDTA. The media were autoclaved at 15 lb pressure for 15 minutes, then adjusted to pH 5.4 with sterile 0.1 N NaOH. Petri dishes, 90 mm in diameter, were filled with 20 ml of medium. Four replicate plates were inoculated with F. *officinalis*, *G. applanatum*, *G. tsugae*, and *Phellinus pini*; five replicate plates were inoculated with *C. puteana*, *E. tinctorium*, *F. pinicola*, and *Perenniporia subacida*. Radial growth of *A. mellea* mycelia was difficult to measure because irregularly shaped colonies developed. We therefore measured the dry-weight gain of *A. mellea* after growing it in tubes 25 mm outside diameter x 200 mm, each

containing 80 ml of medium. A plug of inoculum 4 mm in diameter was taken from the edge of colonies of each fungus and inverted in the center of each agar plate or tube. Inoculated plates and tubes were incubated at room temperature (22 to 24 $^{o}$C). Because of variations in growth rate, radial mycelial growth was measured at three intervals: 12 days for *C. puteana, F. pinicola* and *Perenniporia subacida*; 27 days for *Phellinus pini, F. officinalis, G. applanatum* and *G. tsugae*; and 37 days for *E. tinctorium*. Dry weight of *A. mellea* colonies was determined after incubation for 26 days. Colonies were removed from warm agar, washed in water, and ovendried at 80 $^{o}$C for 48 hours.

The experiment had a completely random factorial design. Unfortunately, some of the fungi grew quickly to the maximum size permitted by the petri dishes; thus, potential growth beyond this size was unknown. Data for these cultures were eliminated from the analyses. A one-way analysis of variance was used for the unbalanced data. The remaining fungi were analyzed in the prescribed manner. Individual differences in both formats were analyzed using Tukey's multiple comparison technique.

## Results and Discussion

The effects of Lauricidin and EDTA on radial mycelial growth are shown in figure 1. Growth of *C. puteana, F. pinicola*, and *Perrenniporia subacida* on media with 10 p/m Lauricidin was significantly lower than for controls, and growth was further significantly decreased on concentrations of 100 and 1,000 p/m. Growth of *F. officinalis* in 10 p/m Lauricidin did not differ significantly from the control but was significantly less on 100 and 1,000 p/m than on controls or 10 p/m Lauricidin. *Ganoderma applanatum, G. tsugae*, and *Phellinus pini* on 1,000 p/m Lauricidin grew significantly less than on controls and on 10 and 100 p/m Lauricidin. Growth of *Echinodontium tinctorium* did not differ on 10, 100, and 1,000 p/m Lauricidin. Except for *P. pini* at 1,000 p/m Lauricidin combined with EDTA, the other Lauricidin-EDTA combinations significantly decreased growth of all fungi compared to Lauricidin alone. Lauricidin at 1,000 p/m plus EDTA significantly reduced mycelial growth of *F. pinicola* and *G. applanatum*, and completely inhibited the growth of *E. tinctorium, F. officinalis*, and *Perenniporia subacida*. *Ganoderma tsugae* failed to grow on media with EDTA or EDTA-Lauricidin combinations. EDTA alone significantly reduced the growth of all fungi. Lauricidin alone, however, increased the growth of *A. mellea*.

