Root rot caused by Armillariella mellea creates roughly circular disease centers of infected dead and dying trees in the young-growth ponderosa pine forest under study. Research objectives concerning chemical control of the disease were: 1) to test the effectiveness of certain chemicals in protecting living pines adjacent to disease centers from lethal attack at the root collar by A. mellea and 2) to test the effectiveness of chemical fumigants in eradicating A. mellea from infected stubs and snags of trees killed by the fungus within disease centers.

The literature revealed the inadequacy of trenching, ring-barking, uprooting, and biological control involving antagonistic organisms in effectively controlling Armillaria root rot in the field, but control with persistent and highly volatile fungicides used as protectants and eradicants holds promise for controlling this disease.

Among seven root collar protectant chemicals tested in vitro, benomyl and copper sulfate were most toxic to A. mellea while captan,
copper metal, and iron sulfate were less toxic. Vorlex and chloropicrin were also highly effective in reducing the growth of *A. mellea* at the concentration tested. One of three isolates of *A. mellea* tested was more tolerant of the chemicals than the other isolates. Protectants tested on potted seedlings showed that captan significantly promoted *A. mellea* infection. Chloropicrin, Vorlex, and high dosages of copper sulfate were phytotoxic to the pine seedlings. Viability of *A. mellea* within alder inoculum segments and production of rhizomorphs was also differentially affected by the applied chemicals. The *A. mellea* isolate least inhibited *in vitro* was significantly more pathogenic than the other isolates. The seven chemicals under test as root collar protectants in the forest showed no conclusive differences because disease severity was too low for evaluation of the treatments at this time.

In a preliminary study of *A. mellea* distribution in unfumigated stumps, the fungus was most frequently isolated from yellow stringy decay and from sound appearing wood primarily within the taproot (lower portion) of the stump. Mycelial fans beneath the bark also frequently yielded *A. mellea*. *A. mellea* was most frequently isolated from stumps lacking visible decay in fresh transverse cuts at the ground line. Isolation frequency declined as the proportion of decay to sound wood on these cuts increased. Recovery was poorest in stumps which were barely intact.
Five chemical fumigants were tested on stumps of ponderosa pine killed by *A. mellea* in typical disease centers. Methyl bromide, Vorlex, chloropicrin, carbon disulfide, and Vapam apparently eradicated *A. mellea* from the treated stumps. The hyphomycete, *Trichoderma*, appeared to be unaffected by fumigants other than methyl bromide and Vorlex. With these, *Trichoderma* incidence increased over the controls. Differences in the effectiveness of fumigation method and the influence of stump size or degree of decay could not be determined at the concentrations of fumigants used.

Because of the apparent effectiveness of all fumigants tested, reductions in amounts and simplification of application should reduce costs of both materials and treatment, bringing costs into the realm of economic feasibility, at least in special cases. Fumigation of *A. mellea* in the infected root residues of disease centers should eliminate disease development at the epidemic level by reducing the inoculum potential. This change should enable further pine production in reasonable safety.
Chemical Applications for Control of
Armillaria Root Rot of
Ponderosa Pine

by

Gregory M. Filip

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Typed by Mary Jo Stratton for Gregory M. Filip
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CHEMICAL APPLICATIONS FOR CONTROL OF ARMILLARIA ROOT ROT OF PONDEROSA PINE

INTRODUCTION

Armillaria root rot caused by the fungus, *Armillariella mellea* (Vahl ex Fr.) Karst., ¹ creates roughly circular openings of understocked land and infected dead and dying trees in the ponderosa pine (*Pinus ponderosa* Laws.) forest. These openings are referred to hereafter as disease centers. Research objectives concerning chemical control of this disease were: 1) to evaluate the effectiveness of certain chemicals in protecting the critical root collar zone of living ponderosa pines as they were reached by the advancing infection front of the expanding disease center, and 2) to test the effectiveness of certain standard soil fumigants in eradicating *A. mellea* from infected pine stubs and snags of killed trees within the disease center.

These field studies were located in an area where damage by *A. mellea* is particularly severe, amounting to 36.8 thousand cubic meters of timber lost annually (unpublished report) on 259,000 hectares. This figure does not represent loss of trees less than 20 cm diameter breast height (DBH) nor growth growth loss from land out of production because of the disease.

¹In this paper the disease is referred to as Armillaria root rot, a common name, and the fungal pathogen as *Armillariella mellea* after Singer (1962) and Pegler and Gibson (1972).
For the most part the chemical tests were made under field conditions and therefore were subject to the extreme variability common to the forest situation. Tests were made in the field so that the results would have validity for problem situations, and also because of the desire to explore, in a preliminary way, problems likely to be met in conducting a control program in the field should there be effective chemicals.

Armillaria root rot is in an epidemic state in the study area. Such behavior is not common for this disease except possibly in plantations. It should therefore be in the reader's interest to describe the field situation in some detail at this point.

Hartig (1874), in European conifer forests, was perhaps the first to notice disease centers caused by Armillariella mellea. In the young-growth pine forest of this study, A. mellea disease centers are characterized by roughly circular (0.1-0.4 ha) openings containing ponderosa pine stubs and snags of various heights which are the remains of trees killed by A. mellea (Fig. 1). Dead and dying trees with discolored foliage occur between the openings and the surrounding, apparently healthy, forest. One or more old-growth pine stumps, approximately 150 cm DBH, usually occur at the middle of each disease center. These stumps represent trees selectively harvested during recent decades. Many of these are stumps of trees with latent infections of A. mellea, and following their harvest, these
Infected old-growth pine stump

Infected young-growth pine stubs and snags

Limit of obviously infected trees

Limit of trees with latent infections

Healthy trees

Direction of spread by *A. mellea*

Fig. 1. Highly schematic diagram of a disease center caused by *Armillariella mellea* in a young ponderosa pine forest.
stumps served as origins for the present disease centers (Hunt, 1958; Shaw and Roth, 1974; Shaw et al., 1976). The disease center therefore represents a chronology of disease spread from origin at the infected old-growth stump through the rings of stubs and snags (Fig. 1) to current active parasitism at the outer margin of the expanding disease center (Shaw and Roth, 1976).

Spread of the disease from tree to tree is believed to be primarily subterranean by root contacts and rhizomorphs rather than aerial by basidiospores (Rishbeth, 1970; Shaw and Roth, 1976). The means of spread along functioning roots of living ponderosa pines, if it occurs at all, is not known. Tree mortality appears to be delayed until attack occurs at the root collar (Day, 1927; Patton and Riker, 1959; Sokolov, 1964; Adams, 1972; Shaw, 1974). Hartig (1874) believed that in European conifers, infection of lateral roots may progress internally to infection of the root collar and thus cause tree mortality, but such spread in American pines is prevented in most cases by the formation of resin barriers within the lateral root (Patton and Riker, 1959; Adams, 1972; Shaw, 1974).

Persistence of *A. mellea* in roots of the stubs and snags kills much of the pine that regenerates in the disease centers. To begin with, pine regeneration at best is poor in the disease center because of grass competition.
This disease configuration provided an appropriate environment in which to conduct the earlier stated objectives of testing the effectiveness of chemical control of Armillaria root rot in a ponderosa pine forest.
LITERATURE REVIEW

**Non-Chemical Control Measures for Armillaria Root Rot**

Although the subject of this thesis is the chemical control of Armillaria root rot, a brief review of other control methods is provided as background material and to acquaint the reader with the need for alternatives to the non-chemical methods. The reader is directed to Sokolov (1964) for a more detailed history of all control measures.

Various control methods have been tested by many investigators in the past. Most of these have proven less than satisfactory. Trenching, the excavation of soil from areas between healthy and infected trees, is the oldest and perhaps most widely reported method and has been recommended by Lawrence (1910) in Washington state, Reitsma (1932) in Germany, Thomas and Raphael (1935) in Tasmania, and Lavrov (1952) in Siberia, among others. The practicality of trenching on a large scale as a barrier to spread of Armillariella mellea, as would be required in the case of the ponderosa pine forest involved in this thesis, would be doubtful.

Ring-barking, the girdling of living trees to prevent infection by *A. mellea*, was first developed by Leach (1939) in East Africa and later explained by Swift (1964) to be concerned with depletion of root
starch reserves that favored saprophytic fungal colonizers rather than \textit{A. mellea}. Redfern (1968) in Britain, however, reported that ring-barking of live trees prior to harvesting failed to prevent \textit{A. mellea} from colonizing the remaining stumps. However, Redfern believed that these infected stumps would not serve as effective food bases for \textit{A. mellea} since few rhizomorphs were produced from sample wood sections in the laboratory.

