AN ABSTRACT OF THE THESIS OF

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Title: INFECTION OF STRESSED PONDEROSA PINES BY ARMILLARIELLA MELLEA FROM INDIGENOUS AND ARTIFICIAL INOCULUM SOURCES

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Armillariella mellea (Vahl. ex Fr.) Karst. was grown aseptically in hazel stems (Corylus cornuta var. californica) which were used in the forest to infect 290 ponderosa pines (Pinus ponderosa Laws.) which had been "stressed" in five different manners plus two control groups. All trees were inoculated twice giving a total of 580 inoculation attempts. Stress treatments consisted of: 1) girdling lateral roots; 2) severing distal portions of lateral roots; 3) removal of fifty-percent of the cross-sectional area of the lateral roots; 4) removal of the bottom two-thirds of the biomass of the live crown; and 5) stressing the roots by felling the tree. Controls consisted of inoculations of the lateral roots and root collars of unstressed trees.

Infection occurred at one-hundred ten (19%) of the inoculation points, of these 97 were by the indigenous population of A. mellea rhizomorphs.

Significantly more trees were infected at the root collar than at lateral roots, but lesion size was uninfluenced by location or

treatment. More infections occurred on trees of excellent than poor vigor, but no differences in susceptibility could be detected based on position of the crown (overtopped, intermediate, codominant, dominant).

Once attacked, lateral roots or root collars showed similar resistance to A. mellea, even while under stress. During the earlier stages of infection establishment, disease is little influenced by stress, crown position and decreased host vigor.

Prevalence of rhizomorphs of A. mellea in eight ponderosa pine stands was found to be influenced by silvicultural histories of the stands. The highest quantities were on sites clearcut in 1965 and sprayed for shrub control. Fewest occurred in young pole-sized stands about 70-years old. The results suggest that A. mellea rhizomorphs will increase with increasing manipulations by man. The greatest increase will occur when a stand is clearcut and little increase will be observed when a stand is thinned.

Internal mycoflora of healthy and declining lateral roots of ponderosa pine respond to changes in host vigor before visual changes are apparent. Microfungi were observed in apparently healthy cambial and xylem tissues. As lateral roots declined in health the mycofloral populations changed. One-hundred twelve days after felling of the tree ten-percent of the xylem and five-percent of the cambium was sterile. The majority of the microfungi isolated from the healthy and declining lateral roots showed no antagonism towards A. mellea in culture.

Infection of Stressed Ponderosa Pines by *Armillariella mellea* from Indigenous and Artificial Inoculum Sources

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INFECTION OF STRESSED PONDEROSA PINES BY ARMILLARIELLA MELLEA FROM INDIGENOUS AND ARTIFICIAL INOCULUM SOURCES

I. INTRODUCTION

Armillariella mellea (Vahl. ex Fr.) Karst., the familiar pathogen of many ornamental fruit and nut trees (Raabe, 1962; Sokolov, 1964) and an important pathogen of commercial forests in North America (Patton and Vasquez Bravo, 1967) is especially virulent upon species of Pinus (Day, 1927).

It is endemic to forests of both temperate and tropical climates (Cartwright and Findlay, 1958; Fox, 1964; Hintikka, 1974) and is widely distributed in forests of the Pacific Northwest (Ehrlich, 1939; Wortendyke, 1972; Baranyay et al, 1972; Anonomous, 1973; Johnson, 1976; Shaw, 1976; Filip, 1977, 1979) where it causes the most serious root rot of ponderosa pine (Pinus ponderosa Laws.) (Johnson, 1976).

Disease expression is either endemic, characterized by scattered mortality of single or small groups of trees, or epidemic, characterized by rapid group killing over fairly wide foci. Where groups coalesce, large areas may be removed from timber production (Hartig, 1874; Pileou, 1965; Shaw, 1974, 1976; Filip, 1977; Roth et al, 1977).

Reasons for epidemic expression of this native fungus are not fully understood but generally damage is associated with modification of the forest by man. Expanding disease foci can usually be associated with buried logging residues, especially stumps (Hartig, 1874; Redfern, 1966, 1973; Shaw, 1974, 1976; Filip, 1976; Roth et al, 1977).

Predisposition of trees to infection and death has been inferred, though less often documented, but many observations of apparently healthy trees dying from attack by A. mellea also are available. The interactions between populations of healthy potential hosts and the populations of endemic potential pathogens offer an extremely challenging area of study. Add to this: 1) physiological complexities of stress in the host, and 2) difficulties of study caused by subterranean development of the pathogen, and the problems become further compounded. For these reasons researchers apparently have yet to systematically and adequately study the relationships between A. mellea and the stressed coniferous host.

Existing studies directed at the stress/A. mellea relationship have been on non-coniferous hosts or were small parts of larger studies which consequently received minor attention (Christensen and Hodson, 1954; Redfern, 1966; Ono, 1970; Wargo, 1972; Wargo and Houston, 1974; Cobb et al, 1974).

The great majority of the literature deals with the relationship in an observational and descriptional way rather than analytical (Baker, 1941; Buckland, 1953; Huntly, Cafley and Jorgensen, 1961; Smith, 1962; Greig, 1962; Leaphart, 1963; Baranyay and Stevenson, 1964; Redfern, 1968; Singh, 1970; Parker and Houston, 1971; Carter, 1975; Johnson and Thompson, 1975; Anderson and Kaya, 1976; Morrison, 1976b; Cambell and Sloan, 1977). In spite of the many reports much is yet to be learned before we reach a comprehensive understanding of the role of host stress in attack by A. mellea. These needs are the justification of the research reported here.

Description of Research Area

Field work for this thesis was conducted at the Pringle Falls Experimental Forest, U. S. Forest Service, Deschutes Co., Oregon.

Edaphic features. Upper soils of the experimental forest are derived from a layer of light colored dacite pumice (Williams, 1942) rich in silicon, aluminum, potasium and sodium but difficient in calcium,magnesium and iron (Dryness and Youngberg, 1966) that orginated from the eruption of Mount Mazama (Crater Lake) approximately 7,300 years ago. The average depth of the pumice layer is 1-$\frac{1}{4}$ meters. The soils are well drained with a pH of 6.0 and an horizon sequence of O1, A1, AC, C1, C2 and D. The D horizon below the pumice is composed of dark red sandy loam, basalt gravels, cobbles and boulders from the original formation of Pringle Butte, a volcanic cone.

Climatic features. Average annual precipitation is 610mm (24 inches), 85 percent of which falls between October 1 and April 30. A snow pack of 610 mm is common from January to March (Barrett, 1970). Summers are warm and dry while winters are cold and wet. Daytime temperatures during the growing season range between 21'C (70'F) and 32'C (90'F) and the nights are cool with occasional frost. The pumice soil offers considerable insulation and soil temperature variations are considerably moderated (Table 1).

Vegetative features. The vegetation is best described as transitional between the communities described by Dryness and Youngberg (1966) as Pinus ponderosa / Purshia tridentata / Festuca idahoensis and Pinus ponderosa / Purshia tridentata - Arctostaphylos parryana var. pinetorum. The major overstory component is a two-storied stand of

ponderosa pine with an old-growth site index of 78-feet at 100 years and an average site quality of IV (Barrett, 1970) on the upper slopes of Pringle Butte. Stands grade into pure lodgepole pine (Pinus contorta Dougl.) at lower elevations where slopes are nearly level and soil less well drained (Frankling and Dyrness, 1973).

Table 1. Average Air and Soil Temperatures for Pringle Butte by Season and Canopy Closure[1].

Summer (June to August, 1976)	Open Canopy	Air	High	23.9'C (75.0'F)
			Low	5.9'C (42.5'F)
		Soil[2]	High	18.9'C (66.0'F)
			Low	13.6'C (56.5'F)
	Closed Canopy	Air	High	22.4'C (72.3'F)
			Low	9.7'C (49.5'F)
		Soil	High	20.2'C (68.5'F)
			Low	16.2'C (61.2'F)
Winter (November to January, 1976)	Open Canopy	Air	High	5.2'C (41.4'F)
			Low	-8.3'C (171.'F)
		Soil	High	2.2'C (36.0'F)
			Low	2.2'C (36.0'F)
	Closed Canopy	Air	High	5.3'C (41.6'F)
			Low	1.1'C (34.0'F)
		Soil	High	8.3'C (47.0'F)
			Low	8.3'C (47.0'F)

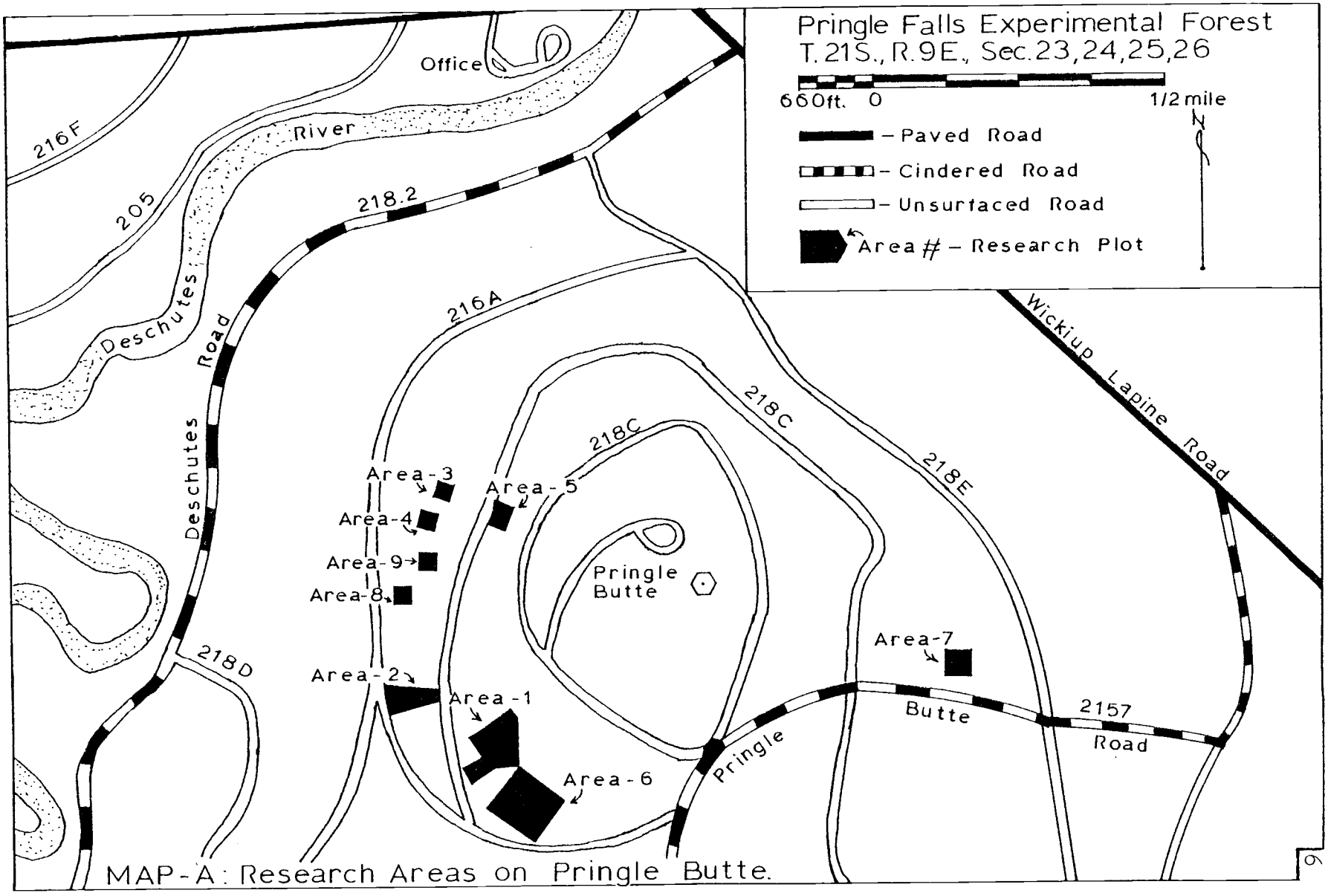
[1] Canopy closure: 75% + = Closed
74% or less = open

[2] Measured at depth of 15 cm.

Major shrub species are Snowbush ceanothus (Ceanothus velutinus Dougl.), Manzanita (Arctostaphylos parryany var. pinetorum (Rollins)

Wiesl. and Schreib.), Bitter-brush (Purshia tridentata (Pursh.) D.C.), and Golden chinquapin (Castanopsis chrysophylla (Dougl.) D.C.). Grasses and forbs compose less than five percent of the ground cover with the major grasses consisting of Idaho fescue (Festuca idahoensis Elmer), Squirrel-tail (Sitanion hystrix (Nutt.) Smith), and Needlegrass (Stipa occidentallis Thurb.).

The data for this thesis was collected at nine separate locations on Pringle Butte (See Map A), eight of which had comparable soil, climate,slope and aspect. Major differences between locations are respective histories of past management (Table 2).



MAP-A: Research Areas on Pringle Butte.

Table 2. Description of the nine locations on Pringle Butte contributing data.

AREA	OVERSTORY TYPE	OVER-STORY AGE	BASAL AREA [1]	AVERAGE D.B.H. (cm)	STEMS PER HECTARE	SITE ASPECT (T°)	PERCENT SLOPE (Topo.)	PERCENT CANOPY CLOSURE	OTHER FEATURES	MANAGEMENT HISTORY
I	Ponderosa Pine	80	5,624	17.2	4,734	225	10	95	Ground-cover is pine needle litter	Pole-sized stand established 80-years ago by fire. No logging activity. 40-years fire suppression.
II	Ponderosa Pine	41	1,912	10.7	2,160	225	20	30	Heavy cover of snowbush and manzanita	A shelterwood cut in 1958, 1969 and 1977.
III	Ponderosa Pine and Lodgepole Pine	12	-	-	477	228	10	5	Ground-cover is grasses and forbs	1965: Clearcut 1967: Planted with native stock 1971: Area III treated with 1.12 Kg/Hectare 2,4,5-T. Area IV untreated.
IV	Ponderosa Pine	12	-	-	477	228	10	5	Heavy cover of snowbush and manzanita	See III above.
V	Old-growth Ponderosa Pine	200+ and 70	Vol. $\bar{=}$ 16.2m^3 per hec.	50.8	-	228	15	80	Ground-cover is grasses and forbs	Natural Area. No management except 40 years of fire suppression.
VI	Ponderosa Pine	70	826	18.5	309	225	10	40	Ground-cover is pine needle litter	Thinned; 5.7m x 5.7m (1957-8) Slash left where felled.
VII	Ponderosa Pine	70	55	15.2	309	150	5	40	Ground-cover is pine needle litter	Same as VI above, except slash 'tommyhawked'.
VIII	Ponderosa Pine	70	5,600	8.1	9,000+	225	10	95	No A. mellea mortality present	Same as II above.
IX	Ponderosa Pine	70	5,600	8.1	9,000+	225	10	95	A. mellea mortality present	Same as II above.

[1] Basal Area is in dm^2 per hectare.

II. LITERATURE REVIEW

Armillariella mellea (Vahl. ex Fr.) Karst. (*Armillaria mellea* (Vahl. ex Fr.) Quel.) has an extremely wide host range (Raabe, 1962; Sokolov, 1964; Shaw, 1973; Laemmlen and Bega, 1974) among woody plants and includes even a few monocotylendenous and herbaceous plants (Pegler and Gibson, 1972). *A. mellea* occurs throughout the temperate and tropical regions of the world (Cartwright and Findlay, 1958) and is well represented in the coniferous forests and plantations of the Pacific Northwest (Ehrlich, 1939; Baranyay *et al*, 1972; Anonomous, 1973; Adams, 1974; Johnson, 1976; Shaw, 1976) and is known to cause severe damage (Wortendyke, 1972; Shaw, 1974; Filip, 1977).

Variability of *A. mellea*

The ubiquity of *A. mellea* has led to studies into the variability of its populations. Variability of the fungus recognized by various authors includes: physiologically distinct strains (Childs and Zeller, 1929); varying ability to produce rhizomorphs (Van Vloten, 1936; Benton and Ehrlich, 1941; Raabe, 1967; Shaw, 1977) and virulence (Day, 1927; Raabe, 1967); and variations in its habitat (Wilbur *et al*, 1972).

Clues to the pathogenicity and virulence of *A. mellea* in the field have been sought in such key cultural characteristics as the ability to produce pseudosclerotia, rhizomorphs and fruiting bodies (Manka, 1961; Raabe, 1967, 1969), ability to produce a wilt inducing protein (Thornberry and Ray, 1953) and optimum growing temperature (Raabe, 1967, 1968), as well as *A. mellea*'s ability to utilize carbon and nitrogen (Rykowski, 1976).

Temperature and A. mellea

There has been a considerable amount of effort put into obtaining information about the response of A. mellea to varying temperatures. A summary of these findings appears in Table 3. Elevated temperatures, as a potential control measure, have been examined in fruit trees in Australia (Birminghan, 1931) and California (Rackham et al, 1966) by exposing the crown roots. The sucess of this control measure is poorly understood in that the cambial temperatures, while not exceeding the cardinal maximums, kill the fungus (Munnecke et al, 1976).

Temperature definitely is a key factor in the growth of A. mellea but is only one of many environmental controlling influences.

Table 3. Cardinal and lethal temperatures for growth of A. mellea.

Author	Reported Temperatures
Bliss, 1946	Rhizomorphs grew best between 19.7'C and 24'C.
Benton & Ehrlich, 1941	Found six isolates grew better at 21'C and four grew better at 25'C.
Reitsma, 1932	Found 25'C optimum for mycellium growth.
Wolpert, 1924	Found 25'C optimum for mycellium growth.
Bohus, 1947	Found optimum for for mycellium to be somewhat above 26'C.
Townsend, 1954	Both mycellium and rhizomorphs grew best at 25'C.
Gibson, 1961	Found mycellial growth of 54 isolates better at 25 - 27'C, 46 better at 22 - 24'C and 16 better at 27 - 29'C.

Table 3. (Continued) Cardinal and lethal temperatures for growth of A. mellea.

Author	Reported Temperatures
Gibson, 1961 (cont.)	Found rhizomorph growth of 48 isolates better at 25 - 27'C, 26 better at 22 - 24'C and 12 better at 27 - 29'C.
Raabe, 1969	Tested 32 isolates and found many had optimum growth at 21'C or 25'C, but this varied according to media used.
Redfern, 1973	In the soil fewer rhizomorphs were initiated at 15'C than 25'C but the total dry weights were the same for the two temperatures.
Rishbeth, 1978	The highest dry weight of rhizomorphs in the soil was produced at 20'C. Isolates varied, however, from optimums of 2'C to 30'C.
Hintikka, 1974	Found 25 - 27.5'C optimum for mycellial growth for pine isolates and 22.5 - 25'C optimum for spruce isolates. Thermal killing would be acheived in a few minutes at 45 - 50'C.
Munnecke et al, 1976	In wood veneer strips mycellial growth ceased at 6', 9' 30' and 36'C but resumed when put at 23'C. Growth diminished at 12'C and 15'C and 39'C was lethal.

Inhibition of A. mellea by host constituents

The importance of the chemical and physical barriers that infected trees present to a pathogen are receiving concentrated attention: Cobb et al (1968) on toxcity of resin of ponderosa pine to Heterobasidion annosum (Fr.) Bref. and Jorgensen (1961) and Shain

(1967) on the accumulation of fungitoxic pinosylvins in infected sapwood.

The volatile constituents, particulary monoterpenes in the oleoresins of pines, have been extensively studied by Mirov (1961, 1967) and Drew and Pylant (1966). However, terpenes, and fatty and resin acids in sapwood and heartwood have been systematically investigated in only a few of the more than 100 species of pine (Anderson et al, 1969a). A list of the fatty and resin acids of ponderosa pine and Jeffrey pine (Pinus jeffreyi Grev. and Balf.) can be found in Anderson (1969b). The triterpenes of pine bark have been studied (Rowe and Bower, 1965), but as yet no studies into their effects on fungal pathogens has emerged.

A. mellea has been shown to be sensitive to many of the terpenes, and especially to Limonene (Hintikka, 1970). Fungi which decay coniferous wood have been found more tolerent of the terpenes than fungi limited to deciduous trees and white rot fungi are generally more tolerent than brown (Väisälä, 1974). A high degree of tolerence to terpenes would be expected of A. mellea since its a pathogen of both coniferous and hardwoods as well as a white rotter. But according to Hintikka (1970) and Väisälä (1974) A. mellea is very sensitive to terpenes. These authors used isolates of A. mellea from hardwoods. In view of the great variability present in the population of A. mellea this author cannot but wonder what the results would have been had an isolate from a coniferous host been used.

In a good review of factors influencing resistance of tree roots to pathogens, Rishbeth (1972) observed that roots heavily impregnated

with resin constitute a considerable mechanical barrier against root pathogens, and in so doing reaffirmed the statements of Zeller (1917) and Verrall (1938). Resin impregnated wood has been suggested as a survival habitat for some root pathogens (Bega and Tarry, 1968).

Stimulation of A. mellea by host constituents

Although the volatiles of resin have been shown to be fungistatic or toxic, the non-volitile components of resin show no evidence of toxicity to A. mellea in naturally occurying concentrations. To the contrary crude resin from ponderosa pine has been used in nutrient media as a growth stimulant of A. mellea in culture (Shaw, 1974; Filip, 1976).

Crude resin preparations are not the only known stimulants of growth of A. mellea. Ethanol was first observed to stimulate rhizomorph growth by Weinhold (1963). Since that time many studies have been undertaken concerning stimulation of rhizomorphs. Incorporation of Disulfiram (Antabuse) and acetate into nutrient media led to the assumption that ethanol is metabolized via the normal biochemical pathways (i.e. via acetate) (Sortkjaer and Allermann, 1972; 1973). Ethanol stimulation of rhizomorphs and of DNA and RNA synthesis has indicated that as long as ethanol is available the growth stimulation is caused by cell division rather than by an accumulation of lipids or polysaccharides (Allermann and Sortkjaer, 1973). Of interest to the ecology of A. mellea in nature is production of ethanol in culture by the ubiquitous fungus Aureobasidium pullulans (de Bary) Arnaud in

amounts adequate to stimulate rhizomorph production of A. mellea (Pentland, 1965, 1967).

Chemicals other than ethanol have been studied for their abilities to stimulate rhizomorphs in culture. Ortho-aminobenzoic acid and para-aminobenzoic acid have been found to stimulate rhizomorph initiation and growth (Garraway, 1969, 1970). Of particular interest concerning A. mellea in conifer roots is Moody's work (1972a, 1972b) on the stimulatory effects of fatty acids, naturally occuring plant lipids, and lipids from tree roots on rhizomorphs. When fatty acids or lipids were the sole carbon source rhizomorphs were not produced, indicating that the chemicals function as growth-promoting substances rather than sources of carbon. The amount of unsaturated fatty acid required for rhizomorph production was found to be from 0.05% to 0.1% (Moody, 1972b). Based on this, the amounts of fatty acids found in ponderosa pine (0.23%) is sufficient to stimulate rhizomorph production, while the levels in various A. mellea tolerant trees (Douglas-fir, 0.01%; White fir, 0.006%; Incense cedar, 0.005%) are insufficient for rhizomorph production (Moody, 1972b). The resin acid portion of hot water extracts of root tissue also contributes to rhizomorph production (Moody, 1972b).

