AN ABSTRACT OF THE THESIS OF

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Numerous isolates of Armillaria mellea were obtained within a 250 by 450 foot Study Area in a ponderosa pine plantation in central Oregon. These isolates were recovered from roots of armillaria-killed young-growth pine, from large stumps of the former pine overstory, including roots away from the stump proper, and from roots of living shrubs.

Characteristic interactions between compatible and non-compatible mycelia in paired culture clearly distinguished group affiliations among the isolates. Three groups (A, B, and C), unequally distributed numerically among the hosts from which they were isolated, were recognized in the Study Area. Members of the same groups and of other groups were found among isolates from roots of shrubs and pine in plots surrounding the Study Area. Isolates of group A were recovered seven-eighths of a mile apart.

Only members of groups A and B were recovered from armillaria-attacked young-growth pine, while C group members were predominately recovered from rhizomorphs on roots of living shrubs.

Inoculation of potted conifer and shrub seedlings with A and C group isolates revealed the presence of physiological strains of A. mellea. Rhizomorphs were found on the roots and root crowns of all plant species tested, but only members of group A were pathogenic on conifer seedlings. Shrub seedlings were not killed by either A or C group members although rhizomorphs of members of both groups were in close physical contact with living root tissue. These results agree with the field occurrence of groups A and C in the Study Area.

Roots of living and armillaria-attacked sapling pines were excavated in the Study Area. Infections of killing potential were found to occur only at the root crown; lesions on lateral roots were common, but did not contribute to the death of the trees examined. There was no evidence in this research of the fungal spread through root contact reported elsewhere in the literature.

Armillaria attack stimulated the host to secrete large amounts of resin at the attack site. Attacks in the root crown region, which ultimately were lethal, caused excessive resin production. The fungus developed very slowly in host tissue during the period of

resin secretion, but thereafter A. mellea quickly ramified the cambium of the now moribund or dead roots killing the tree.

Cold-water extracts were made from foliage, roots, and litter of ponderosa pine and shrubs on Pringle Butte, and from soil under these plants. Extracts of foliage (1:40 and 1:61 dilution) stimulated mycelial growth, and rhizomorph production and elongation over that of the control. Extract dilutions of 1:168 and 1:1600 did not greatly stimulate fungal growth. Extracts of roots, litter, and soil had little effect on growth of A. mellea in culture.

The Relation of Cover to the Distribution of *Armillaria mellea* in a Ponderosa Pine Forest

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INTRODUCTION

The reader is introduced to this study through brief consideration of certain historical and biological features of the rhizomorph, an ectotrophic growth form of some higher fungi. The significance of the rhizomorph in the life history of *Armillaria mellea* (Vahl)Quel. is a focal point of this report.

Armillaria mellea, the honey fungus, is found in temperate and tropical regions throughout the world and is regarded as a root parasite of considerable economic importance. A great variety of higher plants including both annuals and perennials have been reported as hosts (Raabe, 1962a). The fungus was described in 1777 by Vahl as *Agaricus melleus* and renamed *Armillaria mellea* by Quelet in 1872. Forest and orchard trees were reported as the first named hosts for *A. mellea* by Hartig in 1873. A concise synopsis of the important features of *A. mellea* as a pathogen of forest trees is presented by Patton and Bravo (1967).

The genus *Armillaria* contains a variable number of species, the number depending on the criteria used for classification. *Armillaria mellea* is often confused with the closely related fungus *Clitocybe tabescens* (Fr.) Bres. The two fungi are separated principally by the presence of an annulus in *A. mellea* and the lack of

one in C. tabescens (Rhodes, 1945).

Rhizomorphs (Gr. rhiza = root + morphe = shape; Alexopoulos, 1962) are root-like structures produced by A. mellea and are often important in the saprophytic and parasitic activities of the fungus. Rhizomorphs are unique in the genus Armillaria with A. mellea, however, they are formed by other hymenomycetes (including C. tabescens) and are found among Ascomycetous fungi as well.

Rhizomorphs of A. mellea can appear in two different morphological forms depending upon their association with the substrate. Persoon early described these two forms as distinct species: Rhizomorpha subcorticalis and R. subterranea depending upon where they occurred. The creamy white, flattened rhizomorphs of his R. subcorticalis were found beneath the bark, while the dark colored, cylindrical rhizomorphs of R. subterranea occurred in the soil.

As described by Campbell (1934), rhizomorphs arise from pseudosclerotial tissue surrounding the fungal body buried in the substratum. This condition is shown in Figure 1. Campbell notes differences between a true sclerotium and a pseudosclerotium.

Motta (1969) investigated the ultrastructure of rhizomorphs of A. mellea with the electron microscope and described the differentiation of cell types. He found all tissue of the mature rhizomorph to arise from two meristematic regions in the rhizomorph tip: the apical

Figure 1. A piece of well-rotted ponderosa pine root with wood partially removed to expose the dark-colored pseudosclerotium of Armillaria mellea.

center and the lateral meristem. These meristematic regions give rise to the primary and secondary medulla, cortex, subcortex, and apical hyphae. He reported the cortex to become melanized at maturity to yield the dark covering common to rhizomorphs in the soil. He found the subcortex, derived from the lateral meristem to retain its meristematic character throughout the life of the rhizomorph. Lateral branches arise from the subcortex. Vegetative hyphae were found to be uninucleate and lacking clamp connections; cells of rhizomorphs may be uni- or multinucleate depending mainly on their degree of differentiation.

Early investigators believed the rhizomorph to function primarily in affording protection to the traveling mycelium. Garrett (1960), however, believes that the rhizomorph, per se, does not function primarily as a protective device, but rather as an aggregation of hyphae for ". . . pooling of resources of the individual hyphae composing the strand, with a consequent increase in inoculum potential."[1] Thus, successful invasion of roots by A. mellea is not solely a factor of reaching a susceptible host, but is also one of maintaining the capacity to infect at the infection site.

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[1] Inoculum potential may be defined as the energy available to the organism at the site of colonization of a substrate, for colonization of that substrate.

The point is made by Garrett (1951) that the mere presence of rhizomorphs or mycelial strands does not necessarily indicate parasitic habits on the part of the possessor. Rather the degree of association with roots of a potential host must be considered before deciding the saprophytic or parasitic inclinations involved. However, development of the epiphytic growth habit favors the parasitic mode to the degree that competitive saprophytic ability may decline.

Garrett (1951) believes the rhizomorph to be the culmination of a series of types of hyphal extensions found among some members of the higher fungi in which complexity of these organs increases throughout the series. Rhizomorphs are an organ of translocation of the fungal body, and not of nutrient absorption.