Possibly the best method of cultural control is the uprooting of infected stumps which has been recommended by many investigators: McGillivroy (1946), Lavrov (1952), Twarowski (1953), Fassi (1962), and Sokolov (1964). More recently and in the location of this study, Roth (personal communication) has suggested removal of selected entire trees while thinning infected ponderosa pine stands.

Strictly biological methods have been suggested for controlling \textit{Armillaria} root rot, but supporting data have been from laboratory experiments rather than from field tests. Riffle (1973) reported possible biological control by two species of mycophagous nematodes tested in culture flasks with ponderosa pine seedlings. Both species apparently reduced \textit{A. mellea} growth so that root rot development was retarded, but neither species of nematode killed the fungus. The suitability of such a control method in the forest is questionable.

Most other methods of biological control deal with \textit{in vitro} studies of fungi antagonistic to \textit{A. mellea}. Chanturiya and Kakuliya (1953)
recommended the use of *Trichoderma viride*, and Orlos (1957) in Poland suggested applying *Fomes marginatus* spores to the cut surface of stumps to prevent colonization by *A. mellea*. Field data were not reported, however. Manka (1961) tested 91 species of fungi and found only three species which inhibited *A. mellea* in culture. Sokolov (1964) reported six genera of fungi including *Trichoderma*, *Penicillium*, and *Peniophora* which demonstrated antagonistic properties toward *A. mellea* in culture. Conversely, Richard (1971) reported that *A. mellea* produced an unidentified antibiotic to which only one of 21 species tested was resistant. This was a species of *Trichoderma*. Cusson and La Chance (1974) demonstrated antagonism between *Scytalidium lignicola* and *A. mellea* on agar medium and in spruce wood blocks. In general, for all organisms antagonistic to *A. mellea*, failure under field conditions to maintain effective populations of the antagonist is a prime limiting factor on strict biological control. However, effective numbers of antagonists have been artificially sustained in the field for at least partial control of *A. mellea* through the use of chemicals which differentially influence populations of microorganisms (Bliss, 1951).

**Chemical Control Measures for Armillar Root Rot**

Reports on chemical usage to control *Armillariella mellea* prior to 1965 include bordeaux mixture (Barss, 1923), formalin (Sokolov,
1964), potassium permanganate (Thomas and Raphael, 1935), sulfur (De Fluiter, 1939), and lime (Twarowski, 1953). None of these were really effective. However, copper sulfate (Reitsma, 1932; De Fluiter, 1939; Sokolov, 1964), iron sulfate (Gard, 1925; Thomas and Raphael, 1935; Chanturiya, 1947; Sokolov, 1964), chloropicrin (Godfrey, 1936; Bliss, 1951), and carbon disulfide (Fawcett, 1925; Reitsma, 1932; De Fluiter, 1939) were reported to be effective in field trials. Most of these chemicals were applied as soil amendments; however, Sokolov (1964) experimented with chemical injections into infected trees. Godfrey in 1936 and Bliss in 1951 demonstrated the effectiveness of chloropicrin in eradicating A. mellea from woody inoculum buried in jars of soil by applications of 0.08-0.09 ml of chloropicrin per liter of soil. Thomas and Lawyer (1939) and Bliss (1951) applied CS$_2$ to field soils containing buried A. mellea inoculum and reported fungus mortality at 150 cm and 210 cm with 45 ml and 60 ml per injection, respectively. Post et al. (1967) also were successful in eradicating A. mellea from peach stem segments in field soils using CS$_2$.

Since 1965 a variety of newly developed chemicals have been tested both in the laboratory and in the field against A. mellea. Partridge (1966) reported total inhibition of A. mellea growth both in vitro and in infected wood blocks using 50 and 1000 ppm, respectively, of cyclohexamide (actidione). Cheo (1968) tested in vitro 20 chemicals
considered to be systemic in plants. Cyclohexamide (20 ppm) and 2,4-dichlorophenoxy-acetonitrile (50 ppm) were both lethal to *A. mellea* while the others were either only fungistatic or had no effect. Denizet (1971) reported that Armillaria root rot was controlled in a 2500 ha private forest in France using "iron chelate and maneb," but he provided no details of application. Redfern (1971) applied a proprietary compound, "Armillatox" (a phenolic emulsion), to soil containing *A. mellea* inoculated sycamore stem segments. Only at the highest concentrations (10 ml/liter soil) did "Armillatox" kill the fungus or reduce rhizomorph growth. Pawsey and Rahman (1974) applied "Armillatox" to healthy and infected, 9-14 year-old Scots pine saplings. They reported inhibition of rhizomorph growth at 7.6 liters of an 8% "Armillatox" solution per tree but failed to report therapeutic effects on the infected trees. Rykowski (1974) reported sodium penta-chlorophenolate (1 ppm) to be inhibitory to *A. mellea* in agar culture. Applications of 25-100 g active material/tree applied as a soil drench to stumps and to living trees infected with *A. mellea* "stopped the increase in gaps in the plantations" from infected stumps and "weakened the process of dying down" in living infected trees.

Methyl bromide has received much attention as a control for Armillaria root rot. In 1962, Larue et al. demonstrated its effectiveness at 98 g/sq m in eradicating *A. mellea* from infected citrus roots
buried in soils covered with polyethylene tarps. Low soil moisture and soil temperatures above 10°C were suggested as being critical for control. Rackham et al. (1968) reported eradication of A. mellea from large buried citrus stumps treated with methyl bromide (98 g/sq m) under polyethylene tarps. Post et al. (1967) and Kissler et al. (1973) also reported methyl bromide to be effective in eradicating A. mellea from buried inoculum blocks. Munnecke et al. (1969) demonstrated that increasing the dosage of methyl bromide resulted in more rapid diffusion, greater depth of penetration, and a higher concentration of fumigant at greater depths in the soil. Munnecke et al. (1970) later determined the LD<sub>95</sub> values (concentration required to render 95% of infected wood chips non-viable) for A. mellea in citrus root pieces treated with methyl bromide.

Herbicides also have been tested for control of Armillaria root rot. Redfern (1968) frill-girdled and poisoned living trees with 2, 4, 5-trichlorophenoxyactic acid (2, 4,5-T) two years before felling, but stumps became infected with A. mellea after five years. However, treated stumps, as judged from rhizomorph production, were believed to be less effective food bases than untreated stumps. Rishbeth (1972) believes tree poisoning with herbicides promotes rapid invasion by A. mellea and potential fungal competitors as well. Even though stumps became infected with A. mellea following tree poisoning, he feels that treatment reduces the period over which food
bases are available to *A. mellea* because poisoning promotes replacement of *A. mellea* by fungal competitors. Rishbeth (1971) also reported that the kind of herbicide used influences the species of fungi colonizing the dead tree. Several wood-decaying fungi were favored by ammonium sulphamate over 2,4,5-T.

**Integrated Chemical and Biological Control Measures**

Much of the literature dealing with chemical control of Armillaria root rot becomes concerned with the effect of chemical treatment on the microflora and the further effect of the altered microflora on *Armillariella mellea*. In 1951 Bliss demonstrated the ability of the ubiquitous fungus, *Trichoderma viride*, to replace *A. mellea* in artificially infected citrus root segments fumigated with 283 ml/sq m of carbon disulfide. Bliss postulated that one of the possible effects of CS$_2$ was either to damage the protective pseudosclerotium of *A. mellea* or prevent its formation in woody tissue thus facilitating invasion by the antagonist. This postulate was supported by Darley and Wilbur in 1954. Evans (1955) also demonstrated the ability of *Trichoderma viride* to survive in CS$_2$ fumigated soils and also in soils treated with formalin. Garrett (1957) felt that CS$_2$ causes some direct damage to *A. mellea* mycelium, but in 1958 he demonstrated that when *A. mellea* was added to pure cultures of *T. viride*
without CS₂ fumigation, a 95% kill of \textit{A. mellea} resulted. Apparently both direct fumigant toxicity to \textit{A. mellea} hyphae and antibiotic production by \textit{Trichoderma} can contribute to destruction of \textit{A. mellea} in fumigated soils.

Saksena (1960) believed that \textit{Trichoderma viride} was not particularly tolerant to CS₂, but it was more tolerant than the faster growing soil fungi, and faster growing than the more tolerant fungi normally found in the soil. "\textit{T. viride,}" Saksena states, "owes its success in recolonization to the conjunction of a moderate degree of fumigant tolerance and an intrinsically high growth rate." Munnecke et al. (1973) postulated that following fumigation by either carbon disulfide or methyl bromide, a lag period for \textit{A. mellea} growth occurs which indicates a weakening of \textit{A. mellea}. They believe that the more tolerant \textit{T. viride} is able to exploit this lag period and exert its antagonistic action toward \textit{A. mellea} at that time.