Infections by rhizomorphs

Ever since Hartig (1874) first associated A. mellea with disease of forest trees it has been recognized that rhizomorphs are important in epidemiology (Day, 1927; Thomas, 1934; Sokolov, 1964; Swift, 1972; Rishbeth, 1972; Rykowski, 1975; Hintikka, 1974; Shaw, 1977). The

importance of rhizomorphs was seldom questioned, despite instances where root-to-root contact was the only means of spread of the disease (Fox, 1964; Shaw, 1974).

One of the first answers sought was whether or not rhizomorphs could penetrate a host directly or whether wounds were necessary. The question was answered affirmatively by Hartig in 1894(b). Convincing accounts of rhizomorph penetration in conifers in the absence of wounds was given by Day (1927) and Thomas (1934). Thomas (1934) was one of the first to suspect that A. mellea possessed the ability to desuberize cork tissue, a view to be discounted by later authors (Gäumann, 1950; Dickinson, 1960), but ultimately shown true by Swift (1965). The most recent study on the mode of rhizomorph infection is by Rykowski (1975) who studied five levels of disease intensity, described seven forms of infected root systems and discussed the process of infection through healthy tissues.

Effects of substrate on rhizomorph production

It has been long observed that conifers planted on recently cut-over hardwood sites experience severe mortality from A. mellea (Childs and Zeller, 1929; Leach, 1939; Greig, 1962; Singh, 1975). Thus, it has been widely accepted that A. mellea causes disease predominately in areas previously in hardwoods (Peace, 1962). This belief lead Day (1929) to write that;

> "... in natural or semi-natural forests, it (A. mellea) seems to be confined to broad-leaved forests, pure or mixed with conifers. In pure natural conifer forests it seems at least to be rare, but in pure conifer plantations established on the site of old broad-leaved forests it is extremely common at least during

the first rotation."

Such observations have led to a general understanding that hardwoods are a better substrate than conifers, if not the only substrate, from which A. mellea could sustain a parasitic attack. Using isolates collected in East Anglia Rishbeth (1972) concluded that isolates "... of broad-leaved trees produced greater yields of rhizomorphs over a greater period after felling than the isolates from pines." He also observed a general reduction in amount of rhizomorphs produced after prolonged growth in pine tissues.

A. mellea isolates obtained from both conifer and hardwood species have shown variable results in their ability to produce rhizomorphs when inoculated in both conifer and hardwood substrates (Morrison, 1972; Redfern, 1975). After studying rhizomorph production from Picea rubens and Acer rubrum Redfern (1970) stated that the length and weight of rhizomorph systems were not significantly different but that the number of rhizomorph systems and the rhizomorph growing tips were significantly greater for the maple than for the spruce. He further stated that;

> " It is unlikely that the postulated superiority of hardwood roots over conifer roots as food bases for A. mellea can be fully explained by the fact that hardwood root segments apparently permit growth of a slightly greater amount of fungal material than conifer segments of equal volume."

Although A. mellea attacking a susceptible host from a hardwood base seems to be superior to a conifer food base there are still many observations to the contrary.

Adams (1972) studied and reported on infection of young-growth ponderosa pines from stumps of large old-growth ponderosa pines, cut

and presumably infected over 25 years previously. Disease centers in southern Washington state have maintained themselves for an extremely long period of time primarily on ponerosa pine (Shaw, 1974). Certain isolates of A. mellea appear to be more or less host specific (Shaw, 1977).

The preceeding studies, plus others (Boullard and Gaudray, 1975; Rykowski, 1975; Gibson, 1961), showed A. mellea is indeed a pathogen capable of sustaining itself for extended periods in the coniferous ecosystem. This has been realized by one of the advocates of hardwood superiority when he wrote;

> "..., although there is little doubt that hardwood stumps provide the best food base for A. mellea, conifer stumps are probably much more effective than has hitherto been realized. There is certainly sufficient evidence to indicate that A. mellea killing may continue even after successive rotations of conifers... " (Redfern, 1975).

Rhizomorph ecology

Distribution of the rhizomorphs in their natural habitat is an aspect of A. mellea ecology which has received suprisingly limited study. It has been widely observed that rhizomorphs of A. mellea concentrate within 20 cm of the soil surface. In 30 cm^2 pits at the intersections of a 10 m by 10 m grid Redfern (1973) measured the depth of the first 200 rhizomorphs encountered and concluded the distribution varied with the depth but that the main concentration occured between 2.5 cm and 20.0 cm below the surface. Redfern's results agree with those of Ono (1965, 1970) for a poorly drained acid clay soil and those of Morrison (1976) for sands that were well to poorly drained. Both Redfern (1973) and Morrison (1976) recorded a natural lower limit

to rhizomorph growth of 30 to 60 cm. In contrast Hintikka (1974) found in Finland that rhizomorphs of A. mellea permeated the humus layer but seldom entered mineral soil.

Amother area of A. mellea ecology now attracting some interest is the quantitative occurance of rhizomorphs with cover-type and stand history. From 29 separate sites in southern Finland 83 of 232 samples of humus yielded rhizomorphs and the fewest rhizomorphs were in dry pine heaths and the greatest quantity were in moist thinned stands, especially if there were birch stumps present (Hintikka, 1974). In the upper 10 cm of soil in diseased and healthy stands in Japan Ono (1970) noted "..., no remarkable difference of the weight of rhizomorphs between those in the healthy site and those in the damaged site." In Newfoundland rhizomorphs appear more abundant in cutover than in pastureland and most concentrated in the humus layer (Singh, 1977).

Rhizomorph formation in relation to soil types has been little studied. Testing acid and alkaline sands, loams, acid peat and clay at moisture contents between 25 and 100% saturation Redfern (1970) found rhizomorph production from oak pieces to be greater in alkaline soils than acid ones and that moisture between 25 and 75% saturation had little influence. This confirmed Garrett's (1956) findings. Ono (1970) observed that rhizomorph production from infected larch (Larix sp.) roots was abundant in surface soil and soil-compost mix but little growth occured in the subsoil or sand. In another study Redfern (1973) found alkaline loam soils to be superior for rhizomorph production to acid sand and quartz sand, but that dramatic increaces in total dry weight and size of rhizomorphs could be attained when acid

sand or quartz were supplemented with a layer of acid fibrous peat. After studying field expression of A. mellea in 17 Newfoundland sites Singh (1975) concluded that "... there is no direct evidence ... that variations in organic matter, pH, and available nutrients (N and P) affected the incidence of the disease." Gard (1929) concluded that the spread of this disease in France, during 20 years, was due chiefly to a progressive reduction of the lime content of the soil. Reitsma (1932) found that A. mellea occured most frequently on acid sandy soils and least on heavy alkaline or saline soils, while in Florida, Rhoads (1956) found Armillariella sp. to be most prevalent on well-drained, light sandy soils.

Pertinent to this discussion of soil influences is Swift's (1968) report of a yet unidentifiable, water soluble, substance in some Rhodesian soils which totally inhibited the development of rhizomorphs.

Soils clearly are an influencing factor in the epidemiology of A. mellea but the reported effects are not always consistent. Considering the extreme variability possessed by A. mellea it is not unreasonable to expect a particular unoccupied environmental niche to select and support a population of A. mellea which can best occupy that niche whether hardwood or conifer substrate, alkaline or acid soil, high or low soil temperature.

To conclude the review of rhizomrophs in the soil it has been observed that when rhizomorphs are severed they do not die immediately but rather display a flush of regrowth of several to numerous new growing tips (Redfern, 1973; Hintikka, 1974; Pawsey and Rahman, 1976).

This observation led Rishbeth (1973) to state that;

> "Soil disturbance caused by timber extraction and ploughing will clearly be an added stimulus to fresh rhizomorph development and any newly planted trees will be at maximum risk."

The observation that rhizomorphs of A. mellea are able to produce an increased number of growing tips when severed is undoubtedly of great importance to the epidemiology of the disease. Thus it is of little surprise that when Redfern (1973) used 6 cm lengths of severed rhizomorphs as inoculum he was able to kill two out of twelve potted larch trees. He observed regrowth of growing tips from both ends of the severed rhizomorphs thus producing circumstantial evidence that translocation can take place in both directions in the same rhizomorph. This agrees with the Fluorescein studies done by Schütte (1955).

Role of basidiospores

The role of basidiospores in the epidemiology of A. mellea is thought to be minor when compared to spread by rhizomorphs or root-to-root contact (Rishbeth, 1964, 1970), except in rare instances.

Identification of clones of A. mellea

It has been demonstrated that genetically distinct collections of A. mellea can be recognized by pairing isolates in petri dishes and observing the interaction between the two colonies (Adams, 1972, 1974). If the isolates grow together imperceptibly they are interpreted as of the same genotype and therefore members of a clone, but if a "line of demarcation" (Adams, 1974) or a "barrage" (Esser and Kuenen, 1967) forms at the juncture the two isolates are classified as genetically

dissimilar. The capability of identifying individuals and the geographic distribution of such individuals has great practical significance to both the researcher and the field forester. Using the technique of Adams (1972, 1974) it is possible to locate, identify and map the distribution of "giant colonies" thus increasing our knowledge of the epidemiology of A. mellea in nature. This same procedure can provide the field forester with a management tool for root rot control by delineating areas which may contain aggressive colonies of A. mellea capable of reducing the yields of future rotations if adequate control measures are not taken (Shaw, 1974; Filip, 1976, 1979; Roth et al, 1977; Roth and Rolph, 1978). Shaw (1974, 1976) established that the greatest mortality in the pine forest he was studying was caused by a single large clone of A. mellea approximately 1.6 Km in diameter and at least 460 years old.

Genetic status of A. mellea

Armillariella mellea appears to be a genetically aberrant hymenomycete (Raper, 1966). It is considered to be homothallic (Kneip, 1911) but single spore isolates give a tetrapolar pattern when crossed (Hintikka, 1973). The hyphae are heterokaryotic; their nuclei undergo typical meiosis (Ullrich and Anderson, 1977). Hyphal tips have been observed to be uninucleate and diploid (Hintikka, 1973). Tommerup and Broadbent (1975) studying nuclei during growth and differentiation of basidiocarps provided evidence for diploidy, whereas Korhonen and Hintikka (1974) provide evidence for somatic diploidization. Staining tissue by the Feulgen method and comparing nuclear

volumes between dikaryotic and monokaryotic hyphae, the latter presumed to be diploid, showed the nuclei of monokaryotic cells to be approximatly twice the volumes of those in dikaryotic cells (Tommerup and Broadbent, 1975).

Host predisposition and attack by A. mellea

Predisposition of plants to disease is a large and diverse subject beyond the present review. For comprehensive reviews see Schoeneweiss (1975), Colhoun (1973), and Yarwood (1959). The primary concern here is interaction between A. mellea and a host under biological stress. Biological stress can be defined as "any environmental factor capable of inducing potentially injurious strain in living organisms" (Levitt, 1972). The strain placed on an organism is either 'plastic', involving irreversible physical or chemical change, or 'elastic' involving reversible change upon removal of the stress.

Stress by air pollutants

Ozone. Photochemical oxidant air pollution, mainly ozone, was first identified in 1962 as the agent responsible for the slow decline and death of ponderosa pine in southern California (Miller et al, 1963). It has been suggested that since ozone is known to reduce feeder root development in ponderosa pine (Parmeter et al, 1962) it may be possible to predispose the root system to attack by A. mellea (Miller, 1973). A direct correlation has yet to be established for this anticipation of increased disease.

Sulfur Dioxide. In general, exposure to atmospheric pollutants decreases parasitic disease (Heagle, 1973). A. mellea and several wood-rotting fungi are exceptions in that there is increased incidence and severity when trees are weakened by exposure to sulfur dioxide (Heagle, 1973; Schoeneweiss, 1975).

Fluoride. Hydrogen fluoride is generally considered to be the most damaging form of fluoride (Heagle, 1973), however, the interaction between it and A. mellea has yet to be studied. The observation of Heagle (1973) that very little is known of the effects of pollutants on parasitic diseases of plants, especially forest vegetation, seems to be correct.

Entomological stress

Bark beetles (Coleoptera: Scolytidae) appear to be the most damaging insects in the ponderosa pine forest type, but the exact relationship between bark beetles in particular, and insects in general, and A. mellea root rot has been poorly established.

A. mellea Predisposes Trees to Insects. Rudinsky's 1962 review of the family Scolytidae suggests that bark beetles breed mainly in physiologically subnormal trees and are therefore secondary agents. Cobb et al (1974) studying ponderosa pine and white fir (Abies concolor (Gord. and Glend.) Lindl.) stated there to be a high degree of association between root diseases and infestations by the mountain pine beetle (Dendroctonus ponderosae Hopkins; Coleoptera: Scolytidae) and the Western pine beetle (D. brevicomis LeConte; Coleoptera: Scolytidae). Beetles have been observed to kill trees infected by A.

mellea which would normally have survived (Peace, 1962). Infestation of spruce (Picea sp.) by Ips typographus (L.) (Coleoptera: Scolytidae) and Polygraphus polygraphus L. (Coleoptera: Scolytidae) has been associated with prior infection by A. mellea (Wichmann, 1953). Such observations support the observation of Stark and Cobb (1969) that 80% of bark beetle infested ponderosa pines had been infected by root-disease fungi prior to beetle attack, whereas none of an equal number of noninfected trees was diseased. Similar observations have been made by various researchers (Ehrlich, 1939; Hetrick, 1949; Thomas and Wright, 1961).

Insects Predispose Trees to A. mellea. Hudak and Singh (1970) have shown a positive correlation between the intensity of infestations with balsam woolly aphid (Adelges piceae Ratz.) (Homoptera: Eriosomatidae) and the incidence of A. mellea root rot. Infection of undamaged trees was seen to average 2.5% infection by A. mellea and in damaged (aphid infested) living trees the fungus infection was averaging 18.6%.

A survey of plantations for the weevils Hylobius pinicola (Couper) and H. warreni (Wood) (Coleoptera: Curculionidae) and A. mellea revealed the incidence of A. mellea was 4% in Sitka spruce (Picea glauca (Moench)Voss), however, this increased to 15% infection when the trees were infested by the weevils (Singh, 1975). The percent of infection by A. mellea has been shown to increase in trees injured by the weevils as the incidence of weevils increase (Warren and Singh, 1970).

Attacks by the twolined chestnut borer (Agrilus bilineatus (Wever), Coleoptera: Buprestidae) have been shown to occur primarily on weakened trees (Staley, 1965). Dunbar and Stephens (1975) examined 84 dead oaks (Quercus sp.) at 13 different locations which had a previous history of defoliation and found that 47 were infected with both A. mellea and Agrilus bilineatus, 33 were infected with only A. bilineatus, two were infected with A. mellea only, and two had neither organism. They concluded that A. mellea had only a minor role and Agrilus bilineatus a major role in killing the oaks. This association between A. mellea and Agrilus bilineatus has also been observed by Kegg (1973) in oaks in New Jersey.

Stress by defoliation

Stress by defoliation or reduced photosynthesis may be incurred in numerous ways.

Barrett (1968) observed that pruning ponderosa pine beyond a threshold value, dependent upon the percent of total tree height in live crown, reduced diameter and height growth in proportion to the amount of live crown removed. In California Hallin (1956) and Gordon (1959) found that ponderosa and Jeffrey pines with 50% of the live crown removed suffered shock initially but recovered after five years. Removal of three-fourths of the crown length reduced growth drastically and 52% of the trees died from insect attack. Wargo and Houston (1974) while studying the effect of defoliation of sugar maple (Acer saccharum Marsh.) observed that frequency of infection by A. mellea was influenced by season and frequency of defoliation. Trees defoliated in June or

July contained more A. mellea infections than trees treated in August, and trees defoliated in two consecutive years, versus one year, contained a higher percentage of attacks. Wargo and Houston (1974) defoliated their trees by hand, but trees studied by Baker (1941) and Cambell and Sloan (1977) were all defoliated by the gypsy moth (Porthertria dispar L.; Lepidoptera: Lymantriidae). They both observed killing by A. mellea in the defoliated trees and associated it with the weakened condition of the trees. Directly related to this is the work of Redfern (1966), who set up an experiment at the edge of disease gaps caused by A. mellea in 13-year old Pinus nigra var. calabrica Arnold which had a high proportion of incipient or established infections. Some trees were pruned by removing 75% of the live crown and others were left untreated. Twelve of 33 pruned trees were subsequently killed by A. mellea while only one of 25 untreated trees suffered similar fate.

Stress from moisture extremes

Unfortunately, most reports on strain due to water stress are based on field observations and are not substantiated by controlled experimental evidence (Crist and Schoeneweiss, 1975). Most observations are of the nature made by Buckland (1953), "When normal vigor was reduced, through drought or other environmental changes, A. mellea developed...", or Fox (1964) where "...extreme dampness and humidity were associated with increased incidence of mortality."

Studies have been attempted of other root diseases of pines and water relations. Towers and Stambaugh (1968) studying the influence

of moisture stress on parasitism of Loblolly pines (Pinus taeda L.) by Fomes annosus (Fr.) concluded that the rate of fungal penetration was greatly enhanced by induced drought. Conversely, Goheen et al (1978) concluded that wetter soil conditions favored infection of roots of ponderosa pine by Verticicladiella wagenerii Kendrick. Definitive studies of moisture tolerances as they affect A. mellea root rot have not been made.

Stress by silvicultural practices

The greater portion of the North American commercial forest is being managed extensively rather than intensively (Miller, et al, 1975) As land is further converted from extensive to intensive management there is likely to be a major increase in the incidence of A. mellea root rot. One can only agree with Day's (1929) observation that: "... the fungus (A. mellea) is always reported as attacking crops which have been artificially regenerated or grossly interfered with by mankind."

Regeneration and A. mellea. Plants are stressed during and after transplanting, particularly if mishandled, but the nature of the stresses involved are largely unknown (Schoeneweiss, 1975). Increase in the incidence of A. mellea mortality after reforestation has been observed by several workers. Singh (1973, 1975) established a relationship between disease caused by A. mellea and the method of planting in Newfoundland forests and thus concluded that:

> "..., trees in plantations established with bare root stock are much more susceptible to attack by A. mellea than (if) established by container planting or by direct seeding...."

Using artifically inoculated seedlings Ono (1970) established a planting bed in which one group of trees had their roots made into a ball and one group where the root system was fully spread and carefully planted. Two years after planting there were no differences in growth between the two groups, but the incidence of A. mellea on balled roots was twice that found on skillfully planted roots.

Stand Composition and A. mellea. It has long been recognized that host species differ in susceptibility to A. mellea attack and killing and that isolates of A. mellea vary in pathogenicity. Ono (1970) experimentally showed Larix sp., Picea sp., and Pinus sp. to be susceptible and Abies sachalineniss Nast and Cryptomeria japonica D. Don (Todo Fir and Sugi, respectively) to be resistant to A. mellea attack and killing. Ono, like Day (1927), observed that white pine (Pinus strobus L.) was very susceptible to attack but resistant to killing. Adams (1974) and Shaw (1977) found in ponderosa pine stands A. mellea isolates on subordinate vegetation that were non-pathogenic to the pines. Thus the juxtaposition of A. mellea to a pine does not necessarily condemn the pine to death. Death depends on the pathogenicity of the A. mellea isolate(s) and the inherent susceptibility of the host tree.

Differences of pathogenicity among A. mellea isolates can be of great importance to the land manager who has the opportunity to alter stand composition. A considerable amount of mortality in the earlier days of forestry could have been avoided if the land managers had not insisted on planting conifers following rotations of hardwoods. The suggestions of Singh (1975), and others, to replant with the least

susceptible, well adapted native species should go a long way to help reduce root rot in future forests.

Stand Structure and A. mellea. Stand density has been suggested as possitively coorelated with susceptibility to A. mellea. Nelson and Harvey (1974) expressed concern that thinning would increase activity of A. mellea because of the sudden increase in food base available to the fungus. This appears to be consistent with the statement by Smith (1962) that:

> "Thinning may temporarily predispose stands to attack by certain damaging agencies even while it gradually builds up the resistance of the trees enough to reduce harmful effects of the same agencies."

However, in ponderosa pines in Oregon, Johnson and Thompson (1975) reported that instead of an increase in the incidence of disease after thinning there was a reduction in the loss of 'crop trees' from 8.9% in the unthinned control to 4.8% in the thinned plots. They concluded that thinning in diseased stands should not be delayed for fear of increased mortality from A. mellea.

Expectation of increased mortality after thinning seems correct from numerous authors observations that disease incidence is greatest on cutover areas where many stumps remain in the soil (Hartig, 1874; Huntly et al, 1961; Shaw, 1974; Singh, 1970, 1975; Filip, 1976; Filip and Roth, 1977; Roth et al, 1977; Mac Kenzie and Shaw, 1977). The reason that stumps from thinning appear to cause minimal increase in disease while stumps from large crop trees cause severe disease appears to be a phenomenon of inoculum potential (Garrett, 1956) and of differential susceptibility to damage of trees differing widely in

age.

While basic studies into inoculum potential have been started (Garrett, 1956, 1960), a truely adequate experimentally field-tested explanation for the observed differences has yet to appear.

Vigorous hosts and A. mellea

Despite literature of the preceding section, emphasizing a secondary role of A. mellea, considerable evidence is accumulating that A. mellea is an effective primary pathogen.

In his classical work of 1874 on A. mellea Hartig observed vigorous cherry and plum trees in rows dying successively. He further artifically inoculated six-year old Scots pines (Pinus sylvestris L.) and obtained infections without stressing the trees in any mentioned manner. He wrote of the 'Erdkrebs" (Ground canker) when alluding to the habit of A. mellea to attack in an ever increasing arc in the most vigorous fully-stocked plantations.

Lawrence (1910) reported attacks of A. mellea on stocks of apple and berries in the absence of any predisposing factors. Similar statements were made by Long (1914) while observing the death of Chestnuts (Castanea dentata (March.) Borkh.) and white oaks (Quercus alba L.) in New York state. These observations later prompted him to write: "Nothing was found to indicate that the larger white oaks which had been killed were in poor health before they were attacked by this disease." Howard W. Rankin in his 1918 book titled "Manual of tree Diseases" made the statement that:

> "... abundant evidence is at hand that young thrifty trees in the forest ... are often killed when there is no doubt

> that the honey-mushroom (A. mellea) was directly and primarily the cause of the decay of the roots."

Studying the spread of A. mellea in various orchard trees Hendrickson (1925) stated that in connection with prune and apricot trees (Prunus sp.) the young and vigorous trees are as liable to attack as old ones. In conifers, Day (1927) stated that: "... it is definitly true that often among the trees to die of honey fungus ... are some of the best and strongest growing." Although Bliss (1946) observed that the greatest resistance to A. mellea of nine plant species was displayed at the soil temperatures most favorable to root growth he also observed severe attacks on susceptible species regardless of the soil temperature. In Northern Wisconsin Patton and Riker (1959) described areas where A. mellea attacked and killed apparently healthy, vigorously growing trees in a seven to ten-year old coniferous plantation. In subsequent experiments using artificial inoculum Patton and Riker (1959) were able to infect 41 of 58 trees in a greenhouse study and 21 of 79 red and white pines (Pinus resinosa Ait. and P. strobus L., respectively) in a field study, none of which were stressed in any manner. While examining the variation in pathogenicity and virulence of A. mellea Raabe (1967, 1969, 1972) obtained considerable infection in test plants even though care was taken to assure the plants were maintained in good vigor. After studying the spread of A. mellea in a peach orchard Kable (1974) commented specifically on the situation of predisposition in his study by writing:

> " The majority of the trees in this orchard were over 20-years old when attacked, by which time their vigor was declining naturally. However, vigorous replant peach trees aged one to seven were killed with rapidity."

