W. G. Thies and **C. Y. Li**, USDA Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon 97331

Movement of Lauricidin in Douglas-fir Stumps Infested by *Phellinus weirii*

Abstract

Phellinus weirii (Murr.) Gilb., cause of laminated root rot, infects nearly all commercially important conifer species in the western United States and Canada. Infection in a young stand occurs when developing roots of young trees contact residual infested roots and stumps from a preceding stand. Although soil fumigants applied to infested stumps will reduce the amount of inoculum, the chemicals are highly toxic and require special handling. We are interested in developing stump treatments that are safe and environmentally acceptable. Because Lauricidin, a fungicide, has been demonstrated both to inhibit *P. weirii* and to be of low mammalian toxicity, it was tested as a candidate control agent. Two concentrations of Lauricidin in a carrier solution of dimethyl sulfoxide (DMSO) and ethylenediaminetetraacetic acid (EDTA), and the carrier solution alone, were applied to holes drilled in the tops of Douglas-fir (*Pseudotsuga menziesii*) stumps infested by *P. weirii*. Half of the stumps were evaluated after 4 months and the rest after 32 months. Lauricidin did not appear to move significantly in the stumps or roots. Any effect of the treatments on survival of *P. weirii* occurred near the treatment application holes and appeared to be caused by the carrier solution. We concluded that the formulation of Lauricidin tested in this study is relatively immobile in wood and for that reason a poor choice as a chemical for treating stumps to control *P. weirii*.

Introduction

Phellinus weirii (Murr.) Gilb. causes the most important root rot of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the western United States and Canada. Infection in a young stand begins through contact between developing roots and residual infected stumps and roots from a preceding stand. Control of this disease has mainly focused on interrupting the disease cycle at the time of stand regeneration (Thies 1984). One experimental approach has been to chemically treat infested stumps at harvest to eliminate the fungus. Desirable chemical treatments would be those having antifungal properties with low toxicity to nontarget organisms, and ease of handling.

Lauricidin,[1] the trade name of monolaurin with more than 90-percent monoester attached to the first glycerol hydroxyl group, inhibits growth of *P. weirii*, *Fomes annosus* (Fr.) Karst. (Li and Kabara 1978), and other wood-decaying basidiomycetes (Li and Aho 1984) *in vitro*. Lauricidin, alone or in combination with ethylenediaminetetraacetic acid (EDTA), reduced colonization of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stem sections from spores of *F. annosus* (Li and Kabara 1978). EDTA added to treatment solutions improved the ability of Lauricidin to inhibit *P. weirii* (Li and Kabara 1978) and other hymenomycetous fungi (Li and Aho 1984). These results led to a test that demonstrated the usefulness of Lauricidin as a stump surface protectant to reduce infection by *F. annosus* from spores (Nelson and Li 1980).

Lauricidin is nontoxic to humans, is approved by the U.S. Food and Drug Administration as a food emulsifier, and has been classified by that agency as Generally Regarded As Safe (GRAS; reg. No. 21 CFR GRAS 182.4505). Because Lauricidin has been demonstrated to both inhibit *P. weirii* and be of low mammalian toxicity, it was applied to *P. weirii*-infested stumps and its distribution within the roots estimated 4 and 32 months later. This paper reports the results of that field test.

Methods and Materials

Stumps

The study area was a 45-year-old Douglas-fir stand in the Oregon Coast Range near Apiary, Oregon (46°N. latitude, 121°W. longitude). Forty freshly cut Douglas-fir stumps, ranging from 26 to 67 cm in diameter and showing symptoms of *P. weirii* infection, were selected. A disk was cut

[1]This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under The Federal Insecticide, Fungicide, and Rodenticide Act as amended. Also, mention of a commercial or proprietary product does not constitute recommendation or endorsement by the U.S. Department of Agriculture.

from the top of each stump, leaving the stump 30 cm high. Each disk was labeled and stored in a plastic bag. The pretreatment presence of *P. weirii* was later verified by culturing from stained or decayed areas of each disk and examining morphological features of resulting colonies (Nelson 1975). From our experience (Thies unpublished data), *P. weirii* is not present in wood that is not stained or decayed.

Stumps were stratified into five groups of eight stumps based on similarities in stump diameter and amount and stage of decay observed on the stump top. In a natural stand, considerable variation occurs among stumps with regard to size and shape. Additionally, among infected trees there is a continuum of stages of the disease from just infected to standing dead. Stumps were first stratified based on diameter of the stump top: small—less than 29 cm; medium—29 cm to 43 cm; and large—greater than 43 cm. The large stumps were further stratified into three groups based on the amount of stain or advanced decay observed on the stump top: 1. complete or nearly complete ring of stain at the sapwood-heartwood interface; 2. numerous spots or arcs of stain but not forming a contiguous ring; 3. few spots of stain. Stumps were selected so as to achieve five distinct groups and to minimize variability within each group. The eight treatments were assigned at random to the eight stumps in each group, thereby creating a randomized complete block experimental design.