In 1968 Mughogo demonstrated the tolerance of \textit{Trichoderma} to chloropicrin and proposed that successful soil fumigation and resultant biological control depended on chance development of strains of \textit{Trichoderma} spp., which were antagonistic to \textit{A. mellea}, since certain strains were not antagonistic.

Ohr and Munnecke (1972) were perhaps the first to demonstrate a direct correlation between the decline of \textit{A. mellea} and the increase of \textit{Trichoderma} following methyl bromide fumigation under controlled
conditions. Ohr et al. (1973) reported that when *A. mellea* infected roots were treated with sublethal dosages of methyl bromide and buried in non-sterile soils, *A. mellea* died, but when stored in sterile soil the fungus survived. Ohr and Munnecke (1974) later found two isolates of *A. mellea* which produced *in vitro* substances inhibitory to eight species of common soil fungi, confirming Richard's work of 1971. Antibiotic production from *A. mellea* was reduced after fumigation with sublethal concentrations of methyl bromide. The authors suggested that following methyl bromide fumigation and subsequent reduction in antibiotic production by *A. mellea*, the fungus is predisposed to parasitic attack by *Trichoderma* spp. and other antagonists. They proposed an hypothesis that under high concentrations of fumigant (methyl bromide), *A. mellea* is killed directly by the fumigant, and under low concentrations it is neither killed directly by the fumigant nor indirectly by other fungi, but under intermediate concentrations, sublethal to *A. mellea*, it is killed by other fungi.

**Chemical Control Research on Fungal Problems**

**Other than Armillaria Root Rot**

Much of the information concerning chemical application techniques used in this thesis has been derived from articles on chemical application to problems other than Armillaria root rot. In 1969 Houston and Eno tested the hypothesis that a continuous band or
zone of roots rendered unsuitable for invasion by *Fomes annosus* (*Heterobasidion annosum*) will prevent underground spread of this fungus from diseased to healthy roots. They found that soil fumigation with either methyl bromide or sodium-N-methyldithiocarbamate (Vapam) killed roots of red pine (*Pinus resinosa*) when applied as soil injections of 149 g/linear meter and 20 ml/injection hole, respectively, spaced at 0.3 m intervals. When applied to *F. annosus* infected stumps and living infected trees (felled prior to fumigation) as soil injections and top dressings under polyethylene tarps, methyl bromide at 98 g/sq m killed the fungus as determined by isolation on agar medium while Vapam at 19.4 ml/sq m failed to destroy the fungus. In subsequent experiments, utilizing the same treatments as above, both methyl bromide and Vapam reduced *F. annosus* in dead tree roots, only methyl bromide was effective in living roots, and neither fumigant significantly reduced the fungus in well rotted stumps. Well rotted stumps, however, were shown to initially contain less *F. annosus* than infected stumps which were more intact.

In 1975 Houston reported additional work on establishment of barriers to block underground spread of *Fomes annosus* by killing roots with fumigants. Barriers were formed by injecting methyl bromide at 149 g/linear meter into 50 cm deep preformed soil holes at 0.3 m intervals. Red pine roots were killed 0.8 m to 1.8 m on either side of the fumigation line, but roots of white pine (*Pinus strobus*) and
Norway spruce (*Picea abies*) were unaffected by the fumigant. No new roots grew across the fumigation line two years after treatment. Houston reported, however, that in a few cases the fungus (*F. annosus*) traveled across the fumigation line via fumigant killed roots. He explained that healthy appearing trees adjacent to the fumigation zone bore latent infections. Healthy tree roots from such trees which crossed the fumigation line were killed by methyl bromide. *F. annosus* subsequently spread from infected roots not in the fumigation zone to healthy but fumigant killed roots on the same tree. Since *F. annosus* infections were in close proximity to the healthy but fumigant killed roots (on the same tree), it became established in these dead roots before fungal competitors could establish. Houston recommended detection of trees with latent infections before fumigation and subsequent inoculation of fumigant killed roots with antagonistic fungi after fumigation.

Research on prevention of decay by *Poria carbonica* and *P. monticola* in transmission poles by application of fumigants greatly stimulated this research concerning control of *A. mellea* in infected ponderosa pine stumps with chemical fumigants. Cooper et al. (1974) demonstrated that chloropicrin at 475 ml/pole (0.006 ml/cc wood) eradicated all wood decay organisms from a treated transmission pole when injected into four holes drilled in the center of the pole. He found that the diffusion rate of chloropicrin was much
greater longitudinally than transversely, that permeability to chloropicrin increased with decreasing wood moisture content, and that the diffusion coefficient of chloropicrin increased with temperature. He also reported that *Poria monticola* was more resistant to chloropicrin at low temperatures (2°C). Chloropicrin vapor moved more easily in decayed wood than in sound wood but was dependent on wood moisture content. Cooper (1973) demonstrated the slow release of chloropicrin and Vorlex (80% chlorinated hydrocarbons, 20% methyl isothiocyanate) but not Vapam from capped, polyethylene vials and recommended that these be inserted into wood holes to insure periods of slow fumigant release. Graham (1975) treated transmission poles in situ by pouring 950 ml of test chemical into holes made in each pole. Chloropicrin, Vapam, and Vorlex were equally effective in eliminating decay fungi, but chloropicrin was most effective in maintaining the total fungal population at low levels. Methyl bromide was effective initially, but was least effective in controlling the total fungal population. Chloropicrin, Vapam, and Vorlex were all effective after four years and recent data (Graham, unpublished) indicate effectiveness after six years in controlling decay fungi in treated transmission poles.
MATERIALS AND METHODS

Chemicals to Protect the Root Collar

Exploratory Tests in vitro

Response of Armillariella mellea in vitro to the chemicals (Table 1) was evaluated by colony diameter, dry weight, and rhizomorph index (Wargo, 1972) on a medium containing 30 g malt extract, 20 g dextrose, 5 g peptone, 40 g granulated ponderosa pine resin, 19 g agar, and distilled water to make one liter (Shaw, 1974). The concentration of each chemical tested (Table 4) was based on the chemical as commercially formulated except for captan and benomyl that were tested as 50% active ingredients. Solutions of copper sulfate, iron sulfate, captan, and benomyl in sterile distilled water were added to the autoclaved medium at 45°C. Copper metal was tested by pouring 30 ml of the autoclaved medium into each petri plate containing weighed amounts of autoclaved metallic wire bits. There were no significant differences in the pH among the media after autoclaving and adding the test chemicals (pH = 4.4).

Chloropicrin and Vorlex were tested by incubating plates inoculated with A. mellea in the closed atmosphere of plastic crisper boxes (60,000 cc) containing 132 ml of the test chemical distributed equally among four capped 33 ml polyethylene bottles (Cooper, 1973).
<table>
<thead>
<tr>
<th>Common designation</th>
<th>Manufacturer or Source</th>
<th>Active ingredient or chemical formula</th>
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</thead>
<tbody>
<tr>
<td>Copper sulfate, technical grade</td>
<td>Cities Service Co.</td>
<td>CuSO$_4$·7H$_2$O</td>
</tr>
<tr>
<td>Iron sulfate, laboratory grade</td>
<td>Baker Chemical Co.</td>
<td>FeSO$_4$·7H$_2$O</td>
</tr>
<tr>
<td>Captan, 50% wettable powder</td>
<td>Chevron Chemical Co.</td>
<td>N-[trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide</td>
</tr>
<tr>
<td>Benomyl, 50% wettable powder</td>
<td>E.I. duPont de Nemours &amp; Co., Inc.</td>
<td>[methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate]</td>
</tr>
<tr>
<td>Chloropicrin, 99%, Pictume</td>
<td>Dow Chemical Co.</td>
<td>CC$_3$NO$_2$</td>
</tr>
<tr>
<td>Vorlex</td>
<td>Nor-Am Chemical Co.</td>
<td>80% chlorinated hydrocarbons, 20% methyl isothiocyanate</td>
</tr>
<tr>
<td>Copper metal</td>
<td>Electrical wire bits</td>
<td>Cu</td>
</tr>
</tbody>
</table>
Plates of all test media were inoculated with 6 mm plugs cut from the margins of an 11-day-old *A. mellea* plate culture.