The nuclear condition of A. mellea is uncertain, but the fungus is generally regarded as being a homothallic species. This conclusion is based primarily on failure to demonstrate obligatory cross-mating among mycelia of single spore origin. Thus, unlike the great majority of the higher fungi that are heterothallic, i.e., self-sterile and cross-fertile, A. mellea is believed to be self-fertile. This means that a single basidiospore has the potential, upon germination, to account for the complete life cycle of the fungus. Homothallism is considered by Parag (1964) to be an advanced characteristic that could have originated through a "masking" or "loss" of factors

maintaining self-sterility in the heterothallic forms.

A. mellea is endemic in forests of both temperate and tropical climates (Cartwright and Findlay, 1958). Thus, the fungus is often present and established in managed forests and plantations considerably before disease "outbreaks" occur.

Fassi (1959) finds the disease cycle of A. mellea in tea plantations to follow a predictable course of events as based on changes in the inoculum potential. The endemic phase involves scattered killing and latent infection in trees of the natural forest. An epidemic phase develops after large amounts of substrate are made available through falling trees of the natural forest. Colonization of this "new" substrate increases the inoculum potential of root-rotting fungi already present.

Armillaria mellea is significantly involved in the development of western hard pine forests. Ponderosa pine (Pinus ponderosa Laws.) is the predominant and most important coniferous species of the east slopes of the Cascade mountains in Washington and Oregon. Plantations are becoming more prevalent as old-growth pine is utilized. It is desirable therefore, that the disease be studied in this young-growth forest.

Direct references to killing of ponderosa pine by A. mellea are lacking in the literature. However, as based on published reports of armillaria attack of other coniferous species, pines are among

the most susceptible of all conifers. Young-growth pine are especially susceptible to a killing attack, with liability to damage decreasing with increasing host age.

Rhizomorphs of A. mellea are able to penetrate intact, living pine tissue and mycelial transfer between trees can occur through contact of diseased and healthy roots. Both means of attack are well documented in the literature.

Armillaria often becomes established and localized in large roots and stumps of living old-growth timber. After felling of the tree, the fungus quickly ramifies the cambial tissue of the entire root system as a primary invader. Eventually, the stump may act as a "food base" from which A. mellea attacks nearby young-growth.

The period of killing may reach a peak in severity 5-15 years after the overstory is harvested. Activity may decline thereafter to lower levels coincident with increase in stand age, and increase in distance between susceptible hosts and the food base. The presence of the fungus in forest plantations is often particularly noticeable through the killing of young conifers. Centers of infection can be easily observed during the early years of attack; later these centers may coalesce to form larger centers of irregular outline.

In pine plantations, and also in less intensively managed pine forests of the east side of the Cascade mountains in central Oregon, A. mellea can be found decaying stumps of felled old-growth, and

growing on roots of associated shrubs in a presumably weakly parasitic relationship. Young pines in this area seem quite intolerant to attack by A. mellea. Death occurs within 1 to 2 years following attack on seedlings, and 2 to 4 years on saplings.

Above-ground symptoms of armillaria attack may not be present until the host is essentially dead. Symptoms are expressed as poor foliage color, and cessation of growth during the growing season. Resinosus about the root crown is invariably present on dead and dying trees and may be the earliest symptom noted. Mycelial fans, characteristic of A. mellea, are found beneath the bark of the roots and root crown on all armillaria-killed pines.

Local spread of the fungus in the east-side Cascade stands is effectively accomplished by means of rhizomorphs. Spread by root contact may not be important here, but is important in areas of the world where rhizomorphs are not formed. Basidiospores are thought to be important in the establishment of new infection centers, but are of low potential in so doing (Rishbeth, 1964). Sporophores are seldom seen in the pine forests of central Oregon and Washington.

Campbell in 1934 (p. 19) admirably stated the case for armillaria:

> The remarkable feature, however, of the biology of Armillaria mellea is the development of apical growth by which the pseudosclerotium is enabled to bring itself into direct contact with a new host and to implant there its vegetative mycelium. The certainty of this method

> of reproduction over any development of spore production and distribution is amply emphasized by its success in establishing Armillaria mellea as, perhaps, our most serious tree parasite.

This, in essence, is the substance of this thesis. It is an examination of a relationship between a fungus and its host; a relationship made practical only through the presence of a unique structure--the rhizomorph.

This study arose from detailed consideration of what appeared to be several discrete armillaria infection centers in a ponderosa pine plantation in central Oregon. Each center contained several stumps of the former old-growth overstory. Questions immediately apparent regarding these infection centers led to the formulation of hypotheses concerning the distribution of A. mellea in the form of giant colonies within infection centers in particular, and the forest floor in general.

The following hypotheses under consideration are directly related to understanding the relationship between A. mellea and the vegetation complex in and around infection centers. These hypotheses are: 1) distribution of A. mellea is influenced by cover-type, but 2) is independent of host species; 3) infection centers depend on a primary food base (the large stump) for their origin and subsequent development; and 4) armillaria colonies may overlap in geographical area occupied, and are not mutually exclusive.

LITERATURE REVIEW

Members of the genus Pinus are considered to be among the most sensitive of conifers to attack by Armillaria mellea and other root disease fungi (Day, 1927; Gibson, 1960). This sensitivity is early expressed through copious resin exudation at the infection site (Patton and Riker, 1959; Baranyay and Stevenson, 1964; Gibbs, 1968). Recent reports (Jorgensen, 1961; Shain, 1967) indicate that certain phenolics, i.e., pinosylvin and its monomethyl ether, were formed in response to wounding and may represent a chemical barrier augmenting the mechanical barrier presented by pitching.

Gibbs (1968) points to the relationship between drought and reduced resin flow as a factor in the apparent increased susceptibility of conifers to attack by Fomes annosus (Fr.) Cke. Resin yield of Scots pine (Pinus sylvestris L.) was found to vary with the moisture regime of the particular site studied; wet acidic sites were more inductive of high resin yield than were dry acidic or dry alkaline sites. Resistance of pine to attack by F. annosus is highly dependent on host vigor (Rishbeth, 1951; Gibbs, 1967). That resin yield is related to host vigor is shown through a decreasing gradient of resin yield from dominants to suppressed saplings of Scots pine examined by Gibbs (1968).

Above-ground symptoms of armillaria attack vary considerably

with the coniferous species attacked, its age, and the environmental conditions under which attack occurs. A general decline in vigor of the tree may be found or death may be abrupt without preliminary symptoms. Fruit body formation about the base of an infected tree may be common in some localities and rare in others. The characteristic pitching of the root crown may often be the only symptom noted before death of the tree.

Large crops of small cones were found on infected, vigorous, young Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), associated with copious production of resin blisters on small branches. These symptoms are lacking or weakly evident on infected trees of low vigor (Buckland, 1953). Patton and Riker (1959) noted jack pine (Pinus banksiana Lamb.) to die suddenly without obvious crown symptoms of distress from infections of the root crown. Trees with infections of lateral roots did not die during the course of their study, and did not display foliar signs of stress.

