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CHARLES G. SHAW III for the degree DOCTOR OF PHILOSOPHY
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Root rot of ponderosa pine caused by Armillaria mellea was studied in a pine forest that had been under management for 30 years in Klickitat County, Washington. Information sources included disease survey, detailed observation and description of specific disease situations, and examination of roots in situ and removed. Roughly circular infection centers in stands of sapling and pole-sized pines appear to arise regularly from stumps of harvested old-growth trees, some of which have contained viable A. mellea for over 30 years.

Distinctive concentric zones caused by the disease mark the infection centers. These zones differ in above-ground appearance, in degree of root decomposition, and in level of infection. Location of a specific zone depends on the interval since cutting of the old-growth tree, while zone width depends on tree sizes in the young

stand. Distribution of root material in the interior zones is influenced most strongly by rooting characteristics of the former old-growth trees, and by activity of A. mellea and other decay agents over time, while in the young growth pines of the intermediate zones, time since lethal attack by A. mellea and tree size most influence root condition.

Extensive examination of the outer zones disclosed that, in stands containing trees from 3 to 6 inches in diameter, infected root material extended into the healthy-appearing pines 11 ft beyond the outermost dead tree of the infection center, while in stands with trees up to 16 inches in diameter the extent reached 18 ft. A highly significant linear regression with good correlation was found between: 1) the extent of infected root material beyond the outermost dead tree; and 2) mean tree size of the affected stand. Infected root segments from beyond the outermost dead tree, within the region where infected and healthy roots intermingle, produced rhizomorphs, the assumed infection structures, when incubated in bags of moist quartz sand.

The expansion rate of infection centers was calculated to be 2.9 ft (0.88 m)/year.

Cross plating techniques indicated that the 1500 acre study area was occupied by a single strain of A. mellea, suggesting that spores are unimportant to the field disease cycle.

Current loss to disease in the 1500 acre tract is 35 cu ft per acre per yr, three times the loss in 1957. From 1957 to 1971, the proportion of the area in which disease was detectable remained constant. The increased loss resulted from death of fewer, but larger, trees in 1971.

In inoculation tests on ponderosa pine seedlings, A. mellea isolates from old-growth pine stumps and from young pine trees were pathogenic, while isolates from living hardwoods were weakly or nonpathogenic.

Reliable methods are described for isolating A. mellea from infected tissues and of testing the capacity of naturally infected root materials to produce rhizomorphs.

The abundance of A. mellea over a three year interval following five root removal treatments increased in missed root residues.

Resinous lesions were commonly found at the site of A. mellea attack. Rarely does mycelium spread proximally along a living root from one of these lesions, but growth frequently occurs from the lesion toward the root tip following, or concurrent with death of the extremity. Girdling resinous lesions on lateral roots show little effect on tree vigor, while such lesions high on the tap root kill the tree. Soon after tree death, mycelial fans spread under the bark throughout the root system.

To obtain insight on how the resinous lesions retard the fungus, tests were conducted on the effects of resin on growth of A. mellea in culture. Autoclaved resin, acetone extracts of resin, water extracts of resin, and non-sterilized resin all stimulated, rather than restricted, growth over non-resin containing media. Removal of phenols from the water extract of resin with polyvinylpyrrolidone destroyed the growth stimulating effect of the resin. Propylene oxide was effective for sterilization of granulated resin.

Epidemiological Insights into *Armillaria mellea* Root Rot in a Managed Ponderosa Pine Forest

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Chairman of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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EPIDEMIOLOGICAL INSIGHTS INTO *ARMILLARIA MELLEA* ROOT ROT IN A MANAGED PONDEROSA PINE FOREST

I. INTRODUCTION

The root rot caused by *Armillaria mellea* (Vahl ex Fr.) Quél is perhaps the most destructive root trouble of ponderosa pine (*Pinus ponderosa* Dougl.) in the Pacific Northwest. As more intensive forest management is practiced, losses from the disease become more noticeable, if not actually greater. This thesis deals with a particularly severe disease situation where *A. mellea* has caused increasing damage over the past three decades. In this instance basic information concerning disease epidemiology was needed for development of a comprehensive control program. This basic information is sought in this thesis and is integrated into our knowledge of the disease caused by *A. mellea* in ponderosa pine.

This work was conducted in a ponderosa pine forest (the most prevalent and important commercial species along the eastern slopes of the Cascade Mountains in Washington and Oregon) in south central Washington on the southeastern flank of Mount Adams. A major portion of the above forest is within the economic unit known locally as the Klickitat working circle of St. Regis Paper Company. The working circle includes lands held by St. Regis Paper Company,

the U. S. Bureau of Indian Affairs (Yakima Indian Reservation), the Washington State Division of Natural Resources, the U. S. Forest Service, and small private proprietors. For management purposes the working circle has been divided into several blocks by St. Regis, the primary converter of the timber yield of the area. This disease study was mainly conducted in the Glenwood Block, so named for its proximity to Glenwood, Washington.

The Glenwood Block is characterized by gentle to moderate topography, varying in elevation from 1, 900 to 4, 000 ft. Precipitation averaging from 30 to 40 in. /yr on the western side of the block diminishes to 20 to 20 to 30 in. /yr on the eastern side. Much of the precipitation is snow that remains late into June at higher elevations. Light summer rains accompany occasional electrical storms, but mostly summers are dry with warm days and cool nights. The soil is of volcanic origin (olivine-andesite parent material). It is generally deep, but frequently is broken by lava outcroppings, and is covered with a thick litter layer. The western edge of the block is adjacent to the crest of the Cascade Range, where transition occurs between Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) to the west and ponderosa pine to the east.

The area fits into the broadly defined Pinus ponderosa zone of Franklin and Dyrness (1969), who stress the importance of logging history, fire prevention, and site in determining the present stand

composition and structure. Ground cover is mainly pine grass (Calamogrostis spp.) and elk sedge (Carex geyeri Boot.), with bracken fern Pteridum equilinum [L.] Kuhn var. pubescens Underw.), lupine (Lupinus spp.) and other annual and perennial plants not uncommon (see Bailey and Austin, 1937 for additional detail). Brush species, including false rabbit brush (Haplopappus sp.), snow brush (Ceanothus spp.), western hazel (Corylus cornuta Marsh. var. californica [A. DC.] Sharp.), Oregon white oak (Quercus garryana Dougl.), and bitter brush (Pursha tridentata [Pursh] DC.) are scattered. The area has been grazed by sheep and cattle for nearly a century with some over-grazing. Ponderosa pine is the principal forest tree throughout the block. Douglas-fir is common along the western edge. Grand fir (Abies grandis [Dougl.] Lindl.) and lodgepole pine (Pinus contorta Loud. var. murrayana [Balif.] Engelm.) are less common and occur at higher elevations.

Present stand composition over much of the Glenwood Block has been strongly influenced by fire control, a series of cuttings of old-growth timber, and activity of A. mellea. Most of the block has been under tree selection management since the 1940's. The second cutting cycle has now covered most of the block. Topical sanitation-salvage operations have occurred during the past 20 years, with the result that some areas have been cut three or four times.

The 1500 acre "saddle area," on the western edge of the block, has been cut four times. The first cut (1941-42) removed about 1/3 of the old growth (around three trees per acre, each averaging about

2500 bd ft). Sanitation-salvage cuts occurred in 1953 and 1961. In 1965 the planned second cycle cut removed most of the remaining old-growth trees. The present stand consists of a few old-growth trees, patches of both large ponderosa pine poles (8-16 in. DBH) and small poles (4-8 in. DBH), among which occur scattered young Douglas-firs and grand firs, and patches of pine saplings and seedlings with unstocked areas in brush and openings.

A. mellea causes openings or "pockets" throughout the Glenwood block, with most portions light to moderately infected. Mortality was first noted in saplings and small poles around 1945, soon after initial logging. The disease also is found in most other parts of the Klickitat working circle, including state and national forest lands. It is present, but causes minimal damage as yet, on the more recently harvested, adjacent Yakima Indian Reservation. Disease pockets occur between elevations of 4500 ft (on the slopes of Mount Adams) and 800 ft on the floor of the Klickitat River Valley. On the Plateau Block, the flatlands high above and west of the Klickitat River gorge, ponderosa pine appears free of A. mellea, but the fungus is present on the roots of living hardwood shrubs.

From measured periodic mortality on a series of permanent plots throughout the 640,000 acre Klickitat working circle, St. Regis' foresters estimate an annual board foot loss of 6.5 million (roughly 10 bd ft/acre/yr). On the Glenwood Block during the last 10 years, mortality has exceeded 14 bd ft/acre/yr. These figures do not include volume loss in small trees (less than 8 in. DBH), or loss from acres

rendered unproductive. The latter figures are significant in some locations. Losses in the "saddle area" are very severe.

Disease is evident from off-color dead and dying trees, standing dead snags and stubs, and ground littered with stems of fallen trees. Irregular pockets of dead and diseased trees, possibly representing fungal colonies, from several feet to several chains across occur throughout the stand. These pockets are hereinafter referred to as "infection centers."

Infection centers are marked by several recognizable, more or less concentric zones. An old-growth stump(s) and a clump of healthy-appearing saplings frequently are found near the middle of the infection center (Figure 1). Outward from the stump and saplings is an area that is unstocked or poorly stocked, but free of debris (Figure 1). This zone is surrounded by a broad band where the ground is littered by the fallen stems of long-dead trees (Figure 2). Outward from this band is a zone of stubs and standing "snags" of increasing height toward the outer limits (Figures 2 and 3). This orderly increase in size among the residues, along with progressively less weathering, indicates centrifugal growth of the fungus. At the visible limits of the infection center, against the green forest stand, are defoliated, but branchy, snags and more recently killed trees with bright brown foliated crowns (Figure 3). Trees dead a few years or less exhibit typical A. mellea mycelial fans under the bark at the base (Figure 4). Beyond the last dead trees are standing live,

Figure 1. View across an A. mellea infection center showing the clump of relatively healthy saplings near an old-growth stump (second), the presumed disease focus. The poorly stocked belt appears in the foreground and shadow area, right.

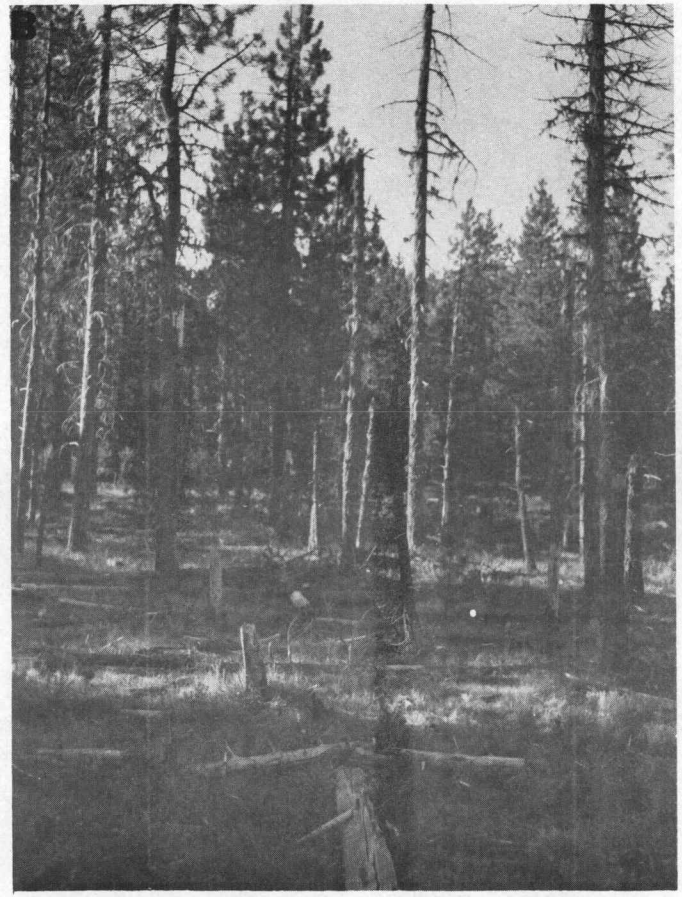

Figure 2. The zones (A) where the ground is littered by fallen stems of long dead trees, and (B) characterized by stubs and tall snags.

Figure 3. Outer limit of an infection center marked by defoliated, but branchy snags, and more recently killed trees with brown foliated crowns (arrows).

Figure 4. A. mellea mycelial fan in the cambial region at the base of a recently-killed tree. Such fans are diagnostic of tree death by A. mellea.

sapling- and pole-sized trees of uncertain health and consequently of uncertain "future" (Figure 3).

These infection centers are unproductive except for infrequent survivors of questionable longevity. As infection centers expand, they often coalesce to form large non-producing areas. In areas of scattered infection the latter has not occurred; however, in parts of the "saddle area" it has.

A. mellea is not uncommon in the ponderosa pine forest type (Weiss and Riffle, 1971; Adams, 1972, 1974), but it is not elsewhere reported to cause major damage, and at low levels may even serve as a beneficial thinning agent. While the ecology of A. mellea in the natural forest has not been adequately elucidated, the fungus must be considered a normal component of the forest community with the system in some form of balance. Otherwise the forest would not have developed so splendidly. Man's timber harvest has evidently had a different effect on the fungus than former tree removal by natural causes. Consequent to logging, large masses of root material have remained in the ground to serve as a food base for saprophytic development of A. mellea. Apparently, through utilization of this substrate in our area, the fungus has developed a high level of inoculum. Stumps from four different cuttings of old-growth trees have perpetuated a major food base, which, joining with an

apparently favorable climate and habitat, has supported an extreme level of forest destruction.

The severity of this epidemic has abundantly merited study. After initial examination of the disease, certain questions arose:

1. What is the present distribution and damage level of A. mellea in the "saddle area," and how have these changed through time?
2. How does the distribution of infected root material relate to the above-ground pattern of disease symptoms in an A. mellea infection center?
3. What is the rate of expansion of A. mellea infection centers?
4. What is the nature and distribution of A. mellea on pine root systems?
5. What types of infected root material are capable of serving as sources of inoculum?
6. How effectively can root material be removed from infected areas?
7. After attempted root removal from infected areas, what is the status of A. mellea in the remaining debris?
8. Are individual infection centers separate A. mellea colonies?
9. What is the distribution of A. mellea colonies in the "saddle area"?
10. What is the pathogenicity on ponderosa pine of A. mellea isolates obtained from different sources?

11. What effect does pine resin have on the growth of A. mellea in culture?

The objectives of this thesis were to answer these questions and integrate the information acquired into our knowledge of the disease caused by A. mellea in ponderosa pine. To obtain this information, three approaches were employed: 1) disease survey; 2) mapping and detailed description of specific disease situations; and 3) examination of root material in situ and removed. The results are presented in sections that individually examine the disease as it relates to the forest, the stand, and the individual tree.

II. LITERATURE REVIEW

Literature concerning A. mellea covers many topics. That relating to parasitism on forest trees and attempted disease control is reviewed here. Literature dealing with resin and its effects on growth of fungi, including A. mellea, is discussed in Chapter V, under "Effects of Resin on the Growth of A. mellea in Culture." Pertinent references are also cited in the Introduction and Discussion of individual sections. While not essential to the body of this thesis, interested readers will find literature on 1) taxonomy of A. mellea, 2) cultural characteristics of A. mellea, 3) the effects of ethanol on the growth of A. mellea, and 4) relations between A. mellea and other fungi reviewed in Appendix I.

Parasitism of A. mellea on Forest Trees

That A. mellea has often parasitized forest stands is evident from the representative examples in Table 1. The fungus has over 600 reported hosts (Raabe, 1962) and is distributed around the world. Historically, the root fungus A. mellea is considered a hearty saprophyte and a moderate parasite mainly of the coarse roots of woody plants. The classic disease problem arises where native vegetation has recently been replaced by exotic forest plantations or orchards. Damage is usual in groups of trees associated with

Table I. Reports of damage attributable to Armillaria mellea in forest situations.

Tree species involved	Geographical location	Reported by	Statement of impact on stand
Conifers	England	Day (1926)	A. mellea in conifer plantations almost invariably causes some losses and under some circumstances this loss may be great.
Exotic softwoods	France	Boullard (1972)	Attacks on exotic softwoods by A. mellea are on the increase. The author considers increase is related to increased planting and use or poorer sites.
Forest trees	USSR	Sokolov (1964)	In places it is a particularly serious disease problem, notably among young trees and seedlings. It is capable of causing root disease that terminates in the death of the tree.
Pine	Poland	Sokolov (1964)	A. mellea is classed as one of the most damaging agents to pine.
Pine	Kenya	Gibson (1960)	Outbreaks occurred in new pine plantations. The disease reached a peak 4-5 yrs after planting.
Conifer plantations	Rhodesia	Swift (1968)	A. mellea caused considerable damage to conifer plantations in eastern and central Africa.
Monterey pine (Pinus radiata D. Don) Ponderosa pine (Pinus ponderosa Laws.)	New Zealand	Gilmour (1973)	A. mellea is potentially the most important root rot organism in exotic pine plantations.
Mixed conifers (Larix leptolepsis) (Sieb. and Ziyz (Gord)) (Picea sitchensis) (Bong. Carr)	Newfoundland	Singh (1970)	Exotic and native conifers were heavily attacked. The incidence of killing ranged from 5% on Larix leptolepsis to 28% on Picea sitchensis in the first 10 yrs of the plantation.

(Continued on next page)

Table I. (Continued)

Tree species involved	Geographical location	Reported by	Statement of impact on stand
Conifers	Ontario	Canadian Forest Service (1972)	A. mellea was one of the chief causes of mortality of young conifers.
Lodgepole pine (Pinus contorta Dougl.)	Alberta	Baranyay and Stevenson (1964)	A. mellea was the most destructive agent on naturally regenerated lodgepole pine.
Eastern white pine (Pinus strobus L.)	New York	Silverbog and Gilbertson (1961)	In a white pine plantation, 38.8% of the trees showed typical A. mellea rot.
Forest trees	Virginia	Skelly (1970)	A. mellea is one of the most inconspicuous and underestimated fungal pathogens of forest trees. It causes a root rot of most major tree species in Virginia, particularly hardwoods.
Western white pine (Pinus monticola Dougl.)	Idaho	Hubert (1950)	Fifty percent of the recently killed trees examined (40 to 90 years old) were infected with A. mellea.
Conifers	Idaho	Partridge (1972)	A. mellea was the most frequent cause of root rot.

residual hardwood stumps (Childs and Zeller, 1929; Greig, 1962; Peace, 1962; Redfern, 1968, 1970). As the food base provided by the hardwood stump disintegrates, losses from A. mellea decrease (Greig, 1962; Peace, 1962).

Numerous cases of predisposition to A. mellea attack are reported. Tarry (1968) summarizes these publications and divides the predisposing factors into: inappropriate silvicultural practices, drought, excessive moisture, insect attacks, other fungi, poor soil conditions, and adverse temperatures. Many reports suggest that A. mellea is a severe pathogen only when its host is in an unfavorable environment, or "artificially regenerated or grossly interefered with by mankind" (Day, 1927). Yet the existence of such contradictory reports as bark beetle infestations predispose trees to attack by A. mellea (Boyce, 1961), and A. mellea predisposes trees to bark beetle infestations (Peace, 1962), along with reports where no predisposing factor could be identified (Hendrickson, 1925; Christensen, 1938; Buckland, 1952; New Zealand Forest Service, 1953; Patton and Riker, 1959; Malek, 1966) leaves the entire question of predisposition unsettled.

Some consider that A. mellea is mainly a problem in young forests with damage dropping off in later years. Gibson (1960) found mortality due to A. mellea in pine plantations in Kenya reached a peak four or five years after planting. Weiss and Riffle (1971)

reported A. mellea may be a significant factor in determining the early survival of ponderosa pine in New Mexico. Ono (1965) showed in larch plantations the disease first appeared within one year after planting, reached a maximum in three or four years, and nearly ceased five to six years after planting.

Kawada et al. (1962) showed larch stands in Japan were less damaged when they reached age 30 to 40 than at earlier ages. In Poland the disease is present in spruce stands of all ages, but reaches a climax in 30-year-old trees (Manaka, 1961). Hubert (1950) found high levels of infection in 40 to 90-year-old, naturally regenerated western white pine (Pinus monticola Dougl.). In contrast, Christensen (1938) found zero percent infection in 5 to 10-year-old jack pine (Pinus banksiana Lamb.) and 100% in 250 to 300-year-old Norway pine (Pinus resinosa Ait.).

Sokolov (1964) stated that the age of the tree is immaterial; trees can be attacked by A. mellea at any age, but young trees become infected more easily and die faster than older trees. In general, it appears that death occurs within two to three years after invasion of young trees, and requires several more years in older trees. However, time until death is related to the position of the attack on the root system (Bliss, 1946; Patton and Riker, 1959). Most frequently, lethal attacks occur in the root collar region (Day, 1927; Patton and Riker, 1959; Peace, 1962; Foster and Johnson, 1963).

Stand density is cited by Baranyay and Stevenson (1964) as an important factor in stand health, with the highest A. mellea mortality occurring among over-topped and suppressed lodgepole pine (Pinus contorta Dougl. var. murrayana [Balf.] Engelm.). Christensen (1938) found in natural forests of jack pine, Norway pine, and northern white pine (Pinus strobus L.) infection was higher in dense stands than in more open stands. In young Douglas-fir plantations, disease incidence was unaffected by tree spacing, as 93% of the trees were in root contact (Pielou and Foster, 1962).

In discussing the impact of A. mellea on forest stands, it is essential to consider the pattern of tree death. Foster (1970) explained that if mortality from any agent occurred on individual trees at random, up to 60% might be beneficial (a natural thinning agent), yet when clumped mortality can be devastating. A. mellea infection centers are an example of the latter. A major component of the disease impact from A. mellea is the increase in the size of the infection center with time (Foster and Johnson, 1963; Pielou, 1965; Foster, 1970). Thus, increases in the level of infection result from the occurrence of new infection centers, the enlargement of old infection centers, and the intensification within old infection centers (Pielou, 1965; Foster, 1970).

Bliss (1946) investigated the relationship between pathogenicity and soil temperature. A. mellea was capable of attacking roots of

the test plants at all temperatures from 7° to 25°C. The greatest resistance to infection was shown by all plants at the temperature most favorable for root growth, while infection was limited to those soil temperatures at which the pathogen produced rhizomorphs. Sokolov (1964) and Rishbeth (1968) give excellent discussions of the effects of soil temperature on the development of A. mellea in field conditions.

The life cycle of the fungus in general follows that of a facultative parasite. It can survive long periods of time as a saprophyte on dead stumps and root material, act as a parasite on living trees, or commonly exist as a parthophyte on dead root material still attached to living trees. Sokolov (1964) stated that the particularly marked growth of A. mellea in many forest soils is explained by the presence in these soils of a large quantity of tree remnants. Pieces of root material infected with A. mellea have long been regarded as the primary source of inoculum (Hendrickson, 1925; Day, 1929; Reitsma, 1932; Peace, 1962). Sporophores are regarded as relatively unimportant in the disease cycle and artificial attempts to infect stumps with spores have met with little success (Rishbeth, 1970).

Rhizomorphs, which extend from infected root material to healthy roots and penetrate these roots to establish infections, are considered the main means of fungal spread (Day, 1927; Campbell,

1934; Sokolov, 1964; Ouellette, 1967; Redfern, 1968; Rishbeth, 1972). In some areas, mainly tropical, rhizomorphs are not found at all, and disease spread is almost solely due to contact between infected and healthy roots (Leach, 1939; Wardlow, 1950; Molin and Rennerfelt, 1959, Gibson, 1960; Swift, 1968). Yet, "There seems little doubt that in temperature countries, by contrast to the tropics, rhizomorphs are regularly associated with disease caused by A. mellea; indeed on the basis of numerous observations Redfern (1968) concluded that this association is invariable" (Rishbeth, 1972).

Early workers concluded that rhizomorphs penetrated through root wounds (summarized in Thomas, 1934), but later investigators (Thomas, 1934; Patton and Riker, 1959) have shown conclusively that A. mellea rhizomorphs can penetrate directly and establish infections on uninjured roots. Penetration is through mechanical destruction augmented by chemical dissolving of suberized cells, with death of host cells always preceding the initial advancement of the rhizomorph into the tissue (Thomas, 1934). After entry, in susceptible hosts, the fungus grows rapidly in the cambial region and later decays the wood (Cartwright and Findlay, 1958; Peace, 1962). In roots expressing a hypersensitive reaction the entry process is the same, but the host response, resin flow in conifers, seems to stop the advancement of the infection and result in a localized lesion (Buckland, 1953; Patton and Riker, 1959; Rishbeth, 1972).

Earlier authors regarded the melanized hard-covered rhizomorph as a means of protecting mycelium as it grew through the soil. Garrett (1960, 1970) explained how the aggregation of hyphae present in the rhizomorph serves to increase the inoculum potential at the point of infection. The successful invasion of a root is not only a factor of the rhizomorph's ability to reach a susceptible host, but also its ability, through translocation of nutrients from a food base, to maintain a high infection potential at the infection site. The ability of the rhizomorph to infect depends on the quantity and quality of the food base from which the rhizomorph has arisen and the distance of this food source from the infection site (Garrett, 1956). The rhizomorph thus serves as a mechanism to transport infective material through the soil, and gives the fungus an increased ability to attack successfully.

Garrett (1951) and Adams (1972) have noted that the mere presence of ectotrophic rhizomorphs on a root system does not indicate that the rhizomorphs are pathogenic on that root system. In fact, rhizomorphs often grow along roots, or through root channels, as well as unattached through the soil (Redfern, 1968; Garrett, 1970).

The ability of infected material to support rhizomorph production has been used to assess the ability of the substrate to serve as a food base for A. mellea (Redfern, 1968). Redfern (1970) showed that

the number of rhizomorph systems and rhizomorph growing tips were greater from hardwood blocks than conifer wood blocks. He considers these characters demonstrative of the greater quality of hardwood stumps as a food base. Redfern states, "Only hardwood stumps can provide suitable bases from which the fungus can grow and infect young trees" (1968) and "conifer stumps apparently do not act as suitable food bases. . . " (1970). This conclusion is in contrast to Gibson (1960), who found infections in pine to have originated from cypress stumps, cut 20 years earlier, and Adams (1972), who demonstrated that large ponderosa pine stumps could serve as an adequate food base from which rhizomorphs extend, infect, and kill young pine trees.

Recently, Redfern (1973) acknowledged that coniferous wood could serve as a lethal food base, although a much poorer one than hardwoods, and that in areas lacking hardwoods coniferous stumps might serve as a means of disease buildup and spread. Rishbeth (1972), using field collected pine and hardwood root segments containing A. mellea and inoculated wood blocks, demonstrated a consistently higher yield of rhizomorphs from the hardwoods. In field trials hardwood stumps maintained the ability to produce rhizomorphs for a much longer time after felling than conifer stumps. Also, the ability of A. mellea isolates to produce rhizomorphs was reduced by prolonged growth in pine tissue. Rishbeth (1972)

concluded:

> By contrast [to hardwoods] pines in the area studied appeared to provide poor substrates for A. mellea, not only for indigenous strains but also for isolates from widely varied sources. . . roots of small pines killed by the fungus would seem unlikely to provide food bases good enough to perpetuate an outbreak of the disease, though the possibility of tree to tree spread in older pines cannot be ruled out.

While the fungus definitely has the ability to survive saprophytically in dead tissue, its ability to colonize dead tissue is relatively low. Garrett (1960) tested this ability by burying autoclaved willow wood segments in soil for different periods of time and then attempting to infect them with A. mellea. The value of the segments as a food source for A. mellea progressively declined. Yet, A. mellea may have a greater saprophytic ability than this experiment indicated, as it can compete with other wood destroying fungi, such as Fomes annosus (Fr.) Cke., and can replace Polyporus versicolor (L.) Fr. in very rotted tissue (Rishbeth, 1972). Garrett (1956, 1960) explained that as a specialized parasite, " A. mellea has an advantage in infecting living tissue, where it benefits from the exclusion of saprophytic competitors by host resistance (in his colonization experiments, green willow shoots constantly provided a better substrate for colonization than dead segments). In saprophytic colonization A. mellea's ability to decompose cellulose and lignin, substrates available to only a minor portion of soil fungi, may add to its competitive saprophytic ability. However, decomposition tests by

Scurti (1956) and Mikola (1955) indicated that lignin was only slightly affected by A. mellea.

The general decline over time in the value of dead segments as a food source supports Leach's (1937, 1939) control methods of ring barking individual trees a year before their removal to establish new tea plantations in tropical regions of Africa. The rather quick decline in resistance and subsequent death of the root system following ring barking allowed for early invasion by saprophytic wood destroying fungi, which seemed to exclude later colonization by A. mellea.