Treatments

The eight treatments are combinations of four solutions with two intervals of exposure. Stump and root excavation and examination is necessarily destructive and hence the need to examine a different set of stumps for each interval of exposure. Lauricidin was dissolved in dimethyl sulfoxide (DMSO) to aid in distributing Lauricidin in the wood. Four treatment solutions were placed in stumps: 1. low—100 g Lauricidin, 1000 ml DMSO, 1.0 g EDTA; 2. high—700 g Lauricidin, 1000 ml DMSO, 1.0 g EDTA; 3. DMSO—1000 ml DMSO, 1.0 g EDTA; 4. check—nothing added. Check stumps were drilled and plugged but nothing was added to the stump.

Treatment holes, 2.5 cm in diameter, were drilled vertically into each stump top, including checks. Two holes extending into the root collar area but not through the bottom of the stump were drilled into each quadrant of the stump top. After treatment, each hole was plugged with a 2.5-cm-diameter by 5-cm-long hemlock dowel.

Data Collection

Stumps were treated in May 1981 and sampled in either September 1981 or February 1984. Of the 40 stumps initially included in this study, 3 did not yield *P. weirii* from the pretreatment sample and were not further evaluated. We conducted assays using *P. weirii* and *Streptococcus mutans* #6715 (Kabara *et al.* 1978) to determine how far biologically active concentrations of Lauricidin had migrated from the treatment holes into stump and root wood.

After each of the exposure intervals, samples were collected from appropriate stumps to determine the presence of viable *P. weirii* within the stump and roots. Stumps were bulldozed from the soil in a manner to retain intact as many major roots as possible, and the root surfaces were cleaned. After removing and discarding the top 5 cm of each stump, we cut an additional 10- to 15-cm disk and split it along radii between treatment holes into blocks. Blocks with stain or advanced decayed wood typical of *P. weirii* surrounding a treatment hole were labeled and stored in plastic bags. Blocks without stain were discarded.

Based on 1981 results, root systems were sampled less intensively in 1984 than in 1981. In 1981, 5-cm-thick disks were removed at 30-cm intervals along each root from the stump to a minimum root diameter of 5 cm. In 1984, a 5-cm-thick disk was removed from each of two infected roots 1 m from each stump. Disks with stain or advanced decay typical of *P. weirii* infection were labeled as to location, placed in plastic bags, and stored at 4°C.

Stump blocks and root disks from both treated and check stumps were sampled similarly. Each stump-sample block was split aseptically along three lines radiating from the hole towards the pith and 120° on either side (Nelson and Thies 1985). Wood chips were aseptically removed midway between the top and bottom of each split face starting 1 cm from the hole and at 1-cm increments across the face of the block in areas of typical stain or of advanced decay. Each root disk was split longitudinally through

stain or decay typical of *P. weirii*. Five chips were removed aseptically from the split face midway between the top and bottom of the disk in areas of typical stain or advanced decay. Each chip was placed on a tube slant of 1.5% malt agar with 1 ppm benomyl. Culture tubes were incubated for 14 days at room temperature. Presence of *P. weirii* was determined from morphological features of developing colonies (Nelson 1975). Tubes without colonies were incubated an additional 14 days and reexamined before discarding.

Samples from the 32-month collection were stored in plastic bags for 14 days at 4°C, and then for another 14 days at room temperature, before examination or isolation. When wood infested by *P. weirii* is stored in a closed plastic bag for 2 weeks at a moderate temperature (between 4°C and 20°C), the distinctive mycelium and setal hyphae of *P. weirii* will commonly grow onto the wood surface to form a thick brown felt on areas with typical stain or advanced decay (Thies and Nelson 1987). After storage, disks with the distinctive felt were recorded as positive for *P. weirii* without an attempt to culture the fungus. Disks lacking a felt or with a weak felt (scattered mycelium and few setal hyphae) were evaluated by culturing.

In addition to isolation of *P. weirii*, a modified bioassay using *Streptococcus mutans* #6715 (Kabara *et al.* 1978) was used to detect the presence of Lauricidin in stump samples collected in 1984. Each stump block was split radially through a treatment hole. Sample locations were marked on a split face 2 and 8 cm from the treatment hole on clear or stained wood but not on areas of advanced decayed wood. A wood sample was collected (Figure 1) by first drilling a 3-cm-diameter hole 0.5 cm deep at each sample point to remove potentially contaminated surface wood. Then, chips were collected from a 1.0-cm hole drilled 2.0 cm deep at the same point. The chips were ground to pass a 40-mesh screen, steam sterilized by autoclaving for 15 min, moistened with sterile distilled water, pressed into a disk 1.0 cm in diameter by 2 mm thick, and placed in the center of a trypticase-soy agar plate previously seeded with 10^8 cells of *S. mutans*. After incubation at 30°C for 24 to 48 hours, each plate was examined for zones of inhibition. As a standard for comparing inhibition zones, 12.7 mm paper disks were seeded with 25-, 50-, or 100-μg of Lauricidin in a water solution and then placed on plates and assayed in the same manner as the ground wood disks.