Ten replicates each of three *A. mellea* isolates were included in each dosage level of chemical. Isolates I2a5-1 and 96 were recovered from dead ponderosa pine trees in Glenwood, Washington, and isolate S8A was from a dead pine tree in Pringle Falls, Oregon. All cultures were incubated for 11 days in the dark at 24°C. Viability of the fungus in plugs showing no growth after 11 days was determined by transferring the plugs to fresh standard medium and incubating for an additional 13 days. Results were recorded in terms of colony diameter, dry weight, and rhizomorph index. Duncan's Multiple Range Test (Steel and Torrie, 1960) was employed for testing significance among treatments throughout the thesis.

**Companion Tests with Potted Seedlings**

Ponderosa pine seedlings (2-0 stock) were individually transplanted in May 1973 into fluted #10 cans containing two liters of pine forest soil. A glass culture tube (25 x 200 mm) was planted along with the seedling extending vertically from deep among the seedling roots to about two inches above the soil surface. Inoculations were accomplished in August 1973 by replacing the culture tubes with cylindrical inoculum pieces consisting of heavily infected red alder (*Alnus rubra* Bong.) stem segments, thus minimizing root
disturbance (Fig. 2). Inoculum was prepared by autoclaving (1 hour, 1.16 atm) the segments (10-25 mm x 100 mm) in 0.95 liter Mason jars filled with distilled water. After autoclaving, the liquid was partially removed (2.5 cm remaining) and each jar was inoculated with a 20 mm agar plug cut from the margin of an 11-day-old A. mellea culture. Three fungal isolates designated I2a5-1, S8A, and 96 were employed. Jars of uninoculated segments provided controls. The alder segments were infected with A. mellea within six weeks at 24°C and then stored at 10°C until used two months later. It is important that A. mellea decay in the inoculum pieces be well advanced before use as inoculum in soil (store at least three months at room temperature in the dark).

Immediately following inoculation, the seedlings were treated with the appropriate chemical (Table 1). Copper sulfate, iron sulfate, captan, and benomyl were applied as a one liter drench per seedling followed by a liter of water to increase percolation of the chemical into the soil (Corden and Young, 1962). Three concentrations of each chemical were tested. Inoculated control seedlings received two liters of water. Seedlings treated with copper metal each received 100 g of wire bits sprinkled on the soil surface of each pot. Chloropicrin and Vorlex were applied by placing 33 ml of each substance into capped 33 ml polyethylene bottles and burying one bottle at the bottom of the can for each seedling tested. Fifteen
Fig. 2. Inoculation of a pine seedling with an alder stem segment infected with *A. mellea* after removal of culture tube from the soil.
replicates, five of each of the three *A. mellea* isolates, were conducted for each chemical treatment (including controls). Uninoculated (sterile alder segment), but chemically treated seedlings served as controls for evaluating chemical phytotoxicity independent of *A. mellea* inoculation. To maintain moderate soil temperatures, all potted seedlings were set in the ground to their rims near the study area. Soil temperatures within each pot reached a summer maximum of 18°C at 15 cm soil depth.

**Tests on Young Pines in the Forest**

The seven chemicals in Table 1 were tested in south-central Washington state southeast of Mt. Adams (Fig. 3). Elevation of the study site is 975 m. Precipitation averages about 76 cm a year, much of which falls as snow. Mean January temperature is about -2°C and mean July temperature is approximately 20°C. The soil (pH = 6.0) is volcanic in origin from olivine-andesite parent material (Shaw, 1974). It is generally deep and is covered with a thick accumulation of litter (Site Index 105). Ponderosa pine (*Pinus ponderosa* Laws.) is the principal tree species in the study area in mixture with Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and some grand fir (*Abies grandis* [Doug.] Lindl.). Understory vegetation includes snowbrush (*Ceonothus* spp.), false rabbitbrush (*Haplopappus bloomeri* Grey), western hazel (*Corylus cornuta* Marsh. var. *californica* [A. DC.])
Fig. 3. (A) Map of Washington state depicting location (star) of study area, and (B) general view of the area surrounding the study site.
Sharp.), and Oregon white oak (*Quercus garryana* Dougl.). The forest has been under a selection cut type of management for 25 years. For a more detailed description of the management operations, see Shaw (1974).

A total of 160 ponderosa pines 7-15 cm DBH in seven locations within the study area were selected on the basis of infection and future probability of becoming infected. Infection probability was regulated by distance from apparently advancing margins of *A. mellea* disease centers (Shaw, 1974). Group One trees included those trees between 0 and 3.1 m from the nearest tree showing symptoms. Group One trees appeared healthy but had a very high probability of having latent infections on lateral roots toward the disease center. To standardize exposure to inoculum, a tree was selected only if the total basal area of dead or dying trees (with *A. mellea* symptoms) located within 3.1 m of the candidate fell between 0.009 and 0.084 sq m. Group Two trees also appeared healthy but had a low probability of latent infections on lateral roots. Group Two trees were selected like Group One trees except that they were located between 3.1 and 6.1 m from the nearest tree with *A. mellea* symptoms. All trees to be treated were at least 1.8 m from their nearest treated neighbor in order to avoid cross influences among treatments.

After the trees were located, treatments were randomly assigned by number and applied in August 1973. Four of the seven
test chemicals (copper sulfate, iron sulfate, captan, and benomyl) were applied in a small moat around the root collar of each tree as a 19 liter drench followed by 19 liters of water to increase percolation of chemicals (Fig. 4). Three concentrations of each material (copper sulfate, iron sulfate, captan, and benomyl) were tested. Control trees received 38 liters of water. Trees treated with copper metal each received 1135 g of metallic bits sprinkled around the base of each tree at the root collar zone. Chloropicrin and Vorlex were applied to each appropriate tree in four capped 132 ml polyethylene bottles buried at a depth of 20 cm at cardinal points around the root collar (Fig. 4). Polyethylene bottles containing water served as controls.

In August 1975, two years after treatment, all treated trees were examined for visible signs and symptoms of *A. mellea* infection which included mycelial fans beneath the root collar (Fig. 5) and tree mortality. Latent infections on buried roots were not determined.

**Fumigants to Eradicate *A. mellea* from Infected Pine Residues in Disease Centers**

Effectiveness of five soil fumigants (Table 2) in eradicating *A. mellea* from root systems of young-growth pines killed by the fungus was measured by culturing the fungus from the main taproot following chemical treatment. For comparison purposes, knowledge
Fig. 4. Application of protectant chemicals as (A) soil drenches and (B) within polyethylene bottles to the root collars of young pines in the forest.
Fig. 5. Mycelial fans of *A. mellea* beneath the bark of a recently killed ponderosa pine.
Table 2. Fumigants tested in young-growth ponderosa pine stumps for effectiveness in eradicating *A. mellea*.

<table>
<thead>
<tr>
<th>Common designation</th>
<th>Manufacturer or Source</th>
<th>Active ingredient or chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vapam</td>
<td>Stauffer Chemical Co.</td>
<td>Sodium-N-methyldithiocarbamate (32.7%)</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>Stauffer Chemical Co.</td>
<td>CS₂</td>
</tr>
<tr>
<td>Vorlex</td>
<td>Nor-Am Chemical Co.</td>
<td>80% Chlorinated hydrocarbons, 20% methyl isothiocyanate</td>
</tr>
<tr>
<td>Chloropicrin, 99%, Picfume</td>
<td>Dow Chemical Co.</td>
<td>CCl₃NO₂</td>
</tr>
<tr>
<td>Methyl bromide, 98%, Dowfume</td>
<td>Dow Chemical Co.</td>
<td>CH₃Br</td>
</tr>
</tbody>
</table>
was needed of *A. mellea* distribution in the main taproot prior to chemical treatment. This was obtained as follows.

**Background Study on Distribution of *A. mellea* in Infected Pine Stumps**

Stubs and snags of 49 pines, 10-38 cm diameter at ground line and thought to be infected with *Armillariella mellea* because they were located within disease centers (Fig. 6), were cut flush with the ground (Fig. 7). The cut surface of the remaining stump was classified as to extent of decay, and the stump was excavated with a backhoe (Fig. 8).

The decay severity classes (stump decay index) were as follows:

- **0** - no visible decay (soundwood)
- **1** - 0-50% yellow decay, otherwise sound
- **2** - 50-100% yellow decay or other decay
- **3** - 100% decay other than yellow decay

Yellow decay in conifers is associated with the presence of *A. mellea* (Partridge and Miller, 1974; Shaw, 1974).

Excavation of the 49 stumps required about two hours. Stumps were promptly transported to the laboratory, kept moist with wet burlap covers, and stored at 16°C until isolations were completed 24-72 hours later.