The ring barking technique did not work in the same manner when tested on hardwoods in a temperature region (Redfern, 1968). The ring barked trees were more quickly colonized than the non-ringed controls. While the stump's effectiveness as a food base probably decreased over time, due to rapid invasion and utilization of the tissue, A. mellea was not inhibited from entering the ring barked trees. The differences may be related to the absence of rhizomorphs in the tropical site. Leach (1939) stated that disease spread was by root contact. Under the temperate condition rhizomorphs were abundant and perhaps even growing epiphytically over the root systems of the ring barked trees; thus, the dying root systems of the ring barked trees could be reached more quickly by the radiating rhizomorph systems than by root contact alone.

Control Measures

Sokolov (1964) gives an excellent summary of control measures for A. mellea, and where and how they have been used. The techniques fall into three main categories: 1) biological control; 2) cultural manipulation; and 3) chemical control.

Biological Control

To the author's knowledge there has as yet been no direct use of antagonistic fungi (see Appendix I) to control A. mellea, although Sokolov (1964) does recommend it. He gives a list of fungi to use, how to obtain them, and how to apply them. The success with Peniophora gigantia (Fr.) Mass. to control F. annosus (Rishbeth, 1973), and the results of Artman and Stambaugh (1970) and Hunt et al. (1971) obtained by applying spore suspensions of antagonistic fungi in chain saw oil during cutting, may hold promise for A. mellea. However, Rishbeth (1973) pointed out that P. gigantia cannot replace F. annosus in very resinous roots, thus limiting effectiveness of the method after clear felling of heavily infected areas. Resinous root systems exist in some pine areas infected with A. mellea, and may limit the possible use of P. gigantia for biological control.

While no direct use of other fungi to control A. mellea has been reported, through ring barking and soil fumigation the population of

antagonistic organisms has increased, resulting in an indirect means of biological control (Leach, 1939; Bliss, 1951; Garrett, 1958; Ohr and Munnecke, 1973; see Appendix I).

Cultural Manipulation

One of the oldest recommendations is the surrounding of disease foci with trenches or ditches and thus blocking underground spread (Horne, 1914; Hendrickson, 1925; Reitisma, 1932; Sokolov, 1964). This technique has been used in orchards and non-forest plantations and, according to Sokolov (1964), was recommended by the (?) "U. S. Forest Commission." The problem is just where to put the ditch; how far in advance of the above-ground symptoms does infected material, capable of serving as inoculum, exist underground? To the author's knowledge, this information is yet undetermined. Also, managerial problems arise in either forests or orchards with ditches scattered hither and yon.

The prophylactic measure of removing diseased trees, uprooting and destroying the stumps, turning the earth over to a considerable depth, and destroying all root remnants has been recommended (Horne, 1914; Hendrickson, 1925; Reitisma, 1932; McGillivray, 1946; Sokolov, 1964). A delay period prior to stand re-establishment after removal of diseased material has also been suggested (Lavor, cited in Sokolov, 1964). The biggest problem with these procedures

is trying to balance the biological level and location of inoculum that must be removed to halt disease buildup and spread (an aspect we know little about) with an economic cost of removal justified by the crop's future value. In a forest situation the economic cost may be limiting; nevertheless, Twarowski (Sokolov, 1964) in Poland and Sokolov (1964) in Russia have recommended removal of inoculum.

A general recommendation in plantation establishment is, where possible, to replace susceptible species with more resistant ones. Rishbeth (1972) and Sokolov (1964) give good general discussions of this practice. Raabe (1960) has compiled a list of relatively resistant tree species that can be used for ornamental purposes where A. mellea is a suspected problem.

The general practice of good silviculture in planting or preferably seeding (Roth, 1964; Singh and Richardson, 1973) the right tree on the right site, or more important not establishing any species where it does not belong is also recommended. As Tarry's (1969) summary indicates, many workers have considered damage caused by A. mellea to be associated with some poor site factor. While the above procedure is simple to state, it may be difficult to practice in the field.

Other suggested control measures, such as pruning or girdling diseased roots, or drying and aeration of the root system around the collar region, may be applicable to individual high-value trees in

parks and gardens, but would generally not be appropriate for forestry practice. However, Sokolov (1964) reports successful control of A. mellea by exposing the root system around the root collar region in a young Siberian larch plantation.

Chemical Control

Since Sokolov's review (1964), more work has been done with methyl bromide (Ohr and Munnecke, 1973). Rishbeth (1972) mentioned chemical treatment of stumps, Denizet (1971) discussed control in a pine forest using iron chelate and maneb, and Cheo (1968) has tested 20 systemic chemicals in vitro. Of these, cycloheximide (actidione), 2,4-dichlorophenoxy-acetronitrile, acrizone chloride, and dimethyl sulfoxide (DMSO) showed promising fungistatic effects. He recommended testing DMSO as a penetrant in field studies.

Recently, a phenolic emulsion, Armillatox, has been placed on the commercial market in Great Britain for control of A. mellea. A test of the compound (Redfern, 1971), while indicating some toxicity to A. mellea, also revealed a phytotoxicity to sycamore seedlings. The effectiveness of Armillatox for control of A. mellea apparently needs additional confirmation.

III. THE DISEASE IN THE FOREST

Comparison of A. mellea Impact over a Fourteen Year Period

In 1957, Hunt[1] surveyed parts of the Glenwood Block, where A. mellea was obviously causing great damage, to determine components of the disease loss. Since Hunt and I had common objectives, the area was re-surveyed in 1971 to determine the present disease condition and changes occurring over time.

Both surveys covered parts of sections 2, 3, 10, and 11 of T6N, R11E, W.M. This is within the "saddle area." The reader is reminded that here distribution of the tree cover following logging of the old-growth pine is more varied than usual for the ponderosa pine forest type due particularly to the successive cuts and Armillaria activity. The present stand consists of a few widely scattered old-growth trees, patches of large ponderosa pine poles and small poles, among which occur scattered Douglas-fir and grand fir, and patches of seedlings and saplings. The 1971 survey of 1,040 acres included the 820 acres (201 plots) surveyed by Hunt. However, as the plots of neither survey were marked, the current data base probably rarely,

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[1] Hunt, J. S. 1958. Armillaria mellea root rot in ponderosa pine. Unpublished office report. U. S. Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

if ever, sampled the actual points providing Hunt's data.

Data were taken from 260 1/100 acre circular plots each located two chains from the next along north-south line transects spaced at 20 chain intervals. All trees were tallied by species, height class (0-5 ft, 5-10 ft, 10-15 ft, 15-20 ft, 20-50 ft, over 50 ft), and vigor (living, killed by A. mellea, dead from some other cause). Hunt's procedures were the same.

All plots that did not have at least one established conifer present were considered unstocked. Plots that contained trees killed by A. mellea (Figure 4) or fell within one of the recognized zones of an A. mellea infection center (Figures 2 and 3) were tallied as infected. The data in the tables labeled "infected plots" come from such plots, while those labeled "noninfected" come from the other (stocked) plots. All trees recorded as dead were considered to have died within the preceding 10 years. For convenience in tabulation of data, the height classes were assigned to maturity classes as follows: 0-5 ft = regeneration; 5-20 ft = saplings; greater than 20 ft = poles and larger.

Several useful comparisons emerged:

1. The proportion of the area with detectable disease remained relatively constant; 25% and 22% respectively (Table II).
2. The number of Armillaria-killed trees per acre in the infected portions of the stand decreased from 374 in 1957 to 230 in 1971.

Table II. Comparison of plots by condition classes, i. e., stocked, nonstocked, infected, and noninfected.

Plot description	1957		1971	
	No. of plots	%	No. of plots	%
All plots	201	100	260	100
Nonstocked	30	15	13	5
Stocked[1]	171	85	247	95
Noninfected[2]	128	75	193	78
Infected[2]	43	25	54	22

[1]At least one conifer (established regeneration or larger) present.

[2]Percentages based on stocked plots = 100%.

3. The volume lost to A. mellea over the entire stand increased from 12.2 cu ft/A/yr to 35 cu ft/A/yr. Volume loss to other causes remained relatively constant; 12.9 cu ft/A/yr in 1957 and 15.1 cu ft/A/yr in 1971 (Table III).

4. Trees killed by the disease were smaller in 1957 (76% were saplings or smaller, 24% poles or larger) than in 1971 (44% were saplings or smaller, 56% poles or larger) (Tables IVA and IVB).

5. Total stocking density (living and dead trees) was greater on infected plots than noninfected in both surveys, but fewer live trees were present on the infected plots than the noninfected plots (Tables V and VI).

6. Stand composition differs between infected and noninfected areas. In 1971, 38% of the stocked plots were represented by pole-sized trees, 40% of these plots were infected. Seventy-two percent of the infected plots consisted of pole-sized trees, while only 29% of the noninfected plots consisted of poles. A comparable breakdown of Hunt's data was not possible.

7. The number of conifers other than pine has increased from 85 per acre in 1957 to 108 in 1971.

8. Disease incidence varied among locations in 1971 (Figure 5). No distribution data are available for 1957.

9. Mortality by agents other than A. mellea in 1971 was the same

Table III. Distribution of stand volume (per acre) between living and dead trees. [1]

	Cubic feet	% of Total	Board feet
Living trees			
1957	1,883	88.2	8,207
1971	2,178	81.3	9,497
Trees killed by *A. mellea*[2]			
1957	122	5.7	537
1971	350	13.1	1,538
Killed by other causes[2]			
1957	129	6.0	570
1971	151	5.6	662
Total loss			
1957	251	11.8	1,107
1971	501	18.7	2,200
Total live and dead			
1957	2,134	100.0	9,314
1971	2,679	100.0	11,694

[1] Based on trees greater than 4 in. DBH (as converted from height classes). Volumes figured from St. Regis volume table for the Glenwood Block.

[2] All trees were judged to have died during the preceding 10 years.

Table IVA. Distribution of Armillaria-killed trees by size classes.

Size class	Trees killed (%)	
	1957	1971
Regeneration	15	10
Saplings	61	33
Poles & larger	24	56

Table IVB. Distribution of mortality (expressed as percent of all trees in each size class), by tree size and cause of death on infected and noninfected plots.

Size class	Infected plots						Noninfected plots	
	Armillaria-killed trees		Dead other causes		Total dead		Dead other causes (total dead)	
	1957	1971	1957	1971	1957	1971	1957	1971
	percent							
Regeneration	21	9	8	5	29	14	10	4
Saplings	35	22	13	6	48	28	23	10
Poles & larger	30	37	5	4	33	41	13	9
All sizes	30	24	10	5	40	29	16	6

Table V. Stocking of living and dead trees on A. mellea infected and noninfected plots.

Plot description	Living trees per plot		Dead trees per plot		Total trees per plot	
	1957	1971	1957	1971	1957	1971
	Average number					
Infected	7.6	6.8	4.9	2.8	12.5	9.6
Noninfected	8.1	7.6	1.5	0.5	9.6	8.1

Table VI. Ponderosa pine stocking (per acre) by size class on infected and noninfected plots in 1971.

	Size class (no. per acre)			Total
	Regeneration	Saplings	Poles & larger	
Infected plots				
Live trees	185	190	196	571
A. mellea-killed trees	24	72	130	226
Trees dead other causes	13	21	13	47
Total trees	222	283	339	844
Noninfected plots				
Live trees	370	170	104	664
Trees dead other causes	16	22	12	50
Total trees	386	192	116	694

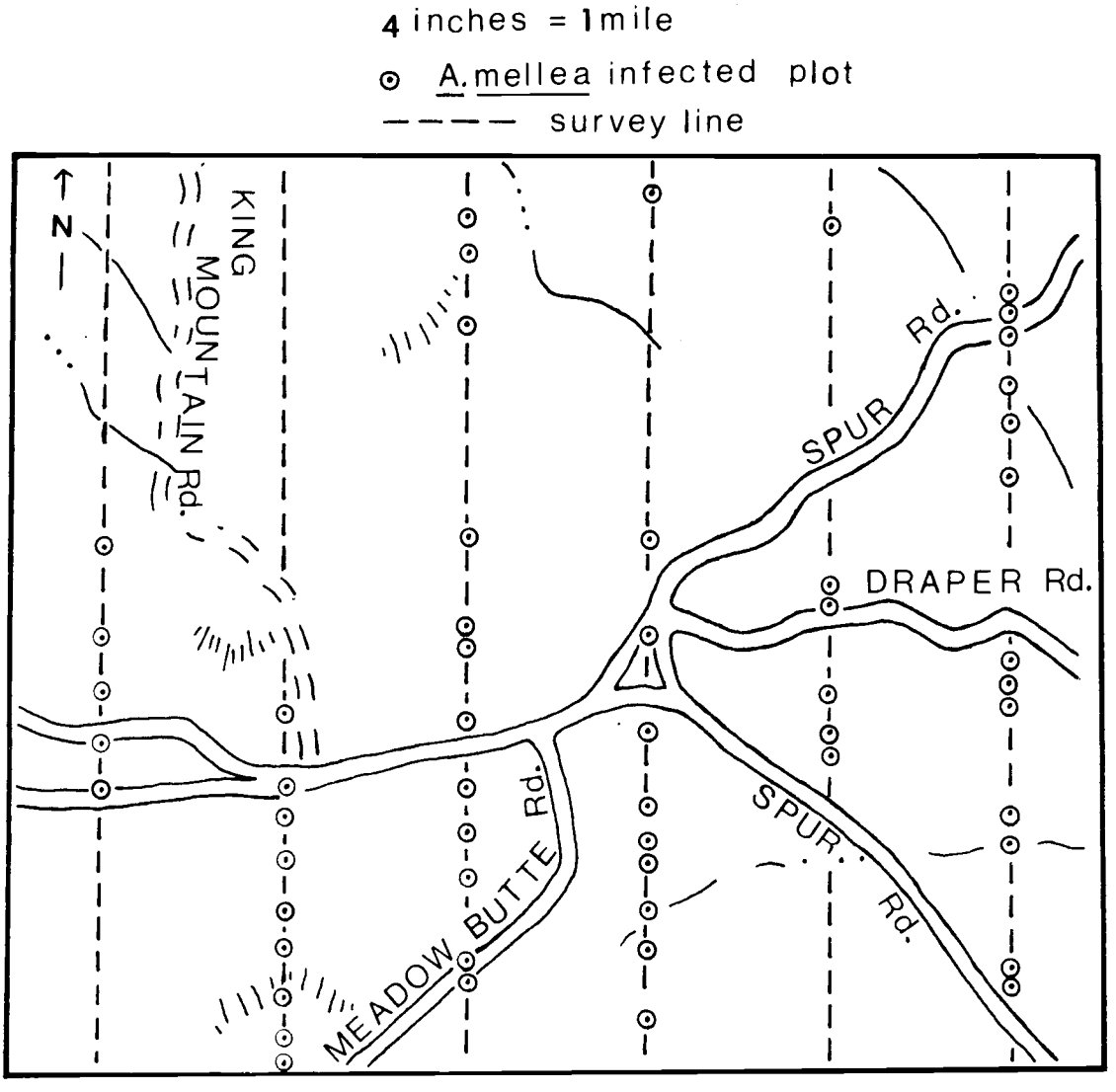

Figure 5. Map of area surveyed showing distribution of infected 1/100 acre plots, and location of survey lines.

on infected and noninfected areas, 47 and 50 trees per acre respectively (Table VI), or 5% and 6% respectively (Table IV).

Discussion

While the amount of area infected was nearly constant from 1957 to 1971 (Table II), the volume lost to A. mellea increased (Table II), and the number of trees per acre killed by A. mellea decreased. This is explained by the shift of mortality from the smaller size classes to the larger with time (Table IVA). To understand why more pole-sized trees and fewer saplings were dead in 1971 than 1957, and why the opposite was true in 1957, one must consider the stand history.

Prior to logging, the stand had a rather low density of old-growth trees (9 to 10 per acre), and the per acre volumes ranged from 15,000 to 25,000 bd ft. Considerable regeneration had become established between the scattered old-growth (see discussion of the "park effect," p. 78). The first logging in 1941-42 removed about 1/3 of the old-growth. A. mellea was first noticed a few years after logging in the abundant regeneration. In 1953 the second harvest removed much of the remaining old-growth. By the time of Hunt's survey (1957), infection centers developing since initial cutting were rapidly expanding, and new centers were becoming noticeable since the 1953 cutting. Consequently, Hunt found an extremely large number

(374 per acre) of trees, but small in size, killed by A. mellea on infected sites. The killing was especially critical since it occurred in patches rather than scattered to cause useful thinning through the stand. However, the volume loss was just beginning to have economic impact (12.2 cu ft/A/yr, Table III as few trees were yet of merchantable size (76% of all the trees recorded as dead from A. mellea in 1957 were saplings or smaller, Table IVA). The volume in 1957 was around 8,200 bd ft/A, about 42% of the original old-growth stand. This reduced volume was being further diminished by 6% (Table III) in patches by A. mellea.

Because of the continuing losses to disease, St. Regis cut nearly all the remaining old-growth between 1960 and 1965.

By 1971, some of the A. mellea infection centers had been active for nearly 30 years, creating openings an acre or more in size. Others had been active since the 1950's and some became active in the 1960's. By 1971, the advance regeneration had grown to near-merchantable size and was being lost to A. mellea at a rate of 35 cu ft/A/yr (Table III). This was three times the 1957 rate, but was accompanied with little increase in infected acreage, through death of 40% fewer, but much larger trees.

The difference in stocking by size classes between infected and noninfected portions of the stand in 1971 (Table VI) reflects general stand conditions. It is important to realize that on one hand the

noninfected stocked portions of the stand (78% of the total stocked area, Table II) consist of three tree cover types: 1) areas of as yet uninfected pole-sized trees; 2) areas formerly infected, when the trees were smaller, that now contain few poles and show almost no disease; and 3) areas released to regeneration by logging the old-growth and thus removing the competition (see discussion of the "park effect," p. 78). On the other hand, infected portions of the stand consist basically of types one and three above, only infected. Areas of pole-sized trees (type 1 above) differ greatly in amount between the infected and noninfected portions. Seventy-two percent of the infected portion consists of pole-sized trees, while 29% of the non-infected portion does so.

The stocking reductions noted are related to the continued killing of trees on present and past infected portions of the stand. While the relation of initial stocking density (as long as it is sufficiently dense to support fungus spread from tree to tree) to disease development is unclear, the fungus will reduce stocking to a point where parasitic activity of the fungus can no longer be supported. This level is a density much lower than the stocking in the presently noninfected portions of the stand.

Present (1971) disease activity is mostly in pole-sized trees on the outer edges of infection centers that are continually expanding. Why then were the percentages of the area stocked and the total area

rated as infected in 1971 the same as that in 1957 (22% versus 25% and 21% versus 21% respectively)? Apparently the portions of the stand presently recognized as diseased are a function of: 1) the rate of infection center expansion (see p. 88); 2) the rate of decline in evidence of infection (a point is reached in disease progress at which areas that were once infected are no longer infected, or at least no longer detectable as being infected by the survey procedures employed); 3) the rate of new tree establishment on past infected areas; 4) the rate of occurrence of new infection centers; and 5) the pattern of distribution of the pole-sized stand.

Since the time of the first cut (1941), the area over which stand destruction has occurred has been cumulative; yet parts of this area now contain living young trees and are no longer recognizable as diseased. While the rate of infection center expansion is such that in two years, 43% of the living trees on the outermost edge of expanding infection centers are killed (p. 91), this potential rate is limited by the extent and distribution of the pole-sized stand in which 72% of the disease activity occurred in 1971. In some instances, infection centers have converged, leaving few trees to be killed. In other cases, expanding infection centers have reached the limits of the patch of poles in which they started. These factors, acting together, have kept the area of current disease activity constant, on the basis of percentage of area involved, with the 1957 level.

Some believe that as the result of exclusion of fire, root rot damage, and the proximity to fir stands, the "saddle area" will change from pine to a mixed Douglas-fir, white fir, pine forest. This trend appears in progress. Eighty-five firs per acre were present in 1957 and 108 in 1971. As Douglas-fir appears less damaged by A. mellea than is ponderosa pine, one wonders what effect, if any, this change might have on future disease development.

Much has been written concerning the association between A. mellea and other causes of death (see p. 16). Some have stated that A. mellea predisposes trees to attack by other agents (Peace, 1962). If this is happening in the "saddle area," one would expect more mortality from other causes on the A. mellea infected portions of the stand than on the uninfected portions. This has not proven to be the case, however, as the number and percent of trees killed from other causes in 1971 was the same in both instances; 50 and 47 trees per acre respectively (Table VI) or 5% and 6% (Table IVB).

While the disease pattern that now exists in the "saddle area" is in part explained by the history of successive logging, this does not imply that other logging schemes would have resulted in less disease loss. In another stand on the Glenwood Block, approximately 7 miles from the "saddle area," the old-growth forest was clearcut in the 1920's. The stand now occupying this site also is riddled with A. mellea, but the pattern of disease is different. Here, the disease

has kept the sapling stand in a chronic, static state with mortality equaling regeneration. The productivity of this area has been reduced at least as greatly as the "saddle area," but the characteristic expanding infection centers are not evident. Instead, mortality is present throughout the lightly stocked stand. While in the "saddle area" some of the trees present in the young stand at the time of initial cutting have continued to develop into their present diseased, pole-sized status, on this other site the fungus has not allowed stand development at all.

It is important to realize that the fungus is native to the forest in which it so "suddenly" appears. In the "saddle area" one can visualized the outline of much older A. mellea infection centers than are now clear. If Adams' (1972) ideas on A. mellea colony development and expansion are correct, the fungus could have been inveterate in the stand for literally thousands of years.

At Glenwood the present A. mellea "outbreak" is an expression of a change in conditions that favor the usually moderately aggressive A. mellea. This condition is evidently related to harvesting of the old-growth, which left a large amount of potential inoculum, root material, in the soil. Yet similar logging procedures have been conducted throughout the pine region, and, while outbreaks of A. mellea do occur elsewhere (Weiss and Riffle, 1971; Adams, 1972), one is

hard pressed to find, under any management scheme, a situation of comparable severity.

The Distribution of A. mellea Clones

Adams (1972, 1974) demonstrated that A. mellea, like many other hymenomycetes, forms lines of demarcation[2] (Figure 6) between paired isolates of genetically distinct origin. Isolates lacking basic genetic differences do not form lines when paired. Adams used line formation to test for the existence and distribution of various A. mellea clones in the pine forest. The method appeared promising for investigation of the historical distribution of A. mellea clones in the "saddle area."

Isolates were obtained from infected pine roots and rhizomorphs scattered throughout a 10-acre control experiment in the "saddle area" in which disease distribution had been mapped (see p. 95). Additional cultures were obtained in the general vicinity from eight trenches where A. mellea infected roots had been extensively excavated (p. 61). These cultures were compared with an isolate (designated H) from hazel (Corylus cornuta Marsh) roots, from the more distant Plateau Block, and one (designated S) from the stipe of

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[2] This is a descriptive term for dark hyphae which appear along the area of meeting of two isolates paired in culture.

Figure 6. Enlargement from the region of meeting (arrows) of paired cultures of A. mellea. Note the lack of a distinct line of demarcation in A (like cultures) and formation of a distinct line in B (differing cultures).

a sporophophore collected from a killed pine sapling from Pringle Butte, Deschutes County, Oregon.

Isolates were paired in petri dish cultures either on a standard growth medium or a 40-gram resin medium (see p. 193). The combination cultures were incubated in the dark at 25°C until the margins met along a broad face (about 3 weeks on the resin medium), and examined with a light box (the type used in virus serology work) for detection of line formation. All pairings were tested twice and most were repeated three or four times.

The H/S pairing regularly formed lines of demarcation. Isolates obtained across individual A. mellea infection centers showed no line formation when paired in all possible combinations against other isolates from the same center. In one instance, the sources of cultures extended over 80 ft from an old-growth stump, the presumed origin of the infection center. Contrarily, all isolates obtained across individual infection centers showed distinct line formation when paired against either the H or S isolate.

None of the 20 isolates tested from the 10-acre mapped area formed demarcation lines when paired among themselves, even though their origins represented several distinct infection centers. All formed lines of demarcation with H or S isolates. Isolates from the four excavated infection centers in the "saddle area" exhibited no line formation when paired against each other, or isolates from the

10-acre area (one of these infection centers was 1/2 mile from the 10-acre area). Isolates from an excavated infection center approximately 2 miles away, outside the "saddle area," formed lines of demarcation when paired with isolates from the "saddle area." Isolates from another excavated infection center, 4 miles away, formed demarcation lines when paired with "saddle area" isolates, and isolates from the previous excavated infection center. These latter two sources were approximately 3 miles apart.

This evidence indicates that the "saddle area" is colonized by a single A. mellea genotype, strengthening the idea that pathogenic spread has been basically vegetative in nature and that spores have played a very minor role in disease development in the "saddle area." Raabe (1966) showed that single spore isolates of A. mellea obtained from a single basidiocarp of A. mellea exhibited as much genetic diversity as did isolates obtained from infected plants throughout the state of California. Thus, one would expect that if spores had served to establish individual infection foci in the "saddle area," lines of demarcation would result between isolates from different infection centers.

If, in times past, the fungus has moved through the stand at the calculated growth rate (see p. 92) of 2.9 ft/yr, then the fungus has been spreading for at least 455 years, the time interval necessary to cross 1/2 the greatest distance between sources of isolates not

forming demarcation lines. Likely, 455 years is a conservative estimate; nevertheless, it still indicates the presence of the fungus in the natural stand prior to modification by man.

Adams (1972, 1974) indicated the presence of three A. mellea clones in his Pringle Butte area study, two of which were isolated from killed pines. We have found only one clone in our essentially equivalent "saddle area." Other isolates were encountered only upon moving out of the "saddle area."

Sporulation of A. mellea

An initial assumption concerning A. mellea in the study area held that sporulation has played little, if any, role in the epidemic disease development. A. mellea sporophores are rare in the pine forest east of the Cascade Mountains (thirty years' observation failed to disclose sporophores at Pringle Falls in central Oregon[3]). In England, Rishbeth (1970) reported that spore infections of stumps were rare, even when high concentrations of spores were available.

Exceptional conditions occurred in the fall of 1973. The October rainfall was exceptionally heavy and A. mellea fruited

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[3] L. F. Roth, unpublished communication.

abundantly throughout the "saddle area," sporophores were found at Pringle Falls for the first time[4] (Figure 7).

The majority of the fruiting was on stump stubs and bases of trees long since killed by A. mellea. However, in one case sporophores were found on two dead trees, that had been alive in 1971. The presence of sporophores on such trees indicates the rapidity with which root systems can be sufficiently colonized to support sporulation.

While it is now known that occasionally abundant fruiting does occur in the pine stands east of the Cascades, there is still no evidence that spores perform a significant role in the disease cycle. Their lack of importance in local dispersal is supported by Adams (1972, 1974) and clonal analysis of the present study. Perhaps spores serve to establish rare infection foci that then expand, possibly for centuries, through host roots and rhizomorphs.

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[4] L. F. Roth, unpublished communication.

Figure 7. Armillaria mellea sporophores on a small stump from a pre-commercially thinned stand of ponderosa pine on Pringle Butte, Deschutes Co., Oregon. Collected by Lewis F. Roth, October 1973, photo by Kay Fernald.

IV. THE DISEASE IN THE STAND

Relation between Above-ground Patterns of Disease Occurrence and Distribution of Infected Root Material in *A. mellea* Infection Centers

The pattern of disease in expanding *A. mellea* infection centers was described earlier. Usually trees expressing similar symptoms occur in a series of more or less complete concentric zones, surrounding old-growth stumps. These stumps are presumed to be the origin of each infection center. While the differing above-ground appearances from zone to zone leads one to speculate on the nature of the root material beneath zones, the condition underground has not been previously described. Root condition among zones in several infection centers was studied by pulling stumps and by root excavation.

Methods

Stump Extraction. In the fall of 1971, on the site of another experiment (p. 95), where cover and infection pattern had been mapped, stumps were extracted with the aid of "chokers" and a rubber-tired log skidder (Figure 8) across an enlarging *A. mellea* infection center that had not yet coalesced with others. These stumps were from a rough sector, laid out from the stump of an old-growth tree cut in 1941, at the presumed origin of the infection

Figure 8. Extraction of stumps for study by pulling with an articulated, rubber-tired skidder.

center, outward across the infection center into the "healthy" stand, a distance of 168 ft. The sector was divided into consecutive 12-ft zones and all living and dead trees, snags, and stubs within each zone were numbered and described by size, condition, and location. The root systems from each of the 12-ft zones were piled separately, and in the summer of 1972, were examined for extent and nature of A. mellea on them. The infection condition of each individual root system was related back to zone location relative to the old-growth stump (Table VII) and to above-ground disease symptoms.

Root Excavation. Roots were excavated under two systems. In the first, the transect was established in 1972 radially from an old-growth stump (cut in 1941, and presumably marking the origin of infection) outward across an active A. mellea infection center into the healthy-appearing stand. The transect was oriented east-west. All living and dead trees, snags and stubs within the transect were recorded by size, condition, and location. A trench was dug (with a "D-7" bulldozer) 13 ft wide, 6 ft deep, and 144 ft long, down the middle of the transect (Figure 9). This provided lateral access to tree root systems to either side and provided a convenient dump for the spoil soil.