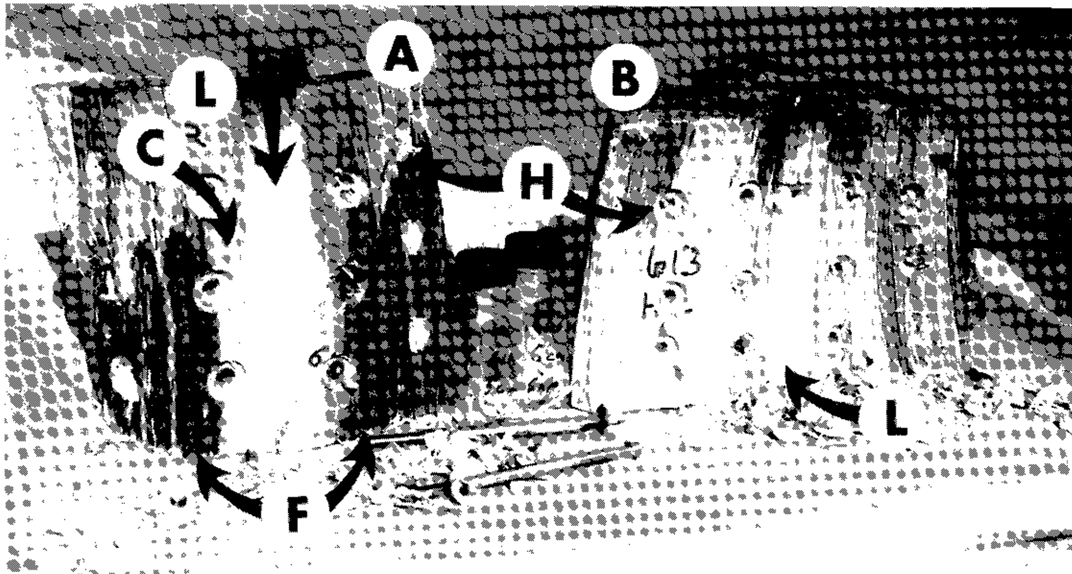

Figure 1. Stump top samples from stumps treated with Lauricidin and the carrier solution of DMSO and EDTA. A—Block infested by *Phellinus weirii*. B—Block not infested by *P. weirii*. H—Holes created while extracting wood samples. L—Lauricidin in treatment holes. F—Felt formed on infested wood by *P. weirii*. C—Zone of clear wood without a *P. weirii*-felt on the infested block.

Results

Lauricidin did not appear to move significantly in the stumps or roots. Stumps split open either 4 months or 32 months after treatment revealed Lauricidin in the treatment holes and active *P. weirii* in wood near these holes.

Lauricidin was found in treatment holes drilled in either sound or stained wood. The amount of Lauricidin that remained in the treatment holes was not measured; however, holes in both sound and stained wood were filled with Lauricidin, without noticeable voids (Figure 1). Treatment holes in advanced decayed wood contained little if any Lauricidin; however, advanced decayed wood less than 2 cm from the treatment holes appeared whitish and infiltrated by Lauricidin. Advanced decayed wood in stumps receiving either the check or DMSO treatment did not have whitish deposit. Advanced decayed wood probably offered little resistance to the flow of the treatment solutions, so that the Lauricidin was carried a short distance into spaces in the wood with the flow of the solution.

At the 4-month evaluation, *P. weirii* was recovered from all stump and root sample disks from both solution- and check-treated stumps. Proximity to the treatment hole did not influence recovery of *P. weirii*. The fungus was cultured from chips collected as close as 1 cm from treatment holes. These samples were not tested with the *S. mutans*.

At the 32-month sampling, a *P. weirii* felt was observed on blocks from 15 of the 17 stumps evaluated. Three stumps, one from each treatment group receiving a solution, were not evaluated; two were lost and one was eliminated because *P. weirii* was not recovered from the pretreatment sample. All five nontreated control stumps had a strong felt on both stump and root samples associated with the presence of stain or advanced decay typical of *P. weirii*. The felt covered only wood with *P. weirii* stain or advanced decay and formed a clear line of demarcation with areas of sound wood. Some areas of advanced decay did not appear to support the felt, and attempts to culture *P. weirii* from these areas were not successful. This finding is consistent with observations made in other studies (Thies and Nelson 1987). Of the 12 treated stumps, 2 were negative for *P. weirii* based on observations for felt or cultures from wood at the 32-month sampling; however, *P. weirii* was isolated from pretreatment samples from both stumps. These stumps were the two smallest (29 cm in diameter) evaluated at the 32-month sampling (mean diameter for stumps in the study was 43 cm) and were treated with solutions containing Lauricidin (one high, one low). Both were from a replicate containing stumps with significant amounts of advanced decay on the stump surface. Because of their size, these two stumps received the highest dosage of treatment solution per kilogram of stump and root biomass. Of the five stumps with a weak felt (suggesting some treatment effect), four were stumps from replicate blocks with significant advanced decay. Two of these five stumps were treated only with the carrier solution (DMSO and EDTA).