Nine isolates of A. mellea collected from ponderosa pine were used to infect ponderosa pine seedlings of 2-0 stock grown from seed and although variation existed among separate isolates collected from pine hosts, all were albe to kill pine seedlings of good vigor (Shaw, 1977).

Few researchers have directly confronted the problem of A. mellea and a coniferous host under stress in the field. Most statements to date are based on emperical observations of experienced personnel. As has been reviewed here considerable evidence may be generated to support both ideas of A. mellea as either a primary or a secondary pathogen. Yet one fact clearly stands out. Despite the long history and extensive host range reporting A. mellea as a pathogen of opportunity, the primary parasitic ability of this fungus in relation to conifers can no longer be ignored or doubted.

Mycoflora of living trees and tree stumps

In recent years researchers have been studying the mycoflora of not only diseased wood but also wood of healthy trees and wood just prior to infection by decay organisms. From these studies is emerging a picture of non-decay organisms not only preparing a substrate for infection by wood rotters but also that a natural, harmless, mycoflora may exist internally in some tree parts. In preparation for a study of the internal mycoflora of roots of ponderosa pine the following literature was assembled.

Relatively few detailed ecological investigations have been made on fungal colonization and habitation of natural woody substrata (Rayner, 1977a).

To expect to find fungi inhabiting tree parts internally and causing no measurable damage is not without precedence. Close examinations have shown that many fungi enter plants which they parasitize to such a limited extent that their presence goes unnoticed unless the invaded tissue is examined microscopically (Wood, 1967). This situation has been observed for deciduous plants (Pugh and Buckley, 1971), broad-leaved evergreens (Rayner, 1948) and evergreen conifers (Kendrich and Burges, 1962; Millar, 1974; Carroll et al, 1977; Bernstein and Carroll, 1977).

Several studies (Riley, 1952; Ethridge, 1961; Good and Nelson, 1962; Shigo, 1972) have shown that non-decay organisms usually preceed decay organisms in some form of succession in branch and stem wood and that they should be considered as part of the decay process (Shigo, 1963).

Studying Heterobasidion annosum in Norway spruce (Picea abies (L.) Karst.) Delatour (1976) distinguished three organizations of organisms as follows:

I. Species located exclusively in the decayed wood. (Scylalidium lignicola Pesante, Trichoderma sp., bacteria)

II. Species located preferentially in the sound wood. (Mollisia sp., Nectria fuckeliana var. fuckeliana Booth)

III. Species located indifferently in the sound or the decayed wood.

(Heterobasidion annosum, Ascocoryne sarcoides (Jacq. ex Gray) Groves and Wilson)

Etheridge (1956) found A. sarcoides in the heartwood of healthy spruces while Roll-Hansen (1962) and Shain (1971) found N. fuckeliana var. fuckeliana in healthy wood of Picea abies. Ricard (1970) isolated A. sarcoides and Cephalosporium sp. from healthy wood at the margins of stabilized decay columns. Observing the mycoflora of living lodgepole pines Bourchier (1961) found that the normal-appearing heartwood of living pines may support a rather constant flora of microfungi and that most of the fungi did not cause appreciable weight loss in wood blocks.

Pertaining to wood in soil Merrill and French (1966) have noted that the number of fungal species recovered from wood is smaller than the number obtained from dilution plates and that the number of species of importance in colonizing wood in soil is even fewer.

The number of studies conducted on the mycoflora of stumps of trees is relatively sparce. Notable studies have been completed on hardwood stumps by Rayner (1977a,b) where he studied the varying mycofloral succession after chemical treatments to the stump surfaces. Käärik and Rennerfelt (1957) investigated rot fungi on one to five-year old stumps of spruce and pine cut from healthy 60 -120-year old trees and found a larger number of fungal species in the upper portion than in the lower portion of the stumps. Meredith (1959, 1960) studied fungi inhabiting pine stumps and concluded that:

> "... initial colonization of stump roots usually proceeds from the body of the stump...and there is often considerable delay between the time of felling and the invasion of

stump roots by fungi present in the soil...."

The stumps and roots remaining in the ground after felling a stand of trees represent a considerable part of the total volume of wood produced by that site. What happens to all this wood is not only of academic interest but should be of interest to the land managers as well. For how this wood is disposed of in the natural ecosystem will determine the likelihood of whether or not a disease cycle may be established or maintained. The importance of this residual wood has for a long time been ignored by both the mycologists and pathologists and is reflected by the dearth of literature relating to the subject.

III. INFECTION OF STRESSED PONDEROSA PINES BY *ARMILLARIELLA MELLEA* FROM INDIGENOUS AND ARTIFICIAL INOCULUM SOURCES

Background

The research on *Armillariella mellea* is extensive, diverse and often conflicting. That part of the research involving use of artificial inoculum is no exception.

Many different substrates have been used in attempts to find the ideal source for rhizomorph formation and infection. Isolates grown on usual laboratory media have been attempted and failed (Rayner, 1930; Plakidas, 1941; Crandall, 1950). Only Guyot (1927) reported any success on pine and acacia (*Acacia* sp.) with rhizomorphs developed on a medium of chestnuts (*Castanea* sp.) or acorns mixed with agar and water. The successful attempts to cause infection have resulted by using cultures grown on root segments or wood blocks. Thomas (1934) infected seedlings of fruit and walnut trees employing inoculum grown in fruit tree prunings. Plakidas (1941) used pieces of artifically infected pear wood to infect healthy pear trees. Bliss (1941a, 1941b, 1946) used segments of citrus roots and pepper-tree stems to infect nine different species of plants. A successful use of pine root segments as a substrate for *A. mellea* resulted in considerable infection on four conifer species which were from seven- to ten-years old (Patton and Riker, 1959).

The success at inciting infection through the use of woody substrates infected with A. mellea is by no measure universal. Using small twigs as a substrate Reitsma (1932) failed to infect one- and two-year old tree seedlings or several herbaceous species. Cooley (1943), using a technique similar to Bliss (1941a, 1941b, 1946), obtained only negative results. Baker (1942) used wood blocks as inoculum but infected only a few western white pines (Pinus monticola Dougl.).

A majority of the studies which resulted in successful infections all had in common a substrate that could serve as a food base for a considerable period. Bliss (1941a, 1941b, 1946) ran experiments from seven to ten months while Van Vloten (1936) infected oaks in five to eighteen months and Patton and Riker (1959) took ten to 96 months.

The above literature convinced me that to have a chance at obtaining meaningful infections a woody substrate was the only choice available.

The light textured pumice soils of Pringle Butte are ideal for inoculation studies with A. mellea because they allow extensive excavation with minimal damage to root systems. The following studies of infection of stressed and unstressed ponderosa pines were conducted to gain insight into the little explored realm of stressed host pathology.

Rhizomorphs of A. mellea for inoculations were from two sources: 1) an artificially infected woody substrate; and coincidentally 2) the indigenous population on the butte.

Materials and Methods

Production of artificial inoculum

Inoculum was grown on 15 cm lengths of hazel stems (Corylus cornuta var. californica (A. DC.) Sharp) 1.5 to 2.5 cm in diameter from wild plants in the school forest. Inoculum sticks were washed in tap water and packed with 100 ml of water into quart canning jars with loosely applied, inverted, self-sealing lids and steam autoclaved at 1.05 Kg/cm^2 for 90 minutes. Several days after cooling exposed ends of the sticks were individually inoculated with a 1 cm cube of A. mellea grown on malt-pitch agar (See Appendix A) for two weeks. In approximately three weeks mycelial fans of A. mellea were visible emerging from uninoculated ends of the sticks (Figure 1). The jars were incubated in the dark at approximately 21'C until used (3 to 7 months).

The isolate of A. mellea used was obtained in Glenwood, Washington (NE $\frac{1}{4}$, SE $\frac{1}{4}$, Sec. 10, T 6 N, R 11 E) from the stump of a ponderosa pine killed by the fungus. The strain is designated GW-C7.

Inoculation procedures

Researchers have emphasized that only infections of the root collar, as opposed to infections on lateral roots, are lethal (Day, 1927; Patton and Riker, 1959; Peace, 1962; Foster and Johnson, 1963; Sokolov, 1964; Adams, 1972; Shaw, 1974). I further examined this phenomenon.

Lateral roots to be inoculated were exposed for 40 cm from the bole. At 20 cm water saturated vermiculite was placed lightly around the area where the inoculum was to be placed. The inoculum stick was placed on top of, and parallel to, the root. Wet vermiculite was added to a depth of 2.5 cm over the stick. The vermiculite was then covered with heavy polyethylene film to retain moisture. Soil was then carefully restored over the entire area (Figure 2a).

Figure 1. Mycellial fans of A. mellea emergeing from inoculum.

A small vertical pit was excavated next to the tap root of each tree to be inoculated at the root collar. The pit was lined with heavy polyethylene film and filled with saturated vermiculite. The inoculum stick was pushed into the vermiculite next to, and level with, the root collar and a plastic flap was placed over the pit. Soil was replaced over the plastic flap (Figure 2b).

Care was taken in all inoculatios to avoid putting humus next to any inoculum stick.

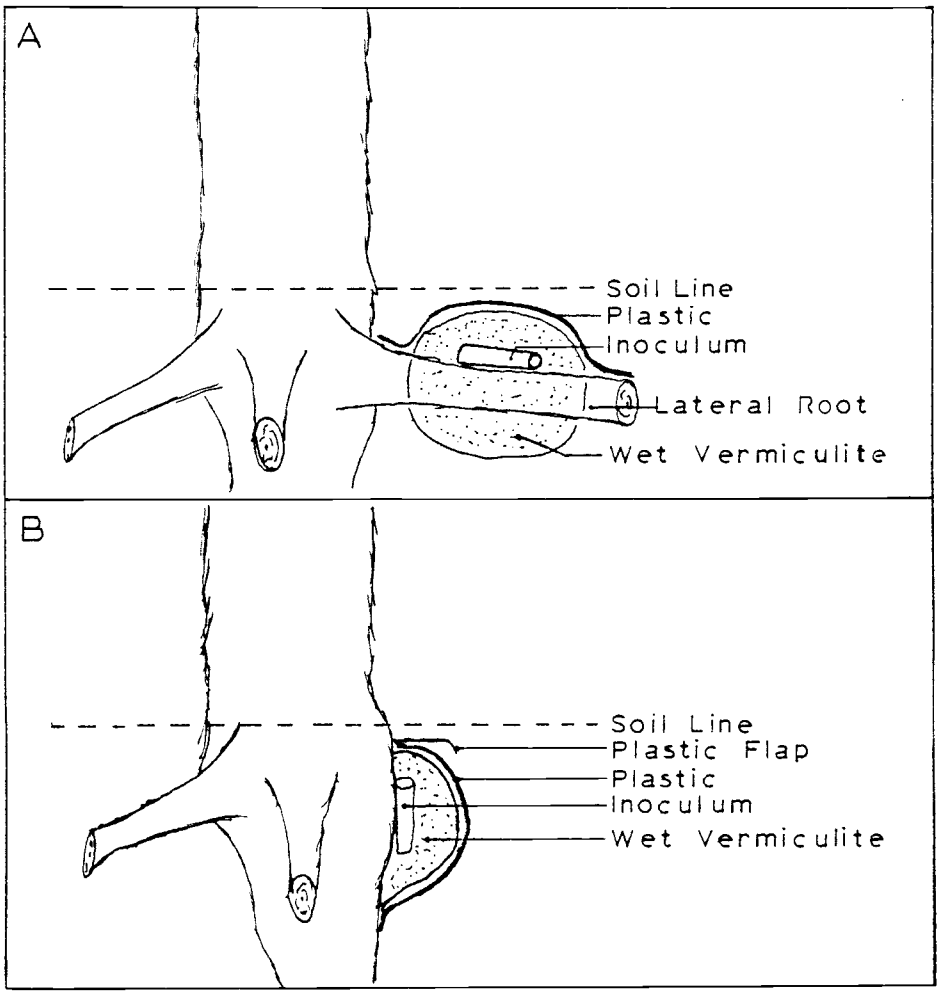


Figure 2. Diagrammatic representation of the method of inoculating a lateral root (A) or root collar region (B) with A. mellea.

Description of treatments

Seven treatments were established comparing infection of stressed and unstressed ponderosa pines: 1) Treatment one consisted of inoculations at root collars of unstressed trees; 2) Treatment two consisted

of inoculations at the lateral roots of unstressed trees; 3) Treatment three consisted of inoculations at lateral roots which were girdled; 4) Treatment four consisted of inoculations proximally to lateral roots which were severed; 5) Treatment five consisted of inoculations at the root collar of trees which had fifty-percent of the cross-sectional area of the lateral roots removed; 6) Treatment six consisted of inoculations at the root collar of trees which had two-thirds of the biomass of the crown removed; and 7) Treatment seven consisted of inoculations at the lateral roots of freshly felled trees.

All trees were selected so as to be randomly distributed through the stand with D.B.H.(o.b.) of 10 to 15 cm (4 to 6 inches) and to represent as much as possible all crown classes (Table 4) (Smith, 1962) and vigor classes (Table 5) (Miller and Keen, 1960).

Treatments one through five and treatment seven were intermixed in the same stand (See Map A, area I) while treatment six was located in a near-by stand (See Map A, area II).

Features of the trees involved in the seven treatments are summarized in Tables six through eleven.

Trees for each treatment were randomly selected, from the population of previously selected trees, in four replications of ten trees each except for the stump treatment (#7) for which trees were selected in four replications of ten trees each plus two replications of five each. Treatment and inoculation dates are shown in Table 12.

The eighty unstressed trees inoculated at the root collar (Treatment 1) or lateral roots (Treatment 2) were not tampered with other than in placement of the inoculum.

Table 4. Classification of crown classes [1]

Classification	Explanation
Dominant	Trees with crowns extending above the general level of the crown cover and receiving full light from above and partly from the side; larger than the average trees in the stand, and with crowns well developed but possibly somewhat crowded on the sides.
Codominant	Trees with crowns forming the general level of the crown cover and receiving full light from above but comparatively little from the sides; usually with medium-sized crowns more or less crowded on the sides.
Intermediate	Trees shorter than those in the two preceeding classes but crowns extending into the crown cover formed by codominant and dominant trees; usually with small crowns considerably crowded on the sides.
Overtopped	Trees with crowns entirely below the general level of the crown cover, receiving no direct light either from above or from the sides.

[1] Smith, D.M. 1962. The Practice of Silviculture, John Wiley and Sons, Inc. 578p.

Each of the forty trees in the root girdling treatment (#3) had two laterals, greater than 1.5 cm in diameter at the point of inoculation, exposed for 40 cm and completely girdled at 25 cm from the bole by a girdle equal in length to the diameter of the root at the point of the girdle. Care was taken to remove all remnants of cambial tissue. The girdle was immediately sealed with asphalt/silicon tree dressing (Morrison's Tree Seal) even though such dressings are reported not to inhibit fungal invasion (Shigo and Wilson, 1971, 1972; Shigo, 1971) (Figure 3). One inoculation of each tree was made proximally to the

(text continued on page 50)

Table 5. Description of Keen's crown-vigor classes[1/].

Character	Class A	Class B	Class C	Class D
Crown vigor	Full, vigorous	Good to fair	Fair to poor	Very poor.
Crown length	Long, 55% or more of total height; or less only if more than average width.	Average length, less than 55% of total height (approximately 30% to 55% if full and wide), or a longer crown if narrower or somewhat thin.	Short (from 10% to 30% of height, if crown of normal density) or long, sparse, and narrow.	Very short (less than 10% of total height), sometimes merely a tuft at top of tree, or somewhat longer when sparse and ragged.
Crown width	Usually average width or wider (narrower if very long and dense).	Usually average width or narrower; may be flat on one side.	Usually narrow or flat on one or more sides.	Usually very narrow and sparse, or limbs all on one side.
Crown density	Usually full and dense or of medium density if longer than 55%.	Usually of full to medium density, not sparse or ragged.	Often sparse and ragged except at very top.	Sparse and ragged.
Foliage	Needles of average length or longer, usually dense and thrifty.	Needles of average length, usually dense and thrifty.	Needles often short and thinly distributed, but of normal length and density when confined to top one-third of crown.	Needles often short, foliage sparse or scattered or only partially developed, but of normal length if reduced in quantity.
Position	Usually isolated or dominant, rarely codominant.	Usually codominant; sometimes isolated or dominant; rarely intermediate.	Usually intermediate; sometimes codominant or suppressed, but rarely isolated or dominant.	Usually suppressed or intermediate, but may occupy other positions if greatly reduced in vigor.
Diameter	Large for age	Average or above for age	Usually below average for age; sometimes larger in decadent trees.	Decidedly subnormal for age, but very old, decadent trees may be of large diameter.

NOTE: The descriptions apply to the usual types of trees found in each class; where exceptions occur, the size of living crown and amount of foliage are the primary considerations in determining the vigor class.

[1/] Miller, J.M. and F.P. Keen. 1960. Biology and Control of the Western Pine Beetle. U.S.D.A. Misc. Pub. 800, 381p.

Table 6. Description of trees used in treatment one according to size (cm), vigor and crown class.

Vigor[1]	Overtopped # Trees	DBH[2]	DAB[3]	Intermediate # Trees	DBH	DAB	Codominant # Trees	DBH	DAB	Dominant # Trees	DBH	DAB
P	8	10.6	15.4	2	12.7	18.3	-	-	-	-	-	-
F	2	11.4	16.2	8	11.7	16.2	3	12.4	17.3	-	-	-
G	1	11.9	17.3	7	11.9	17.0	6	14.2	19.5	2	13.2	19.3
E	-	-	-	1	13.7	19.5	-	-	-	-	-	-
Totals	11			18			9			2		
Means		10.9	15.7		11.9	17.0		13.4	18.8		13.2	19.3

Total number of trees = 40 Average DBH = 11.7 Average DAB = 17.0

[1] Vigor classes: P = Poor, F = Fair, G = Food, E = Excellent (See text for explanation).

[2] DBH = Diameter at breast height (1.37 m).

[3] DAB = Diameter at base of tree (soil level).

The above explanations apply to Tables six through eleven, inclusive.

Table 7. Description of trees used in treatment two according to size (cm), vigor and crown class.

Vigor	Overtopped			Intermediate			Codominant			Dominant		
	# Trees	DBH	DAB	# Trees	DBH	DAB	# Trees	DBH	DAB	# Trees	DBH	DAB
P	3	11.2	15.7	3	10.6	15.7	1	11.7	16.5	-	-	-
F	4	10.9	17.3	8	13.5	18.5	7	14.7	21.6	-	-	-
G	1	12.4	15.2	4	10.9	15.5	5	11.7	18.3	2	15.7	22.1
E	-	-	-	-	-	-	2	11.9	16.8	-	-	-
Totals	8			15			15			2		
Means		11.2	16.5		12.2	17.3		13.2	19.5		15.7	22.1

Total number of trees = 40 Average DBH = 12.7 Average DAB = 18.3

Table 8. Description of trees used in treatment three according to size (cm), vigor and crown class.

Vigor	Overtopped # Trees	DBH	DAB	Intermediate # Trees	DBH	DAB	Codominant # Trees	DBH	DAB	Dominant # Trees	DBH	DAB
P	2	9.6	13.7	7	10.9	13.5	-	-	-	-	-	-
F	4	11.2	14.9	15	11.4	17.3	3	14.2	17.8	-	-	-
G	-	-	-	5	13.2	18.8	3	14.2	19.3	-	-	-
E	-	-	-	-	-	-	1	13.0	16.8	-	-	-
Totals	6			27			7			-		
Means		10.7	14.5		11.7	16.5		14.0	18.3		-	-

Total number of trees = 40 Average DBH = 11.7 Average DAB = 16.5

Table 9. Description of trees used in treatment four according to size (cm), vigor and crown class.

Vigor	Overtopped # Trees	DBH	DAB	Intermediate # Trees	DBH	DAB	Codominant # Trees	DBH	DAB	Dominant # Trees	DBH	DAB
P	3	11.4	15.7	3	13.7	20.8	-	-	-	1	20.1	28.2
F	5	10.7	16.5	1	12.7	19.3	2	14.5	21.8	1	13.5	18.5
G	2	11.4	17.5	12	13.7	19.8	7	12.4	30.2	-	-	-
E	-	-	-	-	-	-	2	15.5	22.4	1	17.8	23.4
Totals	10			16			11			3		
Means		11.2	16.5		13.7	19.8		13.5	27.2		17.0	23.4

Total number of trees = 40 Average DBH = 13.5 Average DAB = 21.6

Table 10. Description of trees used in treatment five according to size (cm), vigor and crown class.

Vigor	Overtopped # Trees	DBH	DAB	Intermediate # Trees	DBH	DAB	Codominant # Trees	DBH	DAB	Dominant # Trees	DBH	DAB
P	12	12.4	17.5	3	11.9	17.8	2	14.7	19.8	-	-	-
F	2	14.2	20.5	7	11.4	19.5	3	12.9	18.0	-	-	-
G	1	12.1	19.5	5	15.7	18.5	2	12.9	19.5	1	17.0	25.4
E	-	-	-	-	-	-	-	-	-	2	18.8	25.4
Totals	15			15			7			3		
Means		12.7	18.5		12.9	18.8		13.4	19.0		18.3	25.4

Total number of trees = 40 Average DBH = 13.2 Average DAB = 18.5

Table 11. Description of trees used in treatment six according to size (cm), vigor and crown class.

Vigor	Overtopped # Trees	DBH	DAB	Intermediate # Trees	DBH	DAB	Codominant # Trees	DBH	DAB	Dominant # Trees	DBH	DAB
P	-	-	-	-	-	-	-	-	-	-	-	-
F	1	10.2	14.7	1	9.9	16.2	3	11.4	16.5	-	-	-
G	-	-	-	3	10.2	15.5	7	10.4	14.9	-	-	-
E	-	-	-	1	9.4	14.5	21	8.9	13.9	3	11.4	16.0
Totals	1			5			31			3		
Means		10.2	14.7		10.2	15.5		9.9	14.5		11.4	16.0

Total number of trees = 40 Average DBH = 13.8 Average DAB = 19.8

Table 12. Dates in 1975 on which trees were treated to induce stress or were inoculated[1].

Treatment	Replication 1	2	3	4	5	6
	Dates[2]					
1	- / 7-4	- / 8-1	- / 8-19	- / 9-13		
2	- / 7-4	- / 8-1	- / 8-21	- / 9-11		
3	7-4 / 7-4	7-29 / 8-1	8-24 / 8-24	9-11 / 9-11		
4	7-4 / 7-4	7-29 / 8-1	8-24 / 8-24	9-11 / 9-11		
5	7-4 / 7-4	7-29 / 8-1	8-24 / 8-24	9-11 / 9-11		
6	7-4 / 7-4	7-29 / 8-1	8-24 / 8-24	9-11 / 9-11		
7	6-6 / 6-9	6-20 / 6-30	7-11 / 7-30	8-1 / 8-28	9-11 / 9-27	9-27 / 9-27

[1] Ten individuals were treated or inoculated on each date except for Treatment 7 on 9-11 and 9-27 when there were 5 each.

[2] To the left of the slash (/) are treatment dates and to the right are inoculation dates.

girdle on one lateral and the other was made distally to the girdle on the other lateral.