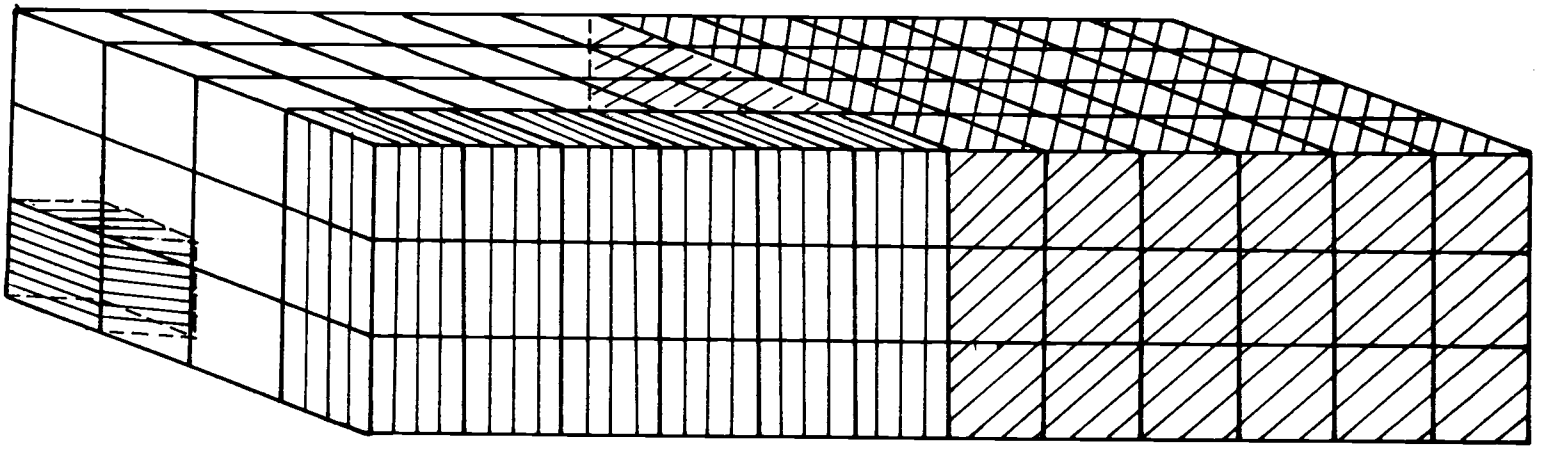
For purposes of orderly procedure among excavation crew members and for subsequent convenience in interpretation of data, the south face of the trench (right side, Figure 9) was divided into 24

Figure 9. View along the large transect from the old-growth stump at the origin (right foreground B) back through the unstocked zone, zone of stubs and snags, zone of present disease activity, and finally the healthy stand. (A) before and (B) after trenching.

major blocks, each 6 ft horizontally (along the trench) by 3 ft (vertically) by 4 ft (back from the face) (Figure 10). Each of these blocks was divided further into four soil units each measuring 6 ft x 3 ft x 1 ft, and numbered consecutively away from the face of the trench. Each soil unit was excavated from the face, one cubic foot at a time; each cubic foot became a numbered sub-sample unit (Figure 10).

Each cubic foot was excavated with a forked hand trowel (Figure 11) with near-archaeological care (Figure 12). To mark the limits of each cubic foot, 16-inch lengths of concrete reinforcing rod were driven into the soil surface and trench face (Figure 12). The upper 3 ft of the entire south face of the trench was excavated, 1 ft at a time, back from the face for 6 ft. As explained below, for the entire trench, the first two 6 ft x 3 ft x 1 ft soil units back from the face were not used. The 1/4 of the trench that lay within the healthy-appearing stand was excavated 10 ft back from the face.

Field data recorded for each cubic foot included: presence or absence of A. mellea infected roots and root lesions, presence or absence of root grafts or root contact at A. mellea attack sites (lesions), presence of insect activity, presence of charcoal, and occasionally other information. All root material from each 1 cu ft was clipped, placed in a bag with the number of the cubic foot from which it came, and air dried (roots were clipped flush to the base of



1 cu ft sub-sample unit

6 x 3 x 1 ft soil unit

6 x 3 x 4 ft soil block

Figure 10. Diagram of excavation system showing two 6 ft x 3 ft x 4 ft soil blocks, eight 6 ft x 3 ft x 1 ft soil units, and 1 cu ft sub-sample unit.

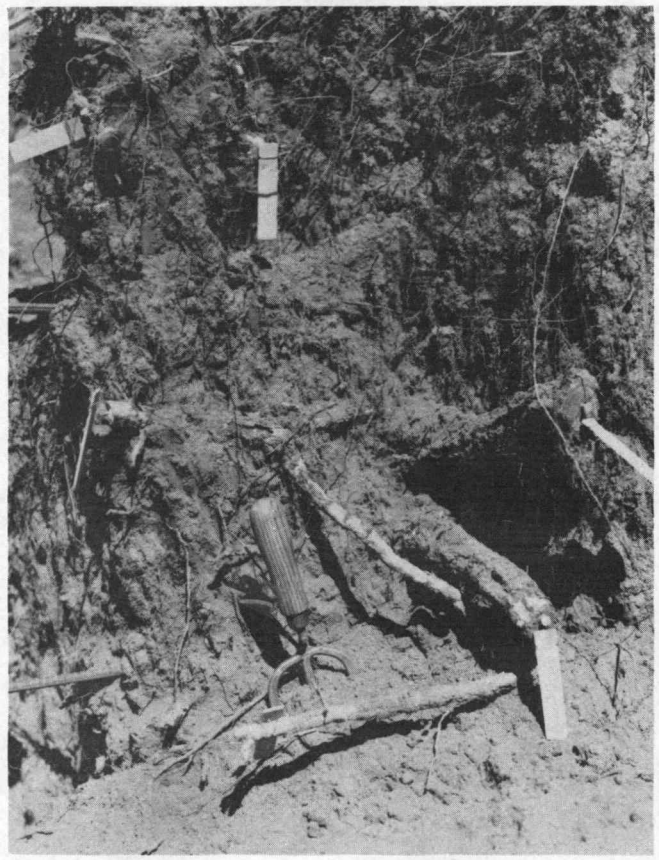


Figure 11. Detail of excavated face showing distribution of roots. Tags bear numbers allowing roots to be traced without confusion. Most excavating was done with a forked hand trowel (arrow).

Figure 12. Excavation procedure. Note 16 in.lengths of concrete reinforcing rod (arrows) driven into the soil to mark the limits of each cubic foot. Paper bags, on the ground, receive root samples, spoil soil falls below the operating face.

the tree's tap root, the tap root itself was not removed). After air drying, the roots were re-examined for the presence or absence of A. mellea infected roots and lesions, divided into three diameter classes (less than 0.5 cm; 0.5 cm to 1.5 cm; and greater than 1.5 cm), and weighed in grams by size class.

To facilitate initial examination of intact root systems and eliminate the possible influence of disturbance caused during bulldozer excavation on root weights,[5] roots within the first 2 ft back from the face of the trench were not bagged or weighed.

Thus, for the entire length of the trench, root weights, determined from approximately 3,000 individual cubic foot sub-sample units, were obtained for consecutive soil blocks measuring 6 ft along the trench, 3 ft in soil depth, and 4 ft back from the face (Figure 10). The root weights presented in Figure 14 (points) are the mean value for the four 6 ft x 3 ft x 1 ft soil units (Figure 10).

Figure 14 does not include data from the most distant soil blocks in the healthy-appearing stand.

The root data obtained were related to above-ground stand conditions along the transect. Stocking was evaluated by calculating basal areas of all living and dead trees, snags, and stubs on each

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[5]The 1972 disease field trip of the Washington and Oregon Pest Action Council included the site of this excavation. The roots within the first 2 ft back from the face of the trench were left intact for examination by participants in this field trip.

consecutive 6 ft x 4 ft surface (same consecutive surface areas as the 6 ft x 4 ft x 3 ft soil blocks from which root weights were determined), plus those trees on an additional 1-ft strip along the north and south side of each block. Thus, the root weights for each block came from a volume with a surface area of 6 ft x 4 ft, while the stocking on each block was determined by basal area present on a 6 ft x 6 ft surface area. In no case did this adjustment result in the counting of the basal area of any tree twice, as consecutive blocks were east or west of each other. The adjustment is intended to account for the influence of major roots extending into the excavated area from trees outside the area.

Trees outside the excavated blocks influenced root mass within the block, and most likely the influence was not the same for healthy trees as for those long-dead. The 1 ft additional enlargement on two sides of each excavated soil block for surface area calculations was chosen as a reasonable estimate. For example (Figure 14), in between locations 84 and 90 ft (distances from the stump), a tree with a basal area of 1.07 sq ft (14 in. DBH) is not included in the basal area figure given (bar) because it was just outside the surface area; yet here in the healthy stand the root mass present (point) was undoubtedly increased by this large tree. The averaging of root weights to obtain a mean value for the four 6 ft x 3 ft x 1 ft soil units

was also intended to reduce the influence of stocking location within each block.

The variations in stocking along the transect somewhat obscure the root weight/distance from the old-growth stump relationship (Figure 14). Figure 15 was arranged to remove the effect of stocking differences by placing the four areas of tree concentration along the transect on an equal basal area basis. The ordinate figures were obtained by dividing the total root weight for each two adjacent 6 ft x 4 ft x 3 ft soil blocks around each area of tree concentration by the basal area calculated for the two adjacent blocks (see above).

Figure 16 compares the data obtained from the stump extraction procedure with the total root mass in each of the first 12 adjacent 6 ft x 4 ft x 3 ft soil blocks outward from the old-growth stump.

As mentioned above, the trench was excavated back 10 ft in the healthy portion of the stand. This was done to obtain information on root distribution in the normal pine forest; a subject about which little is known. For the same reasons as above, the first two soil units back from the trench face were not utilized. Table IX presents descriptive statistics of root weights per cubic foot of soil within the healthy stand by size classes and depth in the soil profile. The size classes are based on root diameters. Table VIII associates the basal area and total root weight by size classes in each successive soil block in the healthy stand. For some of the 6-ft units along the

trench, root weights were recorded back 7 ft and for others, 8 ft; in addition, some root bags were misplaced or destroyed in handling and weights were not recorded. These two factors account for the differences in the number of cubic feet from which data were recorded in each soil block in Table VIII.

In the second system of root excavation, (smaller trench transects), work was concentrated on the transition zone at the limits of the infection center. Data from the stump extractions and the trench transect both indicated that, in passing through the margin of an A. mellea infection center into the surrounding healthy-appearing stand, a rather sharp change occurs underground in the relative amounts of infected and healthy root material. To ascertain if this change is consistent and to determine more precisely the distribution of infected and healthy roots across the border, eight more transects were excavated in sapling and pole-sized trees. Trenches were dug with a bulldozer across the infection center, starting inside the zone containing stubs and long-dead trees (see p. 5) and extending into the healthy-appearing stand (trenches averaged about 40 ft in length). Root systems were excavated by hand (Figure 13) back from the trench face 4 to 6 ft, and to a depth of 2 ft along the entire length of the trench. Roots were left in place rather than being removed and weighed. The distribution of healthy and infected roots was tabulated

Figure 13. Excavation of tree root systems across the border of an A. mellea infection center. Bulk soil from the foreground was removed with a bulldozer.

and graphically recorded in relation to the above-ground disease symptoms.

At various points along seven of the excavated transects, individual infected root segments (4-6 in. long) were clipped, buried in moist quartz sand in plastic bags, incubated at room temperature for 6 months in the dark, and then examined for rhizomorph production. This method is similar to the procedure employed by Redfern (1968) to assay the value of different substrates to serve as food bases for A. mellea. The method demonstrates whether or not the fungus is still viable, and sufficiently active in the substrate to produce the assumed infection structures, rhizomorphs. Since rhizomorphs are considered necessary for infection (Thomas, 1934; p. 133), their formation in this test is taken as evidence that the segment could have served as a source of inoculum for new infections.

Results

Stump Extractions. The stump extraction data (Table VII) show an absence of recent A. mellea mortality in the inner four sample zones (up to 48 ft from the old-growth stump), though some lesions occur on small trees. Number of lesions and tree mortality increase in sample zones 5 and 6, where above-ground cover consists of stubs and broken snags. The percent of trees infected reaches its highest level in zones 7, 8, and 9 where ground cover consists of standing

Table VII. Occurrence of A. mellea on roots of sapling and pole-sized ponderosa pine, tabulated by 12 ft zones along a sector extending across an A. mellea infection center from a point of presumed origin, the stump of an old-growth tree cut in 1941, outward 168 ft into a healthy appearing stand.

Distance from point of origin (ft)	Zone no. from point of origin	Number of					Percent of	
		Trees	Dead trees	Dead with Armillaria	Trees with Armillaria	Lesions	Trees infected	Basal area infected
0-12	1	3	0	0	2	10	66	99
12-24	2	4	0	0	1	8	26	15
24-36	3	9	0	0	1	2	11	2
36-48	4	3	0	0	0	0	0	0
48-60	5	2	1	1	1	2	50	98
60-72	6	4	3	3	3	13	75	98
72-84	7	4	4	4	4	7	100	100
84-96	8	6	3	3	6	21	100	100
96-108	9	11	5	5	9	27	82	93
108-120	10	20	6	4	7	50	35	50
120-132	11	15	3	1	4	20	27	71
132-144	12	21	6	6	7	11	33	18
144-156	13	27	7	7	11	30	41	45
156-168	14	15	0	0	2	7	13	15

snags. Zones 10 and 11 represent the region of present disease activity, the number of attacks (lesions) is high even though the percent of trees attacked is lower than in the preceding region. A marked decline in percent of basal area infected and tree mortality occurs in the transition region (sample zones 12 and 13) and a low level of attack is reached in the healthy-appearing forest, sample zone 14.

Large Trench Transect. Distribution of root material and tree stocking along the excavated transect are shown in Figure 14. The vertical line at 84 ft marks the margin of the infection center as determined above ground from symptoms on trees. Underground, infected roots extend beyond this mark.

Much less root material is present in the infected forest than the healthy. Comparing the peaks in root weights, representing the excavation blocks where trees and thus root material were concentrated, there is a definite decrease in the amount of root material with increasing distance from the healthy forest. The same relationship can be seen by comparing the blocks in which basal area is low or lacking.

From 0 to 24 ft outward from the old-growth stump, the amount of root material progressively decreases. The roots present are either decaying roots of the old-growth stump or small, live roots less than 1.5 cm dia, containing some A. mellea lesions. The

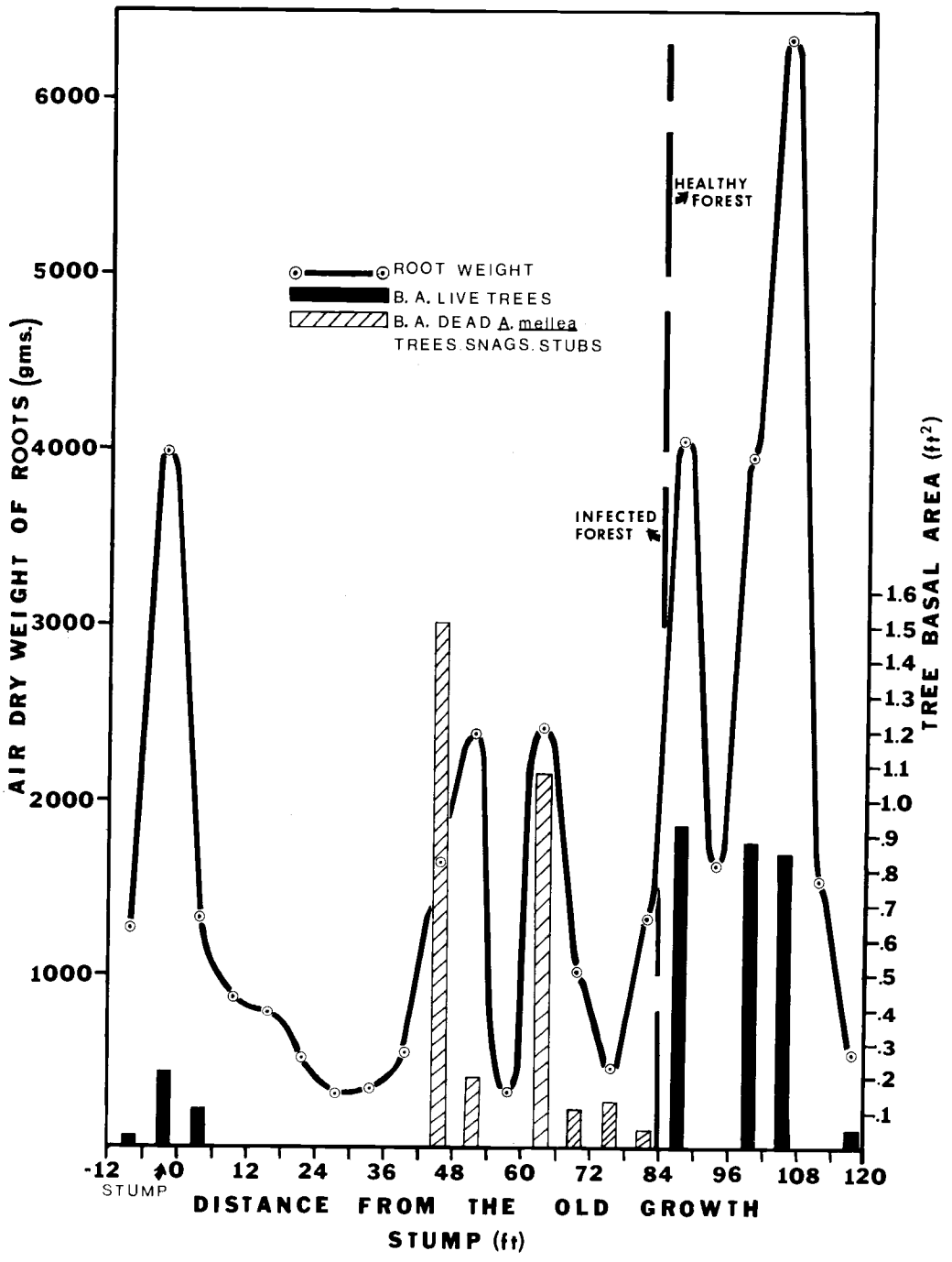

Figure 14. Average root biomass (points) and basal area of living and A. mellea-killed trees (bars) in consecutive 6 ft x 3 ft x 1 ft soil units excavated along a transect extending from an infected old-growth stump, across an A. mellea infection center into the surrounding healthy forest. (For explanation of ordinate and abscissa values, see p. 58).

persisting roots of the old-growth stump (cut in 1941) still contain viable A. mellea after more than 30 years as evidenced by field symptoms and positive isolations.

The area from 24 to 42 ft is essentially free of large pieces of root material. While the root clipping procedure called for cutting the roots flush to the tap root, and not removing the actual tap root (p. 54), in this 24 to 42 ft region all root material was removed as no identifiable tap roots remained. Large roots present are in a state of advanced decay, however, the few that remain still contain A. mellea as evidenced by field symptoms and signs and by positive isolations. The majority of roots present are small (less than 1.5 cm dia), live roots of small recently established trees, relatively few of which support A. mellea.

Between 42 and 72 ft almost no live roots exist and nearly all cubic feet that contained roots, contained infected roots. In contrast to the sparsity of large pieces of root material between 24 and 42 ft, 84% by weight of the root material between 42 and 54 ft was of the largest size class (greater than 1.5 cm dia).

Between 72 and 84 ft, the majority of the root material present consisted of live roots, but nearly all cubic feet of soil that contained roots, had some A. mellea-infected roots. The limit of A. mellea-infected roots was encountered 100 ft from the old-growth stump.

The variations in stocking along the transect somewhat obscure the relationship of root weight/distance from the old-growth stump (Figure 14). In Figure 15, the effect of stocking differences is removed by placing the four areas of tree concentration along the transect on an equal basal area basis. The figure shows an increase in root biomass with distance from the old-growth stump, along with a change in the nature of root material present at each location (see legend for Figure 15).

Figure 16 relates the data up to 66 ft obtained from the extracted stumps (Table VII) to that obtained from the excavated trench (Figure 14). Both sets of data were collected from A. mellea infection centers around 1941 old-growth stumps. Frequency of infection of the extracted stumps parallels root weights from the excavation with increasing distance from the old-growth stump. In the area of lowest frequency of infection, 18 to 42 ft, no large individual pieces of root material are present.

The "healthy forest" portion of Figure 14 shows that the distribution of root biomass is closely associated with the location of the trees. In Table VIII, the root weights are given by root size and the basal area of trees present on each block within the non-infected portion of the transect. The distribution of the small and medium sized roots is independent of tree location (weights are relatively constant from block to block), while the mass of larger roots is highly

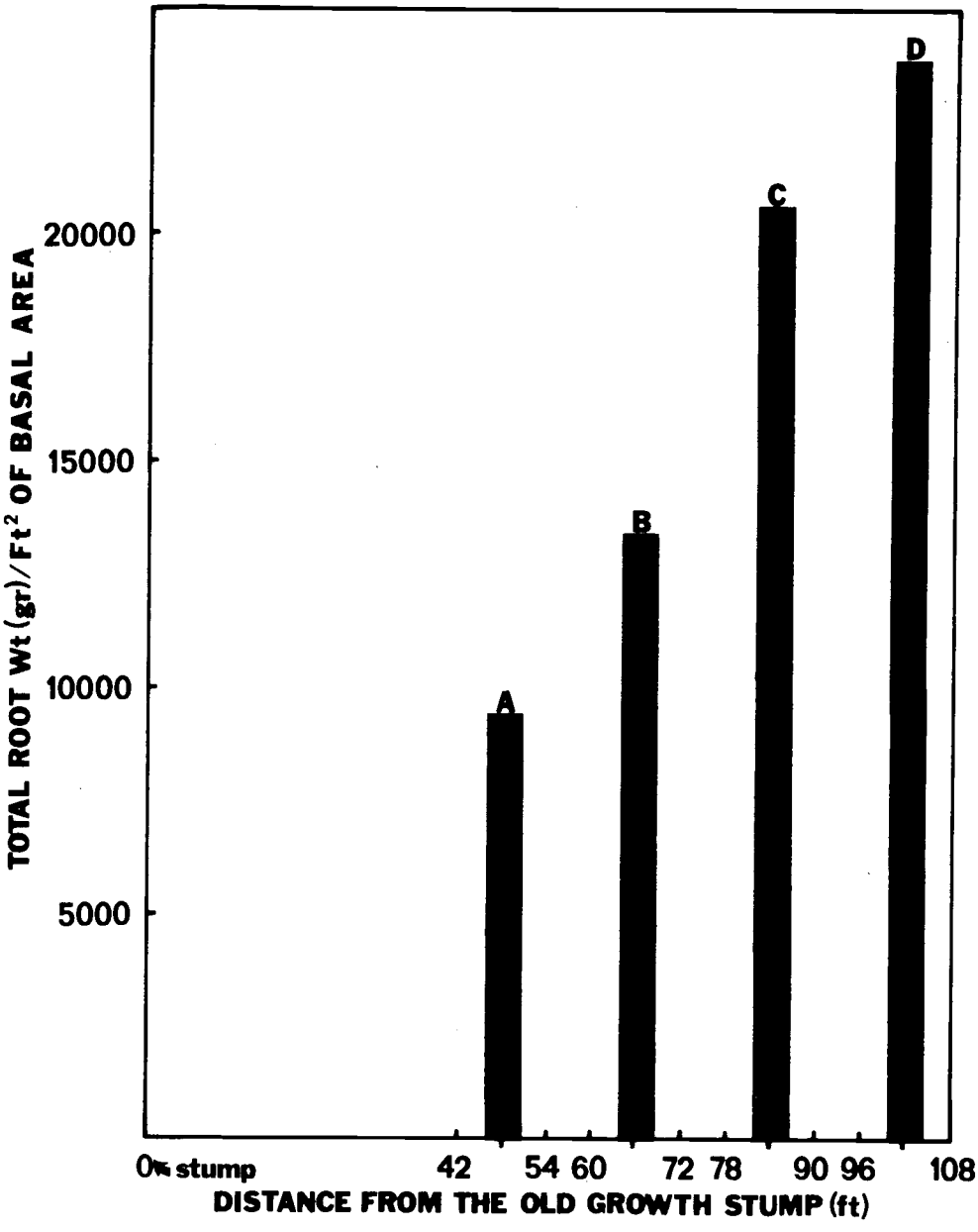

Figure 15. Distribution, after adjustment for basal area, of root biomass at four locations of tree concentration (peaks of the curve in Figure 15) along the transect. (A) Long-dead, A. mellea-killed trees; 90% dead, A. mellea-infected roots. (B) Recently dead A. mellea-killed trees; 90% dead, A. mellea-infected roots. (C) Margin of A. mellea activity; 60% live roots, 40% dead roots, 30% A. mellea-infected roots. (D) Healthy forest; mostly live roots, no A. mellea infected roots. (The break down as to root condition is an estimate.)

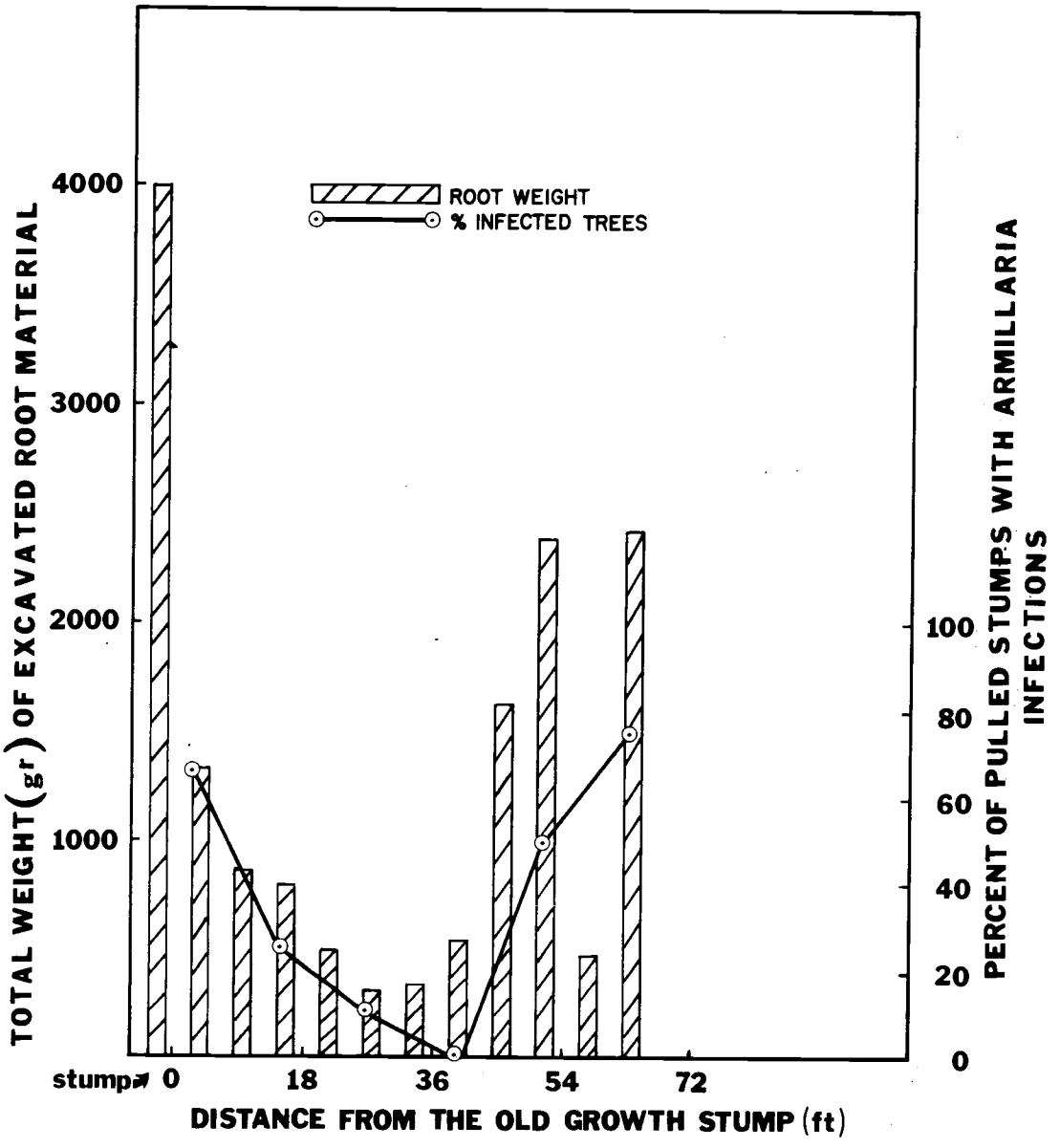

Figure 16. Distribution of total excavated root biomass in relation to frequency of A. mellea infection across two separate A. mellea infection centers. Both centers developed around 1941 stumps.