Felt developed around holes in untreated (check) stumps but not around holes that received a treatment solution. The distinct clear zones appeared on endgrain as well as split surfaces, and extended 2 to 4 cm from each hole (Figure 1). Attempts to isolate *P. weirii* from wood beneath clear zones were unsuccessful, although the fungus could be isolated from beneath adjacent felt-covered wood. The clear zones appeared the same whether or not Lauricidin was part of the treatment solution.

As we dissected the root systems, we could smell DMSO at many points, some as far as 2 m from the stump where the roots were as small as 5 cm in diameter. Many root samples that were returned to the lab had both the stain typical of *P. weirii* and the odor of DMSO. After incubation, stained sections often developed a heavy felt; however, root samples with advanced decay and the odor of DMSO usually developed little or no felt. Root samples, from untreated stumps, that had advanced decay usually developed a felt.

The bioassay for Lauricidin using *S. mutans* appeared to be very sensitive. Zones of inhibition extended a mean of 7 mm from paper disks with 25-μg samples and 26 mm from disks with 100-μg samples. We did not detect Lauricidin in prepared wood samples from any treated stumps. Failing to detect Lauricidin in the stump samples, we did not process root samples.

Discussion

The formulation of Lauricidin tested in this study is relatively immobile in wood and for that reason

appears a poor choice as a chemical for treating stumps to control *P. weirii.* Observed treatment effects on *P. weirii* seem to be due to the carrier solution, which eradicated the fungus only near the treatment holes, even where there was advanced decay. DMSO, however, appeared able to penetrate much of the root system of treated stumps.

Lauricidin has many desirable qualities that may yet allow it to be used in an effective control program. In view of the desirable and relatively safe nature of this compound, more research should be encouraged to develop and field-test formulations and application techniques that will result in better distribution of Lauricidin in stumps and root systems.

Acknowledgments

We thank K. C. VanNatta of Rainier, Oregon, for providing study trees, equipment, and personal services. We also thank Dr. Jon Kabara of Michigan State University, East Lansing, Michigan, for consultation and for providing the Lauricidin, and to Duane Weiss and Michael McWilliams for field assistance.

Literature Cited

Kabara, J. J., P. Lynch, K. Krohn, and R. Schemmel. 1978. The anti-cariogenic activity of a food-grade lipid-Lauricidin. *In:* J. J. Kabara, ed. Symposium on the pharmacological effect of lipids. The American Oil Chemists' Society, Champaign, Illinois, Chapter 3, Pp. 25-36.

Li, C. Y., and J. J. Kabara. 1978. Effects of Lauricidin on *Fomes annosus* and *Phellinus weirii.* In: J. J. Kabara, ed. Symposium on the pharmacological effect of lipids. The American Oil Chemists' Society, Champaign, Illinois, Chapter 5, Pp. 45-50.

Li, C. Y., and P. E. Aho. 1984. Effect of Lauricidin and ethylenediaminetetraacetic acid on growth of nine hymenomycetous fungi. USDA Forest Serv. Res. Note PNW-363. Pac. Northwest For. and Range Exp. Stn., Portland, Oregon.

Nelson, E. E. 1975. Survival of *Poria weirii* in wood buried in urea-amended forest soil. Phytopathology 65:501-502.

Nelson, E. E., and C. Y. Li. 1980. Preliminary test of two stump surface protectants against *Fomes annosus.* USDA For. Serv. Res. Note PNW-363. Pac. Northwest For. and Range Exp. Stn., Portland, Oregon.

Nelson, E. E., and W. G. Thies. 1985. Colonization of *Phellinus weirii*-infested stumps by *Trichoderma viride:* 1. Effect of isolate and inoculum base. Eur. J. For. Path. 15:425-431.

Thies, W. G. 1984. Laminated root rot: The quest for control. J. For. 82:345-356.

Thies, W. G. and E. E. Nelson. 1987. Reduction of *Phellinus weirii* inoculum in Douglas-fir stumps by the fumigants chloropicrin, Vorlex, and methylisothiocyanate. For. Sci. 33:316-329.

Received 19 January 1987
Accepted for publication 14 August 1987