The forty trees of the root severing treatment (#4) had two laterals per tree, greater than 1.5 cm in diameter at the point of inoculation, exposed for 40 cm and severed at 30 cm from the bole. The proximal end of the cut was immediately sealed with asphalt/silicon tree dressing. Each lateral was inoculated 15 cm from the bole.

Figure 3. Appearance of lateral root in Treatment 3 after treatment and before inoculation.

The forty trees in the root removal treatment (#5) had fifty-percent of the cross-sectional area of the roots (excluding the tap root), measured at one-inch from the tap root, severed by excavating a pit around the tree with an approximate radius of 45 cm and a depth of 30 cm (Figure 4). The amount of cross-sectional area needed to be removed was calculated using the regression formula $Y = 3.04x - 16.42$

(R^2 = .96); where 'x' is the diameter of the tree at the soil level (See Appendix B). Soil was replaced prior to inoculation. Trees were supported with guy-wires where needed. Two inoculations per tree were made at the root collar and placed 180' apart.

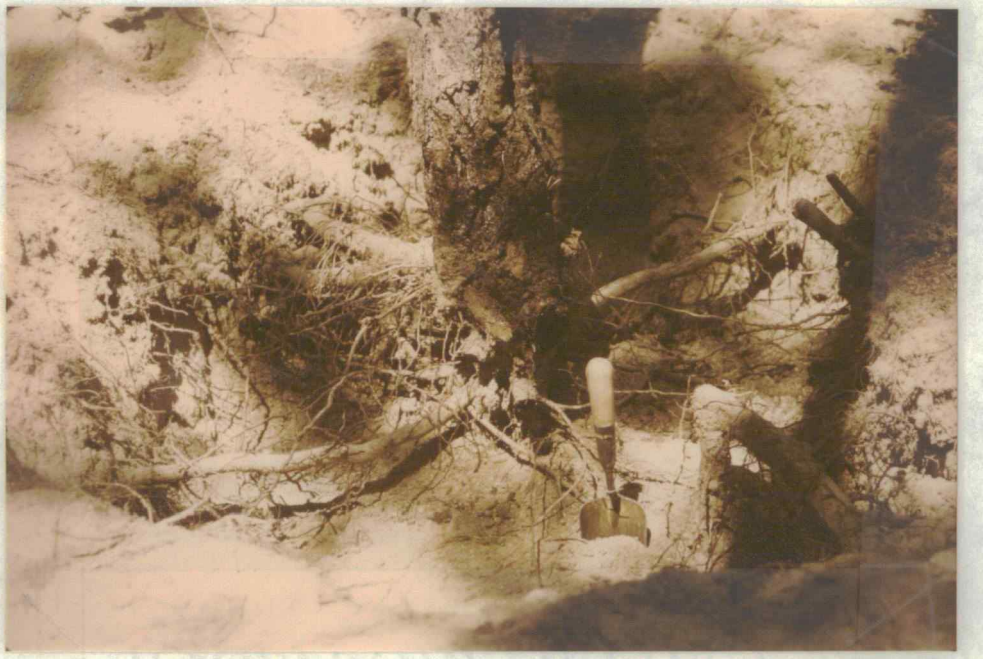


Figure 4. Appearance of the base of a tree in Treatment 5 after treatment and before inoculation.

The forty trees in the crown removal treatment (#6) had two-thirds of the biomass of the crown removed by pruning branches next to the bole for 56% of the linear length of the live crown from the bottom upwards (See Appendix C) (Figure 5). The pruning wounds were left open. Two inoculations per tree were made at the root collar and placed 180' apart.

The fifty trees converted to stumps were cut off 15 to 30 cm (0.5 to 1.0 ft.) above ground level. The stump surfaces were left

untreated (i.e. no chemical treatments). Two inoculations per stump were made 15 cm from the stump on separate lateral roots which were larger than 1.5 cm at the point of inoculation.

Figure 5. Appearance of the crown of a tree in Treatment 6 after treatment.

All inoculations were made the summer of 1975 according to the time schedule in Table 12. Results were recorded during the two summers following inoculation. One of the two inoculation sites on each tree was examined in 1976 and the other in 1977 except Treatment 3

(girdled roots) where both distal and proximal inoculations on the same tree were removed for one-half of the trees in 1976 and the other half removed in 1977.

When examining the individual inoculum sites extreme care was taken not to disturb the relationship of the rhizomorphs to the substrate. Infections were rated by number(s) per inoculation site and size (mm^2).

Results

Performance of artificial inoculum. Production of rhizomorphs from artificially infected hazel was poor in both 1976 and 1977. A total of 580 infected hazel sticks were used to establish the seven treatments. In 99.2% of the inoculum sticks retrieved in 1976 the fungus was viable, and of the inoculum sticks retieved in 1977 91.0% were viable. Viability was determined by visual inspection and by isolation onto Ortho-phenylphenol Malt medium (See Appendix A) as a check. Loss of viability of the inoculum was generally associated with competition from Trichoderma sp. or with desiccation. Eight inoculum sticks produced recognizable rhizomorphs (Table 13) of which 33 were attached to the target substrate. Thirteen of these were possitively identified as causing infection.

Indigenous rhizomorphs as an inoculum source. A. mellea was known to exist on Pringle Butte prior to the installation of the studies included in this thesis (Adams, 1974). What was unknown was the extensive system of A. mellea rhizomorphs in the soil of the study sites which was belied by any above ground indicators.

Table 13. Summary of data collected on two dates along with the totals for each of ten attributes among seven treatments.

ATTRIBUTE OBSERVED	TREATMENTS ONE 76	ONE 77	ONE TOT	TWO 76	TWO 77	TWO TOT	THREE 76	THREE 77	THREE TOT	FOUR 76	FOUR 77	FOUR TOT	FIVE 76	FIVE 77	FIVE TOT	SIX 76	SIX 77	SIX TOT	SEVEN 76	SEVEN 77	SEVEN TOT	# TOTAL	% of 580
Rhizomorphs produced from inoculum	0	4	4	2	3	5	3	8	11	6	3	9	5	5	10	6	12	18	12	11	23	80	13.8
Rhizomorphs produced from indigenous sources	13	9	22	5	7	12	9	12	21	10	6	16	1	13	14	26	15	41	12	31	43	169	29.1
Had rhizomorphs attached to substrate	8	11	19	5	5	10	1	8	9	8	5	13	1	15	16	19	21	40	13	31	44	151	26.0
Infected by *A. mellea*	7	11	18	2	1	3	1	5	6	6	2	8	1	12	13	11	14	25	9	28	37	110	19.0
A. mellea infection by artificial inoculum	0	2	2	0	0	0	0	0	0	1	0	1	0	3	3	2	1	3	1	3	4	13	2.2
A. mellea infection by indigenous inoculum	7	9	16	2	1	3	1	5	6	5	2	7	1	9	10	9	13	22	5	28	33	97	16.7
Had *A. mellea* infection only	7	11	18	2	1	3	1	5	6	4	2	6	1	11	12	11	14	25	7	7	14	84	14.5
Had beetle activity only	0	2	2	0	0	0	0	0	0	1	2	3	2	2	4	0	0	0	11	53	64	73	12.6
Had *A. mellea* plus beetles	0	0	0	0	0	0	0	0	0	2	0	2	0	1	1	0	0	0	2	21	23	26	4.5
Had neither *A. mellea*, beetles, nor rhizomorphs	27	25	52	33	30	63	28	21	49	21	29	50	32	20	52	8	13	21	13	14	27	314	54.1
Total inoculation sites	40	40	80	40	40	80	40	40	80	40	40	80	40	40	80	40	40	80	50	50	100		

The inoculation studies on stressed and unstressed ponderosa pines were initially designed to be conducted using solely artificial inoculum. One year after treatment and inoculation of the trees the extensive distribution of the indigenous A. mellea population became known because the inoculation sites observed during the summers of 1976 and 1977 were heavily infected by this source of the fungus. It was felt that since the results of infection were being evaluated and not the source of the infections, the use of infections from indigenous sources was admissible and, in view of the poor performance of the artificial inoculum, was a fortuitous occurance.

Of the 580 inoculation sites observed indigenous rhizomorphs were present in 169, were attached to the target substrate in 122, and caused recognizable infection in 97.

The combined total for successful infections (both artificial and indigenous) was 110. The efficiency of rhizomorph infection (number of rhizomorphs causing infection divided by the number of rhizomorphs attached to the substrate) for the artificial inoculum was 0.39 and for the indigenous inoculum was 0.80.

Effectiveness of inoculation procedures. The procedure of inoculation utilizing wet vermiculite and a covering of polyvinly sheeting was devised to overcome severe moisture stress common to these pumice soils during mid-summer. After a year, when the first inoculations were removed, the vermiculite was still moist enough that water could be squeezed out by hand.

Although few rhizomorphs were produced by the hazel stick inoculum rhizomorphs arising from indigenous sources flourished in the vermicu-

lite under the conditions provided.

Bark beetle populations. From the inception of these studies I anticipated that working with trees under intentional stresses would encourage insect problems. By chance, this work was done during the dryest year of record which further caused apprehension about insect problems.

Two species of bark beetles were observed in the study trees during the summers of 1976 and 1977. Small non-lethal populations of the mountain pine beetle (Dendroctonus ponderosae Hopkins; Coleoptera: Scolytidae) were present in 12 of the 290 treatment trees observed and the red turpentine beetle (Dendroctonus valens Le Conte; Coleoptera: Scolytidae) was observed only on ponderosa pine stumps, never in the living trees. In 1976 13 inoculation sites on stumps showed activity by D. valens and by 1977 this had increased to 81 inoculation sites.

In 1977, because of the increasing beetle activity, all 100 inoculation sites on stumps (both those scheduled for 1977 and those previously recorded for 1976) were evaluated for the presence of beetles and A. mellea. Eighty-one of the inoculation sites contained D. valens activity; 10 had the beetle alone, one had the beetle plus A. mellea; 46 had the beetle plus an unknown basidiomycete; and 24 had the beetle plus A. mellea plus the unknown basidiomycete.

Statistical analysis. Data on infection was collected in 1976 and 1977 representing: 1) the number of inoculation sites showing infection and 2) the dimensions of the resulting lesions. Infection dimension is expressed in mm^2 and was obtained by peeling away bark plates to the phelloderm in the area of rhizomorph insertion and re-

cording dimensions of the mycelial mat plus the darker discoloration beyond caused by resin exudation.

All stress treatments of the trees and all inoculations were on the same time schedules, thus yielding similar exposure times to probable infection, except for treatment seven (stumps) (Table 12), therefore, treatment seven will be analized separately as needed. A two-way analysis of variance (Snedecor and Cochran, 1973) of the combined 1976 and 1977 data of trees infected, both by artificial and indigenous sources of A. mellea, and the replication in which the tree belonged showed no significant differences (P = .05) among replication means. However, treatment means were highly significnat (P = .01) (Table 14).

Comparisons were planned among only certain means of the study. Since treatment one (unstressed root collar) was designed as the control for treatments six (removed crowns) and five (removed roots) and treatment two (unstressed lateral roots) was intended as the control for treatments three (girdled lateral) and four (severed lateral root) comparisons between the treatments and their respective controls are paramount. Comparisons between treatments one, six and five using the Honestly Significant Difference (Q) test showed no significant differences among their means (P = .05). Comparisons between treatments two, three and four using the same test (Q) showed no significnat differences either (P = .05). However, comparisons based on treatments one + six + five versus treatments two + three + four were significant (P = .05), that is, the number of trees infected at the root collar were significantly greater than the number infected at lateral root inoculations.

Treatment seven (stumps), taken separately, displayed no trends relating the time of treatment or inoculation to the rate of infection.

Table 14. Analysis of variance of a two-way classification based on number of trees infected out of 40 by treatment and replication.

Replication	Treatments 1	2	3	4	5	6	Total	Mean
I	6	0	3	2	3	8	22	3.6
II	6	1	1	1	2	6	17	2.8
III	3	1	2	0	3	5	14	2.3
IV	3	1	0	5	5	6	20	3.3
Total	18	3	6	8	13	25	73	
Mean	4.5	0.5	1.5	2.0	3.2	6.2		

H.S.D. = 2.12

Source of Variation	D.F.	Sum of Squares	Mean Square	F
Replication	3	6.13	2.04	
Treatments	5	84.71	16.94	7.91
Error	15	32.12	2.14	
Total	23	122.96		

Treatment seven (stumps), taken separately, displayed no trends relating the time of treatment or inoculation to the rate of infection.

Since no differences were detected among replication means, that is, neither season nor interval between treatment and inoculation had any effect on rate on infection, data from all replications were pooled thus allowing a more accurate estimate of error.

In these studies infection was caused, exclusively, by rhizomorphs. A t-test for un-paired plots comparing the number of inoculation sites in 1976 versus 1977 which contained rhizomorphs disclosed no significant difference (P = .05) (Table 15).

Table 15. t-Test comparing the number of inoculation sites, by treatments, containing rhizomorphs (both indigenous and artificial) in 1976 and 1977.

Treatment	1976	1977
1	13	13
2	7	10
3	12	20
4	16	9
5	6	18
6	32	27
7	24	42
	Sum = 110	Sum = 139
	Mean = 15.7	Mean = 19.8
	Σx^2 = 2254	Σx^2 = 3891
	t = 0.6529 (n.s.)	

Rhizomorphs causing infection were both indigenous and introduced. A t-test for un-paired plots showed significantly (P = .05) more infections from indigenous than from introduced rhizomorphs (Table 16). This latter statistic must be interpreted with knowledge that significantly (P = .05) more inoculation sites contained indigenous rhizomorphs than contained introduced ones (Table 17) and that the rate of successful infections for indigenous rhizomorphs is higher than for

introduced (page 55).

Comparison of the total number of sites infected in 1976 and 1977 showed no significant differences (P = .05) (Table 18).

Table 16. t-Test comparing the number of infections, for 1976 and 1977 combined by treatments, caused by indigenous and artificial sources of A. mellea.

Treatment	Indigenous		Artificial	
	1976	1977	1976	1977
1	7	9	0	2
2	2	1	0	0
3	1	5	0	0
4	5	2	1	0
5	1	9	0	3
6	9	13	2	1
7	5	28	1	3
	Sum = 97		Sum = 13	
	Mean = 6.9		Mean = 0.93	
	Σx^2 = 1331		Σx^2 = 29	
	t = 3.098 (significant)			

The number of infected trees per treatment provides one way to look at the data but it neglects the influence that target size might have on infection rates. Detection of a change in host response to A. mellea caused by stress requires a more sensitive parameter than number of infections. It was felt that lesion size would yield the desired sensitivity, reasoning that if stress is necessary for infection, it should also influence lesion size, lesions on stressed trees being larger than those on unstressed trees (Towers and Stambaugh, 1968; Wallis, 1961).

Table 17. t-Test comparing the number of inoculation sites, for 1976 and 1977 combined, which contained indigenous or introduced rhizomorphs.

Treatment	Indigenous		Artificial	
	1976	1977	1976	1977
1	13	9	0	4
2	5	7	2	3
3	9	12	3	8
4	10	6	6	3
5	1	13	5	5
6	26	15	6	12
7	12	31	12	11
	Sum = 169		Sum = 80	
	Mean = 12.1		Mean = 5.7	
	x^2 = 2861		x^2 = 642	

t = 2.7226 (significant)

Table 18. t-Test comparing the number of inoculation sites, by treatment, containing infections in 1976 and 1977.

Treatment	1976	1977
1	7	11
2	2	1
3	1	5
4	6	2
5	1	12
6	11	14
7	9	28
	Sum = 37	Sum = 73
	Mean = 5.28	Mean = 10.43
	x^2 = 293	x^2 = 1275

t = 1.3501 (n.s.)

Using the data summarized in Table 19 it was possible to compare the average lesion size between treatments using a t-test for unpaired plots.

Table 19. Data on lesion size, by treatment (excluding stumps), caused by attacks of A. mellea rhizomorphs in both 1976 and 1977 needed to conduct t-tests for unpaired plots.

1	2	3	4	5	6
		Lesion size (mm^2)			
2,500	22,500	2,100	15,000	81,280	162,600
1,500	100	2,000	100	15,000	122,000
900		875	100	6,000	16,000
450		100	100	5,000	15,000
450		100	100	3,200	10,000
300		100	100	2,000	10,000
150		100		1,000	2,500
100		100		750	2,000
100		100		100	1,000
100				100	1,000
100				100	1,000
100				100	150
					100
					100
					100
					100
					100
					4
Totals 6,750	22,600	5,575	15,500	114,630	344,754
n 12	2	9	6	13	19
Means 563	11,300	619	2,583	8,818	18,145

The comparisons tested were: Treatment one versus six; Treatment one versus five; Treatment two versus three; Treatment two versus four; and all collar infections (Treatments 1, 5, and 6) versus all lateral root infections (Treatments 2, 3, and 4). An additional comparison was made between all infections in 1976 and those in 1977. All of the foregoing comparisons were not significant (P = .05). The

average size of infections observed on stumps (Treatment 7) in 1976 was not significantly different (P = .05) from infections resulting from all other treatments. However, for 1977 the increase in average leasion size of Treatment seven was highly significant (P = .01), reflecting the almost complete colonization by A. mellea.

Several authors (Ehrlich, 1939; Wallis, 1961; Baranyay and Stevenson, 1964; Miller and Kelman, 1966; Gibbs, 1967; Gibbs and Greig, 1970) have related disease incidence and susceptibility to the position of the crown in the stand (overtopped, intermediate, codominant, dominant). A Chi-square test of independence between crown position and rate of infection was not significant (χ^2 = 5.28, 3 d.f.), that is, the incidence of infection is acting independently of the crown position (Table 20).

Similarly, several authors have related host vigor to disease incidence and susceptibility (Table 21). A Chi-square test of independence between host vigor (poor, fair, good and excellent) and the rate of infection was significant (χ^2 = 23.62, 3 d.f.), that is, the rate of infection is dependent on the vigor of the host (Table 22). Chi-square is an analysis of proportions and looking closer at the proportions (Table 22a) it is seen that the data runs contrary to almost all that is written about A. mellea, that is, it reveals that the significance of Chi-square is attributable to a higher incidence of infection in the more vigorous classes.

It can be argued, and correctly so, that evaluating whether a tree is infected or not solely on its crown class or vigor class is arfificial since all trees in the study, except treatment one and two, were

Table 20. Chi-square test of independence between rate of infection and crown class, excluding treatment seven.

	Overtopped	Intermediate	Codominant	Dominant	sub-totals
Infected trees	9	44	16	4	73
Non-infected trees	85	191	112	19	407
Totals	94	235	128	23	480

$\chi^2 = 5.38$, 3 d.f. (n.s.)

Table 21. Authors relating host vigor to disease incidence and susceptibility.

Author	Comment
Baker, W.L., 1941	"The shoe-string fungus...hastened the death of weakened trees."
Buckland, D.C., 1953	"Attack by A. mellea resulting in death of the host was most frequent in trees of low vigor."
Carter, C.J., 1975	"Shoestring root rot...is one of the most common root diseases of trees that have been previously weakened."
Christensen, C.M., and A.C. Hodson, 1954	Banding of the tree increased the attack by A. mellea.
Huntly, J.H., J.D. Cafley, and E. Jorgensen, 1961	"All evidence indicates that the fungus is always secondary to some other factor which predisposes the tree to disease."
Johnson, D.W., and J.H. Thompson, 1975	"In most instances, the affected trees (by A. mellea) appeared to be under stress."
Miller, R.S., et al, 1975	"..., root infection by A. mellea is particularily damaging to trees under stress...."
Morrison, D.J., 1976b	"...trees under stress and those nearer a source of inoculum may be successfully attacked (by A. mellea)."
Singh, P., 1970	"The rapid development of the disease (A. mellea) in this plantation can be attributed to...low host vigor...."
Wargo, P.M. and D.R. Houston, 1974	"Characterization of those physical and chemical changes induced by stress that lower resistance of root tissue to A. mellea may permit us to control infection by A. mellea...."

Table 22. Chi-square test of independence between rate of infection and host vigor, excluding treatment seven.

	Host vigor				
	Poor	Fair	Good	Excellent	sub-totals
Infected trees	10	16	25	22	73
Non-infected trees	102	140	121	44	407
Totals	112	156	146	66	480

$\chi^2 = 23.62$, 3 d.f. (significant)

Table 22a. Conversion of Table 22 to percentages.

	Host vigor			
	Poor	Fair	Good	Excellent
Infected trees (%)	8	9	14	27
Non-infected trees (%)	92	91	86	73
Totals	100	100	100	100

stressed in some form and therefore the parameter of what treatment a tree belongs to also has an influence (as shown previously in Table 14).

Constructing a Chi-square R x C contingency table of independence using treatment and crown class as factor effecting infection rate was not significant (χ^2 13.83, 15 d.f.) (Table 23). A Chi-square R x C contingency table of independence using treatment and host vigor as factors effecting infection rate was highly significant (P = .01) (χ^2 = 52.33, 15 d.f.) (Table 24), however, further analysis of the proportions showed that the differences were related to the probability of increased attacks of trees inoculated at the root collar and trees in vigor classes good and excellent. The single greatest contributing factor was the greater than statistically predicted occurance of attacks in vigor class excellent in treatment six.

Discussion

The use of artificial inoculum to infect tree hosts with Armillariella mellea has had a history of variability (Cooley, 1943; Patton and Riker, 1959; Shaw, 1974; Filip, 1975). The use of wet vermiculite under plastic proved to be an aid to rhizomorph growth as attested to by the abundant proliferation of rhizomorphs from indigenous substrata, however, only 29.1 percent of the inoculum sticks used produced rhizomorphs. It is felt that two main factors contributed to the poor performance of the artificial inoculum. The first reason was a probably improper decision to use an isolate of A. mellea not from Pringle Butte and , therefore, not adapted to the site. The reason for

Table 23. Chi-square R x C contingency table relating crown class and treatments to infection rate, excluding treatment seven.

	Treatments						
Crown Class	1	2	3	4	5	6	sub-totals
Overtopped	2	1	0	1	3	2	9
Intermediate	13	1	6	3	6	15	44
Codominant	3	1	0	3	4	5	16
Dominant	0	0	0	1	0	3	4
sub-totals	18	3	6	8	13	25	73

$\chi^2 = 13.83$, 15 d.f. (n.s.)

Table 24. Chi-square x C contingency table of independence relating host vigor and treatments to infection rate, excluding treatment seven.

	Treatments						
Host Vigor	1	2	3	4	5	6	sub-totals
Poor	5	0	0	1	3	1	10
Good	5	1	4	0	4	0	14
Fair	8	2	2	4	6	6	28
Excellent	0	0	0	3	0	18	21
sub-totals	18	3	6	8	13	25	73

$\chi^2 = 52.97$, 15 d.f. (significant)

for this departure from conventional logic was to provide a means of separating infections caused respectively by introduced and indigenous sources. This is accomplished by re-isolating the fungus and pairing in petri plates with known isolates (Adams, 1972, 1974). The second factor being that three to seven months incubation at 21'C probably was insufficient to induce most active rhizomorph formation once inoculum was placed in the soil. In all cases where good rhizomorph formation had occured after one or two years in the soil development was confined to a small section of the inoculum where the A. mellea decay had advanced to the soft white-rot stage. This conflicts with the observations of Redfern (1968) that fewer rhizomorphs were produced in stages of advanced decay but is consistent with observations of earlier students in the Armillaria root rot project at this institution. The majority of the non-rhizomorph producing inoculum was still extremely solid.