Table VIII. Distribution of tree basal area and of total excavated root weight by size classes among the seven consecutive 6 ft x 3 ft x 7(8) ft soil blocks in the "healthy forest" portion of the transect.

Block no.	No. of cubic feet sampled	Tree basal area (sq ft)	Root weights (g) based on root diameter class (cm)		
			Less than 0.5 cm	0.5-1.5 cm	Greater than 1.5 cm
			grams		
1	122	0.400[1]	916	1,942	7,653
2	122	0.394	918	2,200	1,936
3	124	0.049	916	1,790	1,237
4	144	0	1,165	1,838	6,929
5	142	1.64	917	2,497	31,775
6	141	0.88	610	2,735	24,428
7	141	0	761	2,517	10,259

[1] The 0.400 value is an estimate.

dependent on tree location (weights vary substantially between blocks with low and high basal area). Of all root material present, 79% by weight consists of roots greater than 1.5 cm dia.

Table IX presents descriptive statistics for root weights per cubic foot of soil in the healthy portion of the transect, by root size and depth in the soil profile. The variation, as indicated by standard error of mean, standard deviation, coefficient of variation, and the range, is extremely high for the large-sized roots and relatively low for the smaller-sized roots. As with Table VIII, this also indicates the relative independence of the smaller-sized roots, and dependence of the larger roots on tree location. While the smallest roots show a continuous drop in weight with soil depth, only the difference between the 0 to 1 ft depth and the 2 to 3 ft depth is statistically significant at the 95% confidence level. The amount of medium sized root material present at the 1 to 2 ft depth is statistically greater than the amount at either the 0 to 1 ft depth or the 2 to 3 ft depth (95% confidence level). Eighty percent of the total root material was in the upper 2 ft of the soil profile, primarily between 6 in. and 18 in. from the soil surface.

Smaller Trench Transects. The eight transects excavated across the borders of A. mellea infection centers substantiated the near total lack of root material from the sectors corresponding to the 18 to 42 ft region in Figures 14 and 16, and the occurrence of a fairly

Table IX. Average root weights (in grams) per cubic foot of soil within the healthy portion of the transect, arranged by root diameter (cm) and depth in the soil profile (ft). [1]

Root size (cm):	Less than 0.5			0.5 to 1.5			Greater than 1.5		
Depth (ft):	0-1	1-2	2-3	0-1	1-2	2-3	0-1	1-2	2-3
Mean	8.3	7.1	5.2	13.9	25.2	13.4	115	118	32.5
Standard error of mean	0.84	0.84	0.222	1.1	2.1	1.2	61.7	52.8	16.4
Variance	4.3	4.2	0.30	7.4	26.7	9.0	22833	16758	1618
Standard deviation	2.1	2.1	0.543	2.7	5.2	3.0	151	12.9	40.2
Coefficient of variation	0.248	0.290	0.105	0.195	0.205	0.225	1.31	1.100	1.24
Range	5.8	6.0	1.4	7.6	12.4	8.0	408	316	103

[1] Based on average weights per cubic foot of soil as figured from excavated consecutive soil blocks measuring 6 ft x 3 ft by 7(8) ft.

sharp transition between infected and healthy zones at the active pocket margin. The distribution of infected root material in the healthy-appearing stand beyond the outermost dead tree is shown in Figures 17, 18 and Appendix IV. (For further detail, see annual report to St. Regis, 1973.)

There is an apparent effect of tree size on the distribution of infected root material across the border of the infection center. In three of the transects, in which trees ranged from 3 to 6 in. in diameter, infected material ended 10.5 ft outward from the base of the outermost dead tree. In transects with larger trees (up to 16 in. dia), infected material was found as far as 18 ft outward from the last dead tree. In the latter transects, more infected root material generally was present at all distances outward from the last dead tree than in stands of smaller-size trees.

A highly significant linear regression ($F = 29.4$, 1 and 6 degrees of freedom) with good correlation ($R^2 = 0.83$, $R = 0.91$) was found between: 1) the distance from the outermost dead tree to the limit of infected root material, and 2) mean tree diameter for the stand. The equation for this line, $Y = 8.645 + 1.071\ X$, was utilized to estimate limits on this distance (outermost dead tree to limits of infected root material) for infection centers in stands of various sized trees. Confidence limits for distances are given in Table X.

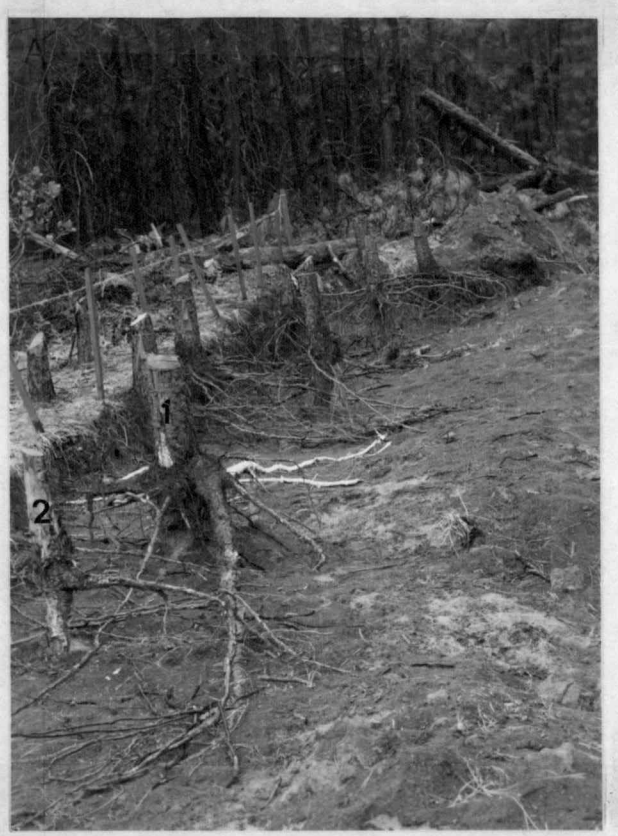

Figure 17. Distribution of roots along an excavated transect passing at a right angle across the perimeter of an A. mellea infection center.

A. Stump 1 represents the outermost dead tree. Stump 2 has been dead longer than 1. Decay following death explains differences in the extent of the two root systems.

B. Stump at left is no. 1 of (A). No live roots exist out of the photo left. Infected (painted white) roots show distribution of infection among roots of adjacent healthy trees. Black strands (right foreground) are bracken fern rhizomes, not A. mellea rhizomorphs. See Appendix IV, map A.

Figure 18. Distribution of roots along an excavated transect passi ng at right angles across the perimeter of an A. mellea infection center.

A. All roots to the left of the outermost dead tree (brown needles) and all roots painted white are infected.

B. Shows how infected roots lie underground, and their relation to the healthy roots. Blue ribbons represent points of new attack (lesions) on living roots. The large piece of residual root material, center foreground, contains A. mellea. Rod markers along the edge of the cut are 3 ft apart. See Appendix IV, map C.

Table X. Confidence intervals at the 95% and 99% probability levels for the distance of extent of infected root material beyond the outermost dead tree of an expanding A. mellea infection center. [1]

Tree size	Confidence interval (distance in ft)	
	95%	99%
Saplings 5 in. DBH or less	12.8-15.2	12.2-15.8
Small poles 6-9 in. DBH	14.6-17.7	13.8-18.5
Large poles 10-16 in. DBH	16.7-22.0	15.2-23.4

[1] Based on the linear equation, $Y = 8.645 + 1.071 X$, that was obtained by regression analysis.

The root distribution maps (Appendix IV) indicate the location of A. mellea lesions (sites of root attack) along each transect. Lesions are located at numerous points on the roots other than those in direct contact with diseased roots.

In addition to location of lesions, the maps show rhizomorphs on roots, distribution of healthy living roots, A. mellea infected roots, and non-A. mellea infected dead root material. Frequently, on a root extending from a live tree, the root segment distal to an A. mellea lesion is dead and infected, while proximally it is still alive and healthy. Rarely is the fungus found proximal to a resinous A. mellea

lesion, unless in another lesion, on a root extending from a live tree (see p. 118).

In seven transects, the sand bagging technique was utilized to test whether infected root segments would support rhizomorph production. Rhizomorphs were produced by samples obtained from infected root material around or from the outermost dead tree in all seven transects (Figure 19). In five of the transects, infected root segments from the region where infected and healthy roots intermingle produced rhizomorphs.

Discussion

In interpreting present patterns of disease incidence and root distribution in A. mellea infection centers, it is important to consider the cover condition of the original old-growth stand. Typically, the widely-spaced, old-growth ponderosa pines have little or no reproduction beneath their crowns. (Occasionally, one or a few small trees establish adjacent to the base of the old tree.) When saplings and poles occur in the forest, they usually are in irregular groups or patches between the old-growth trees. This pattern is known as the park effect, and in the Glenwood area can most likely be attributed to three factors: 1) periodic ground fires that did little damage to established trees, but did not allow reproduction to become established; 2) heavy sheep and cattle grazing which destroyed seedlings, and

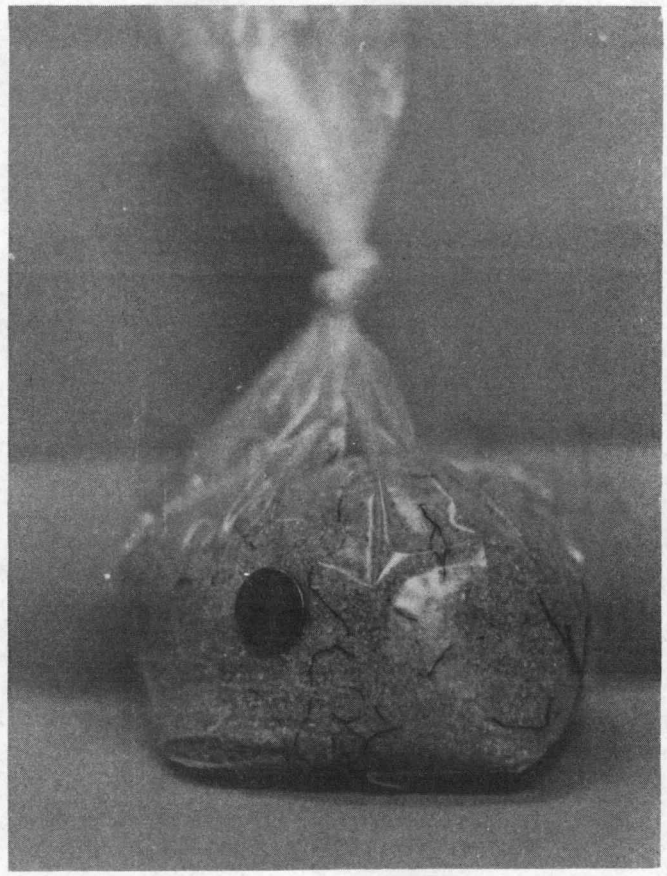

Figure 19. The dark lines seen through the plastic bag are A. mellea rhizomorphs produced from a piece of naturally infected root material incubated in moist quartz sand for six months.

3) the complete utilization of the site around the old-growth tree, mainly for moisture, by the root systems of the old trees. While fires have been curtailed and grazing has been moderated since the 1920's, and successive cuttings have removed many of the old-growth trees, those remaining still exhibit a park effect (Figure 20). Apparently, the competition for moisture is great enough that the park area, once established by a combination of factors, is maintained.

The radius of the park area (distance from the old-growth tree to established saplings or poles) was measured for 20 relatively isolated old-growth trees on three locations in the Glenwood area. Measurements were from trees 28 in. to 50 in. DBH, in areas exhibiting little A. mellea. The average radius was 30 ft (range 20 ft to 39 ft). There was no apparent difference between locations or relation to tree diameter, as long as diameters were within the above range.

Applying the above information to the old-growth stumps associated with the A. mellea infection centers depicted in Table VII and Figures 14 and 16, it is hypothesized that at the time of initial cutting (1941), few, if any, young trees existed within 30 ft. The trees now present and being attacked by A. mellea in this region (zones 1 and 2, Table VII) are of a later generation established since cutting of the old-growth. Late generation trees are also present in zones 3

Figure 20. Old growth ponderosa pine showing the park effect. Area under the crown is free of trees. This effect is believed to be caused by root competition, mainly for moisture. Man (left background) is half way between the old growth tree and smaller trees in the background.

and 4 (Table VII), but here disease activity and root mass are low (Figure 16). This region is beyond the persisting park area and is where the irregular groups or patches of trees present prior to the 1941 cutting started. These were the initial trees killed by the advancing fungus and their remains have decayed away, along with any roots from the old-growth tree that may have been present.

That an appreciable amount of roots from the old-growth tree existed in zones 3 and 4 (24-48 ft) is questionable. While it is reported that lateral roots of old-growth ponderosa pine extend up to 150 ft (Fowells, 1965), the presence of the park area (believed to be maintained by root competition) and the nature of the old-growth root systems examined (p. 109) suggests that few roots extended from the old-growth stump into this area. (Excavations of non-infected old-growth root systems need to be conducted to substantiate this supposition.) Thus, it is hypothesized that the roots of the old-growth stump served both as an inoculum source, and an avenue to bring the fungus to the edge of the park area. Here, the first generation trees were encountered, attacked, and killed. The infection center was established.

While few roots from the old-growth stump were believed present beyond 30 ft, the decrease in root mass from 0 to 24 ft (Figures 14 and 16) is associated with progressive decay of the old-growth stump. A decrease in root material with distance from

the stump would be expected even in the absence of A. mellea. However, that A. mellea is still present and decaying the roots of the old stump was determined by isolation, field symptoms and signs. Thus, the decrease in root weights from 0 to 24 ft is caused by: 1) decay by A. mellea; 2) the normal pattern of root distribution; 3) root taper; and 4) deterioration from other causes. It is not presently possible to apportion the decrease factually among the causes.

From 18 to 42 ft outward from the old-growth stump depicted in Figure 14 (the overlapping area of the two regions discussed above), no large pieces of root material remain from either the old-growth stump or from trees killed by A. mellea. The area can be regarded as formerly infested but now free of A. mellea. As shown in Figure 16, trees are regenerating in the area without becoming infected, while on either side of the area larger amounts of infected root material exist and a high proportion of trees are infected. Thus, the 18 to 42 ft zone may be considered "safe" for stand re-establishment. However, the relationship between stump age and the location of the "infection-free zone" for old-growth stumps of ages other than 31 yrs (1941 cut) has not been determined. The eight smaller excavated transects indicated that the zone does exist in infection centers of different ages, but the location of the zone in relation to the infection center origin was not determined. Undoubtedly, the residual root systems of more recently cut old-growth

trees have deteriorated less, and thus extend further than those of trees cut 31 yrs ago.

In the region between 42 and 72 ft (Figure 14), nearly all remaining root material contained A. mellea. Much of this material was thoroughly decomposed, yet still contined viable A. mellea (as evidenced by field symptoms and signs, and positive isolations). While other unidentified fungi were present in the rotting roots, even the thoroughly decayed material was predominantly occupied by A. mellea. This condition indicates that, after killing the trees, A. mellea remains as the primary, and perhaps nearly sole, root decomposer. As A. mellea is considered to have a relatively low competitive saprophytic ability (Garrett, 1960), the presence of viable A. mellea in the decaying root system of the old-growth tree cut 31 yrs ago supports the supposition that the fungus has remained therein as a primary root decomposer.

Figure 15 shows an increase in root biomass with distance from the old-growth stump.

The pattern is explained by the sequential order of tree death and initiation of root decay. As there is little live root material present at A or B, and the proportion of infected root material is the same (90%), the weight difference is indicative of the amount of root decay with time. Trees at A have been dead longer than those at B and thus root deterioration has occurred over a longer period of

time. The weight difference between C and D (healthy forest) is associated with two factors: 1) initiation of tree mortality by A. mellea and subsequent root deterioration at C, and 2) absence of roots from living trees on the infection center side (left of the graph) influencing C, while live trees in C and the surrounding healthy forest extend roots into D. Thus, the lower weight at C than D is associated with both initiation of decay at C and past tree death within the infection center.

The region in Figure 14 from 0 to 84 ft represents the decay and replacement of root material with time. From 84 to 100 ft is the transition zone of current A. mellea activity in living trees. Beyond 100 ft is the healthy forest.

The main objective of excavating the eight additional transects was to determine the limits of infected root material in the transition zone. These data substantiated occurrence of a fairly sharp transition between infected and healthy zones at the active margin of the infection center. Limits on the distance that infected root material extends into the healthy-appearing stand beyond the outermost dead tree of the infection center were estimated by utilizing the linear regression equation obtained from the data (Table X). Thus, knowing the average sizes of the trees in an A. mellea infection center and the location of the outermost dead tree, the distance that infected root material extends into the healthy-appearing forest can be predicted.

The question now is how can this information be utilized to establish a feasible control program in the managed pine forest? Possibly, a barrier to disease spread could be established over these distances by whole tree removal (see p. 104) or other means, and serve to halt the advance of the fungus.

The maps of root distribution in the excavated transects (Appendix IV) showed that lesions occurred at locations other than where there was direct contact with diseased roots. From this information it is concluded that rhizomorphs have served as the main means of infection. This conclusion is an agreement with other results from temperate regions (Day, 1927; Campbell, 1934; Sokolov, 1964; Ouelette, 1967; Redfern, 1968; Rishbeth, 1972).

Testing the capacity of infected root segments to produce rhizomorphs in moist quartz sand (Figure 19) is a useful tool. The method tests for the causal agent, demonstrates fungus viability and can measure inoculum potential and/or food base quality. It is useful for testing naturally-infected substrates or for experimentation. A point that has caused confusion in the literature (Garrett, 1956, 1970; Rishbeth, 1968; Griffin, 1969) is the necessity to keep the sand moist. If a culture is desired, it can easily be obtained by removing a rhizomorph from the quartz sand, rinsing it in 15% chlorox for 5 min, and placing it on malt agar medium.

The exact source of inoculum for each new root infection, or just what types of infected root material can give rise to a new infection are not known. However, it is known that root systems degenerate subsequent to infection by A. mellea; that trees die in successive order; that transmission is from infected pine to healthy pine; and that the source of inoculum can be traced back to the roots of the most recently killed tree. As rhizomorphs are considered the main means of infection, the ability of diseased roots to produce them would indicate that these roots could serve as a possible source of inoculum. The data show that infected root segments from around the outermost dead tree, and from the region where infected and healthy roots intermingle, produced rhizomorphs. Thus, the outermost infected root material has the ability to produce the infection structures.

While detached segments are capable of rhizomorph production (Figure 19), no doubt infected portions of still complete root systems have even greater ability to produce rhizomorphs, which can establish infections on healthy roots. The infected root system of the outermost dead trees serves concurrently as a food base for buildup of inoculum and as an avenue for fungal spread into the yet uninfected area. The outermost dead tree is initially attacked on its inward side (toward the origin of the infection center). After the tree's death, the fungus spreads throughout the entire root system, including those

roots that extend into the area of healthy trees. The latter roots intermingle with the roots of adjacent healthy trees, through rhizomorphs establish infections thereon, and the process repeats itself.

Rate of Expansion of *A. mellea* Infection Centers

In the preceding section it was noted that infections were transmitted, usually via rhizomorphs, from infected roots of the outermost *A. mellea*-killed tree to uninfected roots extending from adjacent healthy trees. These latter trees eventually were killed, and the process repeated itself, resulting in gradual enlargement of infection centers. The main objective of this section is to discuss the rate of infection center expansion as measured by: 1) the mortality of living trees adjacent to the outermost dead trees of *A. mellea* infection centers; and 2) the expansion rates of the infection center.

Methods

Direct measurement of the rate of increase of infection centers was accomplished by marking, in July 1971, living sapling and pole-sized ponderosa pines on the outermost edge of five separate, actively expanding *A. mellea* infection centers. These trees were the next outward from the last *A. mellea*-killed trees along the infection center margin. While the natural spacing was variable, nearly all marked trees were within 10 ft of the nearest *A. mellea*-killed margin

tree. In October 1973, the trees were re-examined and the ones dead with A. mellea mycelial fans present underneath the bark around the base of the tree were recorded. In addition, living sapling and pole-sized trees within the margins of the infection centers (the outermost dead trees were at least 8 ft beyond these trees) were marked in 1971 and re-examined in 1973.

Indirect estimation of infection center enlargement was achieved by the following procedure, which though admittedly imperfect, is the only attempt known to the author of estimation of the rate of expansion of A. mellea infection centers in a natural forest situation. In the preceding section, the average radius of the "park area" (the region under the crown of old-growth ponderosa pine where no small trees were present) underneath old-growth ponderosa pine in the Glenwood area was determined to be 30 ft (see p. 80).

Accurate cutting records exist for the "saddle area" and adjacent lands. Cutting years were fairly evenly spaced (1941, 1953, 1961-65), and the year of cutting can be determined by field examination of the stumps. The 1941 stumps have a distinctive square undercut, while differences in amount of deterioration allow separation between the 1953 and 1961-65 cuttings. Trees cut between 1961 and 1965 could not be accurately separated in all situations.

Twenty A. mellea infection centers, with which the stump of an old-growth tree could be associated, were selected from within the

"saddle area" and adjacent lands. Infection centers chosen were isolated and had not yet coalesced with other infection centers. The cutting date of each old-growth tree was determined by examination. The distance from each old-growth stump to the margin of disease activity, i. e., to the location of the outermost dead tree, was measured.

From the above distances, 30 ft was subtracted to account for the park area, in which it is hypothesized that sapling and pole-sized trees did not exist prior to cutting the old-growth. The remaining distance is considered to represent the space over which the infection center has expanded. (The initial trees encountered by the infected roots extending from the old-growth stump were the first generation trees on the edge of the park area. These trees were the first killed. Subsequently, the fungus moved throughout their root systems, established infections on adjacent healthy trees, and, by repeating the process, the infection center has expanded by pine-to-pine spread to its present limits [p. 124].)

The date of cutting the old-growth tree was subtracted from the year of measurement (1974). From this figure, five more years were subtracted to allow for the time it took the fungus to spread through the roots of the old-growth stump and encounter and kill the trees on the edge of the park area. The 5 yr interval was chosen on the following two criteria: 1) Hunt's (1957) report (see p. 29)

indicates that infection centers were first noticed 10 to 12 yrs prior to his survey (1945-47), approximately 5 yrs after the initial cutting; 2) infection centers associated with 1965 stumps became noticeable around 1970.

The above adjustments of the distance and time measurements are intended to yield: 1) the distance over which the infection center has expanded, once established by the roots of the old-growth stump encountering and killing the trees on the edge of the park area; and 2) the number of years required to attain present diameter of the infection center. Dividing the distance by the time results in a calculated rate of spread.

Results

An average for the five infection centers of 43% (range 33% to 50%) of the marked living margin trees were dead and had A. mellea mycelial fans after 2 yrs (Table XI). Trees from 2 in. to 18 in. DBH had been killed. In a few instances where the marked trees had been killed, unmarked trees, which were living in 1971 beyond (outward from the infection center) the marked trees, were dead in 1973 and A. mellea mycelial fans had developed.

In the five infection centers, 30 trees within the margins were marked in 1971. Fourteen or 42% were dead and had A. mellea mycelial fans in 1973.

Table XI. Tree mortality on the margins of actively expanding A. mellea infection centers.

Infection center	Marked living margin trees, 1971 (no.)	Dead with A. mellea[1]	
		no.	%
1	36	18	50
2	32	15	47
3	39	13	33
4	39	17	44
5	37	16	43
Total	183	79	43

[1]Includes only trees marked in 1971.

For the 20 infection centers measured, the average expansion rate was 3.3 ft (1.0 m)/yr (range 2.0 to 6.0 ft/yr). For the 11 infection centers associated with 1941 stumps, the average was 2.9 ft (0.88 m)/yr (range 2.0 to 3.8 ft/yr). There was much less variability in the calculated rate of spread for the older infection centers (1941) than the more recent ones.

Discussion

While irrespective of tree size and, to a lesser degree, tree location, the 43% mortality of living border trees over the biennium is indicative of the rapidity of tree death in expanding A. mellea infection centers. Considering the results of the preceding section concerning excavation (p. 72), we know that in 1971 these trees were

already associated with infected root material, and no doubt, many already had infections on lateral roots. All killed trees were within the exposure distances predicted by the regression equation (Table X, p. 77), for substantiating the validity of the equation.

The scattered living trees inward from the infection center margin are considered trees that had, by chance, not yet received a lethal attack, or trees that were more resistant or tolerant to A. mellea. Of the 58% of these trees still alive, some had weak crowns, but others showed no symptoms of decline. The root systems of living trees in similar locations in other infection centers were examined and found to contain A. mellea lesions. It appears that such trees have temporarily escaped a lethal attack. However, it is not known if the escape was by chance, or related to some form of resistance or tolerance. Chance escape appears more likely. The infection centers do not expand equally in all directions at all times, thus making it possible for individual trees to be missed. When such trees are attacked in the critical root collar region (p. 119), they are killed as quickly as trees in the border region, 42% and 43% respectively.

While the infection center expansion rate calculated herein is the only one known for a non-plantation forestry condition, rates have been reported for other situations. Marsh (1952) calculated the rate of spread in apple orchards and black currant plantations in England. While the rates were variable, representative values were 1.5 m/yr

for the apple orchards and 1.8 m/yr in the black currant plantations. Rishbeth (1968) calculated a rate of spread along ash roots in a Cambridge garden at 1.5 m/yr, along roots extending from inoculated Norway spruce stumps at 1.6 m/yr, and in a Douglas-fir plantation at 1.5 m/yr. Swift (1968) observed a rate of 5 m/yr over a 3-yr period in a Pinus elliotti Englem. plantation in Rhodesia. Kable (1974) reported that in peach orchards in Australia the rate varied from 0.8 to 3.2 m/yr with the majority between 0.8 and 1.3 m/yr.

Numerous factors, including soil temperature, tree species, size, root distribution, spacing, soil conditions, and fungus strain present could affect the calculated rate of spread. Even so, most of the calculated rates from the diverse species and conditions represented, compare rather closely. The extremely high rate (5 m/yr) calculated by Swift (1968) must be questioned somewhat as it was for data collected over only a 3-yr period. However, the high temperature of the Rhodesian soil may have created extremely favorable conditions for fungal development. Rishbeth (1968), Kable (1974), and Sokolov (1964) give excellent discussions on how soil temperatures effect the field growth rate of A. mellea. Most likely in the Glenwood situation, the growing season is shorter (the ground is usually covered with snow from November to May) and the soil temperatures are lower than in any of the other areas from which rates of spread have been calculated. Thus, a somewhat slower rate of spread in the Glenwood situation would be expected.

With trees of comparable susceptibility, a slightly slower rate of spread would be expected under natural forest conditions than in plantations or orchards, due to greater variability in tree size, spacing, and genetic constitution. The figure for the 1941 stumps, 0.88 m/yr, is on the lower end of the reported rates. This figure is probably the more accurate of the two given, as it has the least variability, being calculated over the greatest time interval, lessening the effect of year to year variation.

Survival of A. mellea in Root Residues

To test the hypothesis that A. mellea losses could be significantly reduced in a heavily infected forest area by physical removal of the root material providing a food base for the fungus, in 1971 Lewis Roth established an experiment testing the effects of six levels of debris removal on the disease. These six treatments ranged from the most complete removal possible to none.

In the summer of 1971, the 10-acre site for this experiment was selected by the writer and mapped in detail.[6] Figure 21 presents this map in a reduced scale (for further detail see annual report to St. Regis, 1971); Figure 22 cordons the reduction into regions of relatively high and low infection intensity, and depicts the design of the experiment.

[6]The contribution of Gary Fowles, a forestry student at Oregon State University, to the technical mapping is greatly appreciated.