Each stump was scrubbed with water from a firehose and split once with a hammer and wedges (Fig. 9). Isolations were made at
Fig. 6. A representative site for background study concerning *A. mellea* distribution within infected pine stumps and subsequent fumigation studies. Note the broken stubs and snags of ponderosa pine killed by *A. mellea* within a disease center.
Fig. 7. (A) Felling of a pine stub and (B) the cut surface of the remaining pine stump which was classified as to stump decay index. Note the yellow decay on the left side of the cut surface as contrasted to soundwood on the right. Such a stump would receive a rating of one.
Fig. 8. (A) Stump excavation with a backhoe and (B) some examples of excavated and infected stumps of ponderosa pine.
Fig. 9. Preparation of pine stumps for isolation procedures which included (A) scrubbing with a fire hose and (B) splitting with hammer and wedges.
once from 20 stumps according to distribution of the various decay configurations that resulted from attack by a variety of fungi.

Isolations from the remaining 29 stumps followed the formalized sampling pattern in Fig. 10 plus five additional random isolations from the wood surface under the bark where mycelial fans of *A. mellea* normally were present. Wood chips, 5 x 15 mm, were removed with a sterile wood gouge and plated on a selective medium (Shaw, 1974) containing 30 g malt extract, 20 g dextrose, 5 g peptone, and 19 g agar per 1000 ml of distilled water to which 6 mg of the sodium salt of o-phenylphenol (OPP) and 0.1 g of streptomycin sulfate were added after autoclaving. All cultures were incubated for three weeks in the dark at 24°C after which the presence of *A. mellea* was recorded.

**Fumigation Tests**

Stubs of trees killed by *Armillariella mellea* that were to be fumigated were managed as follows. In July 1974, eighteen 37 sq m (0.004 ha) plots were located in typical *A. mellea* disease centers (Fig. 6) and were fenced to exclude range cattle. Each of the 6-11 stubs of *A. mellea* killed trees on each plot was described as to height and to diameter at the ground line and was arranged into one of three diameter classes: 10-18 cm, 20-28 cm, and 30-38 cm. Each stub was cut flush with the ground (Fig. 7) and the cut surface of the remaining stump was rated as to stump decay index (p. 30).
Longitudinal at 9% 2 cm intervals

Transverse at 1 cm intervals

Taproot at 2 cm intervals

Fig. 10. Sampling pattern for isolation of *A. mellea* from split stumps of ponderosa pine.
Stumps on each 37 sq m plot in each of three replicates were treated with one of the following materials: 1) water (control),
2) Vapam, 3) carbon disulfide, 4) chloropicrin, 5) Vorlex, and 6) methyl bromide.

Three methods of application were randomly assigned to the stumps on each plot:

1. Equal amounts of chemical placed in four 2.5 cm holes drilled 46 cm into the soil at cardinal points against each stump. Holes were closed with soil following application of the chemical.

2. Equal amounts of chemical placed in one to three 21 mm holes drilled obliquely (Cooper, 1973) into the cut surface of the stump to depths of 30, 46, and 61 cm (Fig. 11). Number of holes and hole depth depended respectively on stump diameter class. Holes were closed with cork stoppers following treatment.

3. A combination of the preceding.

A "Sentry 1/2 in. Reversible Drill" with a 21 mm x 610 mm bit was used to make all holes in stumps. A reversible drill is recommended to facilitate removal of the bit from the wood. Power for the drill was supplied by a portable electric generator.

All fumigants but methyl bromide were poured into the holes (Fig. 12). Application rates can be expressed as the amount of fumigant (g or ml) per unit of stump wood (cc) (Table 3). Since extracted stumps somewhat resemble a cone, wood volume was
Fig. 11. (A) Drilling holes in a pine stump to accommodate the chemical treatments. (B) Drill holes have been closed with cork stoppers following treatment.
Fig. 12. Application of a liquid chemical fumigant to a prepared stump.
Table 3. Rate of application for chemical fumigants applied in situ to *A. mellea* infected stumps of ponderosa pine. Rate values are chemical amounts\(^a\) (g or ml) per unit (cc) of wood.

<table>
<thead>
<tr>
<th>Number</th>
<th>Stumps treated</th>
<th>Diameter class (cm)</th>
<th>Volume (b) (cc)</th>
<th>Application rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl bromide</td>
<td>All other chemicals</td>
<td>Methyl bromide All treatments (g/cc)</td>
<td>All other chemicals (ml/cc)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>10-18</td>
<td>3078</td>
<td>0.221</td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>20-28</td>
<td>13569</td>
<td>0.100</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>30-38</td>
<td>36310</td>
<td>0.056</td>
</tr>
</tbody>
</table>

\(^a\)Chemical amounts based on formulations as commercially sold.

\(^b\)Stump volumes were computed from mean radii (diameters) for the stump diameter classes and respective heights (stump lengths) of 60, 90, and 120 cm. Volumes of actual fumigant applied can be computed by multiplying application rate by stump volume.
calculated by the formula: \( \text{Vol. of cone} = \frac{\pi}{3} r^2 h \) where "r" is the radius of the stump top and "h" is the length of the stump. For safety purposes a canister type gas mask (MSA industrial) was worn during application of the fumigants.

Application methods for methyl bromide were slightly modified since this fumigant is liquified in canisters under high pressure. After the selected stumps and/or soil were drilled, they were covered with 1.5 x 1.5 m clear polyethylene tarps sealed against a trench floor with soil (Fig. 13). Methyl bromide was then evenly distributed among wood and/or soil holes (depending on which of three treatments was used) with an applicator tube piercing the tarps (Fig. 13). From one to three cans each containing 680 g of methyl bromide were applied depending on stump diameter class. Application holes in the tarp were closed with electrician's tape.

Control stumps were treated with water according to the three methods of chemical application. The stumps of all treatments (including controls) were covered with polyethylene tarps immediately following fumigant injection. Tarps were left in place for four days. At the time of treatment, soil moisture (measured gravimetrically) was 19% by weight at a 30 cm soil depth. Soil temperatures at 30 cm ranged from 7-10°C during the week of treatment.
Fig. 13. (A) Polyethylene tarps which were used to cover each stump following fumigation. (B) Methyl bromide was injected through a slit in the tarp that was later sealed.
RESULTS

Chemicals to Protect the Root Collar

Exploratory Tests in vitro

On the basis of colony diameter and dry weight after 11 days incubation (Table 4, Fig. 14), benomyl and copper sulfate were most toxic to A. mellea while captan, copper metal, and iron sulfate were less toxic. Vorlex and chloropicrin were also highly effective in reducing the growth of A. mellea at the concentration tested. The proportion of rhizomorphs produced to the total fungal colony (rhizomorph index) was not affected by the chemicals.

An increase in colony diameter and dry weight was often noted at the lower concentrations of chemicals over the control (Table 4). This increase was followed by a sharp decrease in growth with increasing chemical concentration.

Of the three isolates of A. mellea tested (Table 5), isolate I2a5-1 was the most tolerant to all of the chemicals; however, for most chemicals, the difference was not statistically significant (P = 0.05).

Companion Tests with Potted Seedlings

In August 1975, two years following inoculation and treatment,
Table 4. Effect of seven protectant chemicals on growth of Armillariella mellea in pure culture. Values are colony diameters and dry weights.

Concentration\(^a\)  | Copper sulfate Dia. (mm) | Copper sulfate Wt. (mg) | Iron sulfate Dia. (mm) | Iron sulfate Wt. (mg) | Captan Dia. (mm) | Captan Wt. (mg) | Benomyl Dia. (mm) | Benomyl Wt. (mg) | Vortex Dia. (mm) | Vortex Wt. (mg) | Chloropicrin Dia. (mm) | Chloropicrin Wt. (mg) | Copper metal Dia. (mm) | Copper metal Wt. (mg) |
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</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>70.5A (^b) 438V</td>
<td>70.5A 438V</td>
<td>68.8A 312W</td>
<td>315W</td>
<td>79.0A 565V</td>
<td>79.0A 565V</td>
<td>87.2A 570V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>25 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>57.4B 296W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ppm</td>
<td>36.2D 200X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38.7D 153XY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ppm</td>
<td>49.5C 227X</td>
<td>75.2A 351W</td>
<td>59.1B 227X</td>
<td>11.0E 26Z</td>
<td>-</td>
<td>-</td>
<td>438V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ppm</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>301W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>68.0B 368W</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2500 ppm</td>
<td>-</td>
<td>-</td>
<td>0, 0F (^d) OZ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>351W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5000 ppm</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>153XY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10000 ppm</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>0, 0F (^d) OZ</td>
<td>42Z</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>33300 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>166700 ppm</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>132 ml(^e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.6B 137W</td>
<td>26.7B 115W</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Concentrations based on commercial formulations except captan and benomyl which are based on 50% active ingredient.