Temperature was ruled out as a significant factor influencing performance of the inoculum since the isolate used had an optimum rhizomorph producing temperature between 10 - 15'C, closely agreeing with the prevailing soil temperatures on the site (Appendix E, and Table 1).

The total number of infections was shown not to have changed significantly from 1976 to 1977. However, there were significant increases in treatments five and seven from 1976 to 1977. In treatment five, where 50% of the cross-sectional area of the lateral roots was removed, an increase in infection rate was probably associated with the manner in which treatment was effected. To get at the lateral roots of a study tree a pit 30 - 60 cm in radius was dug 30 cm deep around

the tree. This procedure unknowingly accomplished two things: First, it effectively sanitized the pit of any existing A. mellea; and second, it severed numerous existing rhizomorphs which subsequently caused a proliferation of new growing tips and reinfecting the site at probably a higher rate than before the treatment. This reinfection, however, was delayed, by the distances involved, for the first year, thus, the apparent increase in infection rate in 1977 over 1976.

The significant increase in infection in treatment seven can be attributed to the mode of spread of A. mellea. In 1976 the number of infections on stumps and their sizes were not statistically different from the values for all the other treatments, indicating that the stumps were retaining some resistance at the end of one year after felling. By the end of two years substrate resistance had declined and A. mellea, now actively growing as a saprophyte, had progressed towards the stump, proximally through infected lateral roots, effectively increasing the total area over which infection could result in the ultimate colonization of the stump.

All treatments, except six (pruned crowns), were conducted in the same stand, and were in reality completely randomized over the study area. Therefore, the differences detected in the rates of infection (Table 13) are felt to be real. The point to be made here is that real differences were disclosed between the number of attacks encountered at the root collar versus those on the lateral roots. I submit that the cause of this difference can be accounted for in the models of soil-borne plant pathogens put forth by Baker (1971) and Baker et al (1967). These two papers describe a model for a moving inoculum (the

rhizomorph in my case) and a moving host (the increment growth of the lateral root or root collar) which would predict the higher incidence of infection in the system with the greatest host movement, other things being equal. The root collar puts on increment growth at a faster pace than do laterl roots and this difference in growth contributes to the difference in disease incidence. Another factor contributing to the increase in disease incidence at the root collar over the lateral roots is that the root collar is orientated perpendicular to the plane of concentrated rhizomorph growth (most rhizomorphs growing nearly parallel to the soil surface) while lateral roots are orientated parallel to the plane of concentrated rhizomorph growth. This orientation of the root collar presents a larger target with a larger apparent motion than do lateral roots. Again, the model of Baker (1971) and Baker et al (1967) would predict the observed increase in infection incidence.

During the summer of 1977 the Pacific Northwest experienced the most extreme drought on record. The drought, plus the added stress of the applied treatments, put realistically severe stresses on all study trees. The drought stress was applied even to the two control groups (Treatments one and two). Despite these stress factors no differences could be detected between the sizes of infections on study trees, whether taken separately, year by year or combined except in treatment seven (stumps). This leaves one with several conclusions from which to choose. Firstly, the stresses applied were not severe enough to generate a real difference. Secondly, the infecting strain of A. mellea, though parasitic to ponderosa pine, was mildly pathogenic. Thirdly, the

non-significance of lesion size is a real phenomenon. One cannot totally discount the first conclusion because of the extreme difficulty in obtaining an accurate estimate of the precise physiological state of a tree. Experimentally, Koonce (personal communication, 1979)[1/] was unable to detect physiological differences among treated and control trees based on root collar and lateral root carbohydrate analysis and on pressure bomb readings of cut twigs. I was able to detect a significant reduction in the increment growth of pruned trees (Appendix F) and to observe gradual loss of vitality in cambial tissue of treatments three and seven. The author feels that the stresses applied were realistic and severe enough to detect any differences had they existed. If the second conclusion were valid one would still expect to be able to detect some differences in the sizes of the lesions and would not expect to detect any mortality separate from the study area. However, mortality in and around the study areas due to A. mellea isolates indigenous to the sites was evident as scattered foci centering around old-growth stumps. Thirdly, the data collected and the observations made can be explained if the size of the individual lesions are not as important as the total numbers and locations of these lesions. Attacks on lateral roots have been shown to be rarely lethal because of the failure of the fungus to move proximally (Shaw, 1974) in infected tissues while any host resistance remains, this fact coupled with the

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[1/] Koonce, A., 1979. Personal communication on work of thesis in progress at Oregon State University.

observation that significantly more attacks are made at the root collar one can envision the host root collar region overcome by numerous coalescing lesions of relatively the same size (Shaw and Toes, 1977; Rykowski, 1975, 1978; Guillaumin and Rykowski, 1978).

Summary. The analyses conducted present a picture of A. mellea attack in the earlier stages of infection of pole-sized ponderosa pines under various stressing influences. While rhizomorphs abound in the soil, few come into contact with a suitable substrate and fewer still cause measurable infections. The root collar region of the tree presents an appreciably larger target for attack than do lateral roots, which is reflected in a higher incidence of successful attacks on the former. Once attacked, lateral roots and the root collar show similar amounts of resistance, even while under stress. During the earlier stages of establishment of infection, stress, crown position and decreased host vigor appear to not play an intergral role in the infection processes.

IV. QUANTITATIVE OCCURANCES OF A. MELLEA RHIZOMORPHS COLLECTED IN FOREST STANDS WITH VARYING SILVICULTURAL HISTORIES

Background

It was realized from previously published work (Adams, 1972) and from observations of scientists and foresters that A. mellea was common on Pringle Butte. It was unrealized, however, that A. mellea rhizomorphs were abundant in areas devoid of pine mortality. The high incidence of indigenous rhizomorphs in the inoculation sites of the previous chapter is notable.

The literature on occurance of rhizomorphs of A. mellea in the soil is limited to reports of variation in the depth of rhizomorph concentration (Ono, 1970; Redfern, 1973; Hintikka, 1974; Singh, 1977) and variation accompanying differences in cover-type (Ono, 1970; Hintikka, 1974; Singh, 1977). No literature could be found, however, which related the occurance of A. mellea rhizomorphs to similar cover-types which had varying histories of management.

Pringle Falls Experimental Forest offers a unique opportunity to study the ecology of A. mellea rhizomrophs in the soil. Within short distances a wider range of silvicultural strategies can be found than occur in the usual production forest. The study reported here relates stand management histories to the quantitative occurances of A. mellea rhizomorphs in the soil.

Materials and methods

Eight forest sites were chosen (Table 2 and 25) (Map A) (Figures 6 through 13), which were as similar as possible in all respects except silvicultural history, for establishment of 4 m x 10 m sampling areas. Each area was selected so that no old-growth stumps were in or within ten-feet of the area, but at least one old-growth stump occured within 20-feet. This was not true of sites 1, 5, and 6 where each area was located at the geometrical center of the stand being sampled because no old-growth stumps occured within reasonable distances. The 4 m by 10 m area was further sub-divided into 10-$2m^2$ plots and inside each $2m^2$ plot one pit ($60cm^3$) was dug by hand. The removed soil was seived through a screen with four openings per 2.5 cm (Figure 14). All rhizomorphs observed were removed to numbered paper bags. The rhizomorphs were subsequently cleaned, dried to constant weight at 102'C and weighed to the nearest 0.1mg.

Results

The dry weights of rhizomorphs collected from the eighty pits are shown in Table 26. An analysis of variance based on this information was highly significant (P = .01).

The comparisons of the means in Table 26 of most interest, tested using the Least Significant Difference (P = .05), are the various silvicultural strategies to the natural area (site 5). Site 5 (1.6712 gr.) displayed a significant difference only when compared to sites 3 (9.9417 gr.)(clearcut: shrubs controlled) and 7 (4.381 gr.)

Table 25. Summary of study sites and their management histories.

Site Number	History[1]
1	Young pole-sized stand established by fire about 70-years ago (Barrett, 1970).
3	Clearcut in 1965 and replanted in 1967 with native stock: Brush was sprayed with 1.12 Kg/hectare 2,4,5-T for control in 1971.
4	Same as 3 above except brush was not sprayed.
5	Area left natural except for selected sanitation salvage and 40-years of fire suppression.
6	Young pole-sized stand established by fire about 70-years ago and thinned in 1965 to 309 trees per hectare: Slash was left where felled.
7	Similar to 6 above but slash was disposed of by use of a 'tommyhawk'.
8	Area cut-over in a three-stage shelterwood in in 1958, 1969 and 1977: A. mellea mortality is absent.
9	Same area as 9 above except A. mellea mortality is present.

[1] For more detailed information see Table 2 in Chapter III.

Figure 6. View of site number one (Young pole-sized stand).

Figure 7. View of site number three (Clearcut; shrubs controlled).

Figure 8. View of site number four (Clearcut: shrubs not controlled).

Figure 9. View of site number five (Natural area).

Figure 10. View of site number six (Young pole-sized stand: thinned and slash left where felled).

Figure 11. View of site number seven (Young pole-sized stand: thinned and slash 'tommyhawked').

Figure 12. View of site number eight (Shelterwood: A. mellea mortality absent).

Figure 13. View of site number nine (Shelterwood: A. mellea mortality present).

Table 26. Dry weights (in grams), and their analysis of variance, of rhizomorphs collected from eight-60cm^3 pits located in eight sites varying in silvicultural histories.

	site number [1]							
	1	3	4	5	6	7	8	9
	1.561	8.1560	3.0159	0.8947	0.350	2.172	1.5355	1.8692
	0.573	8.4009	1.8510	3.6553	0.904	3.162	1.6727	1.1736
	1.812	7.9078	0.7452	4.6467	1.390	2.495	5.2900	2.3614
	0.233	6.8907	3.5421	1.4831	1.478	8.266	1.3307	1.2477
	1.039	6.4237	2.1619	0.6532	0.990	6.631	0.9097	1.6021
	0.523	7.8284	5.4055	2.6947	0.276	5.084	1.0649	1.7134
	0.188	6.6162	3.7146	1.0078	0.898	4.151	4.1467	2.5500
	1.068	23.2379	4.1933	0.2319	0.599	0.537	1.8996	1.5604
	0.813	6.2156	2.7723	0.7127	2.058	10.715	7.2215	1.6617
	0.053	17.7399	7.9385	0.7321	2.000	0.638	3.3663	1.1978
Total	7.863	99.4171	35.3573	16.6122	10.943	43.812	28.4376	16.9373
Mean	0.786	9.9417	3.5357	1.6712	1.094	4.381	2.8438	1.6937

ANOVA

Source	D.F.	Sums of squares	Mean square	F
Treatments	7	619.44	88.49	12.66**
Error	72	503.1		
Total	79	1,122.54		

L.S.D. (P = .05) = 2.36

[1] Refer to Table 25 (page 77) for description of sites.

(thinned and 'tommyhawked'), the latter two areas containing more rhizomorphs than the natural area. Other comparisons of interest tested were: site 9 (1.6937 gr.)(Shelterwood; A. mellea mortality present) versus 8 (2.8438 gr.)(Shelterwood; A. mellea mortality absent) which was not significant; site 4 (3.5351 gr.)(Clearcut; shrubs uncontrolled) versus 3 (9.9317 gr.)(Clearcut; shrubs controlled) which was significant; site 1 (0.7863 gr.)(Pole stand) versus 6 (1.094 gr.) (Thinned pole stand; slash left where felled) which was not significant; site 1 (0.7863 gr.) versus 7 (4.381 gr.)(Thinned pole stand; slash 'tommyhawked) which was significant; and 6 (1.094 gr.) versus 7 (4.381 gr.) which was significant.

Figure 14. Sifting of pumice soils through a wire screen in search of A. mellea rhizomorphs.

Discussion

Unlike several authors' observations (Hintikka, 1974; Singh, 1977), where they observed rhizomorphs of Armillariella sp. only in the humus or upper soil layers, the rhizomrophs of A. mellea on these pumice soils rarely penetrated the humus layer but concentrated at the 10 cm to 30 cm depth. This agrees with the observations of Ono (1970) and Morrison (1976a). Rhizomorphs were found deeper than 60 cm, but in general the occurances decreased with increasing depth.

Quantitatively there were detectable differences in the occurance of rhizomorphs in several sites. Statistically, the differences in rhizomorph weights establishes a trend of increasing weights with increasing management intensity.

The two clearcut areas (sites 4 and 3) were similar except that in 1971 site 3 was treated with the herbicide 2,4,5-T ((2,4,5-trichlorophenoxy) acetic acid) to control the shrubs while site 4 was left natural (Barrett, personal communication)[2/]. Since this was the only difference, the significant difference in the quantities of rhizomorphs extracted (9.947 versus 3.5357 gr., respectively) must be attributed to the loss of the shrub cover resulting in increased soil temperatures and increased available soil moisture, both benificial to A. mellea on this site. All the more interesting about the difference is that the difference must have developed only from the time of herbicide application to observation (1971 to 1978), indicating

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2/ Personal communication with J.W. Barrett, Pacific Northwest Forest and Range Exp. Sta., Bend, Oregon.

rhizomorphs of A. mellea are still capable of response to changing site conditions six-years after the initial flush of growth (i.e. the time of the initial cut in 1965).

One unique observation to emerge was the comparison of the rhizomorph weights between the two shelterwood areas (sites 8 and 9). The mean weights of the two areas were not statistically different, yet one site (9) was experiencing mortality and the other site (8) was not. This is similar to observations made by Ono (1970) in diseased and healthy stands in Japan. Just what these quantities represent have yet to be adequately explained.

Rykowski (1978) and Guillaumin and Rykowski (1978) have reported that infection from rhizomorphs can take place in three manners: 1) penetration of the host by the rhizomorph en masse; 2) penetration of the host by hyphae near the growing apex of the rhizomorph; and 3) penetration of the host by lateral growing tips arising in areas where the rhizomorph has become firmly appressed to the host.

In all of the above three instances the rhizomorph must come into contact with the host. From this it can be deduced that the greater the quantity of rhizomrophs (i.e. meaning length as well as weight) in any given volume of soil the greater is the probability of a susceptible host being attacked. However, this is not what has been observed in this study. In the site experiencing mortality (site 9), the mortality was associated with root-to-root spread and not rhizomorph infection. It appears to this author that, on the sites studied so far, the rhizomorphs of A. mellea function as a means of saprophytic colonization and build-up and occasionally as a means of parasitic attack at

root collars near a source of inoculum (old-growth stumps) where inoculum densities are high.

The above statments must be tempered, however, in the light of several authors' observations that severing viable rhizomorphs will cause a regrowth of several to many new growing tips capable of causing infection (Redfern, 1973; Pawsey and Rahman, 1976) (Figure 15), thus effectively increasing local inoculum densities, and could result in any newly planted trees in an area of recent timber extration being at maximum risk (Redfern, 1973).

Figure 15. Regrowth of severed, viable, A. mellea rhizomorph which displays the typical habit of regenerating more than one new growing tip.

My results support Johnson and Thompson (1978) in their observations that thinning does not appear to be a cause of increased mort-

ality due to A. mellea by adding that thinning does not appear to cause a significant increase in the activity of A. mellea rhizomorphs as well. While fear of A. mellea mortality in thinning operations should not be a major constraint to this type of silvicultural practice much research still needs to be accomplished to pin-point the type of stands for which this holds true and to what levels thinning can be tolerated before A. mellea rhizomorph response causes unacceptable mortality.

Where A. mellea is suspected to be a problem, thinning, combined with any practice which would bury large quantities of wood (i.e. 'tommyhawking'), should be avoided as well as clearcutting and clear-cutting with shrub control. The recommendations of several authors (Hartig, 1874; Shaw, 1974; Roth et al, 1977; Roth and Rolph, 1978) to remove residual woody material by stump extraction should be given considerations provided the site and economics can be justified.

V. INTERNAL MYCOFLORA OF HEALTHY AND DECLINING LATERAL ROOTS OF PONDEROSA PINE ON PUMICE SOIL

Background

A large amount of information is available concerning fungi of the soil and rhizosphere, mycorrhizae of both domesticated and wild plants, and fungi of both agricultural and non-agricultural soils, however, there is almost a total lack of information available concerning the fungi of the larger lateral roots of forest trees, in general, and ponderosa pine in specific. Notable studies have been completed on hardwood stumps by Rayner (1977a, b) where he studied the varying mycofloral succession after chemical treatments to the stump surfaces. Käärik and Rennerfelt (1957) investigated rot fungi on one- to five-year old stumps of spruce and pine cut from healthy 60-120-year old trees and found a larger number of fungal species in the upper portion than in the lower portion of the stumps. Meredith (1959, 1960) studied fungi inhabiting pine stumps and concluded that:

> "...initial colonization of stump roots usually proceeds from the body of the stump...and there is often considerable delay between the time of felling and the invasion of stump roots by fungi present in the soil...."

The following study concerns the mycofloral populations in healthy and declining lateral roots of ponderosa pine. This study also reports on the interaction of these internal microfungi and *A. mellea* in culture.

Materials and methods

The study was conducted in a young pole-sized stand of ponderosa pine (study area I) (Map A) (Table 2). Fifty stumps were created in this stand by felling selected trees in four replications of ten trees each and two replications of five trees each at various dates during the summer of 1975 (Table 27). Five trees at each date containing ten trees per replication were allowed to remain in the soil, before sampling the internal mycoflora, for 53, 40, 20 and 0 days after felling. This group of trees was designated Group I, and all twenty trees were sampled on or near July 30 (mid-summer). Group II was made up of the remaining thirty trees which were left in the soil 112, 98, 77, 55, 15 and 0 days after felling. Group II trees were sampled on September 26 (late -summer).

Each set of data for the number of days in the soil is represented by five stumps, and each stump is represented by no less than two root samples taken near to the root collar. Each root sample ranged in size from 1.0 to 2.0 cm in diameter and 20 cm in length. The root samples were tagged and placed in individual polyethylene bags and kept in a refrigerator (4-7'C) until isolations were made, but no longer than 48 hours.

Before isolations were attempted the roots were scrubbed clean and rinsed in running cold tap water for one hour, dried and flame sterilized with alcohol. Isolations were made from both the cambial and outer xylem tissues. Chips, 3 x 5 mm, taken from both cambium and xylem were plated onto petri plates containing two separate media

Table 27. Dates, number of trees felled and number of trees sampled which represent varying periods the roots were left in the soil after felling.

	Date felled	Number of trees felled	Date trees sampled	Number of trees sampled	Number of days between felling and sampling
Group I	8-2-1975	10	8-2-1975	5	0
	7-11-1975	10	7-31-1975	5	20
	6-20-1975	10	7-30-1975	5	40
	6-6-1975	10	7-29-1975	5	53
Group II	9-27-1975	5	9-27-1975	5	0
	9-11-1975	5	9-26-1975	5	15
	8-2-1975	0	9-26-1975	5	55
	7-11-1975	0	9-26-1975	5	77
	6-20-1975	0	9-26-1975	5	98
	6-6-1975	0	9-26-1975	5	112
		Total = 50		Total = 50	

(Malt extract agar and potatoe dextrose agar, both supplemented with 100 ppm streptomycin sulfate and pH adjusted to 5.5 with NaOH). Two chips per sample from each region (cambium and xylem) were separately plated onto one plate of each medium. The plates were loaded into polyethylene bags and incubated in the dark at room temperature. Sub-cultures were made by hyphal tipping as colonies grew out. The original plates were kept for six months to make sure all organisms possible were isolated.

Several organisms which were isolated were chosen to be tested against A. mellea for evidence of any antagonistic association. The selected antagonists were grown in shaker cultures containing 100 ml of a nutrient medium (See Appendix A) for 16 days. At the same time the shaker cultures were started the selected isolate of A. mellea (GW-C7) which was to act as the test organism was started on Malt-pitch medium (See Appendix A). At the end of the 16-day period the shaker cultures were centrifuged at 50,000g for 30-minutes and the supernatant was drawn off with sterilized pipets, placed in sterile tubes and stored at 5'C until used.

The assay for antagonism was replicated three times using the following procedures:

1) Pour 15ml of Malt agar (Difco) into 15 x 120mm petri plates and allow to solidify.
2) Add one plate of A. mellea to an autoclaved waring blender canister.
3) Add 100ml of Malt agar (Difco) at 45'C to blender with A. mellea.

4) Run blender for 6-seconds.

5) Add 4ml of A. mellea slurry to Malt agar plates (step one) using a sterile pipet.

6) Place three assay disks (Whatman 3MM) on solid medium and immediately add 0.03ml of the selected supernatant onto each disk using a sterile pipet.

7) Incubate, in the dark at room temperature, for 7-days and then read plates for inhibition.

Results

A nearly complete list of fungi isolated and their frequencies of occurance will be found in Tables 28 and 29 (those fungi which were unknown and occured only once have been omitted). A total of 21 species, representing 15 genera, were isolated using the two different media: 13 genera from the cambial tissues and 14 genera from the xylem tissues. Of the 15 genera isolated only eight occured at frequencies which could be considered as dominant. Nine of the 15 genera were of the sub-division Deuteromycotina (Ainsworth, 1971) while three genera were from the sub-division Zygomycotina and one isolate was in the sub-division Basidiomycotina. The remining two genera were associated with bacteria and yeasts, which were not further identified.

It was anticipated at the outset the cambium and xylem tissues would display varying microfungi, if not is species diversification then perhaps in varying frequencies.

A measure of the simularity of species between two tissues can be computed using $2w / a + b$; where a is the number of species in one

Table 28. Frequencies (as percentages) and distribution of organisms observed in mid-summer 1975 (June 6 to August 2) from healthy and declining lateral roots of ponderosa pine (Designated Group I organisms).

	DAYS IN SOIL							
	0		20		40		53	
ORGANISM	C[1/]	X	C	X	C	X	C	X
Absidia spinosa Lendner	-	-	-	18	12	6	9	-
Aureobasidium pullulans (de Bary) Arnaud.	-	-	-	-	-	-	-	-
Bacteria/Yeasts	8	-	-	-	-	-	-	-
Botrytis cinerea Pers. ex Fr.	-	-	-	-	-	-	-	-
Cladosporium sp.	-	-	-	-	-	-	9	11
Graphium sp.	-	-	-	-	-	-	-	-
Mortierella sp.	-	6	9	18	19	6	4	26
Mucor sp.	-	-	-	-	-	-	-	-
Paecilomyces sp.	-	-	-	-	-	-	4	-
Penicillium sp.	-	6	-	9	12	12	14	-
Thysanophora penicilloides(Roum.)Kendrick	-	-	-	9	-	12	4	5
Trichoderma sp.	-	-	-	9	-	6	-	-
Tyromyces subcartilagineus(Overh.)Dom.	13	-	-	-	-	6	18	5
Unknown I	70	-	9	-	13	-	6	-
Sterile (no fungi isolated)	9	88	82	37	44	52	32	53

[1/] C = cambial isolations
X = xylem isolations

Table 29. Frequencies (as percentages) and distribution of organisms observed during summer of 1975 (June 6 to September 26) from healthy and declining lateral roots of ponderosa pine (Designated Group II organisms).