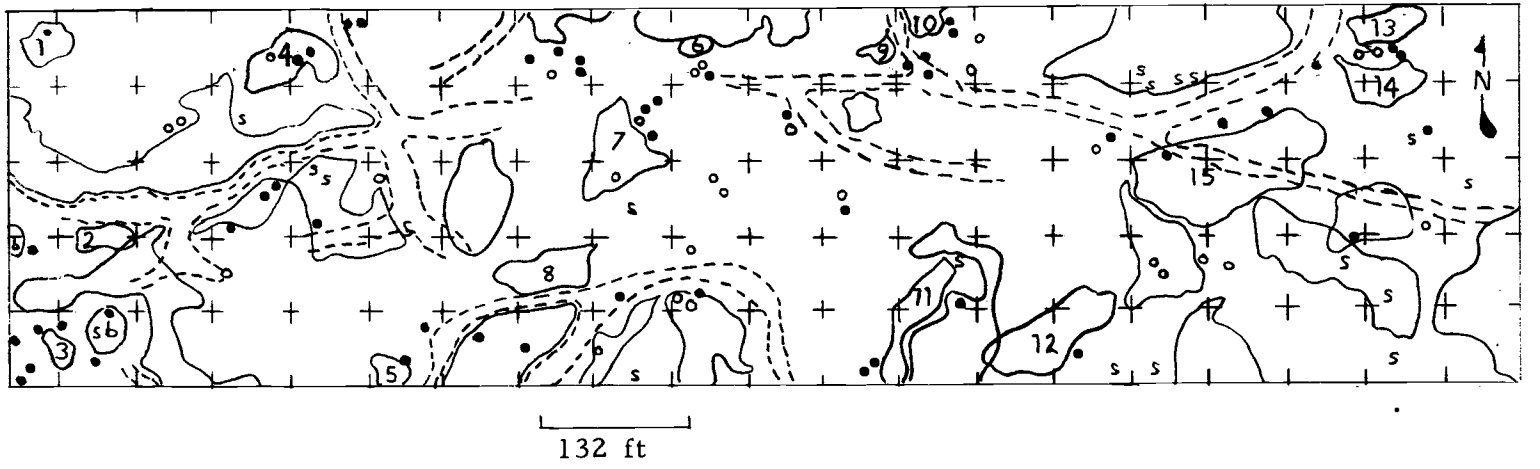

Figure 21. Stand condition map of the pine cover on a 5 x 20 chain (10 acre) tract where the root removal control experiment was established. Solid lines enclose small patches of dense, young-growth pine. Trees in patches numbered 1-15 are saplings, those in unnumbered patches are pole-sized. The rest of the area is open and non-producing. Some of these open areas have resulted from the failure of regeneration to become established following removal of the old stand, e. g. , to the north and east of sapling patch 7; some resulted from skid roads, outlined with broken lines; but most resulted from stand destruction by A. mellea. The areas sb locate slash fires. Dots represent stumps of trees cut in 1941 and 1953; circles mark stumps cut in 1961 and 1965. Small s locates snags, many of which resulted from mortality caused by beetles in the 1930's. Ten residual old trees were scattered over the tract. Crosses are control points located at 1-chain intervals.

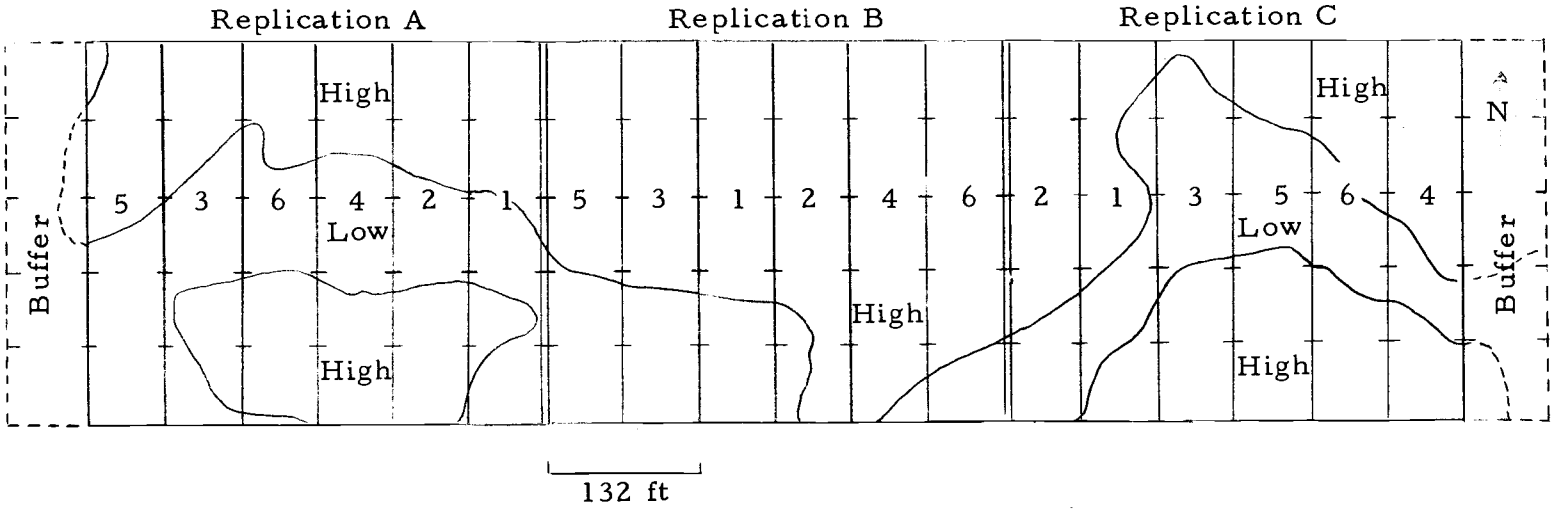

Figure 22. Relative infection intensity and Lewis Roth's plot design on the 10-acre root removal site. The six treatments are given below. The infection intensities are based on the map in Figure 21, an accessory map locating infected trees, and reconnaissance of stand conditions in 1971.

Treatments

1. Trees pushed out, maximum removal of roots by machine, visible remaining roots picked out by hand.
2. Trees pushed out, maximum removal of roots by machine.
3. Trees pushed out, no further removal of roots.
4. Trees pushed out, large stumps left, otherwise maximum removal of roots by machine.
5. All trees felled (low), removed by skidding, area cleaned of trash, sod scalped, no removal of roots.
6. Merchantable overstory logged, no further treatment.

The writer was charged with responsibility for assessing the survival of A. mellea root debris not removed by the treatments, and evaluating the effectiveness of the treatments in removing root debris.

Methods

To evaluate subsequent disease expression among the treatments, each 1/2 acre (1 chain x 5 chains) treatment plot was divided into 112 numbered 11-ft square blocks. These blocks provided a structure for sampling. Six blocks in each plot of treatments 1 to 5 were randomly identified and two were sampled each summer in 1972, 1973, and 1974.

The sample consisted of all of the root material carefully picked from this soil of an 11 x 2 x 2 ft backhoe trench as the soil was filled back into the hole with hand tools.

Roots were placed in numbered paper bags. Within 48 hrs they were sorted into diameter classes, less than 0.5 cm, 0.5-2.0 cm, greater than 2.0 cm, and examined for the presence of A. mellea. Isolations were attempted from locations bearing roots with mycelial fans in the cambial region and from yellow, spongy, decayed wood, typical of A. mellea. No attempt was made to identify or isolate other organisms.

The following medium was used: 30 g malt extract, 20 g dextrose, 5 g bacto-peptone, 19 g agar, and 1,000 ml distilled water; autoclave for 15 to 20 min and then add Streptomycin Sulfate, 100 ppm, and 6 ml of the Sodium Salt of o-Phenylphenol (OPP) solution prepared as described by Russell (1956). OPP is selective for basidiomycetes (Russell, 1956). OPP is somewhat sensitive to light, so after pouring the medium, approximately 30 ml/plate, plates were stored in the dark until used.

Roots from which isolations were attempted either contained still-solid wood with a fair to good degree of integrity, or were rather thoroughly decayed with little integrity remaining.

When isolating from more solid material, a flame sterilized scalpel was used to slice off the outer bark from a root surface area of roughly 2 in. x 1 in. to expose the cambial region and mycelial fans of A. mellea. The flame sterilized tips of a pair of needle-nose pliers, spread slightly apart, were pushed into the exposed surface about 3/8 in. The pliers were squeezed and pulled, resulting in the ripping out of a piece of wood roughly 2 to 3 in. long x 1/4 in. wide x 1/2 in. deep. Flame sterilized bone snippers were used to clip the piece to 1/2 to 3/4 in. long. On occasion, the wood fragment was briefly flamed. The fragment, still held by the pliers, was pushed into the agar, leaving part of the wood exposed above the medium. At times the scalpel tip was used to pry up a piece of wood, followed by

ripping with the pliers. Plates were incubated in the dark at room temperature for up to 3 wks.

If isolations were desired from the interior of large roots, a hammer and flame-sterilized chisel were used to split a sawed root segment.

Thoroughly decayed material often consisted of only a bark tube containing remnants of woody material. Here, a flame sterilized scalpel was used to flake off the external bark and split the tube open. Needle-nose pliers or tweezers were used to pick out a piece of decayed wood. Bone snippers or scissors were used to clip the usually stringy material to about 1/2 to 3/4 in. x 1/2 in. x 1/2 in. This trimmed piece was pushed into the agar surface and plates were incubated as before.

At times, isolations were successful from the inner bark containing visible mycelium and pieces of mycelial fan from the cambial region.

After attempting isolations, root material from all sample locations was air dried and weighed by size classes. In 1972, exhumed material from living and dead trees was fairly distinguishable. In 1973, this distinction was made with difficulty and questionable accuracy. In 1974, separation was not possible. Because the bulk (79%) of the root material was in the upper 18 in. in

1972 (Appendix Table I), the pits were excavated to only 24 in. in 1973 and 1974.

Results and Discussion

Effectiveness of Root Removal Treatments. The air dry weights of roots from each sample location are given in Appendix Tables I, II, and III. Text table XII summarizes appendix tables by presenting the percentages of root material remaining in each of the five food base removal treatments, one, two, and three years after treatment.

While the biological threshold for effective control of the fungus by removal of root material (inoculum and potential inoculum) is unknown, the amount of residual root material recovered in treatments 1 and 2 was higher than anticipated. These treatments were the most thorough and expensive, and were intended to remove the bulk of the food base. The consistency of the values for treatment one (Table XII), however, indicates that the treatment was uniform in thoroughness. The high value, 157%, for treatment 2 (trees pushed out, maximum removal of roots by machine) in 1974 (Table XII) was caused by one pit containing a number of large pieces of root material. Apparently, this was from a region not thoroughly covered by machine raking.

Treatment 3, the least expensive, which simply pushed the trees out whole (Figure 23), is of interest in that it was nearly as effective

Table XII. Percentage of root material remaining after 1, 2, and 3 years in each of five food base removal treatments.

Treatment:	1			2			3			4			5		
Year:	1972	1973	1974	1972	1973	1974	1972	1973	1974	1972	1973	1974	1972	1973	1974
	percent														
Root material remaining	67[1]	59	55	86	75	157	71	80[2] (170)	48	195	46	90	100	100	100

[1] Values are expressed in terms of root weights from treatment 5, which served as a control treatment in that no root material was removed.

[2] Eighty percent excluding the material explained in the text.

Figure 23. Removal of root systems of standing trees by pushing with the blade of a bulldozer.

as the more intensive and expensive type operations. The 170% root material recovered in treatment 3 in 1973 is indicative, however, of the type of material that this treatment does not remove from the soil. One pit in replication C contained one 10,000 g piece and two or three other large pieces of root material, not infected with A. mellea and apparently not connected to pushed out trees. While in this situation the residual material was uninfected, the treatment would not remove similar infected material. If such material is important to the disease cycle (p. 124), then its persistence is one drawback of this treatment. Perhaps this method of root removal could be developed into a useful management tool to retard disease spread by applying it over the distances, as determined by the regression equation (p. 77), that infected root material extends beyond the outermost dead tree in an A. mellea infection center.

The extreme variation in root weights for treatment 4 (trees pushed out, large stumps left, otherwise maximum machine removal of roots) is explained by the random location of the sample plots. In 1972, two of the pits were adjacent to old-growth stumps with massive residual roots. Pits in 1973 were more distant from the stumps, while again in 1974 one pit was near a stump.

Percentages of root material remaining after treatment (Table XII) are of interpretive value only in relation to each other. For example, it would be improper to say that treatment 1 removed only

35 to 45% of the total root material. The masses of roots around old-growth stumps and tap roots of pole-sized trees taken out during treatment certainly account for much more material removed than this figure would indicate. However, when seen in context with values for the other treatments, one can get an idea of the relative effectiveness of each treatment in removing root material.

In treatment 1 (Table XII) the percentage of root material remaining consistently decreased with time. However, the small number of samples, the variability among the treatments, and the relatively short time interval covered limit conclusions concerning amounts of deterioration with time.

Survival of A. mellea in Root Debris. While the 3-yr time interval appeared too short to notably decrease root weight, A. mellea responded interestingly. Nine (68%) more pits yielded positive cultures in 1973 than in 1972, and 10 (69%) more pits yielded positive cultures in 1974 than in 1972 (Table XIII).

That this difference is not due to sampling error is shown by examining the location of the samples on the disease distribution map (Figure 22). Twenty sample pits fell in areas of high disease activity in 1972, 19 in 1973, and 21 in 1974. However, in 1972 90% of the samples that yielded A. mellea isolates came from areas depicted as having a high infection intensity (Figure 22), while only 58% in 1973 and 75% in 1974 were so located. Thus, while there is virtually no

Table XIII. Recovery of Armillaria at yearly intervals following treatments to remove the fungus and its food base.

Year	Sample locations yielding Armillaria cultures		
1972	A-2-30[1]	B-3-98	C-1-45
	A-4-2	B-5-72	C-1-87
		B-5-78	C-2-3
			C-2-47
			C-5-78
1973	A-1-45	B-1-101	C-1-105
	A-1-88	B-2-86	C-3-74
	A-2-81	B-3-72	C-5-106
	A-2-100	B-3-93	
	A-3-22	B-4-97	
	A-3-103	B-5-67	
	A-4-98	B-5-109	
	A-5-45		
	A-5-85		
1974	A-1-29	B-2-63	C-1-58
	A-2-63	B-2-106	C-2-60
	A-2-107	B-3-32	C-2-84
	A-3-23	B-3-59	C-3-26
	A-3-26	B-4-56	C-3-85
	A-4-64	B-5-61	C-5-4
	A-5-1		
	A-5-60		

[1]Replication (A), treatment (2) and sampling location (30).

difference in sampling accuracy, there is an apparent increase in the number of samples yielding cultures of A. mellea in areas originally rated as having a low infection intensity.

Differences in recovery might be due to improved skill in later years in isolating from, and recognition of, infected tissue. Successful isolates were obtained from only 58% of the pits from which they were attempted in 1972, while the success was 90% in 1973, and 100% in 1974. Better handling of the material may also have been a factor. Roots were handled quicker and in cooler weather in 1973 and 1974 than in 1972. However, in both 1973 and 1974 more pits had material judged as suitable for isolation than in 1972 (17 in 1972, 21 in 1973, and 21 in 1974). This difference should not have been caused by inexperience in recognition of A. mellea infected tissue, since the 2 months prior to sampling in 1972 were spent examining A. mellea infected root systems.

As frequency of detection or occurrence is increasing with time, it appears that there has been a saprophytic buildup of A. mellea on the root material remaining in the ground after the root removal operations. If real, this increase has probably arisen through further fungal development on root systems attacked prior to treatment, and possibly through subsequent colonization of previously uninfected material. These judgments are open to question, however, in that the samples, even though randomly selected, are extremely

small in number. For the entire 3 yrs, less than 1% of the treated area was sampled. Such coverage may be suitable for a situation with low variability, but, as the root weights indicate, except possibly for treatment 1, this is a situation with high variability.

If there is a saprophytic buildup of A. mellea in root residues, and if food base destruction becomes a feasible control operation, then how an infected area is managed after treatment becomes important. As substantial amounts of root material remain even in the most thorough treatments, and as the occurrence of A. mellea in this debris is increasing, a good deal of inoculum is present. However, one could expect that over a longer time period, greater than 3 yrs, this material will deteriorate, and the amount and effectiveness of the inoculum will decline. The critical management question is how to plan stand reestablishment so as not to leave the area unproductive, but not to regenerate so soon as to allow the fungal buildup on residual root material to jeopardize the newly developing stand.

Highly reliable field assay methods are essential to progress in pathology. The method described for recovery of A. mellea is highly effective (over 90% success in the last 2 yrs). Efficiency seems to lie in the removal and plating of infected tissue with minimum exposure to possible contamination, either from the air or debris along the root. The procedure is suggested for use with other hymenomycetes associated with the coarse roots of woody plants.

V. THE DISEASE IN THE TREE

Characteristics of *A. mellea* on Individual Pine Root Systems

Root Systems of Old-growth Trees

The 10-acre control experiment involving root removal (p. 95) contained numerous stumps of 30 to 50 in. pines felled in 1941, 1953, 1961, or 1965.

The literature contains contradictory statements concerning the suitability of coniferous species as food bases from which *A. mellea* can attack young trees (Redfern, 1968, 1970, 1973; Weiss and Riffle, 1971; Adams, 1972). Observations regarding the nature of *A. mellea* on root systems of large conifers are scant. However, above-ground observations in the pine region indicate that many *A. mellea* infection centers are associated with the stumps of old-growth ponderosa pine.

Large stumps located within treatments 1, 2, and 3 of the 10-acre control experiment (p. 95) were removed in the course of performing each treatment. The nature and distribution of *A. mellea* on the root systems of these stumps was observed and measured.

The location of each large stump was recorded and date of cutting determined (see p. 89). Removal of the stumps was

accomplished by excavating, often to a depth of 6 ft or more, with the blade of a large (D-8) bulldozer. In this manner much of the root system was removed intact. To facilitate examination, the attached soil and rock was removed with picks or washed away with fire hoses from a 5,000 gal pumper truck. Washing was at times discontinued as the pressure was great enough to destroy the decomposed roots.

In contrast to the well-developed tap root of pole-sized and smaller ponderosa pine, large stumps generally have an extensive locally distributed root system (Figure 24). Apparently, as trees age the tap root becomes a less significant portion of the total root system. In most instances it could not be identified as a separate entity.

The mass of root material removed intact with each stump was often very large (Figure 24). For example, the stump of one tree cut in 1941 had a surface diameter of 39 in., a root spread of intact material of 87 in., and a below-ground depth of 37 in. While part of this volume consisted of rock and soil fused to and entangled with the roots, there is a substantial amount of root material present as a potential food source for A. mellea.

Over half of the stumps examined (14 of 25 including some of all cutting years) had signs of A. mellea present on their root systems. In addition, the root systems of four standing snags of old-growth

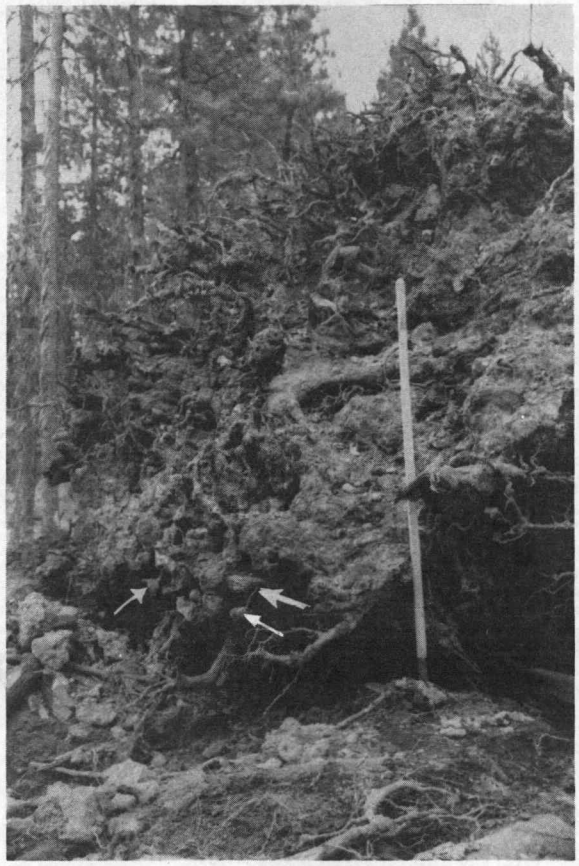

Figure 24. Excavated root system of an old growth tree cut in 1941. Staff is 50 inches tall. Note A. mellea lesions (arrows) on lateral roots.

trees, presumably killed by bark bettle attacks in the 1930's, were found to have little root material remaining, two were extremely resinous throughout, as though attacked by A. mellea, and one contained an A. mellea mycelial fan.

That many of these root systems contained A. mellea prior to cutting was evidenced by the resinous lesions present. As Gilmour (1954) explained:

> In pines particularly, copious resin flow always accompanies an attack of the living cells. Some of the exuded resin solidifies within the bark layers causing them to swell and crack, while the surface of the roots and butt become coated with coagulated resin intermixed with soil, organic matter, and rhizomorphs.

Thus, the present extent of A. mellea in these root systems is a function of both past parasitic attacks on the living trees and saprophytic development subsequent to cutting. It is not clear if all of the stumps that now contain A. mellea were infected prior to cutting; possibly on some stumps A. mellea could have developed solely as a saprophyte.

On infected root systems some interesting characteristics were noted. Resin-bound masses of root, soil, and rock were often encountered. Frequently mycelial fans were present underneath the bark, and rhizomorphs were attached externally along the roots. Both mycelial fans and rhizomorphs were often present on lesions. In some cases sections of the root system consisted of the extremely

moist, decomposed, gelatinized wood characteristic of decay by A. mellea. In other instances, hollow bark tubes were common within the soil-rock-root matrix (Figure 25). These tubes contained white, puffy mycelium, gelatinized wood, and/or small amounts of decayed wood still having some structure. On the inside walls of these tubes blackish strands of old A. mellea rhizomorphs were often present. The extent of these channels in the soil-root-rock matrix is unknown but may be extensive. On one occasion, while utilizing the fire hose to wash rock and soil away from the roots, water squirted out of one of these channels over 2 ft from the surface being washed. The channels offer less resistance to rhizomorph growth than the surrounding medium, and may well serve as avenues for rhizomorph extension (Adams, 1972). (In subsequent excavations, viable rhizomorphs were found inside these tubes.)

Samples of infected roots obtained from various locations on the root systems were incubated in bags of moist quartz sand (see p. 63; Figure 19). As rhizomorphs are considered important in fungal spread and root attack, their production from samples would indicate the ability of the samples to serve as inoculum. Rhizomorphs were produced by samples obtained from trees cut in 1941, and in 1965.

In summary, while extensive examination of the root systems of old-growth trees were not undertaken, it was observed that: they did contain A. mellea; some of them must have contained A. mellea prior

Figure 25. Root channels (arrows) in the soil-rock-root matrix associated with the root system of an old growth tree. These channels are roots from which the woody tissue has decayed away leaving only tubes of bark. They serve as avenues for rhizomorph spread in the soil.

to cutting; infected segments from these root systems, some of which had been dead for three decades, still had the ability to produce the infective rhizomorphs of A. mellea.

Root Systems of Sapling and Pole-sized Trees

Adams (1972) hand-excavated and examined the root systems of 33 living, dying, and A. mellea-killed sapling and seedling ponderosa pine on Pringle Butte in Deschutes County, Oregon. While others have excavated root systems to examine the status of A. mellea thereon (Sokolov, 1964), Adams' work was the only known to the author in the Pacific Northwest and on ponderosa pine. To expand on Adams' observations and see if they applied in general to ponderosa pine, the root systems of over 300 living, dying, and A. mellea-killed sapling and pole-sized ponderosa pines were examined.

Within the 10-acre area of the root removal control experiment (see section on survival, p. 95), four areas were selected for extraction of stumps and examination of their root systems. One of these areas is discussed on p. 50. The other three areas were also expanding A. mellea infection centers. In addition, roots of the trees originally growing in the trench described on p. 52 were examined.

Trees selected for examination included long-dead A. mellea stubs (Figure 2), standing defoliated A. mellea-killed snags, brown foliated A. mellea-killed trees, and living trees. In September, 1971,

the root systems were extracted by pushing the tree or stump base over with the blade of a bulldozer (Figure 23) or by pulling with a choker (Figure 8). The trees from the trench were extracted in July, 1972.

In June and July, 1972, the root systems were examined to determine the nature of A. mellea thereon. To expose roots for observation often bulk soil was removed with hand tools. Samples of infected roots were collected both in the fall of 1971 and the summer of 1972. From these samples, isolations were attampted, and some samples were bagged in moist quartz sand to see if rhizomorphs would develop (see p. 63 , and Figure 19).

In general, the characteristics of A. mellea on these root systems substantiated the observations of Adams (1972).

A. mellea attacks on lateral roots resulted in the formation of resinous lesions (Figure 26). These lesions are easy to recognize as the resin has cemented soil and rock into lumps around the infection site. Rhizomorphs are often embedded in this soil-root-resin matrix. The pitching reaction at the site of infection often girdles the root, resulting in death of locally infected tissue. This is often followed by death and colonization by A. mellea of the distal portion of the root. Occasionally, the root segment distal to a lesion on a lateral root was missing (Figure 26). Whether this had decayed in the soil, or the lesion was a point of weakness at which the root broke

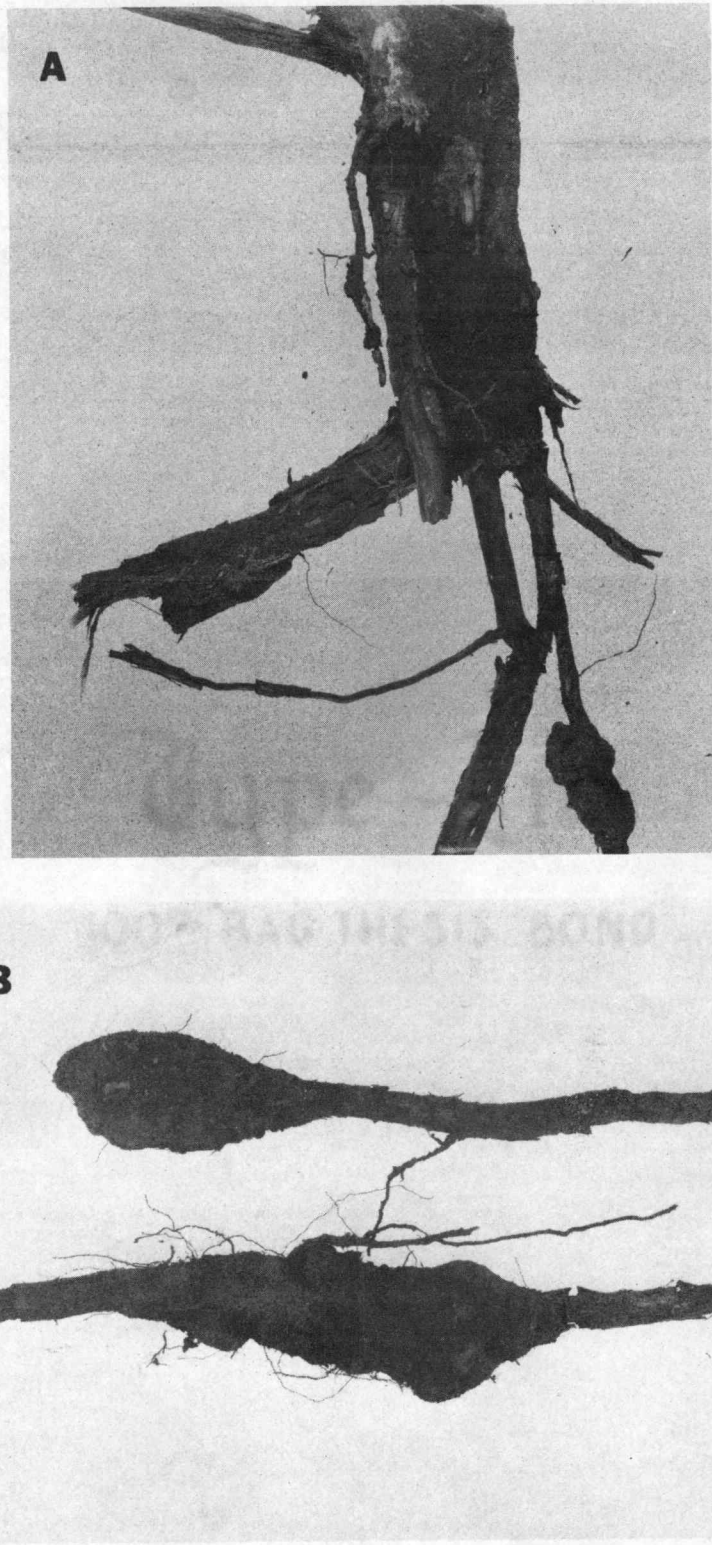

Figure 26. Infected pine roots with occluded lesions. The globular objects are masses of soil cemented to the root by the flow of resin at an A. mellea infection site.

during stump extraction, was not clear. Either explanation is feasible.

In some cases, the lesions were small, although still resinous, and localized on a section of a lateral root. Lesions of this type had not resulted in root girdling or death of the root distal to the infection site.

Only rarely had the fungus advanced in a proximal direction along living lateral roots. In no cases was there evidence to suggest that A. mellea had first attacked and colonized a lateral root and subsequently spread internally throughout the entire root system. The rarity of the fungus spreading proximally from a resinous lesion on a lateral root and the existence of lesions considered non-viable (pitched-out) suggest that the resin serves to occlude the fungus.

The presence of A. mellea lesions on lateral roots and the associated loss of some of these roots did not appear to affect host vigor directly. Some living trees contained 6 to 20 lateral lesions with no noticeable effects on tree growth. (Neither height or diameter growth were recorded; however, no obvious signs of tree decline, such as a weak crown or stress cone crop, were present on these trees. These symptoms were noted infrequently on dying trees in the area.)