\(^b\) Means (30 observations) not sharing a common capitalized letter are significantly different (\(P = 0.05\)).

\(^c\) Values for copper metal (especially control) are disproportionately large because incubation period was 13 rather than 11 days.

\(^d\) Inoculum viable 13 days after transfer to standard medium.

\(^e\) Treatments were 132 ml of chemical in four capped 33 ml polyethylene bottles.
Fig. 14. In vitro growth of three isolates of *A. mellea* on agar medium containing increasing amounts of benzomyl.
Table 5. Growth of three isolates of *Armillariella mellea* on agar medium containing test chemicals. Values are colony diameters and dry weights.

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>Copper sulfate (100)</th>
<th>Iron sulfate (100)</th>
<th>Captan (100)</th>
<th>Benomyl (100)</th>
<th>Vorlex (132 ml)</th>
<th>Chloropicrin (132 ml)</th>
<th>Copper metal (33300)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dia. (mm)</td>
<td>Wt. (mg)</td>
<td>Dia. (mm)</td>
<td>Wt. (mg)</td>
<td>Dia. (mm)</td>
<td>Wt. (mg)</td>
<td>Dia. (mm)</td>
</tr>
<tr>
<td>I2a5-1</td>
<td>62.0A</td>
<td>254X</td>
<td>88.5A</td>
<td>315X</td>
<td>68.3A</td>
<td>252X</td>
<td>20.1A</td>
</tr>
<tr>
<td>96</td>
<td>46.9B</td>
<td>252X</td>
<td>63.1C</td>
<td>285X</td>
<td>54.3B</td>
<td>204X</td>
<td>0.0B</td>
</tr>
<tr>
<td>S8A</td>
<td>39.7B</td>
<td>175Y</td>
<td>74.1B</td>
<td>452Y</td>
<td>54.8B</td>
<td>226X</td>
<td>13.1A</td>
</tr>
</tbody>
</table>

*a* Concentrations based on commercial formulation except captan and benomyl which are based on 50% active ingredient.

*b* Treatments were 132 ml of chemical in four capped 33 ml polyethylene bottles.

*c* Means (10 observations) not followed by a common letter are significantly different (P = 0.05). Comparisons are valid only between values for a particular chemical tested against the three fungal isolates.
pine seedlings were examined for signs and symptoms of *Armillariella mellea* infection and chemical phytotoxicity. The roots of all seedlings were then excavated and examined for resinous lesions, mycelial fans of *A. mellea* (infection), and rhizomorph production from alder inoculum segments (Fig. 15). Each inoculum segment was aseptically split longitudinally, and five wood chips (5 x 15 mm) from the interior were plated on a selective medium (p. 35). Cultures were incubated in the dark at 24°C for three weeks after which the presence of *A. mellea* was recorded.

Of the 15 inoculated control seedlings (no chemical treatment), only two (13%) were infected and one (7%) died from infection (Fig. 16). This low level of infection in the control precluded any conclusions on the relative effectiveness of the chemicals in reducing infection, but captan significantly (P = 0.05) enhanced infection and disease incidence was most severe at the highest concentration of the chemical.

Viable *A. mellea* was isolatable from only 10 (67%) of the 15 inoculum segments in the control (Fig. 17). Captan enhanced the survival of the inoculum to a greater extent than the other treatments. Treatments with the fumigants chloropicrin and Vorlex in polyethylene bottles and with copper sulfate (10 g/liter soil) significantly (P = 0.05) reduced the viability of the inoculum over the control but were all phytotoxic (lethal) to the pine seedlings.
Fig. 15. Healthy (left) and infected ponderosa pine seedlings. Note the mycelial fan of *A. mellea* at the root collar of the infected seedling.
Fig. 16. Effect of chemicals on infection by *A. mellea* in potted ponderosa pine seedlings after two years. Data excludes conspicuously phytotoxic treatments (copper sulfate, 10 g/liter soil; Vorlex, 19.8 g; and chloropicrin, 25.2 g). Only captan at 3.2 g/liter soil was significantly different from the control ($P = 0.05$).
Fig. 17. Effect of chemicals on survival of *A. mellea* in infected alder stem inoculum buried in soil for two years. All treatments of copper sulfate, iron sulfate, Vorlex, and chloropicrin were significantly different from the control (*P* = 0.05).
Rhizomorphs of *A. mellea* were produced from six (40%) of the 15 inoculum segments in the control (Fig. 18). The greatest number of inoculum segments producing rhizomorphs occurred with 3.2 g/liter soil of captan. Vorlex and chloropicrin significantly (*P = 0.05*) reduced the number of inoculum segments producing rhizomorphs.

A comparison of pathogenicity of the three isolates of *A. mellea* when attacking pine seedlings from alder inocula is as follows: of the 85 inoculum segments with isolate I2a5-1, 28 (33%) infected seedlings and 14 (17%) caused death (Fig. 19). These values are significantly (*P = 0.01*) greater than comparable values for isolates S8A and 96. Isolate I2a5-1 significantly (*P = 0.05*) produced more rhizomorphs from alder inocula than did S8A but not more than 96 (Table 6).

**Tests on Young Pines in the Forest**

Two years following treatment with root collar protectants, 23 (28%) of the 80 treated saplings in Group One, those located closest to the inoculum source, had lethal infections of *Armillariella mellea*. Of the 80 saplings in Group Two, those located furthest from the inoculum, only three (4%) had lethal infections, and no control trees died.

Of the five control saplings in Group One treated with 19 liters of water, only one (20%) had a lethal infection (Fig. 20). No mortality (lethal infections) occurred among trees receiving drenches of
Fig. 18. Effect of chemicals on production of rhizomorphs from alder stem inoculum buried in soil for two years. Only Vorlex and chloropicrin were significantly different from the control (P = 0.05).
Fig. 19. Pathogenicity of three pine isolates of *A. mellea* on ponderosa pine seedlings treated with protectant chemicals. Data are from tests employing alder stem inoculum. Isolate I2a5-1 was significantly more pathogenic than the other isolates (*P* = 0.01).
Table 6. Survival, growth, and pathogenesis of three isolates of *A. mellea* in alder stem inoculum buried in soil with ponderosa pine seedlings and treated with protectant chemicals.

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>12a5-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>96</th>
<th>S8A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Mycelium viable</td>
<td>43</td>
<td>51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43</td>
</tr>
<tr>
<td>Rhizomorphs produced</td>
<td>41</td>
<td>48</td>
<td>31</td>
</tr>
<tr>
<td>Seedlings infected</td>
<td>28</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Seedlings killed</td>
<td>14</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Isolate 12a5-1 was significantly (*P* = 0.05) more pathogenic than 96 and S8A and produced more rhizomorphs than S8A.

<sup>b</sup>Values in percent are based on a total of 85 inoculum segments.
Fig. 20. Effect of chemicals applied as root collar protectants on mortality caused by *A. mellea* in young-growth ponderosa pine. Each treatment was applied to five trees. None of the treatments was significantly different from the control ($P = 0.05$).
benomyl, 50 and 100 g/tree or captan, 25 and 50 g/tree. The highest sapling mortality (80%) occurred with chloropicrin where mycelial fans of *A. mellea* were present on all killed trees. None of the chemical treatments were phytotoxic, and there were no significant differences (*P* = 0.05) between the chemical treatments and the control at this point in the study.

In this experiment on root collar protection of forest trees, the underground vegetative spread of *A. mellea* is relatively slow (approximately one meter per year; Shaw, 1974) and necessitates this being a long-term study. Before evaluation of the chemical treatments can be made, sample trees must be exposed to *A. mellea*. Until a substantial number of control trees become infected and die, evaluation of the chemical treatments as protectants is incomplete. Data concerning future infection and mortality especially in trees treated in Group Two, should make it possible to better evaluate the performances of protectant treatment.

**Fumigants to Eradicate *A. mellea* from Infected Pine Residues in Disease Centers**

**Background Study on *A. mellea* Distribution in Infected Pine Stumps**

Disease center excavations of 49 stumps indicated that only three stumps, all living, lacked mycelial fans. *Armillariella mellea*
was isolated on agar medium from 41 (89%) of the remaining 46 stumps. Most of the positive isolations for *A. mellea* were low on the taproot (Table 7) and from characteristic yellow stringy decay (Table 8) but "sound wood" also yielded *A. mellea* at high frequency. The distribution of *A. mellea* as determined by isolation on agar was quite variable and did not conform to any specific pattern within the regions sampled (Fig. 10). However, *A. mellea* was recovered significantly (P = 0.01) more often from taproot isolations than from transverse or longitudinal isolations. Statistical differences between the mean number of *A. mellea* isolations from the various decay configurations (Table 8) were not tested because of the great variation in sample size.