Armillaria mellea was the most damaging among five fungi recovered from roots of diseased trees in a 15-17 year old Douglas-fir plantation (Foster and Johnson, 1963). The fungus caused mortality and increment reduction in 95 percent of the trees examined. Baranyay and Stevenson (1964) cite A. mellea as being "the most destructive agent" among naturally regenerated lodgepole pine (Pinus contorta Dougl. var. latifolia Engelm.) near Robb, Alberta, Canada.

Exotic and native conifers were heavily attacked by A. mellea in a plantation in New Foundland (Singh, 1970). Incidence of killing ranged from 5 percent (Larix leptolepsis (Sieb. and Zucc.) Gord.) to 28 percent (Picea sitchensis (Bong.) Carr.) in the first 10 years of the plantation.

Gibson (1960) in a survey of pine plantations in Kenya found mortality due to A. mellea to reach a peak four to five years after planting. Infection incidence steadily decreases after this period partly through stand thinning, but more important, it is suggested, through increased resistance of the host.

Recently, Barrett (1970) has suggested that A. mellea may play an important role in the ecology of Polyporus schweinitzii Fr. His observations and data suggest that colonization of woody tissue by P. schweinitzii is enhanced through prior colonization of the tissue by A. mellea.

Rhizomorphs have been cited by only several modern workers as the agent responsible for transmission of A. mellea from food base to susceptible host (Day, 1927; Thomas, 1929, 1934; Campbell, 1934). Ouellete (1967) has found rhizomorphs emanating from woody debris, stumps, and dead roots of susceptible hosts. Death occurs within two to three years after invasion of young trees, and within several more years on older trees. Kawada, Takamie and Hama (1962) noted the time between infection and death in larch to be from

the third through seventh year, with the fourth to fifth years being the most damaging. Here, translocation of A. mellea occurred through both rhizomorphs and root contact.

Infectious spread of Armillaria mellea through root contact is more commonly noted in the literature than is spread by means of rhizomorphs. Apparently, some soils possess substances actually inhibitory to rhizomorph formation (Boughey et al., 1964; Swift, 1968). Wardlaw (1950) found spread of A. mellea in an oil palm (Elaeis guineensis Jacq.) plantation to occur only through root contact, however, rhizomorphs formed within host tissue enhanced development of the attack.

Molin and Rennerfelt (1959) found infection to spread exclusively through root contact in pure spruce stands in central Sweden. Armillaria mellea was often found localized in otherwise healthy roots, spreading upward into the stump after felling. Similar observations were made by Leach (1939) who emphasized that trees growing near dead stumps may carry A. mellea in dormant form in localized root lesions and that upon felling the fungus may become active.

Rapid spread of A. mellea in young pine (Pinus longifolia Roxb., P. patula Schiede & Deppe, and P. caribaea Morel.) was noted by Pole-Evans (1933) in northern Transvaal. Spread within stands was by root contact only; rhizomorphs were not found. Trees and stumps of indigenous hardwoods were found as the infection foci in these

plantations. Leach (1939) also found root contact to be the most important means of local spread of A. mellea. He notes rhizomorph development to be generally poor in tropical Africa. Gibson (1960) found spread of A. mellea in Kenya pine plantations to occur primarily through root contact; rhizomorphs were of minor importance only.

Rhizomorphs of A. mellea are not present in Rhodesian soils, and this is attributed by Swift (1968) to an "inhibitor" naturally present in these soils. Rhizomorphs were readily formed on media and in autoclaved forest soils, but not from media incorporating untreated forest soils. All spread in the field is attributed to root contact. Similar observations were made by Dade (1927) for armillaria attack of cacao (Theobroma cacao L.) in the Gold Coast Colony. He found attack to occur exclusively through root contact, and although rhizomorphs were not found in the soil they formed in culture.

Many investigators have observed that A. mellea is found in stands that recently, if not at present, supported hardwoods (Childs and Zeller, 1929; Pole-Evans, 1933; Gibson, 1960; Greig, 1962; Kawada et al., 1962). Mortality of conifers in these stands is usually light at first, but may increase through the first thinning (Greig, 1962), after which mortality usually decreases due to breakdown of the hardwood food bases together with a decline in

the vigor of A. mellea. Baranyay and Stevenson (1964) found armillaria damage to lodgepole pine regeneration most prominent around decayed stumps of aspen (Populus tremuloides Michx.) killed by fire some 21 years earlier. Mortality was greatest in the more dense stands and among overtopped trees, while least in the less dense stands and among dominants and codominants.

Although many workers have found mortality by Armillaria mellea to be especially prevalent on ex-hardwood sites, this is not meant to imply, as does Redfern (1968), that conifer stumps lack the potential to act as effective food bases. Gibson (1960) found armillaria infection of pine to originate from cypress stumps of trees cut up to 20 years earlier. He has also found bamboo (Arundinaria alpina K. Schum.) to actively support A. mellea infection of pine.

Stump colonization by A. mellea is probably most often brought about through enlargement of existing root infections at the time of felling or through rhizomorphs in the soil (Rishbeth, 1964). Dispersal of A. mellea by basidiospores is considered to be of greater importance in long-distance, than in local spread (Gibson, 1960; Greig, 1962; Rishbeth, 1964).

Day (1929) regards armillaria attack of healthy trees to be secondary to some primary factor predisposing the trees to infection. Earlier, Day (1927) noted that where A. mellea acts as a virulent parasite its hosts are "artificially regenerated or grossly interfered

with by mankind."

Armillaria mellea is often cited as a pathogen of trees under stress. Ouellete (1967) finds the fungus attacking feeble or weakened trees in unfavorable environmental situations. High soil moisture, as an unfavorable site factor leading to infection by A. mellea is cited by Kawada et al. (1962) for larch, and Dade (1927) for cacao. Shallow, rocky soils may favor disease spread (Buckland, 1953; Gibson, 1960). Christensen (1954) artificially induced senescence in forest trees by girdling them with metal bands. Armillaria mellea invaded the stressed trees preferentially to adjacent unbanded (control) trees from inoculum endemic within the stand. Stand density was cited by Baranyay and Stevenson (1964) to be an important factor in stand health. Highest mortality as caused by A. mellea in young lodgepole pine forests in Alberta, Canada was among overtopped and suppressed trees. Conversely, mortality among dominants and codominants was quite low. Predisposition of Douglas-fir to armillaria attack through improper planting procedure is cited by Buckland (1953) as a major factor for regeneration loss through attack by the fungus.