However, when attack occurred in the root collar region, or high on the tap root above most or all lateral roots, extreme pitching

occurred. This resulted in girdling of the main tap root and was soon followed by tree death. That death usually is sudden and not a prolonged affair is indicated by the growth rings in cross sections (Figure 27).

Trees killed by A. mellea consistently had either a root collar or high tap root attack. Data were recorded on the presence of resinous A. mellea lesions high on the tap root or in the collar region of A. mellea-killed pine on three of the areas. Forty-four of 50 (88%) A. mellea-killed trees examined had this characteristic attack present. Three of the remaining six root systems were broken during extraction in such a manner that one could not positively determine if a lesion was present in this area or not.

The constancy of attacks high on the tap root or in the collar region on A. mellea-killed pines, and the existence of living, apparently healthy, trees with heavily infected lateral roots, but no infection high on the tap root or in the collar region, suggests that this root area is the critical region for lethal A. mellea attacks on young ponderosa pine.

The literature contains references to A. mellea attacks in the root collar region resulting in tree death (Day, 1927; Patton and Riker, 1959; Peace, 1962; Foster and Johnson, 1963; Sokolov, 1964; Adams, 1972). However, this does not imply that this region is a focal point of attack. Most of the A. mellea-killed pines examined

Figure 27. Living tree (left) and A. mellea killed tree (right) showing that the growth rings have a similar pattern throughout. The lack of a slow down in growth (narrower rings) on the outside of the A. mellea killed tree indicates that death was sudden, not prolonged.

also had many lesions present on lateral roots in addition to the lethal collar attack. Thus, no real specialization to the collar region is indicated. It appears that attack in the collar region is a matter of chance and time. This region presents a reasonably large target area for rhizomorphs and eventually in a heavily infected situation attacks will occur here.

In contrast to the more spreading root systems of the old-growth ponderosa pine examined, these sapling and pole-sized trees exhibited the typical ponderosa pine tap root. A few pole-sized trees were examined that had atypical spreading root systems. Although heavily infected with lateral lesions, none of these trees were killed. While the spreading root systems of the old-growth pine do contain A. mellea, rarely are these trees observed to be killed by the fungus. It appears that in pines with a spreading root system, the collar region, on a comparative basis, does not present as large a target area as the critical region for attack, and such trees are thus more tolerant of A. mellea. Day (1927) ranks seven conifers as to: 1) susceptibility to attack, without considering liability to death afterwards; and 2) likelihood of death after attack. The only two species that were ranked higher (more susceptible) in category 1 than 2 were Thuja plicata Donn and Tsuga heterophylla (Raf.) Sarg. Each of these species is described as having a "wide spreading root system" (Harlow and Harrar, 1958).

In the young pines excavated by Adams, A. mellea was not found decaying internal sapwood tissue. Adams (1972) stated, "the sapwood of the trees killed by A. mellea seems to dry out quickly and as such will not support growth of the fungus." He felt that once killed these small root systems could not act as a food base for subsequent attack on another sapling. While A. mellea-killed pole-sized trees were observed that fit Adams' description, other evidence suggests that in some cases A. mellea does survive in killed tissue.

In the description of the above-ground characteristics of A. mellea infection centers (p. 5), a zone was described that contained stubs broken off near ground level. These represent trees that were killed in the past (perhaps 10 yrs ago or more) by A. mellea. Many of these "ground level stumps" were extracted and examined. Their root systems typically have all of the lateral roots decayed away, leaving only the main tap root. This results in a piece of root material that is carrot-shaped (Figure 28). These "carrots" still contain A. mellea as evidenced by the presence of fresh rhizomorphs and mycelial fans, and cultures obtained from the decaying wood.

In addition to these "carrots," viable A. mellea was demonstrated in roots of pole-sized trees by isolation and the sand bagging technique described on a p. 63 (Figure 19). Both lateral root lesions and root segments that appeared in the field to be infected, produced rhizomorphs after incubation in the bags of moist quartz sand. These

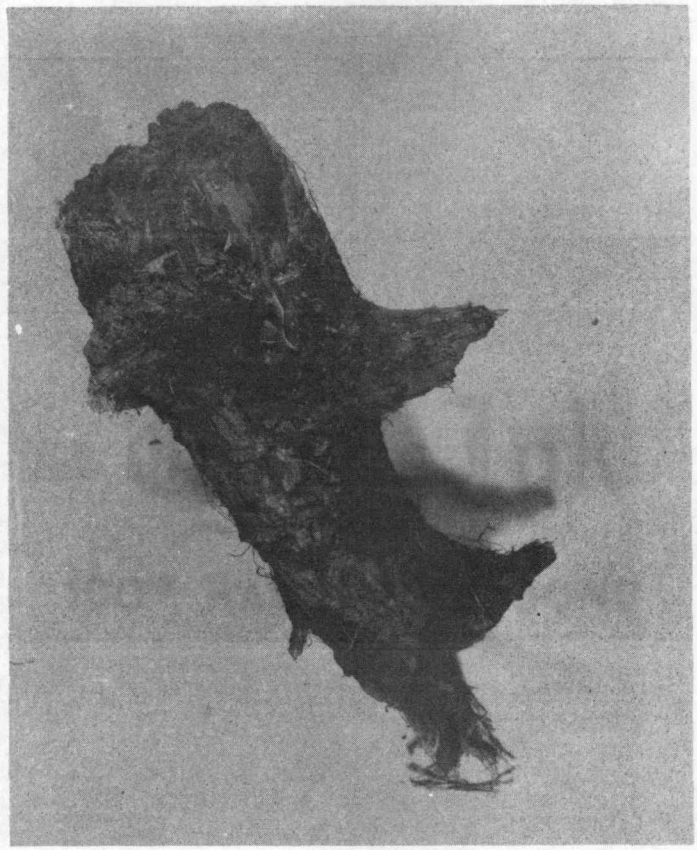

Figure 28. Carrot shaped tap root representing the remaining root system of a pole-sized ponderosa pine killed some years previously by A. mellea. Such material still contains viable A. mellea. Trunks above these root systems are usually rotted or broken off at ground level.

incubated root pieces consisted of lesions from living and A. mellea-killed trees, and non-lesioned root segments from trees killed by A. mellea over a period of 5 yrs or more. Twenty-two percent (35 of 156) of the root segments tested produced rhizomorphs.

Incubated lesions produced rhizomorphs more frequently than the infected root segments without lesions. Bega and Tarry (1966) suggest that resin impregnated wood serves as a survival habitat for Fomes annosus. Perhaps the flow of resin that serves to occlude the fungus in a lateral root lesion while the tree is alive, and is associated with tree death when the fungus attacks in the root collar region, also helps to maintain the fungus after tree death.

In summary, sapling and pole-sized ponderosa pines appear to be killed by A. mellea only when attacked high on the tap root or in the root collar region. Lateral root attacks, even though extensive, are rarely, if ever, lethal. The following chronology is believed to represent the sequence of host fungus interactions in the killing of young ponderosa pines by A. mellea:

1. Rhizomorphs, emanating from infected root material serving as a food base (i.e., a large, old-growth stump, a "carrot," or lateral roots of an A. mellea-killed tree) contact and penetrate healthy lateral roots. The host response involves a flow of resin that results in the formation of a lesion at the infection site. These lesions may girdle the root, and distally it may

die and become colonized throughout by A. mellea. The fungus does not advance proximally within the root from these lesions. A tree may contain numerous lateral lesions and still remain alive.

2. Eventually, rhizomorphs contact and penetrate the host high on the tap root or in the root collar region. The host responds with a copious flow of resin. A girdling, pitchy lesion results, locally killing host tissue and impregnating wood and bark with resin. This effectively "cuts off" the above-ground portion of the tree.
3. Deprived of photosynthate, the vigor of root tissues declines and they become less resistant to mycelial development of the fungus.
4. The above-ground portion of the tree, in essence deprived of its root system, rapidly declines (especially in the drying heat of Glenwood summers). The crown soon turns brown; the tree is dead.
5. Concurrent with or immediately following crown decline, A. mellea quickly ramifies throughout the cambial region of the entire root system.
6. In some cases, most probably in seedlings and smaller saplings, the root system dries out and the tissue will no longer

support the growth of the fungus. A. mellea is replaced by other saprophytic wood decay fungi.

7. In most instances, in the larger saplings, poles and larger trees, the root systems do not dry out appreciably. A. mellea survives and becomes involved in general tissue decay. Eventually this results in "carrots" as depicted in Figure 28.

Interpretation of Pathogenic Resin Flow as a Hypersensitive Reaction

Adams (1972) interpreted the flow of resin at A. mellea infection sites on ponderosa pine roots as a unique type of hypersensitive reaction (HR). A hypersensitive reaction can be broadly defined as a dynamic physiological response on the part of a plant to an infectious biological agent that leads to localized death of host tissue and occlusion of the pathogen.

While the flow of resin at A. mellea infection sites on lateral roots does appear to be followed by pathogen occlusion and localized death of host tissue, the differences between the formation of resinous lesions on pine roots attacked by A. mellea and the HR in a historical sense are seen in the following:

1. Fungi that evoke a HR typically attack green plant parts (i. e., leaves). A. mellea is a root pathogen.
2. Often the pathogens that elicit a HR are obligate parasites (rust fungi). A. mellea is both a parasite and a saprophyte.
3. Most often the HR results in a very small portion of the host

tissue being involved (i. e., "shot holes" in leaves). With lateral root lesions of A. mellea often the entire distal portion of the root may die. When the response is elicited in the root collar region, eventually the entire root system is involved.

4. Most often in a HR the localized death of host cells results in permanent exclusion of that particular infection from other host tissue. A. mellea does remain viable in lateral root lesions, colonizes root portions distal to lesions, and after tree death following an attack in the collar region, the fungus colonizes the entire root system.

5. Most often the host responding with a HR to an attacking pathogen is seen as a form of resistance. In contrast, relative to other conifers, pines are considered more susceptible to A. mellea.

6. The HR is interpreted as a form of resistance because, typically little host tissue is involved, the pathogen is excluded, and rarely, if ever, is the host killed by its own response to one or even to many infections. While the formation of resinous lesions on lateral roots occlude the pathogen and do not appear to directly harm the host, the same response in the root collar region results in tree death.

7. In pines, the flow of resin is a general response to any injury, while typically the HR is a host response to a particular event.

If one can accept these discrepancies between the typical HR and the flow of resin at an A. mellea infection site, then the pine root response can be considered a HR. If one cannot accept the application of this term, which is often used in the context of plant resistance to pathogen attack, to a reaction that, if it occurs in one location the pathogen is occluded, but if in another location the organism dies, while the pathogen survives as a saprophyte, then perhaps HR is inapparopriate.

Comparative Pathogenicity of A. mellea Isolates from Different Sources

Many attempts have been made to artificially inoculate plants with A. mellea (summarized by Raabe, 1967; Wilbur et al., 1972). Adams (1972, 1974) used artificially infected red alder (Alnus rubra Bong.) branch segments to test the pathogenicity on ponderosa pine of A. mellea strains isolated from ponderosa pine and hardwood shrubs in the Pringle Butte study area. The strain of A. mellea that he found predominantly on shrubs was non-pathogenic to ponderosa pine seedlings, while a strain obtained from pine was pathogenic to ponderosa pine seedlings.

In the Klickitat working circle (p. 1), A. mellea damage is noticeably absent in the Plateau Block (the high flatlands above and west of the Klickitat River gorge). Examination of the roots of

willow (Salix sp.), oak (Quercus garryana Dougl.), and hazel (Corylus cornuta Marsh.) on the Plateau Block revealed that A. mellea was present, but apparently not killing these species or the pines.

The objectives of this experiment were to test the pathogenicity on ponderosa pine of A. mellea isolates from old-growth stumps and diseased pines in the "saddle area"; and to test the pathogenicity of isolates from the Plateau Block where disease appears lacking.

Isolates of A. mellea were obtained from: young pines and old stumps of the control experiment (p. 95); young pines from the large trench transect (p. 52); and living hardwoods from the Plateau Block.

Four-inch segments between 3/4 and 1 1/4 in. in diameter, were cut from alder branches and stacked in quart canning jars. The jars were filled with water to just below the tops of the segments, a vacuum seal dome lid was placed on upside down (so it would not seal), and secured with the closure ring (tightened firm and then released 1/2 turn). The jars were allowed to sit for 1 to 3 days, then autoclaved for 1 1/2 to 2 hrs, and cooled for 12 hrs in the closed autoclave. This resulted in slower cooling and less breakage. Three days after removal the liquid was poured off until approximately 1 in. remained in the bottom of the jars,

The segments were inoculated with plugs from individual agar cultures and incubated in the dark at room temperature for 4 months, and then used to inoculate pine seedlings.

To minimize root disturbance during inoculation, a large test tube was placed upside down next to each seedling at potting time (Adams, 1972). Inoculation was accomplished by removing the tube and replacing it with an infected alder segment, which was then covered with soil (Figure 29).

Ponderosa pine seedlings were grown from seed collected at Glenwood in the fall of 1971, designated GW; seedlings purchased from the U. S. Forest Service nursery at Bend, Oregon, designated BN. The BN seedlings were planted (spring 1973), one to a can, in a soil derived from volcanic ash from a pine forest in central Oregon. The GW seedlings were planted two to a can in a greenhouse soil mix. After inoculation the BN seedlings were transferred to Glenwood, where the cans were set in the ground to their rims and watered occasionally through the summer. The cans of GW stock were set in the ground to their rims in a cold frame in Corvallis and watered occasionally.

Ten cans each of the BN stock were inoculated in June 1973 with A. mellea isolates from 11 sources, one from oak, one from willow, an alder check, and eight from pines or old pine stumps (110 seedlings in all). Half of the cans, including all that contained dead and dying

Figure 29. Inoculation of a pine seedling with an A. mellea-infected red alder branch segment. The jar contains inoculum segments. The white tufts visible on the segments are A. mellea mycelium. Photo courtesy Greg Filip.

seedlings, were examined in July, 1974. The rest were left for later examination. GW seedlings were treated like BN seedlings, except that the willow isolate was replaced with one from hazel. Examination was in August, 1974, and included only cans containing dead or dying seedlings.

In the spring of 1974 the experiment was repeated with one-year-old Monterey pine (Pinus radiata D. Dod.) seedlings obtained, through the assistance of Dr. D. W. Adams, from the California State Nursery at Santa Cruz. These had been suggested by Dr. Raabe (personal communication) as highly suitable for pathogenicity tests with A. mellea.

Pairs of seedlings in a 50-50 mix of sand and peat were inoculated at planting time (10 cans of each) with two isolates of A. mellea from old-growth stumps, two from young pines, and one check (100 trees in all). The inoculum had been incubated for 6 months and stored at 5°C for a year. The test was in a greenhouse with daily watering.

The experiment was repeated in June 1974 with one isolate from hazel, one from oak, one from willow, two from old-growth pine stumps, and one alder check. Ten cans were inoculated with each isolate (120 trees in all). The inoculum had been incubated for only 2 months. The cans were set in the ground in a cold frame at Corvallis and watered occasionally.

Raabe indicated that his most virulent strains of A. mellea had killed Monterey pine seedlings of less than 1 yr in age in a months' time. By August 1974, no seedlings were dead or dying. The trees will remain undisturbed for future examination.

In all but one of the 52 BN inoculations examined, inoculum segments appeared viable, and 81% of the segments, including some from all isolates, produced rhizomorphs. Seven trees were attacked by the fungus of which three were killed. In all cases attack was by a rhizomorph arising from the inoculum segment, attaching to the tree, resulting in a resinous lesion and a mycelial fan in the root cambium (Figure 30). Of the dead trees, one isolate was from an old-growth stump cut in 1965, one from a young pine, and one from willow. The resinous lesion on the tree killed by the willow isolate was noticeably the least distinct. None of the other attacked trees had been inoculated with a hardwood isolate. Isolations were made from three of the attacked pines, and all yielded cultures of A. mellea.

Twenty-two cans of the GW stock, all containing dead or dying seedlings, were examined. No trees in either check or hazel cans had A. mellea on their roots. Both hazel inoculum segments produced rhizomorphs but they failed to infect. Dead and dying trees in 14 of the 18 remaining cans had A. mellea infections. Attacked trees, as with the BN stock, had resinous lesions, mycelial fans, and rhizomorphs at the attack site.

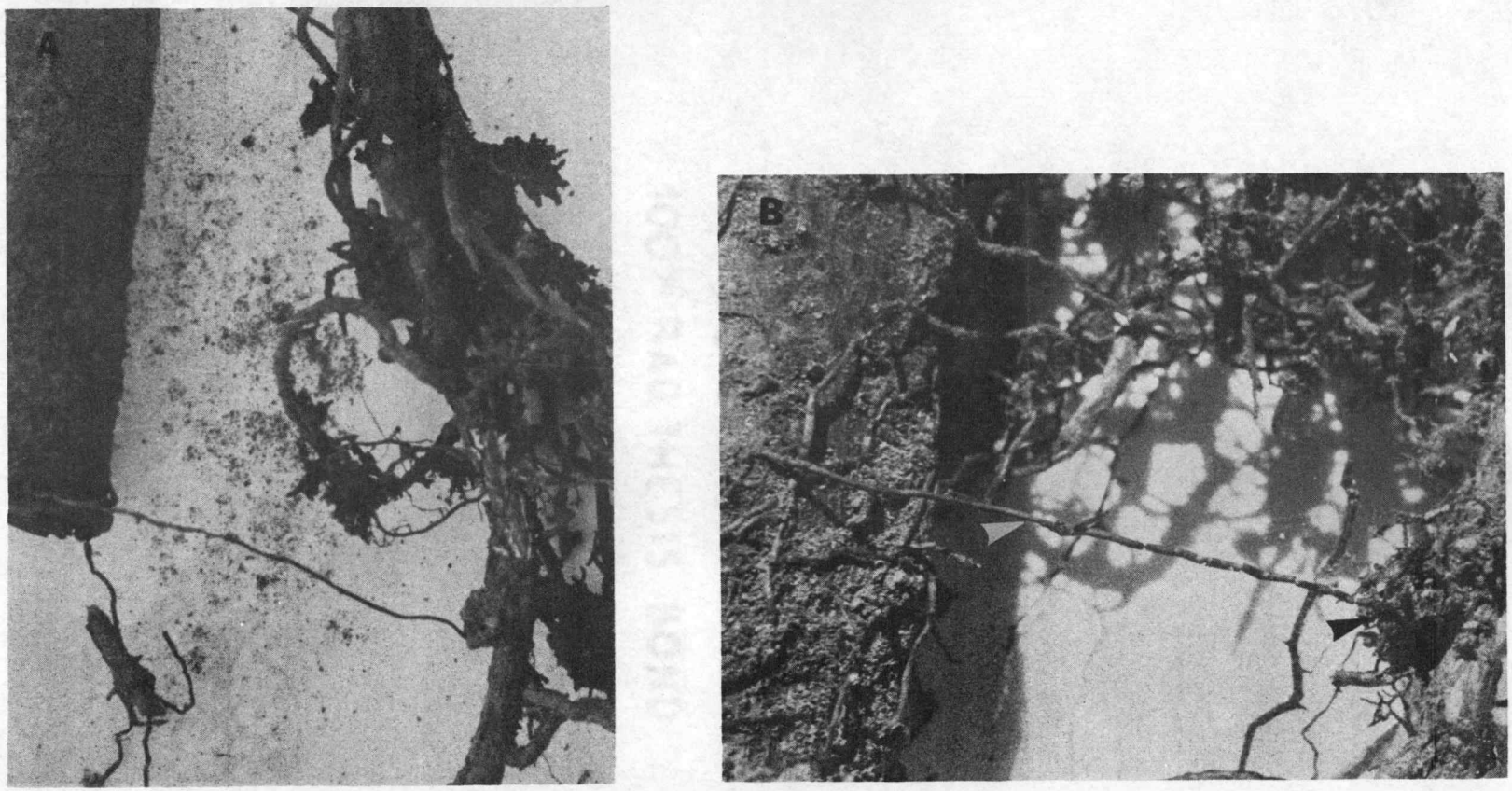

Figure 30. Infection of pine seedlings by rhizomorphs from alder inoculum. (A) Rhizomorph attachment between inoculum and root. Note the white mycelial fan underneath the cut away bark above the lesion. (B) Rhizomorph connection between incoculum and another attacked seedling. White arrow points to the rhizomorph, black arrow to the lesion.

Though the experiment is not yet complete, A. mellea isolates from pine sources in the "saddle area" are indeed pathogenic on ponderosa pine. At the time this experiment was established, the genetic similarity of these isolates was not known. Subsequent tests (p. 46) indicated that all are of the same genotype. It is therefore reasonable that they should behave alike.

While one tree apparently was killed by an isolate from willow, this is the only evidence of damage to pines by hardwood isolates from the Plateau Block. The presence of the resinous lesion indicates that the tree was attacked while alive, and not colonized after death by some other agent (Gilmour, 1954). Further evidence concerning pathogenicity of hardwood isolates from the Plateau Block must await the 1975 examination. Low or no pathogenicity is expected.

Effects of Pine Resin on the Growth of A. mellea in Culture

Parasitic attack by A. mellea on pines, in contrast to saprophytic attack, results in resin exudation (Gilmour, 1954) and lesion formation at the point of attack (Figure 26) (Patton and Riker, 1959; Rishbeth, 1972; Adams, 1972). While resin flow in pines is a generalized reaction associated with wounding (Browning, 1963), the resinous lesions appear in a high degree to prevent the proximal

advance of the fungus on the root (Buckland, 1953; Patton and Riker, 1959; Rishbeth, 1972; Adams, 1972).

Occluding of the fungus by the resin has been thought of as a physical blockage (Zeller, 1917; Verall, 1938), a growth inhibition (see Table XIV), or both. Here, effects of ponderosa pine resin on the growth of A. mellea were tested in culture.

To acquaint the reader with the characteristics of pine resin and with other studies on the effects of resin or resin components on the growth of fungi, the literature on these topics is reviewed.

Literature Review

Table XIV summarizes reports on the effects of resin components on the growth of fungi.

Coniferous resins may be divided into ray parenchyma resin and oleoresin. Oleoresin, sometimes referred to as wound or pathological resin (Browning, 1963), is the viscous liquid secreted from the resin ducts that is present at wounds or infection sites. It is essentially a solution of rosin in a volatile terpene solvent (Mirov, 1961; Mutton, 1962). In pines the rosin portion constitutes 71 to 81%, with resin acids (mostly derivatives of diterpenes) accounting for up to 90% of the rosin. The volatile terpene fraction 19 to 29%, consists mainly of monoterpenoids or sesquiterpenoids. In ponderosa pine the normal composition of this volatile fraction is:

Table XIV. Effects of various components of resin on fungal growth.

Resin component tested	Fungi involved	Workers involved	Findings
gaseous terpenoids longifolene α-pinene β-pinene Δ-3 carene limonene	Coniophora sp. Peniophora sp. Stereum sp. Polyporus sp.	Fries (1973)	Linear growth of wood decomposing fungi was usually retarded.
α-pinene β-pinene limonene Δ-3 carene camphene	38 species of hymenomycetes, including A. mellea	Hintikka (1970)	An exhaustive series of six concentrations of each compound was tested against all 38 fungi. Most species isolated from coniferous wood were able to grow in a saturated atmosphere of these terpenes. In contrast, small amounts of terpenes in the air inhibited the growth of fungi occurring in the wood of deciduous trees. Growth of A. mellea (hardwood isolate) was inhibited at all but the lowest concentration. Hintikka concluded that the terpenes in coniferous wood have a selective effect on invading fungal populations.
volatiles from heat dried wood of various species	Sterum sanguinolentum and other basidiomycetes	Suolahti (1951) in Rice (1970)	Heat dried wood of various species suspended over cultures of different basidiomycetes resulted in an increase in aerial hyphae and a corresponding increase in dry weight. He was not able to identify the volatile substance but implicated the fatty fraction of wood.
volatiles emanating from pine wood	Fomes annosus	Rice (1970)	Wood volatiles promoted the growth of the fungus mainly through formation of dense aerial hyphae. The growth promoting factor may be autoxidation products of the fats contained in the wood.
natural constituents of Pinus sylvestris roots, including Δ-3-carene, α-pinene, β-pinene, limonene, and a mixture	Mycorrhizal fungi: Boletus variegatus; Rhizopogon roseolus	Melin and Krupa (1971)	A 5 day exposure to any of the test substances inhibited the growth of both mycorrhizal fungi 55 to 86%.

(Continued on next page)

Table XIV. (Continued)

Resin component tested	Fungi involved	Workers involved	Findings
monoterpenes, including α-pinene, β-pinene and limonene	Phytophthora cinnamomi and Fomes annosus	Krupa and Nylund (1972)	As a gas each test compound inhibited the extension of growth.
resinous sapwood and extracts thereof	Blue stain fungi (Ceratocystis montia, Europhium sp.)	Shrimpton and Witney (1968)	The addition of oleoresinous sapwood to the medium inhibited growth, while non-resinous sapwood enhanced growth. Volatile components of acetone extracts from both wood types inhibited growth.
α-pinene β-pinene	Fomes pini F. pinicola Ceratocystis pilifera	Keyes (1969)	At four concentrations tested, there was no significant effect on growth. None of these fungi could use pinene as a sole carbon source.
volatile terpenes including α and β pinene, limonen	Trichoderma virdae Lenzites saepiaria Schizophyllum commune	DeGroot (1972)	Growth of each fungus was reduced more than 50% in a saturated atmosphere of the terpene. The relative toxicity of each terpene varied with the fungus.
extracts of the phenol enriched resin soaked reaction zone around root infections in Pinus taeda	Fomes annosus	Shain (1967)	Acetone extracts of the reaction zone inhibited decay of wood blocks and linear growth of Fomes annosus mycelium. Pinosylvin may have contributed, but was not solely responsible for the inhibition. He comments that even if resin acids were only inhibitory at concentrations in excess of 100,000 ppm there still would be sufficient quantity in the wood reaction zone to be effective. The fungus can utilize portions of the oleoresin and modify the phenols present.
resin soaked bioassay discs	Fomes annosus	Bega and Tarry (1966)	Resin had no toxic effects on growth or sporulation. At optimum temperature increased growth and sporulation occurred when the resin was present. They suggested that resin impregnated wood serves as a survival habitat for the fungus.

(Continued on next page)

Table XIV. (Continued)

Resin component tested	Fungi involved	Workers involved	Findings
components of oleoresin from ponderosa pine	Fomes annosus and four blue stain organisms	Cobb et al. (1968)	Volatile constituents of crude oleoresin reduced growth in 3/4 of the blue stain fungi and severely retarded F. annosus. Individual terpenes in general retarded growth and affected colony characteristics.
oleoresin from white spruce roots	white spruce heart rotting fungi: Flammula alnicola, Fomes pini, Stereum sp., Polyporus tomentosus, Coniophora puteana	Whitney and Denyer (1969)	Resin, which accumulated in and around inoculated heartwood of white spruce roots, reduced the amount of infection in all fungi tested except P. tomentosus and C. puteana in which it had no effect.
aromatic components which may or may not be present in oleoresin	Polyporus versicolor and other hymenomycetes	Fahraeus (1962)	Aniline and p-hydroxybenzoic acid stimulated growth only in laccase producing fungi. Author suggests that laccase plus phenol play a special role in the metabolism of these fungi.
lipids and resin acids from tree roots, including ponderosa pine	Armillaria mellea	Moody and Weinhold (1972)	Saponified lipids from tree roots, including ponderosa pine, stimulated rhizomorph production in a strain of A. mellea that produced no rhizomorphs on their standard medium. The resin acid fraction of an extract from 1-5 g of root tissue stimulated approximately twice as much rhizomorph tissue as fatty acids from the same amount of roots. With filter sterilized abietic acid, at concentrations from 0.1 to 10 g/liter, few rhizomorphs were produced. Abundant rhizomorphs were formed if the abietic acid was autoclaved; perhaps indicating the formation of a breakdown product from autoclaving.

(Continued on next page)

Table XIV. (Continued)

Resin component tested	Fungi involved	Workers involved	Findings
levopimaric acid (a major component of the resin acid fraction)	Fomes pinicola Haplosporella sp.	Shriner and Merrill (1970)	Both fungi could use the acid as a sole carbon source. When glucose was present as a carbon source the addition of levopimaric acid had no significant effect on growth. Wood inhabiting fungi can not utilize monocyclic or bicyclic monoterpenoids, but can utilize diterpenoids (diterpenes are the major components of the resin acid fraction of oleoresin).
pinosylvin	Fomes annosus	Jorgenson (1961)	Sapwood surrounding infections that contained the phenol, pinosylvin, did not contain F. annosus. Sapwood without the phenol contained F. annosus.
fungitoxic phenolic compounds from Pinus contorta heartwood, including pinosylvin and its monomethyl ether	Fungal microflora of P. contorta heartwood, including both ascomycetes and basidiomycetes	Loman (1970a)	Predominating species of fungi in P. contorta heartwood possess enzyme systems capable of detoxifying fungitoxic phenolic compounds, including pinosylvin and its monomethyl ether.
fungitoxic phenolic compounds from Pinus contorta heartwood, including pinosylvin and its monomethyl ether	Peniophora pseudo-pini Coniophora puteana Fomes pini	Loman (1970b)	Bioassay of fungitoxic phenolic compounds on 2% malt agar cannot be extrapolated to natural substrates. Cultural tests showed fungi surviving on natural substrate medium containing four times the toxic concentration of the phenolic compound in the standard bioassay medium.
oleoresin volatiles from Pinus sylvestris	Armillaria mellea	Rishbeth (1972)	The growth rate on malt agar was halved by exposure to vapors from the terpentine fraction or from resinous bark of pine trees attacked by the fungus. Some isolates failed to form rhizomorphs in the presence of the vapors.