Recovery of *A. mellea* from mycelial fans as shown in Table 7 (34%) differs from recovery rate in Table 8 (83%). The difference is thought to result from a decline of *A. mellea* during storage at 16°C. Colonies of *Trichoderma* developed occasionally on the stumps during storage period.

A negative correlation was found between the extent of stump decay as observed from the cut stump surfaces (stump decay index, p. 30) and the frequency of *A. mellea* recovery (Fig. 21). From 29 stumps examined (Table 7), *A. mellea* was isolated most often from those without visible decay and least often from those which were barely intact (100% decay other than yellow decay).
Table 7. Distribution of *A. mellea* in young-growth ponderosa pine stumps as determined by isolations according to the formalized scheme in Figure 10.

<table>
<thead>
<tr>
<th>Stumps examined</th>
<th>Total isolations</th>
<th>Transverse isolations</th>
<th>Longitudinal isolations</th>
<th>Taproot isolations</th>
<th>Fan isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>29</td>
<td>23</td>
<td>79</td>
<td>7</td>
<td>24</td>
<td>6</td>
</tr>
</tbody>
</table>

*Taproot isolations are significantly greater (*P* = 0.01) than either transverse or longitudinal isolations.*

Table 8. Influence of the different types of wood decay as noted from fresh stump surfaces of ponderosa pine on rate of recovery of *A. mellea* by culturing.

<table>
<thead>
<tr>
<th>Total examined</th>
<th>Stumps positive for <em>A. mellea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Description of decay</td>
</tr>
<tr>
<td></td>
<td>Sound wood</td>
</tr>
<tr>
<td>Number</td>
<td>18(20)</td>
</tr>
<tr>
<td>Percent</td>
<td>90</td>
</tr>
</tbody>
</table>

*Values in parentheses are the basis (number of stumps) for percentage calculations.*
Fig. 21. Rate of recovery of *A. mellea* from stumps of ponderosa pine in various stages of decay. Stump decay index is as follows:

0 - no visible decay (soundwood)
1 - 0-50% yellow decay, otherwise sound
2 - 50-100% yellow decay or other decay
3 - 100% decay other than yellow decay
Fumigation Tests

Fumigant treated stumps infected with Armillariella mellea were evaluated for effectiveness of eradication as follows. In July 1975, one year after treatment, 132 stumps were excavated with a backhoe as in the preliminary study. In some cases a strong odor of fumigant was detected upon breaking the ground with the hoe. To avoid prolonged exposure of the stump prior to excavation, stumps were excavated in three lots of 45 stumps each, one lot a week. After excavation, the stumps were promptly taken from the field site to the laboratory in moistened burlap bags, and stored at 16°C until isolations were complete 24-72 hours later. Each stump was scrubbed with water and split longitudinally. The strong odor of fumigant, especially chloropicrin and Vorlex, was persistent in many of the dissected stumps.

After stump dissection, Trichoderma was often found sporulating in the application holes of fumigated stumps, especially those treated with either methyl bromide or carbon disulfide (Fig. 22). It was not determined whether this sporulation occurred prior to stump excavation or following excavation. A pseudosclerotium (black zone line) of A. mellea was often noted surrounding the application holes in control stumps but generally was not found in fumigated stumps (Fig. 23).
Fig. 22. Dissected pine stumps which received treatment with chemical fumigants one year prior to excavation. Note the sporulation of *Trichoderma* fungi in application holes.
Fig. 23. Dissected stump which received treatment with water (control) one year prior to excavation. Note the pseudosclerotium (black zone line) of *A. mellea* completely surrounding the application hole.
Immediately following the dissection of each stump, 15 isolations were made from yellow decay in the interior taproot region (Fig. 10) because the mycelium of *A. mellea* was most prevalent in this region (Table 7). An additional ten isolations were made from the wood surface immediately below the bark where mycelial fans of *A. mellea* normally were present. A total of 3300 isolations (550 per treatment) were made on a selective medium (p. 35) from 132 stumps excavated. After three weeks incubation in the dark at 24°C, the presence of *A. mellea* and *Trichoderma* was recorded.

Of 550 isolations from 21 control stumps, 104 (19%) were positive for *A. mellea* (Fig. 24). Methyl bromide, Vorlex, and chloropicrin apparently eradicated *A. mellea* while carbon disulfide and Vapam were nearly as effective. *Trichoderma* was unaffected by all fumigants except methyl bromide and Vorlex, which increased its incidence over the control. This was a statistically significant (P = 0.05) increase in the case of methyl bromide.

Since *A. mellea* was practically eliminated in all treatments, differences in the effectiveness of fumigation method, whether into the wood, the soil, or both could not be determined. The same is true for stump size and degree of decay (stump decay index) although the chemical application rate varied with stump size and the preliminary study indicated that the more decayed stumps may have contained less viable *A. mellea* prior to fumigation (p. 57).
Fig. 24. Recovery of *Armillariella mellea* and *Trichoderma* from naturally infected ponderosa pine stumps treated *in situ* with five chemical fumigants.
DISCUSSION

The results of experiments presented here are not convincing in all cases, largely because of extreme variability of the experimental material. The young-growth ponderosa pine forest, varying greatly in tree size, age, and spacing, is a difficult environment for experimentation concerning features of the forest *per se*. Difficulties are compounded where the concern is with secondary influences such as disease. Disease is commonly very unevenly distributed and sometimes varies along gradients creating difficult sampling problems, as is the case with Armillaria root rot. *Armillariella mellea* itself has a history of producing inconsistent results in repeated experiments. Even though studied more than 100 years, effective inoculation procedures have been available for only a relatively short time (Patton and Riker, 1959). These procedures still require as inoculum, the use of natural plant substrates which are unpredictable in performance.

Experiments on woody plants are necessarily long term, a situation that may, depending on the availability of time, prevent repetition of experiments, as has been the case here. These difficulties have been reduced as much as possible by replication and randomization within the experiments and by inclusion of a moderately large number of treatments. In some cases, comparisons among treatments may be more meaningful than comparisons between a particular treatment and the control.
Tests of chemicals employing seedlings inoculated and treated in pots were intended 1) as an experimental control on root collar applications of young-growth pines in the forest, and 2) to correlate results from *in vitro* and *in vivo* studies of chemical toxicity.

Such a correlation was observed with captan, which was one of the least toxic chemicals to *A. mellea* *in vitro* (fungus growth at 10,000 ppm captan, Table 4), and which also allowed significant increase in seedling infection over the control. Since *A. mellea* is highly tolerant of captan, perhaps in this study, as has been suggested for captan elsewhere (Kendrick and Middleton, 1954; Ranney and Bird, 1956), the chemical was effective against the flora of soil fungi by removing them from competition with *A. mellea* thus allowing the latter, unharmed in the inoculum segments, to freely attack the seedlings.

Stimulation of *A. mellea* growth was often noted at the lower concentrations of some of the test chemicals *in vitro*. This response is consistent with the Arndt-Schutz Law, which states that any toxicant in low dose is stimulatory (Horsfall, 1945). Rykowski (1974) noted a similar increase in growth with *A. mellea* in cultures with low concentrations of sodium penta-chlorophenolate. Englander and Corden (1971) demonstrated that the fungus, *Endothia parasitica*, secreted when grown in toxicant free culture, large amounts of oxalate which precipitated primarily as calcium oxalate at the periphery of the
They further demonstrated that mycelial growth was retarded when calcium oxalate accumulated in toxicant free culture, but retardation was absent in media containing metallic salts that prevented the accumulation of calcium oxalate crystals. Hamada (1940) also demonstrated that certain levels of dextrose stimulated the growth of *A. mellea* by solubilizing calcium oxalate as it was formed in the culture medium.

In vitro studies also indicate that of the three isolates of *A. mellea* tested, 12a5-1 appeared to be the most tolerant to the test chemicals. Inoculation of pine seedlings with 12a5-1 caused significantly more seedling infection and mortality in the presence of the chemicals than did the other isolates. Since the three isolates were equally pathogenic to control seedlings, greater pathogenicity of 12a5-1 on chemically treated seedlings may result from chemical tolerance. The three isolates were from ponderosa pines in different geographic locations. Since there was no difference in pathogenicity among the isolates on control seedlings, but differences were noted in tests with chemically treated seedlings, perhaps variation in field performance of promising chemicals may be expected depending on the geographic distribution of strains of *A. mellea*.