Christensen (1938) found infection of jack pine, Norway pine (P. resinosa Ait.), and northern white pine (P. strobus L.) in natural forests to be higher in dense stands than in more open stands. The vigor of the tree in dense stands did not appear to determine susceptibility. Stands under 30 years old and those over 200 years

appeared to be more susceptible to attack than stands of intermediate age.

Disease incidence in young Douglas-fir plantations as caused by A. mellea was unaffected by tree spacing as 93% of the trees were in root contact (Pielou and Foster, 1962). Later, Pielou (1965) noted healthy and diseased patches of timber to exist within the same stand. During a period of three years' duration armillaria centers enlarged and coalesced to form fewer, but larger centers in these Douglas-fir plantations in British Columbia. Buckland (1953) found killing attack by A. mellea to occur in trees of all vigor classes in immature Douglas-fir, but was most frequent in trees of low vigor. Armillaria mellea was often checked in its advance through roots in trees of high vigor by resin excreted at the infection site. Cankers were formed at these sites and the fungus either remained dormant or grew into the wood causing a "yellow spongy butt and root rot", not leading to an active parasitism of living tissue. Armillaria mellea spread from the cankers to the living cambium under conditions of reduction of normal host vigor.

Decay of woody tissue by A. mellea has also been described as a fibrous, stringy white rot (Cartwright and Findlay, 1958, p. 73), and a yellow-brown stringy rot (Bassham and Morawski, 1964), and is confined mainly to cellulose.

The root crown of a young conifer is frequently the point

attacked by rhizomorphs in bringing about death of the tree (Day, 1927; Patton and Riker, 1959; Peace, 1962; Foster and Johnson, 1963). Older trees are invaded less rapidly; the attack may be localized and prolonged over many years while the healthy roots maintain the tree (Gibson, 1960; Peace, 1962).

Thomas (1934) concluded that branches of the parent rhizomorph directly penetrate sound, healthy tissue of either susceptible or resistant plants. He regards host resistance, if present, as a phenomenon of abating fungal development within the plant tissue once penetration to living tissue has occurred. Similar observations and conclusions were reported for infection of young conifers (Day 1927, Campbell, 1934).

Penetration of Douglas-fir roots by A. mellea was never direct (Buckland, 1953). He found the fungus to gain entry only through weakened or damaged tissue, or through root contact with a diseased tree. Hubert (1950) describes infection of western white pine (Pinus monticola Douglas) as originating in "root spurs" with subsequent advancement to the root crown. Early colonization of discs cut from woody stems found A. mellea invading the cambial and bark tissues, while colonizing the wood later in the decay process (Rishbeth, 1964).

Patton and Riker (1959) observed initial penetration sites on 58 infected potted jack pine to be as follows: "stem above root collar, 11 trees; root collar, 16 trees; main roots 2 mm or more in

diameter, 27 trees; and small roots 1 mm or less in diameter, 4 trees." The actual infection site was deemed to be most important in the subsequent health of the tree. Attack at or above the root collar was usually followed quickly by death of the tree, even before foliar symptoms appeared. Armillaria mellea developed very slowly in lesions on roots. Mycelium had not reached the root collar in 35 months after the initial inoculation. Pitching was associated with all lesions.

Hendrickson (1925) found that spread of A. mellea in a prune and apricot orchard in California was mainly by means of rhizomorphs and secondarily by root contact. Host vigor did not appear to influence disease susceptibility, nor did soil type. The fungus was believed to have been on roots of oak which were felled during establishment of the orchard. Four or five infection centers initially present coalesced in 23 years' time to encompass some 860 trees.

Marsh (1952) however, did not find consistent patterns of spread of A. mellea in black currant and apple orchards in England. His data suggested that new infections could be expected near to previous attacks, but the relationships between dead (or dying) hosts and latent attack was not clear. It was thought that killing of apple trees is a slow process and that complete killing of all roots may take many years. Spread was believed to be mainly by root contact.

Garrett (1960) postulates that as distance from the food base

to susceptible host increases several factors occur which may tend to negatively influence the chance for infection. Most important among these factors is the effective decrease in rhizomorph apices per unit of circumference as the distance between rhizomorph apices and food base becomes greater. Secondly, he finds the depletion of the food base to be a limiting factor over the level of nutrient translocation through the extending rhizomorph system. And thirdly, he believes the rate of nutrient translocation to the rhizomorph apex tends to be inversely related to rhizomorph length. The last two factors, i.e., depletion of the food base, and growth related reduction of nutrient translocation, would greatly influence the inoculum potential of the rhizomorph unit at the potential infection site.

In investigating the relationship between pathogenesis and soil temperature, Bliss (1946) found A. mellea capable of attacking roots of the test plant at all temperatures from 7-25 C. Greatest host resistance against attack was displayed at those temperatures most favorable to root development. He found two critical thermal regimes to exist for any suscept; these lie at the extremities of the range for pathogenesis. The host has little resistance at the lower critical temperature for pathogenesis as the temperature lies below the minimum for root growth. Susceptibility is offset at the higher critical temperature by the vigorous root activity at this temperature and higher.

Snider (1959) has precisely described the development of A. mellea in pure culture. He identifies five stages: 1) development of the "central mycelium" preliminary to rhizomorph "initial" development, 2) a brief period in which the rhizomorph initials are formed from microsclerotial tissue, 3) a short period in which rhizomorph growth rate is increasing, yet the tips have not passed beyond the extremities of the central mycelium, 4) the period in which the rhizomorphs have reached their linear growth rate (usually 5 or more times faster than the growth rate of the central mycelium), and 5) the final stage in culture in which rhizomorphs with apparently unlimited growth potential are restricted by the sides of the container. Concurrent development of microsclerotial tissue on the medium surface directly over the rhizomorph gives the entire colony a lobed appearance.

Detection of clonal relationships among isolates of a species through cultural pairing has been attempted for only a few forest fungi. Mounce (1929) separated clones of Fomes pinicola (Sw.) Cooke, recovered from dead Douglas-fir. Childs (1963, 1970) distinguished clonal lines of Poria weirii Murr. attacking Douglas-fir in western North America. In Britain clones of Polyporus schweinitzii recovered from unnamed coniferous host(s) have been distinguished (The Commonwealth Forestry Institute, 1963-1964, p. 26-27). Recently, Adams and Roth (1963, 1965) demonstrated the presence of multiple

clones of Fomes cajanderi Karst. in single trees of living Douglas-fir.