ORGANISM	DAYS IN SOIL: 0		15		55		77		98		112	
	C[1]	X	C	X	C	X	C	X	C	X	C	X
Absidia spinosa Lendner	-	-	-	-	-	-	-	-	-	-	-	-
Aureobasidium pullulans (de Bary) Arnaud.	6	-	-	5	4	5	-	-	6	-	3	-
Bacteria/Yeasts	3	8	19	20	-	5	13	16	21	12	28	30
Botrytis cinerea Pers. ex Fr.	-	4	-	-	-	-	-	-	-	-	-	-
Cladosporium sp.	-	-	9	10	4	-	9	5	-	-	-	-
Graphium sp.	-	-	-	-	4	-	-	-	6	-	-	-
Mortierella sp.	12	4	5	-	14	10	-	5	-	17	17	-
Mucor sp.	-	-	-	-	-	-	-	-	-	-	3	9
Paecilomyces sp.	-	-	-	-	-	5	-	-	-	-	3	-
Penicillium sp.	21	4	23	20	7	-	30	26	18	-	7	22
Thysanophora penicilloides (Roum.) Kendrick	-	-	-	-	4	-	-	-	3	12	10	17
Trichoderma sp.	-	-	-	-	-	-	-	-	-	-	-	-
Tyromyces subcartilagineus (Overh.) Dom.	3	-	27	-	15	5	4	-	12	4	3	9
Unknown I	41	19	14	-	44	-	26	5	24	4	21	4
Sterile (no fungi isolated)	14	61	3	45	3	70	18	43	10	51	5	9

[1] C = cambial isolations
X = xylem isolations

population, b is the number of species in the other population, and w is the number of species found in both populations (Christensen et al, 1962). This formula calculates a 'coefficient of simularity' where the least similar populations are indicated by a low coefficient (i.e. zero indicates no species in common and 1 indicates all species in common).

The 'coefficient of simularity' was calculated comparing Group I cambium and xylem microfungi and Group II cambium and xylem microfungi (Table 30). The least similar populations were existing in mid-summer

Table 30. Coefficients of simularity comparing cambium and xylem isolations for both Groups I and II by number of days roots were in the soil after felling of the trees.

Group	Number of days roots in soil	Coefficient of simularity[1]
I	0	0.0
I	20	0.28
I	40	0.60
I	53	0.67
II	0	0.73
II	15	0.60
II	55	0.46
II	77	0.80
II	98	0.66
II	112	0.80

[1] See text pages 92 and 95 for explanation.

(Group I) when the lateral roots were first opened to colonization (trees were felled) and gradually progressed towards a more uniform distribution of populations as time in the soil increased. Group II isolations (representing a longer time span and isolated in late summer) indicated a much more uniform distribution of microfungal populations

through time than was observed in Group I.

The behavior of a couple of the isolated organisms is of interest. The imperfect microfungus identified as Unknown I was observed regularly in the cambial isolations in both Group I and II, being denser in the former. In the xylem isolates Unknown I was not isolated in Group I but was isolated frequently in Group II, suggesting that this unknown fungus might migrate between the cambium and xylem as the season changes. Aureobasidium pullulans (de Bary) Arnaud. was not isolated in Group I but was common in Group II cambium and xylem, being denser in the cambium. Bacteria were evenly distributed between the cambium and xylem tissues, and in general, the densities increased with increasing time the roots were in the soil. Absidia spinosa Lendner was not isolated in late summer (Group II) but was common in mid-summer (Group I). Tyromyces subcartilagineus (Overh.) Dom. was isolated in both groups but was denser in late summer and concentrated in the cambial tissues.

Eight organisms were tested for antagonism against A. mellea (Table 31). Antagonism was rated as: 1) No reaction; 2) Mild reaction (inhibition zone around disk was from 0.5 to 3.5 mm); and 3) Strong reaction (inhibition zone around disk was 4.0+ mm). The distances of inhibition chosen were subjective and chosen after observation of all replications, which were averaged.

Table 31. Results of tests for antagonism to growth against one isolate of A. mellea by eight genera of microfungi isolated from healthy and declining lateral roots of ponderosa pine.

ANTAGONIST	REACTION OF A. MELLEA 1/
Absidia sp.	No reaction
Aureobasidium sp.	Strong
Graphium sp.	Mild
Mortierella sp.	No reaction
Thysanophora sp.	Mild
Trichoderma sp.	Strong
Tyromyces sp.	No reaction
Unknown I	No reaction

1/See text for explanation.

Discussion

Thirteen genera, excluding bacteria and yeasts, is relatively a small number compared to the number which may be obtained from the soils or rhizosphere of forest trees in general (Harley and Waid, 1955; Meliszewska and Moreau, 1960; Iverson and Kalznelson, 1960; Mishra and Kanaujia, 1973; Wicklow et al, 1974) or form the pumice soil in specific (Fuller, 1979, unpublished data). Even fewer organisms than the thirteen isolated can be considered important, due to the lack of consistency in occurances, or used as indicators of succession, which agrees with Merrill and French (1966).

The isolation of Absidia sp., Mortierella sp., Mucor sp., and Thysanophora sp. should not be surprising because these species are known to be common inhabitants of the soil as well as reported to invade pine blocks in the soil (Merrill and French, 1966) and cause weight loss due to decay in the stem bark of loblolly pine (Pinus

taeda L.) (Kuhlman, 1970) and in general able to decompose cellulose (Waid, 1974).

The antagonism to A. mellea growth displayed by four of the eight organisms tested indicated that, in part, they possessed some attributes which might deter A. mellea in nature. The strong inhibition to A. mellea growth caused by Trichoderma sp. is a widely observed occurance (Chanturiya and Kakuliya, 1953; Manka, 1961; Sokolov, 1964; Richard, 1971). Pentland (1965, 1967) showed that Aureobasidium pullulans produced ethanol which stimulated the growth of A. mellea rhizomorphs and mycelium in culture, however, this stimulatory effect of A. pullulans has over-shadowed her observations that in paired culture A. mellea was inhibited and that there was: "...a zone of mutual antagonism between the A. mellea mycellium and the A. pullulans colony." My research conflicts with Pentland (1965) in that she observed stimulation of A. mellea when incorporating a Millipore filtrate in which A. pullulans was grown, whereas, I observed strong inhibition when using a supernatant of the medium in which the fungus was grown. Although I cannot resolve these conflicting reports I must speculate that the differences probably lie in the different techniques involved to obtain the results.

In general, the following were found to be true: 1) cambial tissues contained more diverse and denser quantities of microfungi than the xylem tissues; 2) late summer populations were more diverse than mid-summer populations; and 3) the longer the lateral remained in the soil after felling of the tree the more diverse and denser became the microfungal populations.

The fungi which apparently are a resident population in the lateral roots of ponderosa pine are not inhibitory to A. mellea and therefore cannot be cited as the explanation why some infections are successful and other attempts at infection fail. The fungi which showed strong inhibition of A. mellea showed up in isolations in low densities and after a period of decline of the lateral roots.

This sutdy reports the presence of microfungi in the healthy and declining lateral roots of ponerosa pines in pumice soils. The microfungi varied quantitatively and qualitatively according to the time of year observed and the physiological state of the root. Before any visible signs of root decline are evident the internal mycoflora are responding to substrate decline. A difference between cambial and xylem microfungi was clearly evident. Meredith (1959, 1960) concluded that roots of stumps were colonized from above (i.e. that the cut surface was colonized first) and that root colonization from the soil was delayed several months. The data from this study suggests that colonization of roots from the soil is immediate, even prior to root decline.

A summary note

Interest in the practical question of the extent to which A. mellea can colonize dead woody material in competition with other soil fungi was aroused by Leach (1937, 1939) and further researched by Garrett (1960). Garrett (1960) showed a distinct decline in the ability of A. mellea to colonize a dead substrate after the substrate had been in the soil longer than 28 to 35 days.

Observations from this study indicate that after 112 days in the soil almost 10 % of the xylem and 5% of the cambium are still sterile and the microfungi invading the non-sterile tissues during this period are not particularily detrimental to A. mellea. These observations suggest that A. mellea is a better competitive saprophyte than previously believed and that woody substrata left in the soil are probably not immune from colonization by A. mellea for several months.

BIBLIOGRAPHY

Adams, D.H. and L.F. Roth. 1967. Demarcation lines in paired cultures of Fomes cajanderi as a basis for detecting genetically distinct mycelia. Can. J. Bot. 45: 1583-1589.

Adams, D.H. and L.F. Roth. 1969. Intraspecific competition among genotypes of Fomes cajanderi decaying young-growth Douglas-fir. For. Sci. 15: 327-331.

Adams, D.H. 1972. The relation of cover to the distribution of Armillaria mellea in a ponderosa pine forest. Ph.D. thesis, Oregon State Univeristy, 115,.

Adams, D.H. 1974. Identification of clones of Armillaria mellea in young-growth ponderosa pine. Northwest Science 48(1): 21-28.

Ainsworth, G.C. 1971. Ainsworth and Bisby's Dictionary of the Fungi. Commonwealth Mycological Inst., C.A.B., 603p.

Allermann, K. and O. Sortkjaer. 1973. Rhizomorph production in fungi: II. The effect of 12 different alcohols on growth and rhizomorph formation in Armillaria mellea and Clitocybe geotropa. Physiol. Plant 28(1): 51-55.

Anderson, A.B., R. Riffer, and A. Wong. 1969z. Mono-terpenes, fatty and resin acids of Pinus lambertiana and Pinus monticola. Phytochem. 8: 869-872.

Anderson, J.F., H.K. Kaya. 1976. Perspectives in Forest Entomology, Academic Press, 428p.

Anonomous. 1973. Forest Insect and Disease Survey: Annual Report, Dept. of the Environment, Can. For. Ser., 101p.

Baker, L.F. 1942. Inoculation of western white pine with Armillaria mellea. M.S. thesis, Univ. of Idaho, 69p.

Baker, R.C., C.L. Maurer and R.A. Maurer. 1967. Ecology of Plant Pathogens in Soil. VII. Mathematical Models and Inoculum Density. Phytopath. 57: 662-666.

Baker, R. 1971. Analyses Involving Inoculum Density of Soil-Borne Plant Pathogens in Epidemiology. Phytopath. 61: 1280-1291.

Baker, W.L. 1941. Effects of gypsy moth defoliation on certain forest trees. J. For. 39: 1017-1022.

Baranyay, J.A. and G.R. Stevenson. 1964. Mortality caused by Armillaria root rot, peridermium rusts, and other destructive agents in lodgepole pine regeneration. Forestry Chronicle 40(3): 350-361.

Barabyay, J.A., and M.G. Bauman. 1972. Distribution maps of common tree diseases in British Columbia. Can. For. Ser., Info. Report BC-X-71, 79p.

Barrett, J.W. 1968. Pruning of ponderosa pine: Effect on growth. U.S.D.A. For. Ser. Res. Paper PNW-68, 9p.

Barrett, J.W. 1970. Ponderosa pine saplings respond to control of spacing and understory vegetation. U.S.D.A. For. Ser. Res. Paper PNW-106, 16p.

Bega, R.V. and J. Tarry. 1968. Influence of pine root oleoresins on Fomes annosus. (Abst.) Phytopath. 56: 870.

Benton, B.L., and J. Ehrlich. 1941. Variation in culture of several isolates of Armillaria mellea from western white pine. Phytopath. 31: 803-811.

Bernstein, M.E., and G.C. Carroll. 1977. Internal fungi in old-growth Douglas-fir foliage. Can. J. Bot. 55(6): 644-653.

Birmingham, W.H. 1931. Armillaria root rot of fruit trees. N.S.W. Dep. Agric., Pl. Dis. Leaflet No. 18, 4p.

Bliss, D.E. 1941a. Relation of soil temperature to Armillaria root rot in California. (Abstr.) Phytopath. 31: 3.

Bliss, D.E. 1941b. Artificial inoculation of plants with Armillaria mellea. (Abstr.) Phytopath. 31: 859.

Bliss, D.E. 1946. The relation of soil temperature to the development of Armillaria root rot. Phytopath. 36: 302-318.

Bohus, G. 1947. Néhány gomba mćeliumánuk höigénue. (Heat required for the mycelium of some fungi.) Acta Mycol. Hungarica 4: 12-15, (Abstr. Bil. Abs. 23: 16276).

Boulard, B. and D. Gaudray. 1975. A pros D'une attaque de résineus Hauts-Normands par L'Armillaire Couleur de Miel (Armillariella mellea (Fr.) Karsten)... ou L'ecologie au service de la Phytopathologie. Botaniste, serie LVII: 119-151.

Bourchier, R.J. 1961. Laboratory studies in microfungi isolated from the stems of living lodgepole pine, Pinus contorta Dougl. Can. J. Bot. 39: 1373-1385.

Buckland, D.C. 1953. Observations on Armillaria mellea in immature Douglas-fir. Forestry Chronicle, 29: 433-246.

Caldwell, R. 1963. Observations on the fungal flora of decomposing beech litter in soil. Trans. Brit. Mycol. Soc. 46(2): 249-261.

Campbell, R.W. and R.J. Sloan. 1977. Forest stand responses to defoliation by the gypsy moth. For. Sci. Nomo. 19, 23: 2-34.

Carroll, F.E., E. Müller, and B.C. Sutton. 1977. Preliminary studies on the incidence of needle endophytes in some European conifers. Sydowia 29: 87-103.

Carter, C.J. 1975. Diseases of Midwest Trees. Univ. of Illinois Press, 168p.

Cartwright, K. St. G. and W.P.K. Findlay. 1958. Decay of Timber and its Prevention. 2nd. ed. Her Majesty's Stationary Office, London, 332p.

Chanturiya, N.N. and M.A. Kakuliya. 1953. Ispytanie biologicheskogo metoda bor'by s vozbuditelem kornevoi gnili shelkovitsy. (Tests of a biological method of controlling the causative agent of root rot in mulberry trees). Report of the Academy of Sciences of the Georgian Soviet Socialist Republic, No. 4, 14p.

Childs, L. and S.M. Zeller. 1929. Observations on Armillaria root rot of orchard trees. Phytopath. 19: 869-873.

Christensen, C.M. and A.C. Hodson. 1954. Artificially induced senescence of forest trees. J. of For. 52: 126-129.

Christensen, M., W.F. Whittingham, and R.O. Novak. 1962. The soil microfungi of wet-mesic forests in southern Wisconsin. Mycologia 54: 374-388.

Cobb, F.W., Jr., J.R. Parmeter, Jr., D.L. Wood, and R.W. Stark. 1974. Root pathogens as agents predisposing ponderosa pine and white fir to bark beetles. In: Proc. of the 4th. Intern. Conf. on Fomes annosus. Athens, I.U.F.R.O. Sec. 24, 8-15.

Colhoun, J. 1973. Effects of environmental factors on plant disease. Ann. Rev. Phytopath. 11: 343-364.

Cooley, J.S. 1943. Armillaria root rot of fruit trees in the eastern United States. Phytopath. 33: 812-817.

Crandall, B.S. 1950. Cinchona root and collar rot in Peru and Bolivia. U.S.D.A. Cir. No. 855, 16p.

Crist, C.R. and D.F. Schoeneweiss. 1975. The influence of controlled stresses on susceptibility of European white birch stems to attack by Botryosphaeria dothidea. Phytopath. 65: 369-373.

Day, W.R. 1927. The parasitism of Armillaria mellea in relation to conifers. Quart. J. For. 21: 9-21.

Day, W.R. 1929. Environment and Disease: A discussion on the parasitism of Armillaria mellea (Vahl.) Fr. Forestry 3: 94-103.

Delatour, C. 1976. Microflore interne des tissus ligneus de l' épicéa commun sur pied. Ann. Sci. Forest. 33(4): 199-219.

Dickinson, S. 1960. Plant Pathology (Ed. by Horsfall and Dimond). Academic Press, New York. (2) 217p.

Doty, J.E. and P.C. Cheo. 1974. Light inhibition of thallus growth of Armillaria mellea. Phytopath. 64(5): 736-764.

Drew, J. and G.D. Pylant, Jr. 1966. Terpentine from the pulpwoods of the United States and Canada. TAPPI 49(10): 430-438.

Dunbar, D.M. and G.R. Stephens. 1975. Association of two-lined chestnut borer and shoestring fungus with mortality of defoliated oak in Connecticut. For. Sci. 21: 169-174.

Dyrness, C.T. and C.T. Youngberg. 1966. Soil-vegetation relationships within the ponderosa pine type in the central Oregon pumice region. Ecology 47(1): 122-138.

Ehrlich, J. 1939. A preliminary study of root diseases in western white pine. U.S.D.A. North. Rocky Mnt. For. and Rng. Exp. Sta. Paper No. 1, 10p.

Esser, K. and R. Kuenen. 1967. Genetics of Fungi. (Trans. by E. Steiner), Springer-Verlag, Berlin, 499p.

Etheridge, D.E. 1956. Occurrence of Coryne sarcoides with heartrot fungi on spruce in Alberta, Canada. (Abst.) Brit. Mycol. Soc., Trans. 39(3): 385-386.

Etheridge, D.E. 1961. Factors affecting branch infection in aspen. Can. J. Bot. 39(4): 799-816.

Etheridge, D.E. and L.A. Morin. 1967. The microbial condition of wood of living Balsam fir and Black spruce in Quebec. Can. J. Bot. 45: 1003-1010.

Filip, G.M. 1976. Chemical applications for control of Armillaria root rot of ponderosa pine. Ph.D. thesis, Oregon State Univ., 83p.

Filip, G.M. 1977. Evaluation of timber loss due to Armillaria root rot and suggested management alternatives to minimize future loss on the Klamath Ranger Distric, Winema National Forest, Oregon. U.S. For. Ser., Region-6, 6p.

Filip, G.M. and L.F. Roth. 1977. Stump injections with soil fumigants to eradicate Armillariella mellea from young-growth pine killed by root rot. Can. J. Bot. 7(2): 226-231.

Filip, B.M. 1979. Location, extent, and cause of tree mortality in a mixed conifer forest on the Kettle Falls Ranger District, Colville National Forest, Washington. U.S. For. Ser., Region-6, 16p.

Fox, R.A. 1964. A report on a visit to Nigeria undertaken to make a preliminary study of root diseases of rubber. Research Archives of the Rubber Institute of Malaya, Doc. 27, 34p.

Franklin, J.F. and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. Pacific Northwest Forest and Range Experimental Sta. U.S.D.A. For. Ser., General Tech. Report PNW-8, 417p.

Freese, F. 1967. Elementary statistical methods for foresters. Agr. Handbook 317, 87p.

Gard, M. 1929. Pourridié et carbonate de chaux. Comp. rend. Acad. Sci., Paris, 189: 497-498.

Garraway, M.O. 1969. Influence of compounds related to the shikimic acid pathway on rhizomorph initiation and growth in Armillaria mellea. Phytopath. 59: 1027(Abstr.).

Garraway, M.O. 1970. Rhizomorph initiation and growth in Armillaria mellea promoted by o-aminobenzoic and p-aminobenzoic acids. Phytopath. 60(5): 861-865.

Garrett, S.D. 1956. Rhizomorph behavior in Armillaria mellea (Vahl.) Quèl. II. Logistics of infection. Ann. Bot. 20(78): 193-209.

Garrett, S.D. 1960. Rhizomorph behavior in Armillaria mellea (Fr.) Quèl. III. Saprophytic colonization of woody substrates in soil. Ann. Bot. 24: 275-285.

Gaumann, E. 1950. Principles of Plant Infection. 44 (Crosby Lockwood, London).

Gibbs, J.N. 1967. The role of host vigour in the susceptibility of pines to Fomes annosus. Ann. Bot. 31(124): 803-815.

Gibbs, J.N. and B.J.W. Greig. 1970. Fomes annosus: A fungus causing butt rot, root rot and death of conifers. Forestry Comm. Leaflet No. 5, 10p.

Gibson, I.A.S. 1961. A note on variation between isolates of Armillaria mellea (Vahl. ex Fr.) Kummer. Trans. Brit. Myc. Soc. 44: 123-128.

Goheen, D.J., F.W. Cobb, and G.N. McKibbin. 1978. Influence of soil moisture on infection of ponderosa pine by Verticicladiella wagenerii. Phytopath. 68: 913-916.

Good, H.M. and J.I. Nelson. 1962. Fungi associated with Fomes igniarius var. populinus in living poplar trees and their probable significance in decay. Can. J. Bot. 40(4): 615-624.

Gordon, D.T. 1959. Ten-year observations on pruned ponderosa and Jeffrey pine. U.S.D.A. For. Ser., PSW Res. Note 153, 4p.

Greig, B.J.W. 1962. Fomes annosus (Fr.) Cke. and other root rotting fungi in conifers on ex-hardwood sites. Forestry 32(2): 164-182.

Guillaumin, J.J. and K. Rykowski. 1978. Etudé de l'infection du noyer par les rhizomorphes de l'Armillaria mellea (Vahl.) Quèl. dans le modele d'experiance controlee. Folia Forestalia Polonica (in press).

Guyot, R. 1927. Mycelium lumineux de L'Armillaire. Compt. rend Soc. Biol. 46: 114-116.

Hallin, W.E. 2956. Pruning ponderosa and Jeffrey pine. U.S.D.A. For. Ser. PSW Res. Note 115, 4p.

Hartig, R. 1873. Vorläufige Mittheilung über den Parasitismus von Agaricus melleus und dessen Rhizomorphen. Bot. Ztg. 31: 269-297.

Hartig, R. 1874. Wichtige Krankheiten der Waldbäume. Beiträge zur Mykologie und Phytopathologie für Botaniker und Forstmanner. Berlin, J. Springer, 124p.

Hartig, R. 1894a. Text-book of the Diseases of Trees. Transl. by W. Summerville. (Rev. and Ed. by J.M. Ward), 331p.

Hartig, R. 1894b. Die Ausschlagfähigkeit der Eichenstöcke und deren Infection durch Agaricus melleus. Forstl. Naturw. Ztschr. 3: 428-432.

Harley, J.L. and J.S. Waid. 1955. The effect of light upon the roots of beech and its surface population. Plant and Soil 7(1): 96-112.

Heagle, A.S. 1973. Interactions between air pollutants and plant parasites. Ann. Rev. Phytopath. 11: 365-388.

Hendrickson, A.J. 1925. Oak fungus in orchard trees. Univ. Calif., Col. of Agri., Agri. Exp. Sta., Berkeley, Calif., Circular No. 289, 13p.

Hetrick, L.Z. 1949. Some overlooked relationships of southern pine beetle. J. Econ. Ent. 42: 466-469.

Hintikka, V. 1970. Selective effect of terpenes on wood decomposing hymenomycetes. Karstenia 11: 28-32.

Hintikka, V. 1973. A note on the polarity of Armillariella mellea. Karstenia 13: 32-39.

Hintikka, V. 1974. Notes on the ecology of Armillariella mellea in Finland. Karstenia 14: 12-31.

Hudak, J. and P. Singh. 1970. Incidence of Armillaria root rot in balsam fir infected with balsam woolly aphid. Can. Pl. Dis. Survey 50: 99-101.

Huntly, J.H., J.D. Cafley, and E. Jorgensen. 1961. Armillaria root rot in Ontario. Forestry Chronicle 37(3): 228-236.

Iverson, K.C. and J. Kalznelson. 1960. Studies on the rhizosphere microflora of yellow birch seedlings. Plant and Soil 13: 30-40.

Johnson, D.W. and J.H. Thompson. 1975. Effect of pre-commercial thinning on ponderosa pine, Pinus ponderosa, infected with Armillaria mellea. Pl. Dis. Rpt. 59(4): 308-309.

Johnson, D.W. 1976. Incidence of diseases in national forest plantations in the Pacific Northwest. Pl. Dis. Rpt. 60: 883-885.