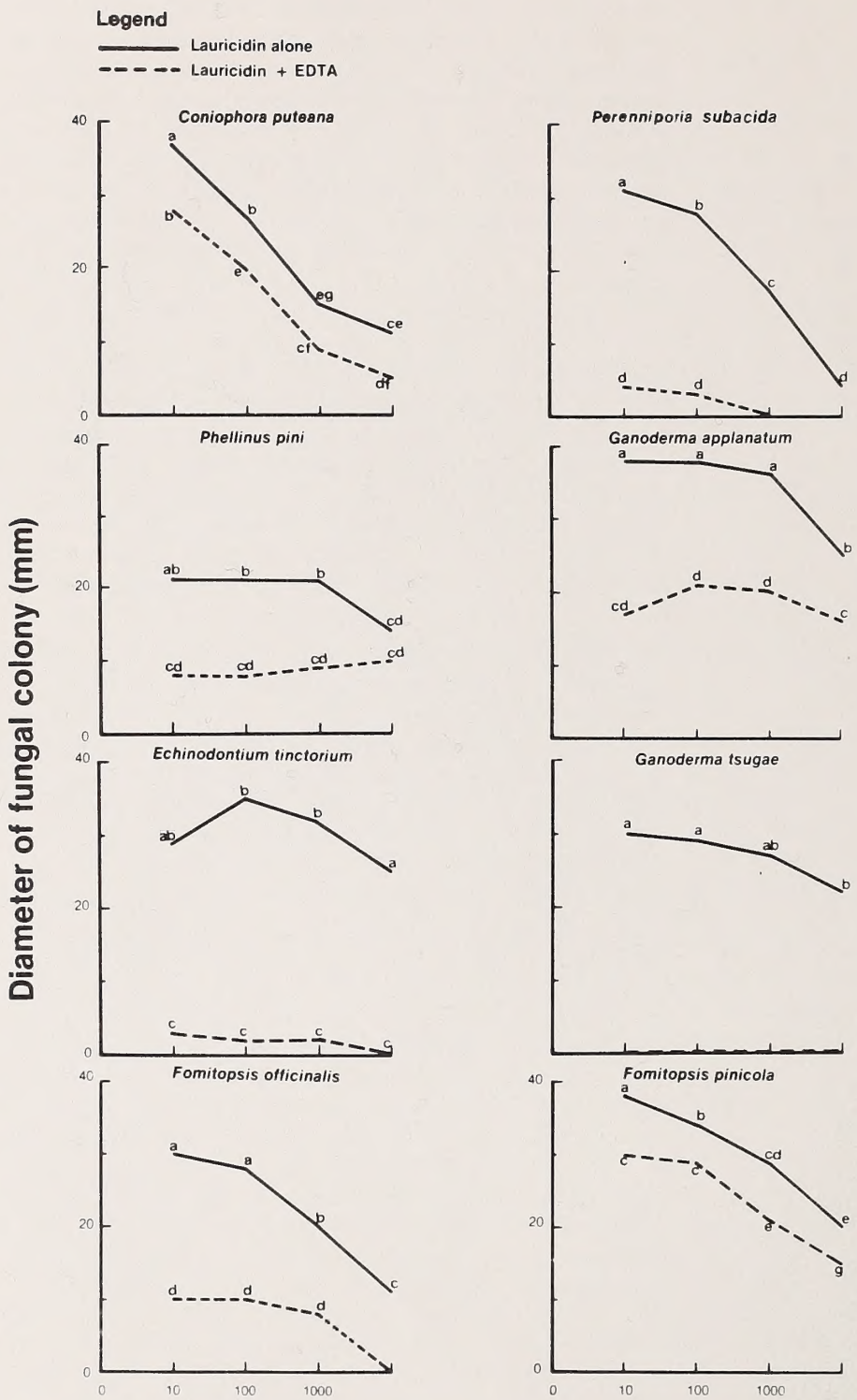


Figure 1. Effect of Lauricidin and EDTA on mycelial growth of eight hymenomycetous fungi. Means not sharing a common letter significantly differ at the 95-percent confidence level with the Tukey test.

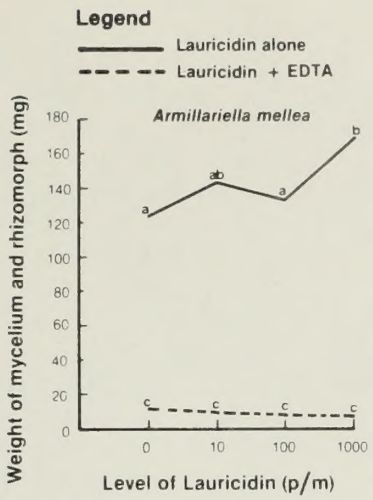


Figure 2.--Effect of Lauricidin and EDTA on growth of Armillariella mellea. Means differ significantly at the 95-percent confidence level with the Tukey test.

These data indicate that EDTA enhances the antifungal activity of Lauricidin on *C. puteana, E. tinctorium, F. pinicola, F. officinalis* and *Perenniporia subacida*, as was shown for *Phellinus weirii* and *Heterobasidion annosum* in earlier studies (Li and Kabara 1978). EDTA either inhibited or reduced fungal growth. The mechanism by which these compounds inhibit growth of fungi is unknown. Shibasaki and Kato (1978) reported that bacterial cells treated with EDTA released lipopolysaccharides from the outer cell membrane, allowing monolaurin to penetrate easily into the inner membrane, a primary site for its antibacterial action. Some wood-destroying fungi were reportedly inhibited by metal-complexing agents, such as EDTA, because essential elements are not available for metal-requiring fungal enzymes (Highley 1975, Mandels and Reese 1963). Bohne (1973) reported that chelation of metal elements by EDTA can inhibit deoxyribonucleic acid synthesis.

Our study indicates that growth of most test fungi was reduced significantly, and four were completely inhibited by one or more of the treatments. Two important invaders of tree wounds, *G. applanatum* and *F. pinicola*, are the least affected by Lauricidin and EDTA. Their growth was unaffected or only delayed. Malt agar is an excellent growth medium for these fungi, however, and the size and type of inoculum used is probably more effective than that found under natural conditions.

Lauricidin and EDTA show promise for interfering with the growth of decay fungi. These compounds may also effectively inhibit germination of basidiospores on the surface of wounds (Nelson and Li 1980). Further testing in combination with other chemicals or at higher concentrations is necessary, however, to establish these compounds as attractive alternatives to the use of petrochemicals in forest or urban environments. Field tests on wounds are also needed to establish what works in practice.

## Metric Equivalents

1 millimeter (mm) = 0.039 inch
1 millimeter (mm) = 0.001056 quart (U.S. liquid)

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Pacific Northwest Forest and Range
Experiment Station
319 S.W. Pine St.
P.O. Box 3890
Portland, Oregon 97208