Host specificity has been reported for A. mellea by Childs and Zeller (1929). They indicate the presence of two physiological strains in orchards in Oregon. One, the fir strain, is found on roots of conifers and did not parasitically attack apple; while the other, the oak strain, whose source is the native oaks, is strongly pathogenic in apple orchards. Rhizomorphs of the fir strain are commonly found on roots and root crowns of apple in the orchards observed. No killing or infection of apple was observed through either naturally or artificially inoculated trees. However, Raabe (1967a, b) did not find host specificity through cross-inoculation among isolates from gymnosperm, and woody and herbaceous angiosperm hosts. Oak and pine isolates of A. mellea were both effective in establishing infection of conifer seedlings in pots.

Growth of Armillaria mellea in culture occurs over broad optimum ranges of pH and temperature. Both mycelial growth and rhizomorph development have pH optima of 5.0-6.0 (Reitsma, 1932; Lisi, 1940; Benton and Ehrlich, 1941; Jennison, Newcomb and Henderson, 1955). Snider (1959) reported abundant rhizomorph development on various media with reactions ranging from 4.0-7.0.

In general, the optimum temperature for mycelial growth of A. mellea in culture is 25 C (Reitsma, 1932; Townsend, 1954; and

Rishbeth, 1968), or slightly lower (Benton and Ehrlich, 1941). The optimum range is broad, however, and growth exceeding 50 percent of the optimum within the 15-30 C range is reported by Rishbeth (1968) for colonies developing from basidiospores.

There is considerable variation in cultural appearance among isolates of A. mellea (Raabe, 1966). Yet Gibson (1961) and Raabe (1967) both found morphological consistency within clonal lines throughout periodic transfer of isolates during maintenance of cultures. Marked variation among single spore colonies derived from the same fruit body has been noted by MacLean (1950), and Raabe (1966).

Benton and Ehrlich (1941) reported finding three morphological classes among the A. mellea colonies they studied: isolates with profuse mycelial and rhizomorphic growth, others with thin mycelial growth and little or no rhizomorphic development, and colonies consisting of a sclerotial mat with subtending short and long rhizomorphs.

One of the most fascinating and most investigated aspects of A. mellea biology is concerned with factors affecting the production and development of rhizomorphs.

Rishbeth (1968) found that rhizomorph elongation through a light loam soil in tubes from woody inoculum, had a broad optimum temperature range of from 18-25 C. Above 25 C rhizomorphs were not initiated, while rhizomorph elongation ceased at 30 C. Reduction

in rhizomorph growth at higher temperatures is thought to arise from a reduction in translocation.

Light was found by Townsend (1959) to inhibit elongation of rhizomorphs in culture, while not inhibiting the formation of rhizomorph initials. Her oft-cited study consisted of placing an unspecified number of plates of A. mellea on a "well-lighted window sill"; some plates were wrapped in black paper while others were left unwrapped. Though poorly documented, light does seem to adversely affect mycelial and rhizomorphic growth; several workers cite their cultures of the fungus as dark grown (Patton and Riker, 1959; Swift, 1968; Motta, 1969).

Rhizomorph production in culture is variable. Raabe (1966) found about two-thirds of 84 isolates recovered from tissues of infected hosts to produce rhizomorphs on PDA, while all produced rhizomorphs on wood inoculum blocks buried in a planting mix. Virulence and pathogenicity, however, could not be related to rhizomorph production in culture on PDA (Raabe, 1967, 1969).

The C:N ratio is important in rhizomorph growth. A carbohydrate-nitrogen relationship was found for both the initiation of rhizomorph initials and rhizomorph development in A. mellea (Garrett, 1953), and a relative increase in both nitrogen and carbohydrate levels was associated with increased rhizomorph development. At suboptimal carbohydrate levels, supraoptimal nitrogen

levels (peptone nitrogen) depressed formation of rhizomorph initials, while at higher carbohydrate levels excess nitrogen depressed rhizomorph growth while having little affect upon development of rhizomorph initials.

Hamada (1940) found a C:N ratio of 2:1-4:1 to provide the most optimum ratios for rhizomorph growth. Aerial mycelial growth had a broad optimum C:N ratio of 2:1-1:2 with dextrose and peptone. He also found the color and form of rhizomorphs to be influenced by the C:N ratio. With decreasing C and increasing N rhizomorph color changed from yellow to brown. At low C:N ratios the R. subcorticalis form occurs, while at high N:C ratios the R. subterranea form occurs.

Glucose appears to be the most easily assimilated of the many carbon sources that have been tested with A. mellea (Reitsma, 1932; Jennison et al., 1955; Weinhold and Garraway, 1966). Ammonium nitrogen in ammonium tartrate form is the most utilizable inorganic nitrogen source for growth of A. mellea found thus far; nitrate nitrogen apparently cannot be metabolized (Garrett, 1953; Nykvist, 1962; and Weinhold and Garraway, 1966). Casein provides the most utilizable organic nitrogen source and is superior to any inorganic nitrogen form tested (Weinhold and Garraway, 1966).

Lindeberg (1944) found Ca and Mn related to utilization of thiamin in species of Marasmius that are heterotrophic for thiamin. Growth

increased (dry weight basis) 2-2.5 times over that of controls containing no Ca or Mn, when these elements were present in the medium. Garrett (1953) has demonstrated A. mellea to be heterotrophic for thiamin.

Naturally occurring organic substrates have received attention in developmental studies of A. mellea. Waksman and Tenny (1927) define the water soluble fraction of naturally occurring organic materials as containing the substances most readily acted upon by decomposing organisms. Cold-water extracts contain simple carbohydrates and nitrogenous substances. Starches, various nitrogen compounds, some pectin and certain hexoses are extracted with hot water.

Lipid extracts from ponderosa pine roots among other plants tested, stimulated rhizomorph production. Removing the fatty acid fraction through saponification inactivated the remaining extract. Roots of ponderosa pine were found to have relatively high amounts of unsaturated fatty acids believed to contribute to rhizomorph production in A. mellea (Moody and Weinhold, 1970).

Cold-water extracts of incense cedar heartwood were used by Bynum (1965) in growth studies of Polyporus amarus Hedge. He found P. amarus to grow best on unsupplemented medium with 2.27 mg/ml extractive; higher extract concentrations resulted in a near-cessation of colony growth. Addition of extract did not stimulate

growth on supplemented media (1 percent malt extract) at low extract concentrations and had a depressing effect at higher concentrations.

Hot-water fig-wood extract supplemented with dextrose was found by Raabe (1962b) to greatly stimulate growth of A. mellea over that of the PDA control. Further investigation by Weinhold, Hendrix and Raabe (1962) has led this group to believe the growth stimulative substance in fig-wood to be IAA or an IAA-like material. Weinhold (1963) has also noted rhizomorph production to be stimulated with 25 ppm ethanol; 500 ppm being close to optimum. Ethanol concentrations to 2000 ppm did not significantly increase rhizomorph production, and even higher ethanol concentrations led to malformation of developing rhizomorphs. Rehill (1968) has found hot-water extracts of red alder wood to stimulate rhizomorph production. However, lesser stimulation was noted with IAA (5 ppm) and ethanol (500 ppm).