Jorgensen, E. 1961. The formation of pinosylvin and its monomethyl ether in the sapwood of Pinus radiata. Can. J. Bot. 39: 1765-1772.

Käärik, A. and E. Rennerfelt. 1957. Investigations on the fungal flora of spruce and pine stumps. Meddelanden Från Statens Skogsforskningsenstitut, Band 47(7): 1-7.

Kable, P.F. 1974. Spread of Armillariella sp. in a peach orchard. Trans. Bri. Mycol. Soc. 62(1): 89-98.

Kegg, J.D. 1973. Oak mortality caused by repeated gypsy moth defoliations in New Jersey. J. Econ. Ent. 66: 639-641.

Kendrick, W.D. and A. Burges. 1962. Biological aspects of the decay of Pinus sylverstris litter. Nova Hedwigia 4: 313-344.

Kniep, H. 1911. Über das Auftreten von Basidien im ein kernigen Mycel von Armillaria mellea. Autschr. Bot. 3: 529-553.

Kniep, J. 1920. Über Morphologische und Physiologische Geschlechtsdifferenzierung. Verh. Phys.-med. Ges., N.F. 46: 1-18.

Kniep, H. 1922. Über Geschlechtsbestimmung und Reduktion-steilung. Verh. Phys.-med. Ges., N.F. 47: 1-28.

Korhonen, K. and V. Hintikka. 1974. Cytological evidence for somatic diploidization in dikaryotic cells of Armillariella mellea. Arch. Microbiol. 95: 187-192.

Kuhlman, E.G. 1970. Decomposition of loblolly pine bark by soil- and root-inhabiting fungi. Can. J. Bot. 48: 1787-1793.

Laemmlen, F. and R.V. Bega. 1974. Hosts of Armillaria mellea in Hawaii. Pl. Dis. Rpt. 58: 102-103.

Lawrence, W.J. 1910. Root diseases caused by Armillaria mellea in the Puget Sound country. State College of Wash., Ag. Exp. Sta. Bull. No. 3, 16p.

Leach, R. 1937. Observations on the parasitism and control of Armillaria mellea. Proc. Roy. Soc., B, 121: 561-573.

Leach, R. 1939. Biological control and ecology of Armillaria mellea (Vahl.) Fr. Trans. Br. Mycol. Soc. 23: 320-329.

Leaphart, C.D. 1963. Armillaria root rot. U.S.D.A. For. Pest Lft. 78, 8p.

Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press, Hew York, 697p.

Long, W.J. 1914. The death of chestnuts and oaks due to Armillaria mellea. U.S.D.A. Bur. Pl. Industry Bull. No. 89, 9p.

MacKenzie, M. and C.G. Shaw III. 1977. Spatial relationships between Armillaria root-rot of Pinus radiata seedlings and the stumps of indigenous trees. New Zealand J. For. Sci. 7(3): 374-383.

Manka, K. 1961. Rozwaj opienki miodowej (Armillaria mellea (Vahl.) Quèl.) na tle bioycznech wlasciwosci mikoflory korzeniowej swierka (Picea excelsa L.). (The development of A. mellea on the basis of the biotic properties of Norway spruce (P. excelsa) root microflora.) Roczn. Wyzsz. Szkol. Rol. (Poznan), 10: 21-74, (Abstracted in Rev. Applied Mycology 41: 342. 1962).

Meliszewska, W. and R. Moreau. 1960. A study of fungal mycoflora in the rhizosphere of fir (Abies alba Mill.), In: The ecology of soil fungi (Ed. by Parkinson, D. and J.S. Waid), Liverpool Press, 209-220.

Meredith, D.S. 1959. The infection of pine stumps by Fomes annosus and other fungi. Ann. of Bot. 23(91): 455-476.

Meredith, D.S. 1960. Further observation on fungi inhabiting pine stumps. Ann. of Bot. 24: 63-78.

Merrill, W. and D.W. French. 1966. Colonization of wood by soil fungi. Phytopath. 56: 301-303.

Meyer, W.H. 1961. Yield of even-aged stands of ponderoas pine. U.S.D.A. Tech. Bull. 630 (rev.), 59p.

Millar, C.S. 1974. Decomposition of coniferous leaf litter. In: Biology of plant litter decomposition, Vol. 1, (Ed. by C.H. Dickinson and D.J.F. Pugh), Academic Press, N.Y., 105-128.

Miller, J.M. and F.P. Keen. 1960. Biology and control of the Western Pine Beetle - A summary of the first fifty years of research. U.S.D.A. Misc. Pub. 800, 381p.

Miller, P.R., J.R. Parmeter, O.C. Taylor, and E.A. Cardiff. 1963. Ozone injury to the foliage of Pinus ponderosa. Phytopath. 53: 1072-1076.

Miller, P.L. 1973. Oxidant-induced community change in a mixed conifer forest. Advances in Chemistry Series No. 122: 101-117.

Miller, R.S., et al. 1975. Pest Control: An assessment of present and alternative technologies. Vol IV - Forest Pest Control, Nat. Acad. of Sci., 170p.

Miller, R. and A. Kelman. 1966. Growth of Fomes annosus in roots of suppressed and dominant loblolly pines. For. Sci. 12(2): 225-233.

Mirov, N.T. 1961. Composition of gum turpentines of pines. U.S.D.A. Tech. Bull. 1239.

Mirov, N.T. 1967. The genus Pinus. The Ronald Press, N.Y., 486p.

Mishra, R.R. and R.S. Kanaujia. 1973. Investigations into rhizosphere mycoflora: XII. Seasonal variation in the mycoflora of certain gymnosperms. Sydowia 27: 302-311.

Moddy, A.R. 1972a. Fatty acids and naturally occuring plant lipids as stimulants of rhizomorph production in Armillaria mellea. Phytopath. 62: 264-267.

Moddy, A.R. 1972b. Stimulation of rhizomorph production by Armillaria mellea with lipids from tree roots. Phytopath. 62(11): 1347-1350.

Morrison, D.J. 1972. Studies on the biology of Armillaria mellea. Ph.D. thesis, University of Cambridge, 168p.

Morrison, D.J. 1976a. Vertical distribution of Armillaria mellea rhizomorphs in soil. Trans. Brit. Myc. Soc. 66(3): 393-399.

Morrison, D.J. 1976b. Armillaria root rot. For. Pest Leaf. No. 35, Canada, 5p.

Munnecke, D.E., W. Wilbur, and E.F. Darley. 1976. Effect of heating or drying on Armillaria mellea or Trichoderma viride and the relation to survival of A. mellea in soil. Phytopath. 66: 1363-1368.

Nelson, E.E. and F.M. Harvey. 1974. Diseases, In: Environmental effects of forest residues management in the Pacific Northwest. U.S. For. Ser., PNW F.P.R.E.S. S-1-11.

Ono, K. 1965. Armillaria root rot in plantations of Hokkaido. Effects of topography and soil conditions on its occurance. Bull. of the Bov. For. Exp. Sta., Meguro No. 179, 62p.

Ono, K. 1970. Effect of soil conditions on the occurrence of Armillaria root rot of Japanese larch. Bull. of the Bov. For. Exp. Sta., Japan, No. 229: 123-228.

Parker, J. and D.R. Houston. 1971. Effects of repeated defoliation on root and root collar extractives of sugar maple trees. For. Sci. 17(1): 91-95.

Parmeter, J.R., R.B. Bega, and T.Neff. 1962. A chlorotic decline of ponderosa pine in southern California. Pl. Dis. Rpt. 46: 269-273.

Patton, R.F. and A.J. Riker. 1959. Artificial inoculations of pine and spruce trees with Armillaria mellea. Phytopath. 49: 615-622.

Patton, R.F. and R.B. Bravo. 1967. Armillaria root rot, Armillaria mellea (Vahl. ex Fr.)Kummer. In: Important forest insects and diseases of mutual concern to Canada, the United States and Mexico (Ed. by A.G. Davidson and R.M. Prentice, Ottawa, pg. 37-38).

Pawsey, R.G. and A. Rahman. 1976. Field trials with Armillatox against Armillariella mellea. PANS 22(1): 49-59.

Peace, R.T. 1962. Pathology of Trees and Shrubs. Oxford at the Clarendon Press, 753p.

Pegler, D.N. and I.A.S. Gibson. 1972. CMI Descriptions of pathogenic fungi and bacteria No. 321 - Armillariella mellea.

Pentland, G.D. 1965. Stimulation of rhizomorph development of Armillaria mellea by Aureobasidium pullulans in artificial culture. Can. J. Micro. 11: 345.

Pentland, G.D. 1967. Ethanol produced by Aureobasidium pullulans and its effect on the growth of Armillaria mellea. Can. J. Micro. 13: 1631-1639.

Pielou, E.C. 1965. The spread of desease in patchily-infected forest stands. For. Sci. 11: 18-26.

Plakidas, A.G. 1941. Infection with pure cultures of Clitocybe tabescens. Phytopath. 31: 93-95.

Pugh, H.J.F. and N.G. Buckley. 1971. Aureobasidium pullulans: An endophyte in sycamore and other trees. Trans. Brit. Mycol. Soc. 57: 227-231.

Raabe, R.D. 1962. Host lists of the root rot fungus Armillaria mellea. Hilgardia 33: 25-88.

Raabe, R.D. 1966. Variation of Armillaria mellea in culture. Phytopath. 56(11): 1241-1244.

Raabe, R.D. 1967. Variation in pathogenicity and virulence in Armillaria mellea. Phytopath. 57(1): 73-75.

Raabe, R.D. 1969. Cultural variations of Armillaria mellea not related to pathogenicity and virulence. Proc. 1st. Inter. Citrus Sympos., Vol. 3: 1263-1272.

Raabe, R.D. 1972. Variation in pathogenicity and virulence in single-spore isolates of Armillaria mellea. Mycologia 64: 1155-1159.

Rackham, R.L., W.D. Wilbur, M.P. Miller, and A.O. Paulus. 1966. Control of oak root fungus in citrus. Calif. Pl. Dis. - 26. Univer. Calif. Agr. Ext. Ser., Berkeley, 2p.

Rankin, W.H. 1918. Manual of Tree Diseases. The MacMillan Company, New York, 398p.

Raper, J.R. 1966. Genetics of sexuality in higher fungi. N.Y., 283p.

Rayner, M.C. 1930. Observations on Armillaria mellea in pure culture with certain conifers. Forestry 4: 65-77.

Rayner, R.W. 1948. Latent infection in Coffea arabica L. Nature (London), 161: 245-246.

Rayner, A.D.M. 1977a. Fungal colonization of hardwood stumps from natural sources - I. Non-basidiomycetes. Trans. Br. Mycol. Soc. 69(2): 291-302.

Rayner, A.D.M. 1977b. Fungal colonization of hardwood stumps from natural sources - II. Basidiomycetes. Trans. Br. Mycol. Soc. 69(2): 303-312.

Redfern, D.B. 1966. Root infection by Armillaria mellea. Ph.D. thesis, Univ. of Cambridge, 148p.

Redfern, D.B. 1968. The ecology of Armillaria mellea in Britain biological control. Ann. Bot. 32: 293-300.

Redfern, D.B. 1970. The ecology of Armillaria mellea: rhizomorph growth through soil. In: Root diseases and soil-borne pathogens. Vol. I. (Ed. by T.A. Tossous et al), Univ. Calif. Press., 147-149.

Redfern, D.B. 1973. Growth and behavior of Armillaria mellea rhizomorphs in the soil. Trans. Br. Mycol. Soc. 61(3): 569-581.

Redfern, D.B. 1975. The influence of food base on rhizomorph growth and pathogenicity of Armillaria mellea isolates, In: Biology and control of soil-borne pathogens (Ed. by G.W. Bruehl), The American Phytopathological Society: 69-73.

Reitsma, J. 1932. Studien über Armillaria mellea (Vahl.) Quel. Phytopath. Z. 4: 461-522.

Rhoads, A.S. 1956. The occurrence and destructiveness of Clitocybe root rot of woody plants in Florida. Lloydia 19(4): 193-240.

Ricard, J. 1970. Biological control of Fomes annosus in Norway spruce (Pinea abies) with immunizing commensals. Studia forestalia succia 84, 50p.

Richard, C. 1971. Sur l'activite antibiotique de l'Armillaria mellea. Can. J. Micro. Biol. 17: 1395-1399.

Riley, C.G. 1952. Fomes igniarius decay of poplar. Can. J. Bot. 30: 710-734.

Rishbeth, H. 1964. Stump infection by basidiospores of Armillaria mellea. Trans. Brit. Mycol. Soc. 47: 460.

Rishbeth, J. 1970. The role of basidiospores in stump infection by Armillaria mellea. In: Root diseases and soil-borne pathogens (Ed. T.A. Toussoun, R.B. Bega, and P.E. Nelson), Berkeley, Univ. of Calif. Press, pg. 141-146.

Rishbeth, J. 1972. The production of rhizomorphs by Armillaria mellea from stumps. Eur. J. For. Path. 2(4): 193-204.

Rishbeth, J. 1978. Effects of soil temperature and atmosphere on growth of Armillaria rhizomorphs. Trans. Bri. Mycol. Soc. 70(2): 213-220.

Roll-Hansen, F. 1962. Nectria cucurbitula sensu Wollenweber, its Cephalosporium state and some other Cephalosporium spp. from stems of conifers. Meddelelser fra Det Norske Skorforsoksvesen 27(3): 293-312.

Roth, L.F., C.G. Shaw III, and L. Rolph. 1977. Marking ponderosa pine to combine commercial thinning and control of Armillaria root rot. J. For. 75(10): 644-647.

Roth, L.F. and L. Rolph. 1978. Marking guides to reduce Armillaria root rot in ponderosa pine are effective. For. Sci. 24(4): 451 454.

Rowe, J.W. and C.L. Bower. 1965. Triterpenes of pine barks: Naturally occurring derivatives of Serratenediol. Tetrahedron Leter 32: 2745-2750.

Rudinsky, J.A. 1962. Ecology of Scolytidae. Ann. Rev. Ent. 7: 327-348.

Russell, P. 1956. A selective medium for the isolation of basidiomycetes. Nature 177: 1038-1039.

Rykowski, P.K. 1975. Modalité d'infection des pins sylverstres par L'Armillariella mellea (Vahl.) Karst. dans les cultures forèstieres. Eur. J. For. Path. 5: 65-82.

Rykowski, P.K. 1976. Recherche sur la nutrition azotée de plusieurs souches de L'Armillaria mellea - II. L'influence de différentes concentrations du carbone et de l'azote (C:N). Eur. J. For. Path. 6: 264-274.

Rykowski, K. 1978. Infection biology of Armillaria mellea (Vahl.) Karst. In: Proc. V Inter. Conf. on root and butt rot in conifers. IUFRO, Kassel, Germany, pg. 215-233.

Schoeneweiss, D.F. 1975. Predisposition, stress, and plant disease. Ann. Rev. Phytopath. 13: 193-211.

Schütte, K.H. 1955. Translocation in the fungi. New Phytologist 55: 164-182.

Shain, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by Fomes annosus. Phytopath. 57: 1034-1045.

Shain, L. 1971. The response of sapwood of Norway spruce to infection by Fomes annosus. Phytopath. 61(3): 301-307.

Shaw, C.G. 1973. Host fungus index for the Pacific Northwest - II. Fungi. Wash. Agr. Exp. Sta. Bull. No. 766, 162p.

Shaw, C.G., III. 1974. Epidemiological insights into Armillaria mellea root rot in a managed ponderosa pine forest. Ph.D. thesis, Oregon State University, 201p.

Shaw, C.G., III. 1976. Armillaria root rot. What's new in forest research. For. Res. Instit. N.Z. No. 36, 4p.

Shaw, C.G., III. 1977. Armillaria isolates from pine and hardwoods differ in pathogenicity to pine seedlings. Pl. Dis. Rpt. 61: 416-418.

Shaw, C.G., III and E.J.A. Toes. 1977. Impact of Dothistroma needle blight and Armillaria root rot on diameter growth of Pinus radiata. Phytopath. 67: 1319-1323.

Shigo, A.L. 1963. Fungi associated with the discolorations around rot columns caused by Fomes igniarius. Pl. Dis. Rpt. 47(9): 820-823.

Shigo, A.L. 1971. Wound dressing research on red maples. Inter. Shade Tree Conf., Proc. 47: 97a-98a.

Shigo, A.L. and C.L. Wilson. 1971. Are tree wound dressings beneficial? Arborist's...News. 36:8.

Shigo, A.L. 1972. Successions of microorganisms and patterns of discoloration and decay after wounding in red oak and white oak. Phytopath. 62: 256-259.

Singh, P. 1970. Armillaria root rot in Newfoundland conifer plantations. Bi-mon. Res. Notes, Dept. of Fish and For., Ottawa 26(1): 5-6.

Singh, P. and J. Richardson. 1973. Armillaria root rot in seeded and planted areas in Newfoundland. The For. Chronicle, August. 49: 180-182.

Singh, P. 1975. Armillaria root rot: Distribution and severity in softwood plantations in Newfoundland. Acta Phytopath. Acad. Scie. Hungaricae 10(3-4): 389-406.

Singh, P. 1977. Growth and distribution of rhizomorphs of Armillaria mellea in Newfoundland forest soils. (Abstr.) In: Proc. of the Amer. Phytopath. Soc. 4: 89.

Smith, D.M 1962. The Practice of Silviculture. John Wiley and Sons, Inc. 578p.

Smith, A.M. and D.M. Griffin. 1971. Oxygen and the ecology of Armillaria elegans Heim. Australian J. Soil Sci. 24: 231-262.

Snedecor, G.W. and W.E. Cochran. 1973. Statistical Methods. The Iowa State University Press, Sixth Ed., 593p.

Sokolov, D.V. 1964. Root rot caused by Armillaria mellea and its control. English translation No. 37 by Can. Dept. of For.

Sortkjaer, O. and K. Allermann. 1972. Rhizomorph formation in fungi I: Stimulation by ethanol and acetate and inhibition by disulfiram of growth and rhizomorph formation in Armillaria mellea. Physiol. Plant. 26: 376-380.

Sortkjaer, O. and K. Allermann. 1973. Rhizomorph formation in fungi III: The effect of ethanol on the synthesis of DNA and RNA and uptake of asparagine and phosphate in Armillaria mellea. Physiol. Plant. 29: 129-133.

Staley, J.M. 1965. Decline and mortality of red and scarlet oaks. For. Sci. 11: 2-17.

Stark, R.W., P.R. Miller, F.W. Cobb, Jr., D.L. Wood, and J.R. Parmeter, Jr. 1968. Photochemical oxidnat injury and bark beetle (Coleoptera: Scolytidae) infestation in ponderosa pine. I: Incidence of bark beetle infestation in injured trees. Hilgardia 39: 121-126.

Stark, R.W. and F.W. Cobb, Jr. 1969. Smog injury, root diseases and bark beetle damage in ponderosa pine. Calif. Agr. 23(9): 13-15.

Strand, M.A. and L.F. Roth. 1976. Spread and intensification of western dwarf mistletoe in thinned ponderosa pine saplings as examined by a simualtion model. Phytopath. 66(7): 888-895.

Swift, M.J. 1965. Loss of suberin from bark tissue rotted by Armillaria mellea. Nature 207: 436-437.

Swift, M.J. 1968. Inhibition of rhizomorph development by Armillaria mellea in Rhodesian forest soils. Trans. Brit. Myc. Soc. 51: 241-247.

Swift, M.J. 1972. The ecology of Armillaria mellea Vahl. (ex Fr.) in the indigenous and exotic woodlands of Rhodesia. Forestry 45: 57-86.

Thomas, G.M. and K.H. Wright. 1961. Silver fir beetles. U.S. Dept. Agr. For. Pest Leaf. No. 60, 7p.

Thomas, H.E. 1934. Studies on Armillaria mellea (Vahl.) Quél., infection, parasitism, and host resistance. J. Agr. Res. 48(3): 187-218.

Thornberry, J.J. and B.R. Ray. 1953. Wilt-inducing protein-like pigment from Armillaria mellea (Vahl.) Quél. isolated from peach roots. Phytopath. 43: 486 (Abstr.).

Tommerup, I.C. and D. Broadbent. 1975. Nuclear fusion, meiosis and the origin of dikaryotic hyphae in Armillariella mellea. Arch. Microbiol. 103: 279-282.

Towers, B. and W.J. Stambaugh. 1968. The influence of induced soil moisture stress upon Fomes annosus root rot of loblolly pine. Phytopath. 58: 269-272.

Townsend, B.B. 1954. Morphology and development of fungal rhizomorphs. Trans. Brit. Mycol. Soc. 37: 222-233.

Ullrich, R.C. and J.B. Anderson. 1977. A sexual cycle in Armillaria mellea. (Abstr.) Proc. of the Amer. Phyto. Soc. 4: 133.

Väisälä, L. 1974. Effects of terpene compounds on the growth of wood-decomposing fungi. Ann. Bot. Fennici 11: 275-278.

Van Vloten, H. 1936. Onderzoekingen over Armillaria mellea (Vahl.) Quél. fungus. Vageningen 8: 20-23.

Verrall, A.F. 1938. The probable mechanism of the protective action of resin in fire wounds on red pine. J. For. 36: 1231-1233.

Waid, J.S. 1974. Decomposition of roots. In: Biology of plant litter decomposition (Ed. C.J. Dickinson, and G.J.F. Pugh) Vol. I. Academic Press, London, pg. 175-211.

Wallis, G.W. 1961. Infection of Scots pine roots by Fomes annosus. Can. J. Bot. 39: 109-121.

Wargo, P.M. 1972. Defoliation-induced chemical changes in sugar maple roots stimulate growth of Armillaria mellea. Phytopath. 62(11): 1278-1283.

Wargo, P.M. and D.R. Houston. 1974. Infection of defoliated sugar maple trees by Armillaria mellea. Phytopath. 64(6): 817-822.

Warren, G.L. and P. Singh. 1970. Hylobius weevils and Armillaria root rot in a coniferous plantation in Newfoundland. Bi-monthly Res. Notes 26: 55.

Weinhold, A.R. 1963. Rhizomorph production by Armillaria mellea induced by ethanol and related compounds. Science 142: 1065-1066.

Wichmann, H.E. 1953. Rindenbrüter und Hallimasch. Forstwiss. Cbl. 72: 57-63. For. Abstr. 14, No. 2498.

Wicklow, M.C., W.B. Bollen and W.C. Denison. 1974. Comparisons of soil microfungi in 40-year-old stands of pure alder, pure conifer, and alder-conifer mixtures. Soil Biol. Biochem. 6: 73-78.

Wilbur, W., D.E. Mannecke, and E.F. Darley. 1972. Seasonal development of Armillaria root rot of peach as influenced by fungal isolates. Phytopath. 62: 567-570.

Williams, H. 1942. The geology of Crater Lake National Park. Carnegie Institute, Washington, Pub. No. 540, 162p.

Wood, R.K.S. 1967. Physiological Plant Pathology. Botanical Monographs. Vol. 6. Blackwell Scientific Pub., Oxford, 570p.

Wortendyke, J. 1972. A biological evaluation of an Armillaria mellea-infected Douglas-fir plantation. U.S.D.A. For. Ser., Region-6, inter-office report, 9p.

Yarwood, C.E. 1959. Predisposition. In: Plant pathology, (Ed. by J.G. Horsfall and A.E. Dimond), Academic Press, New York, 674p.