Performance of the chemicals as root collar protectants of young pines in the forest varied in Group One (trees within 3.1 m of the nearest infected dead tree). As was the case with potted
seedlings, some examples of increased infection following chemical
treatment were evident. Chloropicrin was the most notable in this
regard. From Shaw's 1974 work in the study area of this thesis, the
average distance of extent of *A. mellea* infected roots from dead trees
can be estimated from the average DBH of the stand. In a stand of
7-15 cm DBH, the DBH of the treated trees, infected roots may
extend 4.1 m beyond the nearest infected dead tree. Since all trees
treated in Group One were between 0 and 3.1 m from the nearest
infected dead tree, the roots of all of these trees may have been in
contact with infected roots and thus have a high probability of being
infected (latent infections) with *A. mellea* prior to treatment. If such
infections were not at the root collar, these trees would still be alive
and symptomless above ground (Day, 1927; Patton and Riker, 1959;
Sokolov, 1964; Adams, 1972; Shaw, 1974). These infections remain
dormant on pine roots largely as a consequence of host resistance
mechanisms (Patton and Riker, 1959; Adams, 1972; Shaw, 1974).
However, upon treatment with chemicals such as chloropicrin,
infections on the lateral roots could, as a result of chemical injury to
host root tissue and disruption of host resistance mechanisms, spread
to the root collar beneath root bark. Root collar infection would then
result in tree death.

Data will be needed during a few more years before final effects
of all treatments can be known. This information should enable
evaluation of the chemicals and perhaps interpretation of chemical effects on host resistance mechanisms influencing internal spread of root infections.

In preliminary isolation studies on dead, naturally infected, young-growth ponderosa pines, *A. mellea* was recovered at a high frequency throughout the "soundwood" as well as the characteristic yellow stringy *A. mellea* decay. Recovery was highest from stumps lacking visible decay and decreased as invasion by decay and soil-borne fungi other than *A. mellea* increased. *A. mellea* recovery was least from stumps which were barely intact. This suggests that *A. mellea* upon killing the pine host ramifies throughout the stump within a few years and then, with time, is slowly replaced by other decay and soil-borne fungi.

Fumigation of *A. mellea* infected stumps within disease centers demonstrated that methyl bromide, chloropicrin, carbon disulfide, Vapam, and Vorlex all effectively eradicated *A. mellea*. It appeared also that some of the treatments increased *Trichoderma* populations as is commonly reported in the literature. These *Trichoderma* increases, except possibly for methyl bromide, appeared not to be of a magnitude sufficient to influence *A. mellea* through their antibiotic action. Many of the fumigated stumps upon dissection still contained a noticeable odor of fumigants one year after treatment. Fumigant persistence may have limited growth of larger *Trichoderma*
populations. It appears, therefore, that the noted decrease in
*Armillaria mellea* resulted directly from chemical toxicity rather than
antagonistic fungal action.

One of the possible toxic effects of chemical fumigants to
*Armillaria mellea* is either to damage the characteristic protective pseudosclerotium or prevent its formation. Damage to the pseudosclerotium exposes the fungus to the toxicant and also facilitates invasion by
*Trichoderma* or other antagonistic fungi. A pseudosclerotium of
*Armillaria mellea* was often noted surrounding the application holes drilled in
control stumps but generally was absent in fumigated stumps. This
observation supports the findings of Bliss (1951), Darley and Wilbur
(1954), and Ohr et al. (1973) regarding the damaging effect of carbon
disulfide and methyl bromide on the pseudosclerotium of *Armillaria mellea*.

The fumigants were studied for their biological effectiveness in
controlling Armillaria root rot in the forest. No attention was given
the matter of cost. Since soil fumigation generally is economically
feasible only on the most productive agricultural lands producing high
value crops, some consideration of costs for its application in the
forest is of interest. Very rough cost estimates of treating a hectare
(approximately 2285 stubs/snags) of *Armillaria mellea* infected young-growth
pine stubs/snags by several methods are provided in Table 9.

Application methods used in this research were unreasonably
specialized for commercial use. The toxicity results showed that soil
Table 9. Comparative costs of chemical methods of sanitizing pine sites where young-growth timber has been killed by *Armillariella mellea*.

<table>
<thead>
<tr>
<th>Fumigation treatment</th>
<th>Cost per hectare(^a) (dollars)</th>
<th>(2285 stubs/snags)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Materials and labor</td>
<td>Chemicals</td>
<td>Total</td>
</tr>
<tr>
<td>Experimental methods (^b)</td>
<td>5560</td>
<td>1975(^e)</td>
<td>7535</td>
</tr>
<tr>
<td>Conventional injection (^c)</td>
<td>740</td>
<td>1975</td>
<td>2715</td>
</tr>
<tr>
<td>Adapted procedure (^d)</td>
<td>740</td>
<td>990</td>
<td>1730</td>
</tr>
</tbody>
</table>

\(^a\) One hectare = 2.471 acres

\(^b\) Two stubs/snags per hour (includes felling, drilling, pouring, and tarping).

\(^c\) Twelve stubs/snags per hour (soil injection only).

\(^d\) Applied at 1/2 of the experimental rate.

\(^e\) Estimated per hectare cost of chloropicrin. Carbon disulfide is $1235 and methyl bromide, $8650.
application (applied against the stump) alone was effective at the rate of fumigant applied. Application, therefore, probably could be accomplished with injectors available for this purpose (MacClean Equipment Co., Los Angeles) at great savings in time resulting from elimination of the need to fell, drill, and perhaps tarp infected stumps (conventional injection, Table 9). Apparently, also, more chemical was used than was required. Reduction in application rates could also decrease treatment costs (adapted procedure, Table 9). Future testing is needed to ascertain the limits of both treatment simplification and dosage reductions.

Most diseased hectares in the pine forest under study have fewer stubs/snags per hectare than the 2285 on the hectares employed for the experiments. Where infection is widely scattered, per hectare treatment costs could drop considerably and the chemical methods show an economic advantage over mechanical destruction of the food base. For example, with chloropicrin and 50 infected stubs/snags per hectare, costs would approximate $45 per hectare.

Chemical treatment was sufficiently effective when applied to young-growth stubs/snags (10-38 dm dia. at ground) as to indicate the desirability of tests on large stumps. Also the suitability of fumigants for specialized application in establishing chemical barriers to vegetative fungal spread should be studied (Houston, 1975).
In conclusion, the possible effects of chemical fumigation on the epidemiology of \textit{A. mellea} in the forest situation merits some attention. Hartig (1874) was perhaps the first to recognize two distinct expressions of Armillaria root rot. Day (1929) noted that in one case \textit{A. mellea} attacked only weakened trees while in another case it attacked healthy, vigorous trees. Fassi (1959) also noticed this dual nature and was perhaps the first to apply the terms "endemic" and "epidemic" disease phases to Armillaria root rot. The endemic, latent, or chronic phase in the forest is characterized by a slow decline often associated with butt rot or perhaps even death of individual trees following infection by \textit{A. mellea}. The epidemic phase, conversely, is characterized by relatively rapid decline of the individual as a result of cambial destruction and by spread to adjacent trees resulting in death of a group of trees to form disease centers.

The epidemic disease phase typifies the study area. Harvesting of the often infected old-growth pine has left the large root systems as a massive food base for the fungus and an effective inoculum source from which to infect the surrounding young pines. Roth (personal communication) has suggested that the explosive shift from parasitism of the living tree to saprophytism on the dead (harvested) tree causes an increase in energy (inoculum potential) for infection, especially in trees of large size. Adjacent, vigorous trees are subsequently infected via root and/or rhizomorph contact from the old-growth.
stump. With time the characteristic disease center develops centrifugally with the infected old-growth stump at the origin. In brief, the endemic (latent) phase as characterized by isolated infections on large, mature pines was transformed into the epidemic phase as a result of harvesting and tree death. Each tree infected by inoculum from the old-growth stump, becomes part of the disease center, increases the total food base of the fungus, and contributes to the inoculum potential necessary to maintain the epidemic. For a detailed discussion of the continuum between the epidemic and endemic disease phases in general, the reader is directed to Van Der Plank (1975).

The possible effect of fumigation on epidemiology of *A. mellea* infected stumps within the disease center becomes of interest. While the fumigants tested were highly effective in eradicating *A. mellea* from treated stumps, it cannot be safely assumed that the fungus was completely eradicated from the site. Numerous buried infected smaller roots were undoubtedly present and not treated. This material, however, appears to be inadequate to supply the inoculum potential required to maintain the epidemic disease phase and should result only in limited and isolated *A. mellea* infections on mostly weakened or suppressed trees (latent disease phase). These isolated infections resulting in occasional tree mortality may in fact, in young
stands, be beneficial as a natural thinning agent (Roth, personal communication).
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