GENERAL DESCRIPTION OF STUDY AREA

Field studies reported here were conducted in the Pringle Falls Experimental Forest, Deschutes County, Oregon. The Forest is a facility of the Pacific Northwest Forest and Range Experiment Station, Pine Silviculture Laboratory, U.S. F.S., Bend, Oregon.

Topography of the Forest is dominated by two adjacent cinder cones: Pringle Butte and Cruiser Butte. Pringle Butte rises almost 800 feet above the Deschutes Plateau and has a basal circumference of about 4.5 miles. Data were collected from two study areas on the east and south intermediate slopes of Pringle Butte.

Nearly pure stands of ponderosa pine dominate the higher and steeper slopes while lodgepole pine is abundant in stands on the lower slopes. Shrub cover, abundant in open and young ponderosa pine stands, relates to past fire history and recent logging operations. Snowbrush ceanothus (Ceanothus velutinus Dougl.) and antelope bitterbrush (Purshia tridentata (Pursh.) DC.) are the principal species on the eastern intermediate slopes. This vegetation complex corresponds closely to the Pinus/Ceanothus-Purshia community of Dyrness and Youngberg (1966).

Pine manzanita (Arctostaphylos parryana var. pinetorium (Rollins) Wiesl. and Schreib.) and snowbrush ceanothus predominate following fire and are abundant on the higher and more open slopes.

Vegetation on the lower slopes of Pringle Butte resembles the Pinus/Purshia/Festuca community (Dyrness and Youngberg, 1966).

The pine and shrub names will be abbreviated hereafter to PP (ponderosa pine), SB (snowbrush ceanothus), BB (antelope bitterbrush), and M (manzanita).

Except for two light sanitation cuts, the east side of Pringle Butte had no logging history prior to 1957. In 1957, 140 acres of old-growth PP, averaging 17 M.b.f. per acre, was logged under intensive care to minimize damage to understory saplings (Barrett, 1960). These saplings were thinned in early 1958 to approximately 13 × 13 feet. My Study Area I was established in this released stand.

Study Area II was on the south slope of Pringle Butte in a pine plantation established in 1966 following removal of an extensive M/SB brush field and a few scattered old-growth pines. An armillaria infection center associated with stumps in the plantation comprises Study Area II.

Climate in the Experimental Forest is characterized by an average annual precipitation of 24 inches, with 85 percent falling from October 1 through April 30 as rain or snow. Snowpacks commonly reach 24 inches (61 cm) through January to March (Barrett and Youngberg, 1965). Summer temperatures seldom exceed 95 F (35 C). Occasional frosty nights occur during the summer. Infrequent summer rains occur as thunderstorms.

Soil in the Experimental Forest is primarily of the Lapine series, with occasional areas of Longbell soil (Barrett and Youngberg, 1965). Lapine series soils are immature soils developed from dacite pumice deposited during the eruption of Mt. Mazama some 6500 years ago to form Crater Lake (Williams, 1942; Libby, 1955). The pumice mantle on the slopes of Pringle Butte averages 33 inches in depth and overlies a layer of cinders and basalt fragments (the D horizon) originating during the earlier volcanic activity of Pringle Butte itself. These light textured soils lend themselves ideally to excavation of PP root systems revealing infection loci, and host/rhizomorph relationships.

Soils on Pringle Butte reach field capacity in early spring from snow melt, but become deficient in moisture by mid-summer. The infrequent summer rains do little to replenish soil moisture lost during the growing season.

METHODS

By both direct and indirect methods this thesis examines interrelationships between vegetative cover of the pine stand and distribution of clones of A. mellea. A resume of detailed procedures follows.

Distribution of Armillaria mellea

Field recovery of A. mellea was from study areas designated I and II. Area I, 250 feet by 450 feet, was established to enable a systematic sampling for A. mellea on roots of PP and associated shrubs. Area II, encompassed an infection center in a young pine plantation. Armillaria mellea was also recovered from a series of small "peripherial" sites located within a one-half mile radius of Area I. Isolates of A. mellea recovered in culture were from mycelium in infected wood of large stumps and roots; mycelial fans in the cambium of large stumps and roots of dead saplings; and rhizomorphs epiphytic on roots of large stumps and living shrubs, from within hollow roots of the large stumps, and in the soil not in contact with plant tissue.

In order to achieve a spatial study of distribution of armillaria colonies in the released stand a grid was established over the area (Figure 2). Map locations of isolate recovery or host locations referred to hereafter are derived from Figure 2, and relate to