Zeller, S.M. 1917. Studies in the physiology of the fungi. III. Physical properties of wood in relation to decay by Lenzites saepiaria Fr. Ann. Missouri Bot. Garden 4: 93-164.

APPENDICES

APPENDIX A

MEDIA USED DURING STUDIES

MEDIA USED DURING STUDIES

The following formulae apply when the specified media were used.

1) Potatoe Dextrose Agar (Difco)

39 gr. P.D.A. (Difco)
1000 ml Distilled water

2) Malt Agar IDifco)

45 gr. Malt Agar (Difco)
1000 ml Distilled water

3) Ortho-phenylphenol Malt Extract Agar (Modified, Russell, 1956)

30 gr. Malt Extract
20 gr. Dextrose
5 gr. Bacto-Peptone
19 gr. Agar
984 ml Distilled water

Mix the above and autoclave at 1.05 Kg/cm^2 for 20 minutes.

Cool to 50'C

Add 0.1g Streptomycin sulfate (Dissolved in 10 ml of sterile distilled water).

Add 6 ml OPP solution*.

*Make OPP solution by adding 1 gr. O-phenylphenol salt to 99 ml of 95% ethanol and mix until dissolved. Store in dark.

4) Pitch-Malt Agar

30 gr. Malt Extract
20 gr. Dextrose
5 gr. Peptone
19 gr. Agar
20 gr. Crude pine pitch
1000 ml Distilled water

Mix and place in steamer for 1-hour.

Filter thrqugh double-thickness cheese cloth and autoclave at 1.05 Kg/cm^2 for 20 minutes.

5) Nutrient medium for shaker cultures

30 gr. Malt Extract
20 gr. Dextrose
5 gr. Bacto-Peptone
1000 ml Distilled water

APPENDIX B

DETERMINATION OF THE CROSS-SECTIONAL AREA OF THE LATERAL ROOTS OF PONDEROSA PINES WITH A DIAMETER AT THE SOIL OF 15 TO 26 CENTIMETERS

DETERMINATION OF THE CROSS-SECTIONAL AREA OF THE LATERAL ROOTS OF PONDEROSA PINES WITH A DIAMETER AT THE SOIL OF 15 TO 26 CENTIMETERS

Background

To treat trees in Treatment five (Chapter III), which were to have fifty-percent of the cross-sectional area of the lateral roots removed, it was necessary to establish the cross-sectional area of the lateral roots on trees similar in size as those scheduled to be treated.

Materials and methods

Five trees of comparable size, vigor and stand position were felled. The resulting stumps were hand excavated for the entire length of their tap root (Figure 16). The tap roots were extracted from the soil and all lateral roots greater than 2.5 mm in diameter, at 2.5 cm from the tap roots, were cut-off. The tap root was divided into six-inch lengths, starting at the soil line, and the cross-sectional area determined in square-inches by the six-inch groups.

Results

Based on the information gathered it was possible to establish a regression formula to predict the amount of cross-sectional area representing fifty-percent of the total.

Two regressions were established, one using D.B.H. as the independent variable and the other using diameter of the tree at soil level. The regression based on D.B.H. resulted in an R^2 of .40, which was unacceptable. The regression based on the diameter of the tree at soil level yielded an equation ($Y = 3.04x - 16.42$) with an R^2 of .96 (Figure 17).

Figure 16. Excavated stump prior to extraction from the soil for determination of the cross-sectional area of the lateral roots.

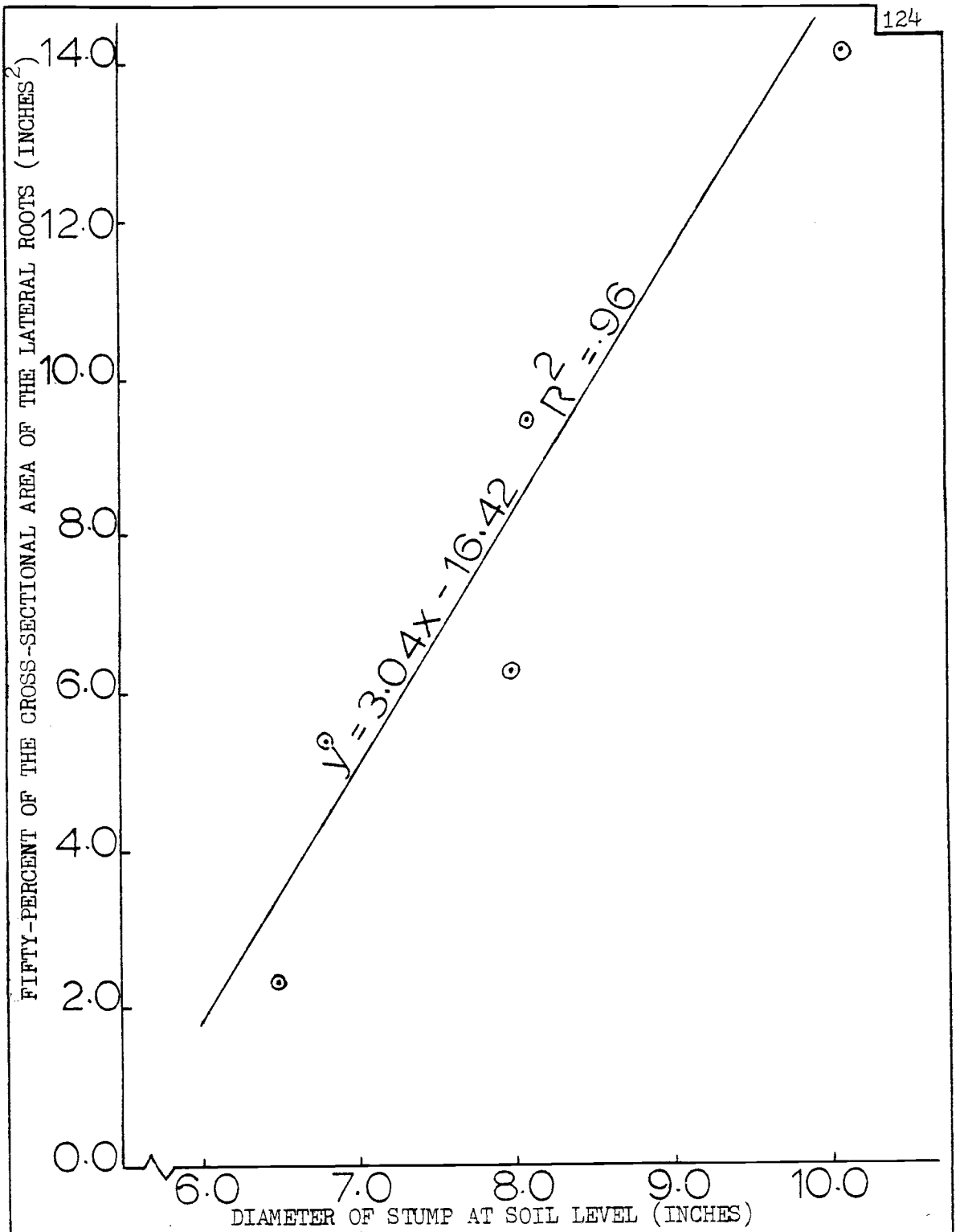


Figure 17. Regression of fifty-percent of the cross-sectional area of the lateral roots on diameter of stump at the soil level.

On the average, fifty-percent of the cross-sectional area of the lateral roots lies within the first six-inches of the tap root (Figure 18), however, this criterion was not consistent (i.e. it varied from as little as 18% to as much as 97%).

Discussion

There is a lack of information concerning the cross-sectional area of lateral roots of ponderosa pines on pumice soils. For this reason it was necessary to establish a model which could accurately predict the cross-sectional area contained within the lateral roots of pines with a diameter at the soil level from 15.0 to 26.0 cm. The equation $Y = 3.04x - 16.42$ ($R^2 = .96$) accurately describes the relationship of diameter of the tree at the soil and cross-sectional area of the lateral roots.

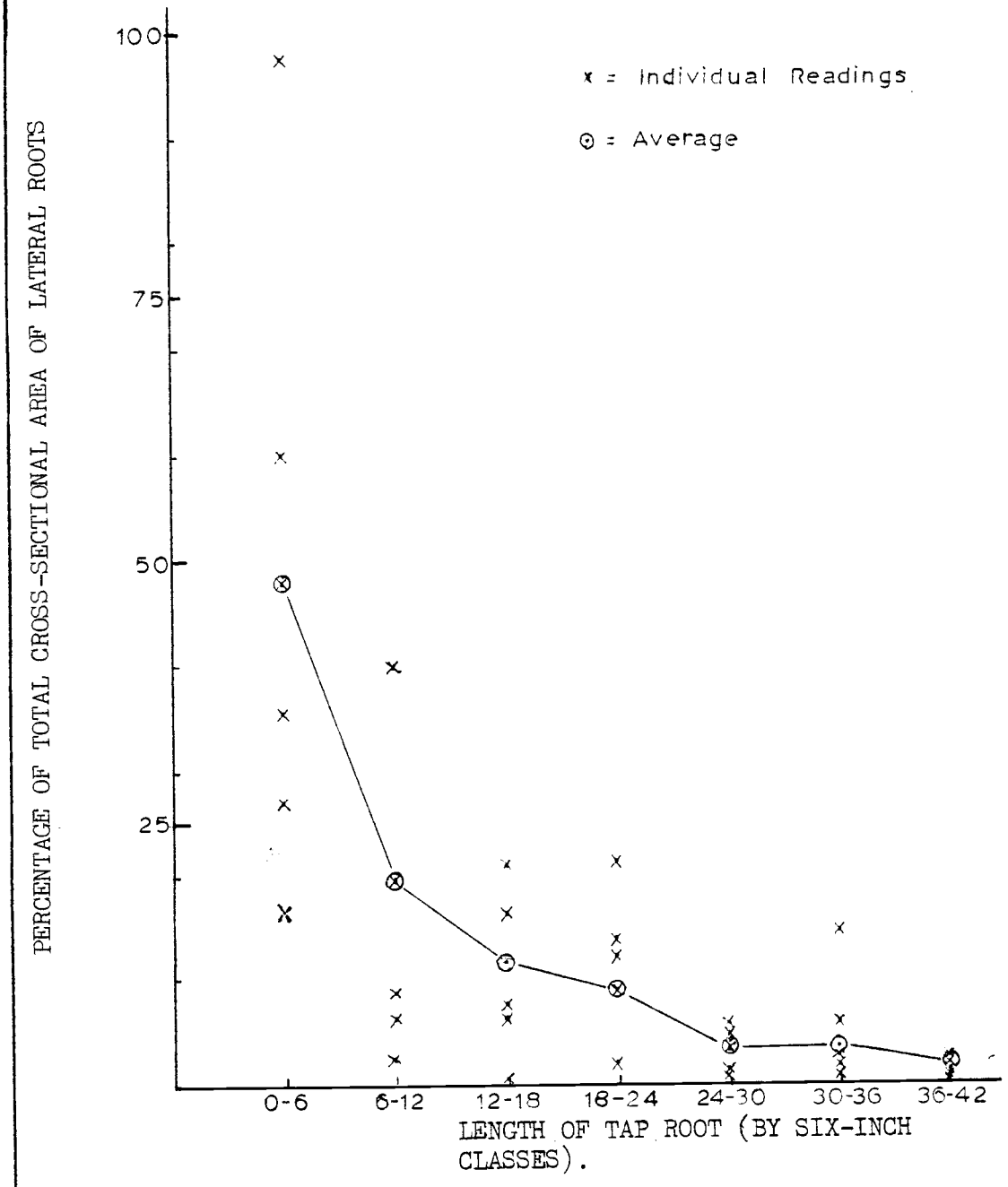


Figure 18. Individual and averaged figures, based on six-inch length classes, representing the total cross-sectional area of the lateral roots of five ponderosa pine tap roots grown on pumice soils.

APPENDIX C

DETERMINING BIOMASS OF LIVE CROWN FOR TREATMENT SIX (REMOVAL OF TWO-THIRDS OF THE BIOMASS OF LIVE CROWN)

DETERMINING BIOMASS OF LIVE CROWN FOR TREATMENT SIX (REMOVAL OF TWO-THIRDS OF THE BIOMASS OF LIVE CROWN)

One of the treatments carried out to stress trees was the removal of two-thirds of the biomass of the live crown, from the bottom upwards. To remove this biomass it was necessary to have a model of the live crown of ponderosa pine.

A model of ponderosa pine crowns, based on trees on Pringle Butte, was developed for evenly spaced (2 to 6 m) trees of uniform height (3.5 m) by Strand and Roth (1976). This model describes the outside surface of the tree crown. To use the model for crown volumes one assumption must be made - that is, the biomass within the crown is directly proportional to the surface area of the crown.

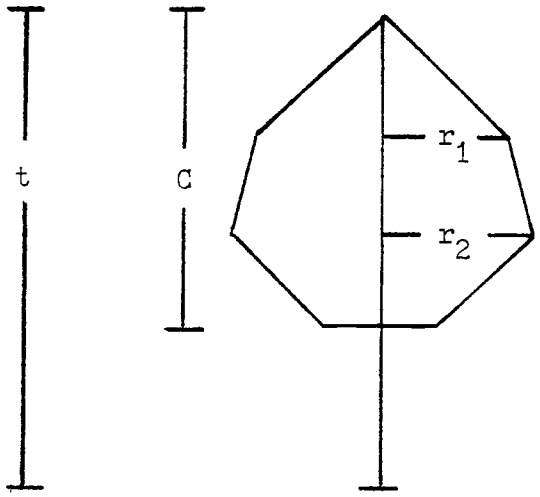
The model developed by Strand and Roth (1976) is as follows:

1. $C = -0.218 + 0.718t$ $(R^2 = .83)$
2. $r_1 = 0.112 + 0.126t$ $(R^2 = .90)$
3. $r_2 = 0.083 + 0.163t$ $(R^2 = .91)$

where; t = tree height (m)
C = length of foliated crown
r_1 = crown radius at t - (C/3)
r_2 = crown radius at t - (2 · C/3)

By turning this two-dimensional model into a three-dimensional model it was possible to determine the volume of the crown occuring in each third of the crown by using the formula for the volume of a cone for the top 1/3 of the crown and the formula for the volume of a truncated cone for the middle and botom 1/3 of the crown.

Based on the sub-division of the crown as above it was possible to determine that two-thirds of the biomass of the crown was contained in 56-percent of the linear length of the live crown, if measured from the base of the crown upwards.



While the assumption that the two-dimensional model of the surface of the crown is directly proportional to the volume of the three-dimensional crown is not proven, the benifit from using this type of procedure is that the treatment is applied uniformly to all treatment trees.

APPENDIX D

DATA SHEET USED TO EVALUATE

ARTIFICIAL INOCULATIONS

DATA SHEET USED TO EVALUATE

ARTIFICIAL INOCULATIONS

Treatment_____Block_____Tree No._____
Date: Treated_____Inoculated_____Observed_____
Days between: Treatment and Inoculation_____
Inoculation and Observation_____

TREE STATUS:

Living: Yes_____No_____
D.B.H._____Dia. at base_____

Crown Class: Dominant___Codominant_____
Intermediate___Overtopped_____

Tree Vigor: Excellent_____Good_____
Fair_____Poor_____

INFECTION STATUS:

Inoculum: Rhizomorphs produced: Yes___No_____
Length of longest rhizomorph_____mm
Rhizomorphs attached to substrate: Yes_____
No_____
Inoculum viable: Yes___No_____

INFECTION: Infection initiated: Yes_____No_____
Infection successful: Yes_____No_____
Dimensions of infection _____mm by _____mm
Able to recover A. mellea from substrate: Yes_____
No_____

NOTES:

APPENDIX E

ABILITY OF *A. MELLEA* ISOLATE GW-C7 TO PRODUCE RHIZOMORPHS UNDER VARYING TEMPERATURES

ABILITY OF A. MELLEA ISOLATE GW-C7 TO PRODUCE RHIZOMORPHS UNDER VARYING TEMPERATURES

Background

The two factors which exert the most influence on the growth of rhizomorphs of A. mellea in the soil is temperature and moisture (Rishbeth, 1978). One, apparent, universal requirement for adequate rhizomorph growth is high relative humidity (Smith and Griffin, 1971; Morrison, 1976), however, temperature does not seemingly produce such consistent results. Temperature requirements for adequate rhizomorph growth have been shown to vary widely among different isolates of A. mellea (Benton and Ehrlich, 1941; Bliss, 1946; Gibson, 1961; Hintikka, 1974; Rishbeth, 1978).

The moisture provided by the wet vermiculite during the course of the inoculation studies (Chapter III) indicates that this was not a limiting factor.

The use of an isolate of A. mellea not native to the study site for artificial inoculum production indicated to this author that the influence of soil temperature would be a more plausible answer as to why this isolate produced rhizomorphs so reluctantly.

To try and answer this question of temperature influence and poor inoculum performance the following study was conducted.

Materials and methods

Forty-nine A. mellea infected segments which were buried in the summer of 1975, and which had produced rhizomorphs by the summer of 1977, were removed from the soil and vermiculite. All adhearing rhizomorphs were removed and the inoculum sticks were individually loaded into polyethylene bags containing saturated vermiculite. The bags were sealed and placed in the dark for one year (Jan. 1, 1977 to Jan 3, 1978) at the following seven temperatures (seven bags per temperature): 5'C, 10'C, 15'C, 20'C, 25'C, 30'C and room temperature (approximately 21'C). At the end of one year the bags were opened and all rhizomorphs were removed, washed, lengths and branching pattern recorded, and dried at 60'C for 12 hours. After drying the rhizomorphs were weighed to the nearest 0.1 mg.

Inoculum segments which did not produce rhizomorphs at the end of the one year incubation period were reloaded into polyethylene bags and wet vermiculite and reincubated for six months at 15'C.

Results

The data collected is summarized in Table 32. Rhizomorphs were produced from 29 out of 49 inoculum segments. Based on a one-way analysis of variance the variation among the means of weight was significant ($P = .05$). There was a definite trend for rhizomorphs to increase in average weight and length up to 15'C, but above this temperature the average weights and lengths declined.

Table 32. Number of inoculum pieces producing rhizomorphs at seven different temperatures, the average weight of rhizomorphs per segment, the rhizomorphs average length and the length needed to equal one gram.

Temperature ('C)	# of segments out of seven producing rhizomorphs	Average weight (mg) of rhizomorphs per inoculum sigment	Average length (mm) per segment	mm needed to make one gram
5	5	5.6	62.2	11,187.0
10	7	112.7	54.0	479.1
15	7	172.6	84.6	490.5
20	5	166.8	76.0	455.6
21	5	4.7	47.6	10,214.6
25	0	-	-	-
30	0	-	-	-

The twenty segments, spread among temperatures 5', 20', 21',25' and 30'C, which did not produce rhizomorphs after one year and were subsequently reincubated at 15'C, all produced rhizomorphs except those which were previously at 30'C.

The length in millimeters of rhizomorphs needed to equal one-gram was used as an aid to describe their structure. At the temperature extremes (5' and 21'C) where growth occured a considerable length of rhizomorph would be needed to make one-gram, thus confirming the visual observation that these rhizomorphs were extremely small and fragile.

Branching of rhizomorphs was profuse and similar for all temperatures at which growth occured.

Discussion

The data indicates that the isolate of A. mellea used had an optimum temperature for rhizomorph development of 15'C. At this temperature the rhizomorphs are thicker, denser, longer, and weigh more per unit of length than those grown at the six other temperatures.

The uniform, profuse branching of rhizomorphs is different from the results obtained by Redfern (1973) where he observed sparce branching of rhizomorphs produced at 15'C and profuse branching at 25'C. My results also differ from Redfern (1973) in that I observed no rhizomorph production at 25'C whereas Redfern observed considerable rhizomorph production at this temperature.

The temperatures of 5', 20', 21' and 25'C were all inhibitory to A. mellea rhizomorph growth as indicated by the production of rhiz-

omorphs from all segments reincubated at 15'C for six months. The inoculum which had been treated at 30'C was killed by this temperature as subsequent attempts at recovering the fungus were all negative. This can be compared to the work of Munnecke et al (1976) where they observed that 30'C, applied to A. mellea for three days on citrus agar, caused cessation of growth but was resumed if the plates were incubated subsequently at 23'C.

Summary. This study reaffirms the variability which exists in the population of A. mellea at large.

The initial impetus for this study was to try and explain, through temperature response, the lack of rhizomorph production in the artificial inoculum used in Chapter III. However, re-examining Table I (page 4) it is seen that the prevailing temperatures during the summer are ideal for rhizomorph production. While those during the winter are less so, they are still sufficient to allow for rhizomorph growth. Clearly the explanation for the lack of rhizomorph production lies in other areas.

If I were to speculate as to the reason for the poor inoculum performance I would attribute it to the inoculum being insufficiently decayed at the start of the study. In all cases, in the field, rhizomorphs grew from portions which were in an advanced stage of decay (soft white-rot stage). Apparently the study site is good for rhizomorph production but elicits slow decay processes.

APPENDIX F

REDUCTION IN RADIAL INCREMENT RELATED TO PRUNING OF CROWNS

REDUCTION IN RADIAL INCREMENT RELATED TO PRUNING OF CROWNS

Background

Determining the physiological state of a tree has been a persistent problem. While this thesis deals with trees under stress, evaluating the severity of this stress is outside its scope. However, it was felt that some effort was needed, at least, to get a feel for the effectiveness of the applied treatments. To this end the trees in the treatment involving crown removal were evaluated for reduction in radial increment growth two years after pruning.

Materials and methods

Fifty trees, twenty-five pruned and twenty-five similar but unpruned, were selected. Radial increment of all trees over the last four years was established using increment cores collected from each bole at one meter above ground. The cores were collected using an increment hammer. All cores were numbered and placed in polyethylene bags.

The data collected represented the increment growth of all trees two years before and two years after the date of pruning.

Results

The increment rates for pruned and unpruned trees is shown in Table 33.

Bartlett's Test of Homogenicity of variance (Freese, 1967) was insignificant (P = .05), indicating that the variances of the pruned and unpruned trees could originate from the same population and that valid comparisons using standard statistical formulae could be made.

Table 33. Mean radial increment for pruned and unpruned trees two years before and after the date of pruning.

	------ UNPRUNED ------		-------- PRUNED ------	
	Two years before	Two years after	Two years before	Two years after
n	25	25	25	25
$\bar{x}$	3.33mm	3.14mm	4.23mm	2.36mm

The differences between means for radial increment in unpruned trees before and after the pruning date were insignificant (P = .05). The radial increment of pruned trees two years before pruning was significantly greater (P = .05) than the radial increment for unpruned trees. The differences in radial increment two years after pruning in the pruned trees was significantly (P = .05) less than that increment for unpruned trees and significantly less when compared to the radial increment of the pruned trees the two years prior to pruning.

Discussion

Pruning has been shown before to cause a decrease in diameter growth (Barrett, 1968). What this study shows is that while the study trees (pruned) were growing more rapidly than non-study trees (due to a bias in tree selection towards vigorous looking, open grown and non-

dwarf mistletoe infested trees), once the study trees were pruned their radial increment declined significantly below that of the non-study trees.

While a reduction in radial increment due to crown pruning is not concrete evidence of a physiological stress being placed upon a tree (but only that a trees growth rate has been lowered) there is a precedence to believe that such a reduction may be detrimental to a trees ability to withstand attack from diseases and insects (Hallin, 1956; Gordon, 1959; Redfern, 1966; Wargo and Houston, 1974).