α -pinene, 6%; β -pinene, 21%; Δ -3 carene, 51% (the latter accounts for the distinctive "sweet" odor of ponderosa pine oleoresin; Mirov, 1961); myrcene, 10%; and limonene, 9% (Smith, 1973). These amounts vary substantially, as individual trees may have no Δ -3 carene and 60% limonene (Smith, 1973).

This is a general view of pine oleoresin composition. Exactly how many different compounds are present and in what combinations, concentrations, and forms is not adequately known. For example, Jorgenson (1961) and Shain (1967) indicated that certain phenolic compounds (e.g., pinosylvin and its monomethyl ether) are formed in response to wounding. These free phenols apparently become incorporated in the oleoresin, and are suggested as possible chemical constituents which augment the proposed mechanical barrier that resinous wood presents (Cobb et al., 1968). Yet, Loman (1970a) showed that certain wood rotting fungi break down these toxic phenolic compounds.

Oleoresin, in contrast to ray parenchyma resin, is very low in fatty acids. In Pinus palustris Mill. the composition of oleoresin and wood resin (mainly ray parenchyma resin) respectively are 90% resin acids, 6% fatty acids, 4% unsaponifiables and 36% resin acids, 54% fatty acids, and 9% unsaponifiables (Mutton, 1962). Thus, the reported stimulation of A. mellea growth by fatty acids obtained from root extracts of ponderosa pine and other species (Moody and Weinhold,

1972; see Table XIV) is most likely unrelated to any effects of the oleoresin present in lesions on pine roots at points of A. mellea attack.

While the reported results of laboratory tests on the effects of resin on the growth of fungi are perplexing (Table XIV), there seems to be good field evidence of inhibitory effects of oleoresin. However, it is unclear if the resin accumulation is acting as a mechanical barrier, or if the latter, is reinforced by chemo-toxic effects (Cobb et al., 1968). In pines oleoresinous lesions appear to prevent or retard proximal spread of the fungus along the root for some period of time. If there is a chemical effect augmenting the physical presence of the resin as a barrier, then it would seem logical that either more persistent type compounds are involved or a continuous flow of volatiles is maintained. Many of the compounds tested (Table XIV) are highly volatile. How long these would persist and could act as a fungus deterrent in the soil root atmosphere, if not continuously replenished, is certainly open to question.

Resistance to entry of fungi and various insects has been related to the amount of resin flow in a tree. Gibbs (1968) showed that the resistance of pines to Fomes annosus is correlated with the ability of the tree to produce resin. He also suggested that the increase in complexity and activity of the resin system from larch to Douglas-fir to spruce to pine is related to the relative resistance of these species to F. annosus. Reid et al. (1967) showed that in

ponderosa pine trees injured by air pollution the incidence of bark beetle attack was much higher and resin flow from beetle wounds was reduced by 2/3. Marthre (1964) presented evidence that trees with high oleoresin exudation pressure are more resistant to blue stain fungi than those with low pressure.

Shain (1967) concluded that both the survival and penetration of F. annosus was inversely related to the physiologic state of the host. Resistance to F. annosus in pines is highly dependent on host vigor (Rishbeth, 1951; Gibbs, 1967), and Gibbs (1968) showed that there is a decreasing gradient in resin yield from dominant to suppressed Scots pine saplings. Sokolov (1964) admitted that resin can serve as a hindrance to advancement of A. mellea, but found no difference in the ability of diseased and healthy trees to produce resin.

Rishbeth (1972) observed that F. annosus spread rapidly on pine roots with intermittent resin flow, and much slower on those with a copious flow. He also noted that the greater resistance of Pinus nigra var. calabrica over Pinus sylvestris is correlated with greater resin yield from P. nigra. However, this relationship is apparently not uniform, as Hodges (1969) shows an inverse correlation between resin exudation and the degree of infection by F. annosus on three species of southern pines. When considering effects of resin on pathogens, the variability in its chemical composition between and

within species may be more important than the actual amount of resin present (Cobb et al., 1968; Rishbeth, 1972).

General Methods

Resin was collected from pre-existing stem wounds on pole-sized and larger ponderosa pines in the Glenwood area and stored at 5°C until used (see Discussion, p. 151, for justification of collection and storage of resin in this manner). Nearly all the resin collected and used was not hard and brittle, but pliable and sticky.

Crude resin pieces (up to 10 g, but most often much smaller) were placed in a mortar. Liquid nitrogen (-180°C) was poured over the resin. Immediately after the nitrogen evaporated (about 10 sec) the frozen resin was ground with a pestle and sifted through a 16 mesh/in. screen. The still frozen resin particles (now powder and granules) were then used as described in Appendix III for preparation of the various media.

Unless otherwise stated, the control medium used was: 20 g malt extract, 19 g agar, 30 g dextrose, 5 g bacto-peptone, and 1,000 ml distilled water. This was autoclaved for 15 to 20 min. This medium is referred to throughout as the regular medium. All other media used contained these same ingredients in the same amounts plus the resin or an extract thereof as described in Appendix III. All petri plates were poured with approximately 30 ml media/plate.

All experiments used an isolate of A. mellea, from a killed, pole-sized pine at Glenwood, Washington. The various test media were inoculated with plugs (no. 4 cork borer) of regular or water extract of resin medium containing aerial mycelium and rhizomorphs from fringes of new cultures.

All cultures were incubated in plastic crisper boxes, in the dark, at 25°C for 21 days, unless stated otherwise. To avoid possible effects of volatile compounds, test plates of the regular medium were always incubated separately from plates containing resin or resin extract media.

The widest colony diamter present in each plate was measured as one parameter of growth. This measurement was between rhizomorph tips, mycelial limits or both.

Dry weight also was used as a growth parameter. After diameters were measured, cultures were steamed for 30 to 60 min. Colonies were removed from the melted agar with forceps, rinsed in hot tap water, and placed on filter papers. These were dried in a forced-air oven, remaining there 24 hrs at 90°C, and weighed. With the medium containing acetone dissolved resin, globules of the heated resin adhered to the colony and would not rinse off by the above procedure. To obtain most accurate colony weights, the solid agar containing the colony was removed intact from the petri dish. Most of the resin remained in the bottom of the petri plate. What did not was

easily scraped from the lower agar surface without loss of mycelium or rhizomorphs. The agar containing colony was then placed in another petri dish, heated, and treated as above.

Results

Within 48 hr of inoculation, a deep red ring appeared around the inoculum on the autoclaved resin plates, but not on the regular medium controls.

After 21 days, growth on the autoclaved resin plates significantly (.05 level) exceeded growth of the controls (Table XV), rhizomorph initiation and development were more rapid, and contamination was less prevalent.

Pine resin sterilized in Propylene Oxide (PO) stimulated growth at concentrations less than 2 g per plate. Higher concentrations frequently resulted in no growth, probably due to incomplete removal of the PO. Inoculum plugs from the 2 and 3 g resin plates showing no growth failed to grow on fresh plates of regular medium, whereas fresh inoculum grew well on re-inoculated PO sterilized resin plates. This response is considered later.

Non-sterilized resin in amounts of 1 and 2 g stimulated growth of A. mellea over the regular medium, and the higher concentration appeared to retard contamination. However, after 13 days all non-sterilized resin plates were badly overgrown with contaminants.

Table XV. Growth of A. mellea on regular medium and autoclaved resin media.[1]

	Regular medium		Autoclaved resin media			
			40 g resin/liter		80 g resin/liter	
	Mean dry weight (mg)	Mean colony diameter (cm)	Mean dry weight (mg)	Mean colony diameter (cm)	Mean dry weight (mg)	Mean colony diameter (cm)
Replication 1 (10 plates)	0.18	3.8	0.81	9.0	0.94	9.0
Replication 2 (10 plates)	0.19	3.7	0.76	9.0	0.89	9.0

[1] All plates were incubated for 21 days.

Growth on all acetone-resin media was significantly greater (.05 level) than on regular medium (Table XVI). Both colony diameters and dry weights consistently increased between concentrations of 3 and 7 ml of the filtrate, but neither measurement consistently changed between 7 and 10 ml (Table XVI).

On the water extract of resin medium a brownish ring, less intense than the red ring on the autoclaved resin medium, appeared around the inoculum plugs. Growth was significantly increased over the regular medium (Table XVII, Figure 31), and rhizomorph initiation and development were more rapid.

Growth on the water extract of resin medium with the phenols removed (treated with polyvinylpyrrolidone, PVP), and the gallic acid medium showed no significant difference from the regular medium, and all three had less growth than the non-treated water extract of resin medium. A light brown band was present around the inoculum plug in the gallic acid medium.

As a general observation, the resin media tested, except for the non-sterilized resin plates, were less contaminated than the regular medium.

Discussion

The resin employed in these tests, even though collected from pre-existing stem wounds, is considered very similar to that found

Table XVI. Growth of A. mellea on the regular medium and the acetone extract of resin media.

	Mean colony diameter (cm)				Mean culture dry wt. (mg)			
	Acetone filtrate (ml/plate)				Acetone filtrate (ml/plate)			
	0[1]	3	7	10	0[1]	3	7	10
	------- cm -------				-------- mg ---------			
Replication 1 (5 plates)	3.6	5.6	6.5	7.2	0.16	0.39	0.59	0.75
Replication 2 (6 plates)	3.3	6.8	8.0	7.7	0.10	0.31	0.37	0.48
Replication 3 (6 plates)	4.4	6.7	8.8	8.4	0.26	0.48	0.78	0.59

[1]Regular medium.

Table XVII. Growth of A. mellea on regular medium and the water extract of resin medium. [1]

	Regular medium		Water-resin medium	
	Mean dry weight (mg)	Mean colony diameter (cm)	Mean dry weight (mg)	Mean colony diameter (cm)
Replication 1 (4 plates)	0.16	3.4	0.28	4.5
Replication 2 (8 plates)	0.15	3.2	0.33	5.3

[1]Replication 1, plates incubated 21 days; replication 2, plates incubated 22 days.

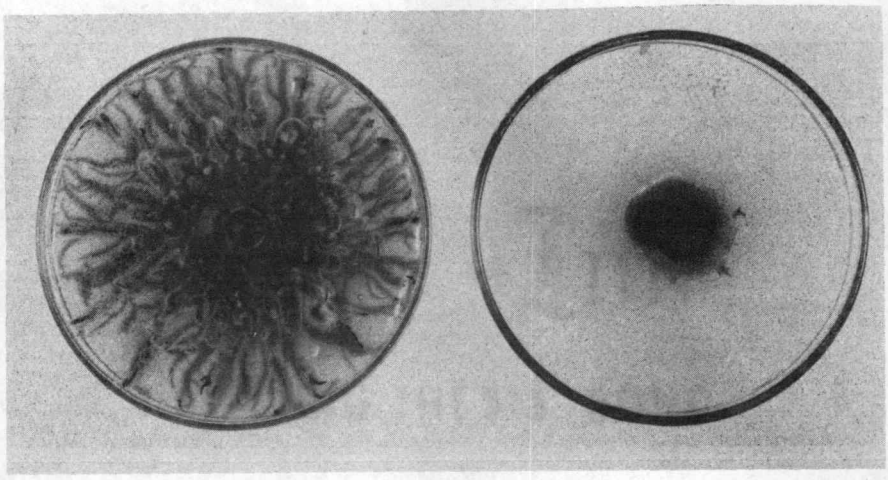

Figure 31. Growth of A. mellea after 21 days. Regular malt agar medium on right, medium containing water extract of resin on left.

on roots at A. mellea infection sites. The flow of oleoresin in pines is considered a generalized reaction to any wounding (Browning, 1963) and the resin duct system in pines is considered functionally interconnected (Gibbs, 1968). Both probably are very low in volatiles because of prolonged exposure, and consist primarily of resin acids and other less volatile compounds. Cobb et al. (1968) emphasized this difference in composition between fresh and exposed resin when noting the variation between their tests and those of Bega and Tarry (1966).

As field evidence indicates that resinous lesions inhibit proximal spread of the fungus along a root even when the resin present is dry and crumbly (thus not freshly and continually produced, nor high in volatiles) any long term effects of the resin on the fungus were considered to be caused by the non-volatile, or only slightly volatile, compounds remaining.

Stimulation of growth by autoclaved resin may be caused by a breakdown product formed during autoclaving. Moody and Weinhold (1972) found that in medium containing abietic acid, a commonly available resin acid, few rhizomorphs formed if the material was filter sterilized, but if autoclaved, rhizomorphs developed in abundance.

That the growth stimulation found in my tests was caused by a breakdown product resulting from heat (autoclaving) seems unlikely

when one considers that acetone dissolved resin, water extracts of resin, and non-sterilized resin also increased growth. Shain (1967) suggested that resin acids may be the component of reaction zone wood that inhibited growth of Fomes annosus. He commented that even if the resin acids were only inhibitory in concentrations in excess of 100,000 ppm, there still would be sufficient quantity in the wood reaction zone to be effective. My tests indicate that stimulation of A. mellea in culture occurs with concentrations of resin acids around 40,000 ppm.

Propylene Oxide (PO) has been used for sterilization purposes with many types of biological materials (Hansen and Snyder, 1947). Use of PO under vacuum enhances the penetrating ability of the gas into finely ground material, and as such the gas serves as more than a surface sterilant. Incomplete removal of PO prior to inoculation, rather than any effect of the resin, is considered to have caused the lack of growth on the 2 and 3 g resin plates. Failure of inoculum plugs from these plates to grow when placed on fresh regular medium, while upon re-inoculation good growth occurred on the PO treated resin plates from which the initial inoculum plugs had been removed, supports this assumption (the re-inoculated plates had already set for 21 days and any remaining PO had had a chance to dissipate). The method employed herein is seen as applicable to sterilizing any material that can be finely ground (perhaps by first freezing the

material with liquid nitrogen and grinding in a mortar and pestle, as described for the resin) when one does not wish to use heat.

The acetone dissolved resin media did not show a consistent increase or decrease in growth with higher resin concentrations. The autoclaved resin media did show a consistent dry weight increase with the higher resin concentration, but colony diameters were the same. However, colony expansion was limited, as all colonies had grown to the edge of the petri plate. A more complete series of resin concentrations, perhaps grown in larger petri plates, should be tested in both cases.

The PVP treatment of the water resin solution reduced the phenolic content of this extract to 1/10 of the original and resulted in the loss of growth stimulation. While indicative, this does not prove that the phenolic containing compounds removed from the extract were responsible for growth stimulation. The compounds absorbed onto the PVP need to be removed from the PVP and tested to determine their effect on growth of the fungus. Also, other tests with PVP suggested a possible interaction with the regular medium.

Gallic acid medium is often used to test if a fungus is a brown rot or a white rot (Nobels, 1948). Typically, the white rot fungi, including A. mellea, form a dark colored ring around the inoculum plug and growing colony, while the brown rots do not. Gallic acid medium used for this purpose is more concentrated than the gallic

acid medium used here (5 g/liter vs. 0.2 g/liter). However, even at this low concentration a light colored brown ring was noticeable on the gallic acid medium.

The presence of the deep red ring around the inoculum plug and the red band around the developing colonies on the resin media is considered to be this same reaction. The extracellular oxidases of A. mellea are reacting with resin components, most likely phenolics, and causing a color change in the medium.

The data presented represent the condition of the colonies at the end of their respective incubation periods. Throughout the growth periods, colony diameters were recorded and other observations made in an attempt to identify any changes in the stages of colony development on the different media. Snyder (1959) described five stages in the development of A. mellea colonies on solid agar medium. These were: 1) production of "central mycelium" prior to development of rhizomorph initials; 2) a brief period during which the rhizomorph initials formed from pseudoscierotical tissue; 3) a short period when rhizomorph growth rate increased, but the tips were within the extremities of the central mycelium; 4) a period during which the rhizomorphs reach their maximum linear growth rate (this rate is usually five or more times faster than the growth rate of the central mycelium); 5) a stage when the rhizomorphs, with apparently unlimited growth potential, were restricted by the walls of the petri plate.

Pseudosclerotical tissue and aerial hyphae developed concurrently on the agar surface during these stages, and, along with the rhizomorphs, gave the entire colony a lobed appearance (Figure 31).

All of these stages were recognizable in the resin and regular media plates. The resin media that increased growth in comparison to the regular medium appeared to: speed up stage 1; shorten the times of stages 2 and 3; and reach stage 5 sooner. Stage 5 was rarely encountered on the regular medium during incubation for 3 wks. If plates were left longer, on occasion this stage was reached. Often the resin media plates, especially the autoclaved resin media, would reach this stage in 3 wks or soon thereafter. Often resin media plates also showed an increase in the amount of aerial mycelium present. This was especially noticeable with another isolate (from hazel) that developed more aerial mycelium on all media than the isolate used in these tests.

It was not determined if there was a difference in the linear growth rate of the rhizomorphs, once they were beyond the extremities of the central mycelium (stage 4), on the two types of media. There were occasional individual plates of the regular medium on which rhizomorphs did not develop at all. Nondevelopment of rhizomorphs was extremely rare on the resin media plates (see Table XIV; Rishbeth, 1972). The resin media seem ideal for culture of A. mellea. They are promising for acceleration of all sorts of isolations and

in vitro studies. Filip (personal communication) utilizes the 40 g resin medium (described in Appendix III) as the standard medium for his cultural tests.

In these tests ponderosa pine wound resin treated in various manners stimulated the growth of A. mellea. Field evidence indicates that the growth of the fungus is inhibited in the resinous lesions present on pine roots at sites of A. mellea attack. The seeming paradox in these observations might be explained as follows: 1) the tests did not deal with the highly volatile compounds present in fresh resin; 2) a breakdown product was created in all instances; and/or 3) the resin present was unrepresentative of that present in A. mellea root lesions. These possibilities have been discussed and are regarded as unlikely explanations.

Two other alternatives are suggested: 1) in the field resin serves solely as a mechanical barrier, or 2) the resin concentrations tested are unrepresentative of the resin content in A. mellea root lesions. The first of these alternatives, that resin would physically retard fungal development and physiologically stimulate growth, also seems unlikely. Bega and Tarry (1966) suggest that resin impregnated wood serves as a survival habitat for Fomes annosus, Shain (1967) found that F. annosus could utilize portions of the oleoresin, and Shriner and Merrill (1970) have shown that F. pinicola (Schwartz ex Fr.) Cke. can utilize diterpenes (the major component of the resin

acid fraction of pine oleoresin). However, no literature suggests that the same concentration of resin concurrently serves both to stimulate and retard growth of the same organism.

The second alternative seems more feasible. The oleoresin concentration at a resinous root lesion is not known, but the resin is present in excess. It penetrates the root and surrounding soil. The concentrations of resin in the various media tested were undoubtedly much lower than those in contact with the fungus in a resinous lesion. Hueppe's principal (Arndt-Schutz law; Horsfall, 1945) states that every substance, which in definite concentrations will kill protoplasm, will inhibit development in smaller amounts, and in still greater dilution will act as a stimulant. Perhaps the concentrations of resin tested in these experiments correspond to this lowest level, and the higher resin concentration in the lesion is serving to both physically and physiologically retard fungal development. This explanation emphasizes the need for determining a complete dosage response curve when attempting to determine the effects of supposedly inhibitory compounds.

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APPENDIX I

SUPPLEMENTAL LITERATURE REVIEW

Taxonomy of *Armillaria mellea* (Vahl ex Fr.) Quél.

The species was first technically described by Vahl in 1777 as *Agaricus melleus*, though it may have been noted earlier (Sokolov, 1964). Fries (1821) placed the fungus in the tribe *Armillaria*. Fries' *Armillaria* was reduced to generic rank by Quelet (1872), who included the honey fungus as *Armillaria mellea*.

In 1879 Karsten, on the basis of several morphological features, united certain laminate fungi into the new genus *Armillariella*; *Armillaria mellea* was among these. This transfer resulted in the name *Armillariella mellea* (Vahl ex Fr.) Karst. Karsten's classification, however, did not become established and remained initially unused by mycologists. Singer (1949, 1962) accepted Karsten's usage, with the result that *Armillariella mellea* is now being utilized with increasing frequency in the literature.

Another taxonomic consideration arises from the relation between *A. mellea* and *Clitocybe tabescens* (Scop. ex Fr.) Bres. *C. tabescens* is often confused with *A. mellea* as they resemble each other very closely in both morphological features and biological properties. Rhoads (1945) separated the two primarily on the lack of an annulus in *C. tabescens* and its presence in *A. mellea*. Richards (cited in Gilmour, 1954) stated that *C. tabescens* and *A. mellea* were the only fungi in America that produce similar rhizomorphs in

culture. She separated the two by examining cultures in the dark, where A. mellea cultures were luminescent and C. tabescens were not.

C. tabescens does not usually produce rhizomorphs under natural conditions, and this characteristic was used by Rhoads (1945) to distinguish C. tabescens from A. mellea. The absence of rhizomorphs in the field, however, may be related to environmental conditions rather than to the fungus involved (Bliss, 1946; Swift, 1968; Rishbeth, 1968). Swift (1970; also see Olembo, 1972) has found a water leachable soil factor that inhibits field and cultural production of rhizomorphs (it is suggested by Boughey et al., 1964, that this factor may be related to alleopathic relations between higher plants). When this factor is removed, isolates have the ability to produce rhizomorphs.

As most C. tabescens descriptions come from warmer areas (Gainesville, Florida, for example), Rishbeth (1968) suggested that high soil temperature (greater than 25°C, which occurs at Gainesville for at least part of the year) may inhibit field production of rhizomorphs, and that there may be no real difference between the two species. Recently, Billie-Hansen (1973) summed up the state of confusion between Armillaria and Clitocybe:

> But if we compare the diagnosis of Clitocybe with that of Armillariella character against character, they overlap each other so completely that the only distinctive remaining

> character is that Clitocybe has clamps at every septum, while Armillariella only bears clamps at the base of the basidia. I think like Smith (1968) that presence/absence of clamps cannot be taken that serious.

Cultural Characteristics of A. mellea

Nobles (1948) had the following to say about the appearance of A. mellea cultures:

> Cultures of Armillaria mellea may be readily recognized on the basis of macroscopic appearance alone, their reddish-brown crustose areas, their rhizomorphs, and their frequent luminosity of young actively growing colonies providing a group of characters unique among the [126] species studied.

Snider (1959) described five stages in the development of A. mellea in pure culture; these are discussed on p. 154. Pseudosclerotical tissue and aerial hyphae develop concurrently on the agar surface during these stages, giving the entire colony a lobed appearance (Figure 32).

Considerable variation exists in cultural appearance among isolates of A. mellea (Raabe, 1966), and yet, within a single isolate, morphological consistency is maintained through periodic culture transfers (Gibson, 1961; Raabe, 1967).

A. mellea will grow on many solid and liquid media, over wide ranges of pH and temperature. Snider (1959) reported abundant rhizomorph formation from pH 4.0 to 7.0 on various media. Mycelial growth is said to occur from pH 2.5 to 8.5 (Sokolov, 1964).

Development of both mycelia and rhizomorphs has a fairly broad pH optima, between 4.0 and 6.0, somewhat dependent on the different medium (Reitsma, 1932; Lisi, 1940; Benton and Ehrlich, 1941; Sokolov, 1964). These optima agree with field observations that best development occurs in light, slightly acid, soils (Sokolov, 1964).

Temperature optimum for mycelial growth and rhizomorph formation in culture is 25°C or slightly lower (Reitsma, 1932; Townsend, 1954; Rishbeth, 1968). Rishbeth (1968) found rhizomorph production was at least 50% of the optimum at any temperature between 15°C and 25°C. The optimum temperature for rhizomorph development from wood blocks was found to be lower than that on culture medium (22°C vs. 25°C). Interestingly, Bliss (1946) showed that infection was limited to those temperatures at which the pathogen produced rhizomorphs.

The effects of light on the development of the fungus in culture are inconclusive. Townsend (1959) considered light to have an adverse effect on growth of both mycelia and rhizomorphs. Sokolov (1964) found no conclusive evidence on the effect of light, but noted that in nature A. mellea normally developed in complete darkness. Many workers have elected to grow their cultures in the dark (Patton and Riker, 1959; Swift, 1968; Motta, 1969; Adams, 1972).

Atmospheric humidity between 90 and 100% favors rhizomorph development, while production ceases below 50 to 60% (Sokolov, 1964).

Benton and Ehrlich (1941) found optimum growth of the fungus in wood of Pinus monticola Dougl. to be at a moisture content as high as 150%. Naturally infected wood is reported as being extremely moist (Peace, 1962).

A. mellea can remain viable for at least 2 months in an atmosphere void of oxygen, and growth is retarded only when the barometric pressure is less than 40 mm (Sokolov, 1964). Growth is inhibited at high levels of atmospheric carbon dioxide (60-80%), retarded at moderate levels (20-60%), and favored by normal (0.03%) or low levels (up to 19%) (Sokolov, 1964; Raabe and Gold, 1967).

Glucose appears to be the best sugar tested as a carbon source for growth of A. mellea in culture (Reitsma, 1932; Weinhold and Garraway, 1966; Garraway, 1973). Garraway (1973) indicated that both concentration and type of carbohydrate present are important to fungal growth. Recent evidence showed that defoliation of sugar maples, which may predispose them to attack by A. mellea (Houston and Kuntz, 1964), followed by production of new leaves, brings about a decrease in the starch content and increases the amount of glucose and fructose in the outermost root wood (Parker and Houston, 1971; Wargo, 1972). Extracts of this glucose rich wood stimulated growth of A. mellea in culture (Wargo, 1972).

In ring barking experiments, a depletion in root starch reserves was considered important to control success (Leach, 1939), and the

decreased value of the stumps to serve as a food base (Redfern, 1968). Thus, laboratory findings on sugars most suitable for fungal growth may well help explain changes in host root physiology, initiated by defoliation, ring barking, etc., that create conditions more or less favorable for A. mellea.

Peptone is a suitable nitrogen source for A. mellea (Hamada, 1940; Garrett, 1970). Ammonium nitrogen in the form of ammonium tartrate was the most suitable inorganic nitrogen source tested, while Casene was the most utilizable organic nitrogen source (Weinhold and Garraway, 1966). Apparently nitrate cannot be utilized (Garrett, 1953).

Changes in the carbon-nitrogen ratio (C:N) effect the growth of A. mellea. Hamada (1940) found that changing the ratio effects the color and form of rhizomorphs. A C:N ratio of from 2:1 to 4:1 was best for rhizomorph growth, while aerial mycelium developed from 2:1 to 1:2 (Hamada, 1940). Dextrose and peptone were used as a source of carbon and nitrogen. Townsend (1954) gives a ratio of glucose to peptone of 3:1 to 5:1 as the best for growth. Garrett (1953) observed that a relative increase in both C and N resulted in increased rhizomorph development, while at suboptimal carbohydrate concentrations an abundance of N (peptone) depressed formation of initials, and at high carbohydrate concentrations excess N depressed

rhizomorph growth, but did not effect the development of new rhizomorph initials.

Suitable medium sufficient for culture growth can be made utilizing peptone, glucose, and malt extract (Hamada, 1940; Garrett, 1970; Adams, 1972).

Effects of Ethanol on the Growth of A. mellea

The stimulatory effects of certain alcohols, mainly ethanol, on rhizomorph formation in A. mellea have received much attention. Weinhold (1963) and Weinhold and Garraway (1966) showed stimulation of mycelial growth and rhizomorph development with as low as 50 ppm ethanol, and no inhibition was observed at concentrations as great as 10,000 ppm. They suggested that a suitable carbon source, such as glucose, is sufficient for growth initiation only if the medium contains a low concentration (100 to 500 ppm) of a growth promoting substance such as ethanol. Ethanol alone, at a high enough concentration, is a suitable carbon source (Weinhold and Garraway, 1966).

Garraway and Weinhold (1968) used C^{14} labeled glucose to show that ethanol has a definite effect on the metabolism of glucose. In the absence of ethanol, C^{14} was predominately detected in respiratory CO_2, whereas in the presence of ethanol radioactivity was more prevalent in cell wall constituents. With C^{14} labeled ethanol they detected a great deal of radioactivity in the lipid fraction. Moody et al.