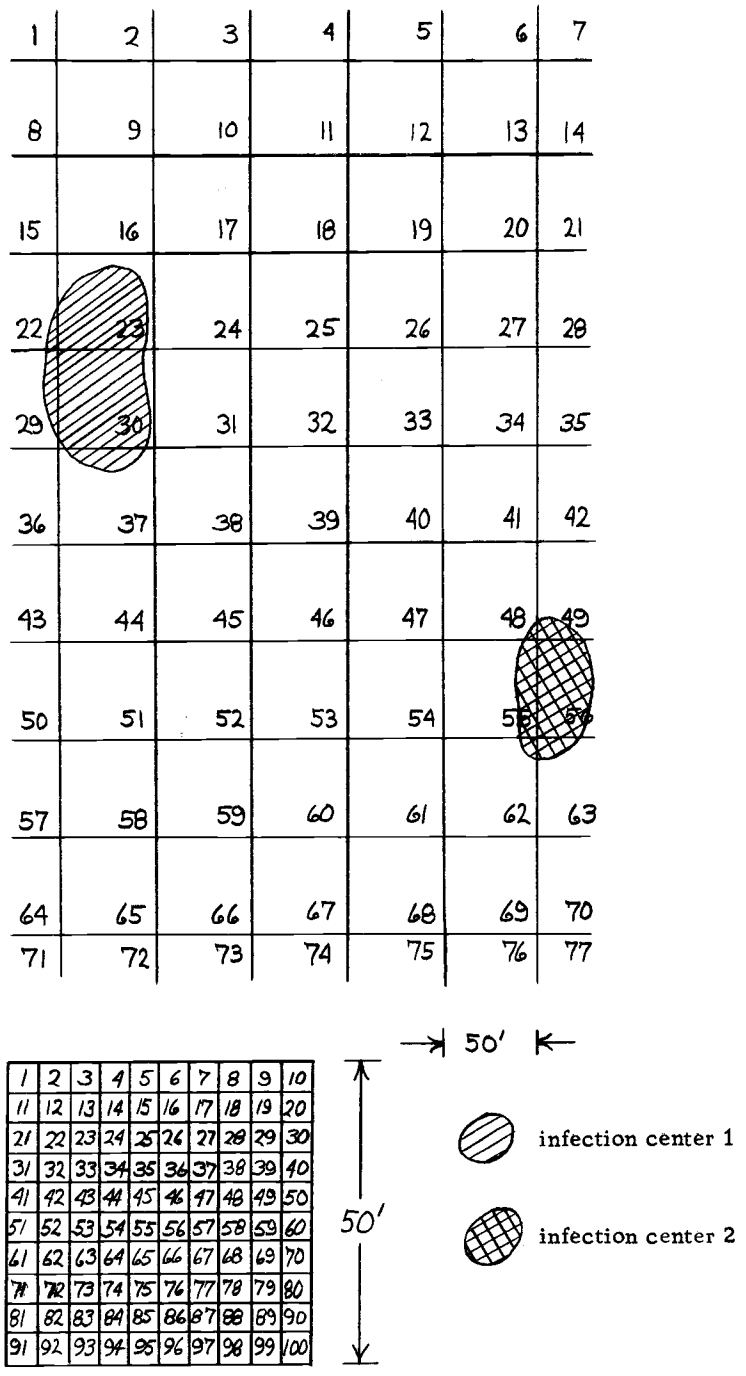


Figure 2. Grid diagram of Study Area I with numbering system used to record location of hosts and isolates. Infection centers 1 and 2 are identified.

Study Area I only. For example, an armillaria isolate with the number 27-92S was recovered from SB in 50 foot square number 27, and 5 foot square number 92 of Study Area I.

Vegetation within 10 foot wide transects centered on each of the six longest grid lines (Figure 2) was examined for A. mellea. Twenty percent of the area within the grid was thus sampled.

Field recovery of A. mellea was made from the most convenient source available along the grid lines (Figure 2). Since the fungus was known to be consistently associated with roots of living BB, M and SB plants, these plants were examined as they occurred on the grid line. Armillaria mellea was recovered, as present, by removing the plant from the soil and recovering rhizomorphs from the root surface; mycelium and sporophores were not common on shrubs. Rhizomorphs were also recovered from the surfaces of roots of large stumps of the former overstory, and in the soil away from direct root associations. Recovery of A. mellea from stumps consisted of isolating the fungus from wood of both the lateral roots and the lower stump. Armillaria mellea was recovered from roots of dead saplings through transfer of mycelial tissue directly to an agar medium favorable for growth of the fungus.

Rhizomorphs, because of their root-like appearance are occasionally difficult to visually separate from roots of higher plants. However, the two can be readily distinguished in the field by two

easily performed tests: 1) living rhizomorphs are much more resistant to breakage than are roots (it is difficult to break a rhizomorph through either pulling or bending, while living roots of rhizomorph size are easily snapped, and 2) the melanized rhizomorph cortex can be scraped off with the fingernail to reveal the very white medullary tissue on living specimens. Comparable features are not found in roots of higher plants.

Use of the word "stump(s)" in this thesis always refers to stumps of the former old-growth PP overstory. These stumps resulted from a harvest of commercial sized timber in clearing the land for the plantation in which this study was performed. The word "sapling(s)" always refers to young pine of the plantation.

Each pine and shrub examined was given a map location number with species designation (BB, SB, M, or PP). Armillaria tissue recovered was identified by a map location number, host designation and additional notation relating to tissue recovered (rh = rhizomorph, m = mycelium, fb = fruit body). Viable tissue recovered from roots were labeled and stored up to 48 hours in moist paper towels under refrigeration until cultural isolations could be made.

Rhizomorphs recovered for isolation purposes were washed with agitation in cold, running water for 6-12 hours. After washing, the rhizomorphs were surface sterilized in 20 percent Clorox for 10-15 minutes. They were then cut into 10-15 mm lengths and aseptically

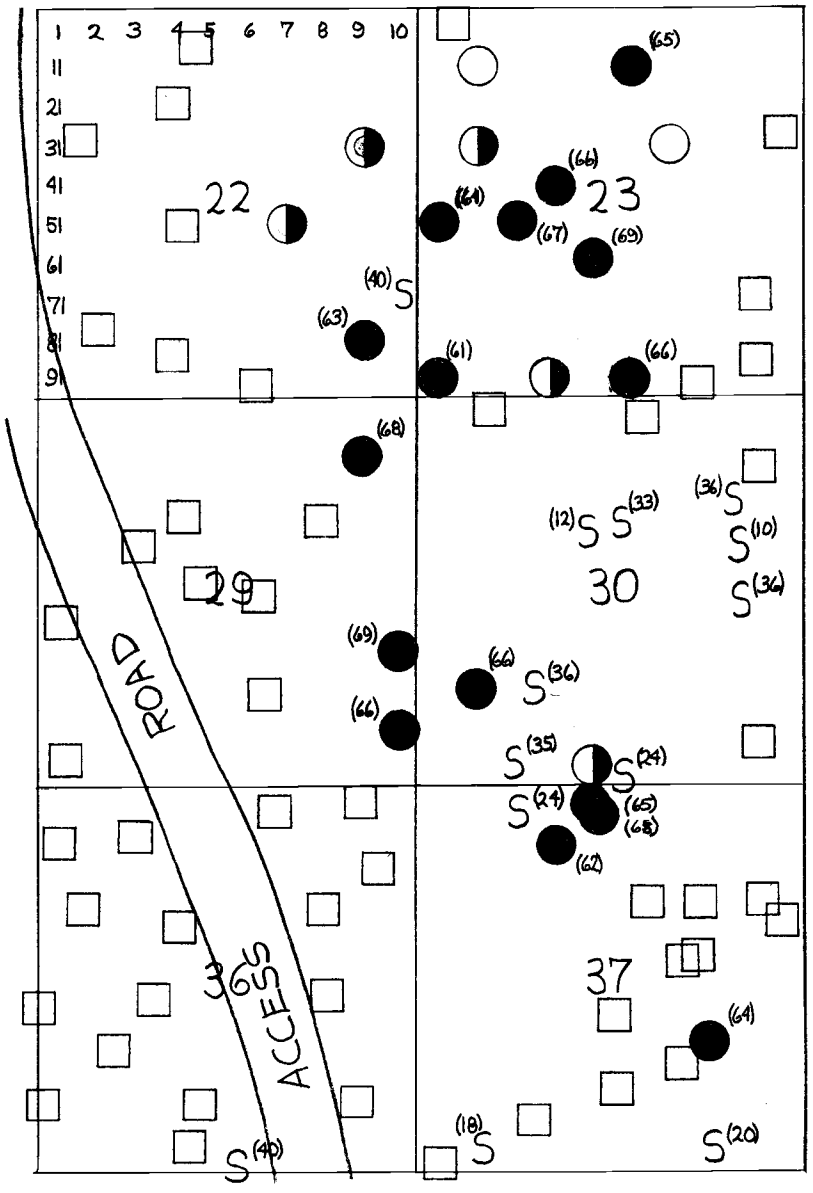
placed onto the isolation medium (3 percent malt-extract, 2 percent dextrose, 0.5 percent peptone (MDP medium), and 0.006 percent orthophenylphenol (OPP)(Russell, 1956), in 2 percent agar). The pH was adjusted with dilute NaOH or HCl to 5.0-5.3 prior to autoclaving. All cultures were incubated at 25 C in the dark. Bits of mycelium from mycelial fans in recently killed pines, and armillaria infested wood from large stump roots were put directly onto MDP medium with OPP.