(1968, 1970, 1971, 1972) demonstrated a stimulatory effect of oils and fatty acids on A. mellea growth. Sortkjaer and Allermann (1972) interpreted the above information, along with their own work, to mean that ethanol is metabolized by A. mellea by the normal pathway (via acetate into fatty acids) and that this metabolism has a regulatory effect on the metabolism of glucose.

Recently, Sortkjaer and Allermann (1973) showed that as long as ethanol was present in the medium there was a continuous increase in dry weight and DNA and RNA content. Further, upon depletion of ethanol from the medium there is a stagnation in the increase in dry weight, DNA, and RNA. If ethanol is added again, all three again begin to increase in amount. They conclude by saying, "these results indicate that growth (dry weight) stimulated by ethanol in A. mellea is caused by cell division and not the accumulation of lipids or polysaccharides."

Garraway and Weinhold (1968) concluded that ethanol is necessary only for initiation and early development of rhizomorphs on a synthetic glucose medium. Contrarily, Sortkjaer and Allermann (1972) concluded that ethanol is a limiting factor during the entire growth period. Pentland (1965, 1967, 1968) showed that ethanol supplied daily at 50 ppm stimulated rhizomorph development to a greater extent than an initial supply of 700 ppm. She also concluded that a substance stimulatory to Armillaria mellea produced by the fungus Aureobasidium pullulans (de Bary) Arnaud was ethanol.

Many soil inhabiting microorganisms and wood rotting fungi present in the natural environment of A. mellea are known to produce ethanol as a result of their metabolism (Perlman, 1950; Pentland, 1967). Krupa and Fries (1971) have shown that ethanol and other volatile compounds are produced by mycorrhizial roots. Bolton (1966) has shown that roots, under certain conditions, may produce and secrete ethanol. Tarry (1968) showed that A. mellea itself could produce small amounts (25 ppm) of ethanol when in pure culture. Thus, it is apparent that in its natural environment A. mellea could encounter small, possibly continuous amounts of ethanol.

Rishbeth (1972) isolated from infected Pinus sylvestris L. some strains of A. mellea that would not produce rhizomorphs. However, when grown in the presence of the yeast Hansenula holstie Wickerham, a common yeast in pine resin, most of the isolates regained their rhizomorph producing ability. Although he does not cite ethanol as the cause, he does discuss the phenomenon in context with Pentland's experiments involving Aureobasidium pullans.

Tarry (1968) reviewed the effects of other chemical substances (IAA, for example; Weinhold et al., 1962) that have been shown to have a stimulatory effect on the growth of A. mellea.

Relations between A. mellea and Other Fungi

Many studies in addition to Pentland's work with Aureobasidium

pullans and Rishbeth's findings on Hansenula holstie (see above) have dealt with the effects of other fungi on the growth of Armillaria mellea. Haskins and Thorn (Sokolov, 1964) found an antibiotic secreted by the bunt fungus (Ustilago maydis [DC.] Cda. that inhibits growth of A. mellea. Leach (1939) noted in Africa that Rhizoctnia lamellifera in tea roots prevented A. mellea from settling in them. Often A. mellea can be isolated in conjunction with other root rot fungi such as Fomes annosus (Fr.) Cke. (Kallo and Norokopi, 1972).

Sokolov's summary (1964) stated that Darpoux et al. showed an antagonism between A. mellea and Peniophora gigantia (Fr.) Mass., Hypholma fasciculare (Huds. ex Fr.) Quél., and Trichoderma viridae Pers. ex S. F. Gray.

Much has been written of the antagonism of T. viridae to A. mellea; particularly following soil fumigation. Bliss (1951) found that T. viridae grew exceedingly well in soils recently fumigated with carbon disulfide, and that T. viridae, not direct fungicidal action of the fumigant, suppressed the growth of A. mellea in citrus plantations in California. Garrett (1957, 1958) found that, while A. mellea could be killed by direct fungicidal action, 95% of his inoculated root segments failed to produce A. mellea when incubated for 23 days in soil inoculated with T. viridae. He concluded that under optimum conditions T. viridae might provide some degree of post fumigation biological control of A. mellea.

Recently, Ohr and Munnecke (1973) reasoned that sublethal fumigation with methyl bromide stressed A. mellea by reducing its "antibiotic production" and causing possible cellular damage. Concurrently, the population of T. viridae quickly increased following fumigation, due to the elimination of less fumigant tolerant competitors. The increased population of T. viridae was able to kill the weakened A. mellea. In non-fumigated soils, however, competition among microorganisms probably would not allow T. viridae to build to a level high enough to curtail A. mellea.

Orlos (1957, cited in Sokolov, 1964; Redfern, 1968) suggested that on the basis of his laboratory experiments it should be possible to control A. mellea by using Fomes pinicola (Schwartz ex Fr.) Cke. Sokolov (1964) cites Manka (1961) as testing 91 fungi as possible inhibitors to A. mellea, with only a few having inhibited growth. In general, fungi which Manka isolated from dead roots were inhibitory, and those from living roots stimulatory to A. mellea. Apparently Manka found seven other fungi that severely inhibited the growth of A. mellea, but was unable to identify them.

Sokolov (1964) found 15 fungi antagonistic to A. mellea in culture. From field observations (not experiments) most of these were expected to be antagonistic. For example, he had observed that stumps colonized by Lenzities saepiaria (Wulf. ex Fr.) Fr. and Peniophora gigantia were not invaded by A. mellea.

Rather than being antagonistic, A. mellea may predispose woody tissue to attack by other wood decay fungi. Barrett (1970) has shown that colonization of woody tissue by Polyporus schweinitzii Fr. is enhanced through prior colonization by A. mellea. In fact, Barrett suggested that the infection of conifers on old hardwood sites by P. schweinitzii may reflect the natural abundance of A. mellea on such areas!

APPENDIX II

TABLES PRESENTING AIR DRY WEIGHTS OF ROOTS FROM EACH SAMPLE LOCATION IN THE FIVE ROOT REMOVAL TREATMENTS IN 1972, 1973, AND 1974

Appendix Table I. Air dry weights of roots obtained from each 66 cu ft soil pit sampling the amount of root material remaining 1 yr after treatment (1972) in five different treatments of the root removal control experiment.

Replicate	Root condition and stratum	Root size	Root residue removal treatment									
			1		2		3		4		5	
			Number of sample pit									
			1	2	1	2	1	2	1	2	1	2
		(cm)	grams									
A	Upper living	<0.5	80	42	80		191	127		50	109	294
		0.5-2.0	494	191	46	49	522	542		434	404	
		>2.0	630	63	432		1,565	97		392	470	
	All		1,204	296	558	49	2,278	766		876	983	294
	Dead	<0.5	106	40	43	45	37	53	62	63	44	658
		0.5-2.0	295	70	60	246	161	64	494	234	276	
		>2.0	382	118	246	250	1,238		1,949	2,983	1,534	1,142
	All		783	234	349	496	1,436	117	2,505	3,343	2,504[1]	1,800
	All lower		361	182	76	845	195	206	292	690	820	228
B	Upper living	<0.5	39	214	30	160	44		152	76	132	28
		0.5-2.0	148	384	160	648	66	69		241	98	
		> 2.0				1,730						
	All		187	598	190	2,538	110	69	152	317	230	28
	Dead	< 0.5	33	30	148		40	30	46	28	36	243
		0.5-2.0	276	296	780	86	396	192	1,378	318		1,150
		> 2.0	246	916	2,812		3,310	2,126	6,951	264		814
	All		555	1,242	3,819	86	3,947[1]	2,528[1]	8,573	610	36	2,207
	All lower		363	465	1,310	1,890	858	70	825	855	214	167

(Continued on next page)

Appendix Table I. (Continued)

Replicate	Root condition and stratum	Root size	Root residue removal treatment									
			1		2		3		4		5	
			Number of sample pit									
			1	2	1	2	1	2	1	2	1	2
		(cm)	grams									
C	Upper living	< 0.5	27	38	80	29	30	182	48	127	113	64
		0.5-2.0	45	180	202	92	89	130	154	282	285	118
		> 2.0				131		312	10,658	129	134	
	All		72	218	282	252	119	624	10,860	538	532	182
	Dead	< 0.5	70			163	27		144	56	152	65
		0.5-2.0	644	463	30	328	124		303	176	560	306
		> 2.0	1,612	2,502	670	1,495	242			816	696	7,395
	All		2,588[1]	2,965	700	2,058[1]	393		447	1,077[1]	1,538[1]	7,938[1]
	All lower		372	374	120	1,426	176	253	6,138	787		186
Total living			1,463	1,408	1,030	2,839	2,507	1,459	11,074	1,731	1,745	504
Total dead			3,926	4,441	4,868	2,640	4,776	2,645	11,525	5,030	4,078	11,945
Total upper			5,389	5,849	5,898	5,479	8,283	4,104	22,599	6,761	5,823	12,449
Total lower			1,096	1,021	1,506	4,161	1,229	529	7,265	2,332	1,034	581
Grand total			6,485	6,870	7,404	9,640	9,512	4,633	29,864	9,903	6,857	13,030

[1] Includes unsized material.

Appendix Table II. Air dry weights of roots obtained from each 44 cu ft soil pit sampling the amount of root material remaining 2 yrs after treatment (1973) in five different treatments of the root removal control experiment. [1]

Replication	Root condition	Root size	Root residue removal treatment									
			1		2		3		4		5	
			Number of sample pit									
			1	2	1	2	1	2	1	2	1	2
		(cm)	grams									
A	Living	< 0.5	246		20	40		50			60	
		0.5-2.0	498	20	10			82	28	60	28	
		> 2.0	172	1,054	410							
	All		916	1,074	440	40		132	28	60	88	
	Dead	< 0.5	128		74		92	76			50	66
		0.5-2.0	264	190	412	710	276	316	200	364	124	188
		> 2.0	612	610	1,492	2,872	372	3,932	2,748		5,432	10,548
	All		1,004	800	1,978	3,582	740	4,324	2,984	364	5,606	10,802
B	Living	< 0.5	22		20	24			10			40
		0.5-2.0	50									
		> 2.0										
	All		72		20	24			10			40
	Dead	< 0.5	56	40	150	36	116	72	48	28	80	142
		0.5-2.0	132	68	260	106	36	106	98		110	136
		> 2.0	3,244	1,152	2,032	3,338	3,486	584	3,386	272	262	306
	All		3,432	1,260	2,442	3,480	3,638	762	3,532	300	452	584

(Continued on next page)

Appendix Table II. (Continued)

Replicate	Root condition	Root size	Root residue removal treatment 1		2		3		4		5	
			Number of sample pit 1	2	1	2	1	2	1	2	1	2
		(cm)	grams									
C	Living	<0.5	6			36	28	72	46	66		
		0.5-2.0				120		110		52		52
		>2.0				230		290		552		
	All		6			386	28	472	46	640		52
	Dead	<0.5	54	70	18	56	150	150	28	42	192	
		0.5-2.0	206	162	74	168	524	348	50	160	370	280
		>2.0	1,900	284	728	804	18,322	2,820	572			650
	All		2,160	516	830	1,028	18,996	3,318	650	202	562	930
Total living			994	1,074	460	450	28	604	84	700	88	92
Total dead			6,596	2,576	5,250	8,090	23,374	8,404	7,130	866	6,602	12,316
Grand total			7,590	3,650	5,710	8,540	23,402	9,008	7,214	1,566	6,690	12,408

[1]Since, in 1972, roughly 80% of the roots occurred in the upper 18 in. of soil, samples in 1973 and 1974 were taken only to a depth of 2 ft.

Appendix Table III. Air dry weights of roots obtained from each 44 cu ft soil pit sampling the amount of root material remaining 3 yrs after treatment (1974) in five different treatments of the root removal control experiment.[1]

Replicate	Root size	Root residue removal treatment 1		2		3		4		5	
		Number of sample pit 1	2	1	2	1	2	1	2	1	2
	(cm)	grams									
A	< 2.0	465	135	475	625	230	625	150	320	615	800
	> 2.0	2,920	555	3,625	1,340	1,165	7,575	3,270	8,070	1,095	2,855
	All	3,395	690	410	1,965	1,395	1,480	3,420	8,370	1,710	3,655
B	< 2.0	250	270	660	430	380	858	445	760	475	220
	> 2.0	3,005	4,550	29,350	8,945	2,205	935	5,880	3,315	10,595	670
	All	3,255	4,820	30,010	9,375	2,585	1,790	6,300	4,075	11,070	890
C	< 2.0	230	305	385	170	310	645	655	60	795	645
	> 2.0	830	1,750	980	2,495	2,015	3,860	3,225	255	6,265	4,120
	All	1,060	2,055	1,465	2,605	2,325	4,305	3,880	315	7,060	4,765
Total		7,710	7,975	31,885	13,945	6,305	7,575	13,680	12,760	19,840	9,310
Total of each treatment		15,965		45,890		14,080		26,360		29,150	

[1]Since, in 1972, roughly 80% of the roots occurred in the upper 18 in. of soil, samples in 1973 and 1974 were taken only to a depth of 2 ft.

APPENDIX III

PREPARATION OF MEDIA INCORPORATING PINE RESIN IN VARIOUS FORMS

Preparation of Media Incorporating Pine Resin in Various Forms

Autoclaved resin medium was made by adding 40 g or 80 g of prepared resin to 1 liter of the regular media prior to autoclaving.

To avoid heat sterilization of prepared resin, sterilization was attempted using Propylene Oxide (PO). One, two or three grams of prepared resin was evenly distributed in petri dish bottoms. These were stacked four to a level, with each level offset 90° from the one below it, in a 250 mm desiccator. The desiccator contained 13 ml of PO in a dish on the bottom. A vacuum of 25 lb was drawn for 4 min. The desiccator was sealed and left overnight under a hood.

A sterile air filter was placed on the desiccator and it was opened. After 30 min air from a compressor was forced through the filter into the desiccator. Air flow was maintained until the distinctive odor of PO could no longer be detected.

The petri dish bottoms were removed and immediately covered with sterile lids. The plates were poured with the autcoaved regular medium held at approximately 44°C.

Nonsterilized resin medium was made by sprinkling 1 or 2 g of the prepared resin into the sterilized bottom of a petri dish and immediately covering with a sterile lid. The regular medium at approximately 44°C was poured over the resin.

An acetone extract of resin medium was made by adding 40 g of prepared resin to 250 ml of acetone in an Erlenmeyer flask. This was mixed on a wrist-action shaker until the resin was dissolved. The solution was filtered through a doubled glass filter pad. The filtrate appeared brilliant amber in color (the resin appeared completely soluble in the acetone as only small pieces of bark, wood, and debris remained on the filter pad).

Three, seven, or ten milliliters of the filtrate were pipetted into sterile petri dish bottoms. These were stacked four to a level, with each level offset 90° from the one below it, in a 250 mm desiccator. A sterile air filter was placed on the vessel, and air from a compressor was forced through the filter and into the desiccator with the lid left ajar to let the circulated air escape. Air flow was maintained until no odor of acetone could be detected and the nonevaporated portion of the filtrate had solidified. This resulted in a film of rather uniform thickness over the bottom of the petri plate. The bottoms were removed and immediately covered with sterile lids. The plates were poured with regular medium held at approximately 44°C.

A water extract of resin medium was made by adding 40 g of prepared resin to an Erlenmeyer flask containing 500 ml of distilled water at room temperature. This mixture was agitated overnight on a wrist-action shaker. The solution was filtered through a doubled

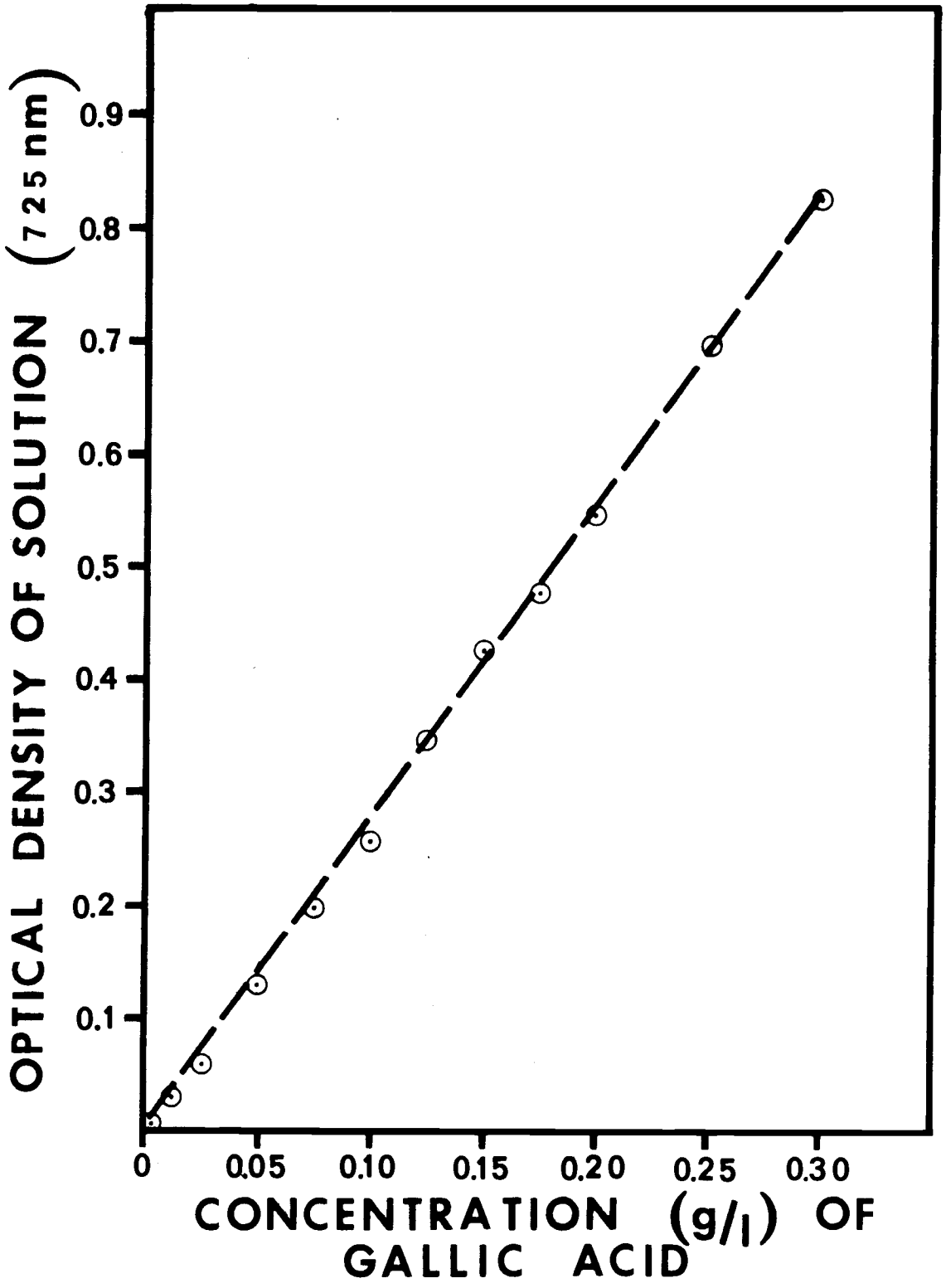
glass filter pad, and then sequentially through 1.2 μ, 0.8 μ, and 0.45 μ millipore filters. The filtrate was brilliant, light amber in color, and smelled distinctively of pine resin.

The dry ingredients for a liter of the regular medium were autoclaved in 500 ml of distilled water and held at approximately 44°C. To this 500 ml of the filtrate was added, and immediately the medium was poured into petri plates.

The phenolic concentration of the filtrate from the water-resin solution was determined in gallic acid equivalents using a Folin-Dennis test (Swain and Hills, 1959, p. 64). The procedure was modified as follows: 20% $CaCo_3$ was utilized instead of a "saturated solution"; to avoid clouding of the mixture, absorptivity readings at 725 mμ were recorded 10-25 min after final mixing instead of after 1 hr (H. A. Melouk, personal communication).

A standard curve was established using known concentrations of gallic acid varying from 0.00625 g/liter to 0.3 g/liter (Appendix Figure 1). Utilizing this data and the formula $K = \frac{OD}{C}$ (K = constant; OD = optical density of solution as read at 725 mμ; C = concentration [μg] of gallic acid in sample tested), a value of K = 0.011 was determined.

Using this constant, the concentration of phenols in gallic acid equivalents of the filtrate from the water-resin solution was calculated to be between 0.17 and 0.20 g/liter of filtrate.



Appendix Figure 1. Standard curve for Folin-Dennis Test. Points are mean values of two replicates.

Polyvinylpyrrolidone (PVP) is a substance insoluble in water used to remove phenols from plant extract solutions (Loomis and Battaile, 1966). Various methods of using PVP were tested; the following was found to reduce the phenol concentration in gallic acid equivalents, as determined by the modified Folin-Dennis Test, to about 0.025 g/liter of filtrate (1/10 of that in the non-PVP treated filtrate). Prior to agitation on the wrist-action shaker, 40 g of PVP powder were added to the resin (40 g) and water (500 ml). The solution was then handled in the same manner as the water-resin solution. To 500 ml of this filtrate 40 more grams of PVP were added. This solution was placed on the wrist-action shaker for 2 hr and filtered as before. Later it was determined that treating the water-resin filtrate with only this final PVP treatment was nearly as effective in removing phenols.

Medium was prepared from the PVP treated filtrate in the same manner as from the water-resin filtrate.

A medium containing 0.2 g/liter of gallic acid was made by the procedure of The Mycological Society of America Guidebook (preliminary edition, 1970, p. 921). All other medium ingredients were the same as the regular medium.

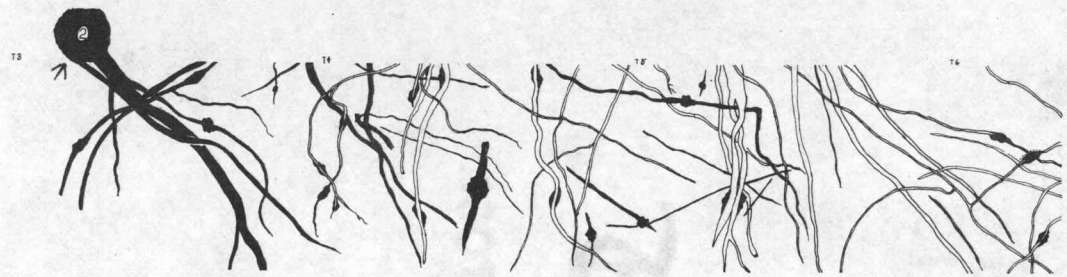
APPENDIX IV

MAPS OF DISTRIBUTION OF INFECTED ROOT MATERIAL BEYOND THE OUTERMOST DEAD TREE IN EIGHT TRANSECTS EXCAVATED ACROSS MARGINS OF *A. MELLEA* INFECTION CENTERS

KEY TO ROOT DISTRIBUTION MAPS

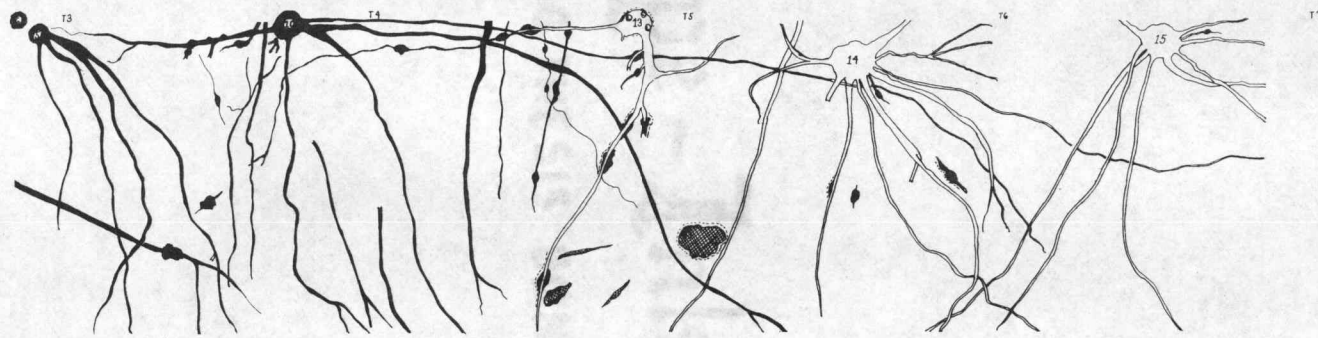
- HEALTHY ROOTS
- ARMILLARIA INFECTED ROOTS
- DEAD, NON ARMILLARIA INFECTED ROOTS
- 14 PINE STUMP
- C CEANOTHUS STUMP
- RHIZOMORPHS
- LESION ON LIVE ROOT
- LESION ON INFECTED ROOT

Arrows point to the outermost dead tree of the infection center. Infection center is to the left, healthy appearing stand to the right.

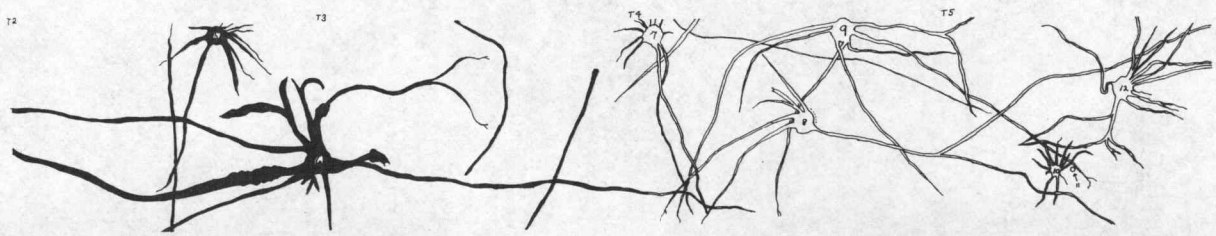


B Trench

0 1 2 3
SCALE IN FEET



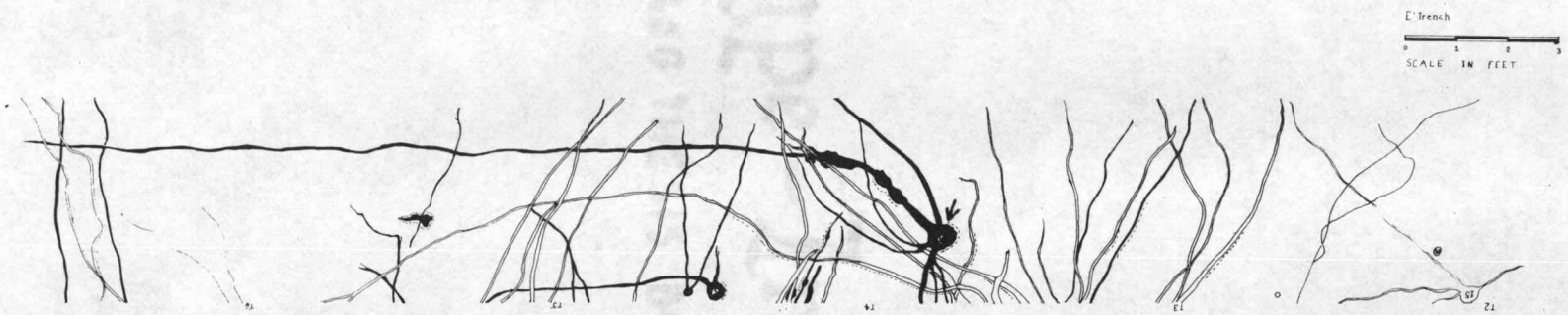
C Trench

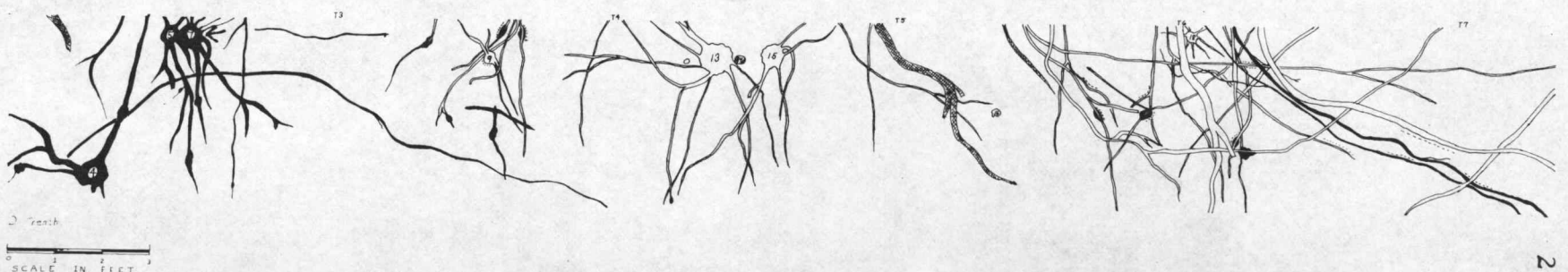


F Trench

0 1 2 3
SCALE IN FEET


A Trench
SCALE IN FEET


E Trench
SCALE IN FEET


D Trench
SCALE IN FEET