All isolates recovered (of either rhizomorphic or mycelial origin) on MDP/OPP medium were transferred to MDP medium without OPP in screw-top slant tubes with loosely tightened caps. Cultures were incubated at 25 C for 2-3 weeks, at which time the caps were tightened and the tubes stored at 5 C for later use. Under airtight seal the cultures remained viable for a year or longer.

The presence of infection centers within the Study Areas, recognizable by dead pines (Figure 3-7), required adapted procedures. In infection center 1 (Figure 3, 4) roots of all living shrubs within 10 feet of killed sapling 29-90PP were examined to recover A. mellea as present. Fungal tissue was taken from all armillaria-killed pine saplings (14), seedlings (2), and one large stump (22-80PP). Viable rhizomorphs growing within hollow roots were also collected for recovery of A. mellea. The fungus was recovered from wood of lateral roots of the stump, and wood of the stump root crown.

Figure 3. Part of infection center 1. Flags mark trees killed by A. mellea.

Figure 4. Plat of infection center 1 showing locations of stumps, excavated trees, and healthy appearing, unexcavated trees over 6 feet tall. Five foot square subdivisions are numbered as in block 22.



Key

Excavated trees:

- ○ A. mellea free
- ◑ A. mellea attacked, pitching, living
- ● A. mellea attacked, pitched, dead (year died)

□ Unexcavated, healthy appearing PP

S Stump with diameter (inches)

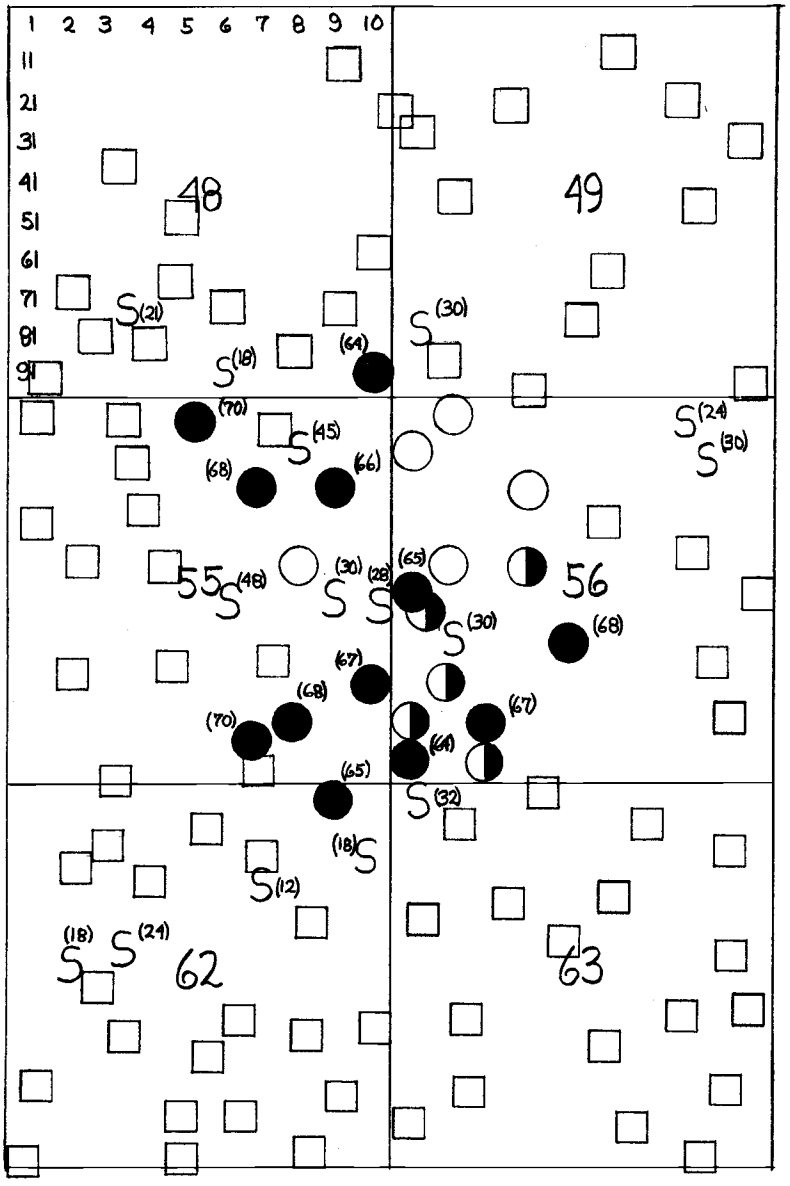
Vegetation in infection center 2 (Study Area I, Figure 5) was sampled through recovery of fungal tissue from roots of saplings and stumps. Shrubs were not sampled. Mycelial tissue was taken from 10 of 12 armillaria-killed saplings in this center. Wood of four oppositely facing roots on each of seven stumps of old-growth PP was collected for sampling.

Mortality in infection center 3, Study Area II (Figure 6, 7) consists entirely of seedling PP. Mycelial tissue taken from the cambium of the killed seedlings (16) was used for recovery of A. mellea. Stumps and shrubs were not examined. Twenty-one PP seedlings were killed by A. mellea in this center, however, five had been previously removed leaving 16 for isolation purposes.

In addition to the above isolate sources twelve sampling sites were established around Study Area I. These sites are one-eighth, one-fourth, and one-half mile distant, in the cardinal directions, from the center of the Study Area. Sampling involved recovery of armillaria tissue (rhizomorphs and mycelium) from several shrubs or pine at each location. Armillaria mellea was recovered from 10 of the 12 sites examined. These isolates recovered are designated as "P" isolates.

Armillaria isolates recovered in central Oregon outside the Pringle Butte area were used as controls in all tests of clonal relationships. These collections were recovered from 1) PP, 75 mi N,

Figure 5. Plat of infection center 2 showing locations of stumps, excavated trees, and healthy appearing, unexcavated trees over 6 feet tall. Five foot square subdivisions are numbered as in block 48.



Key

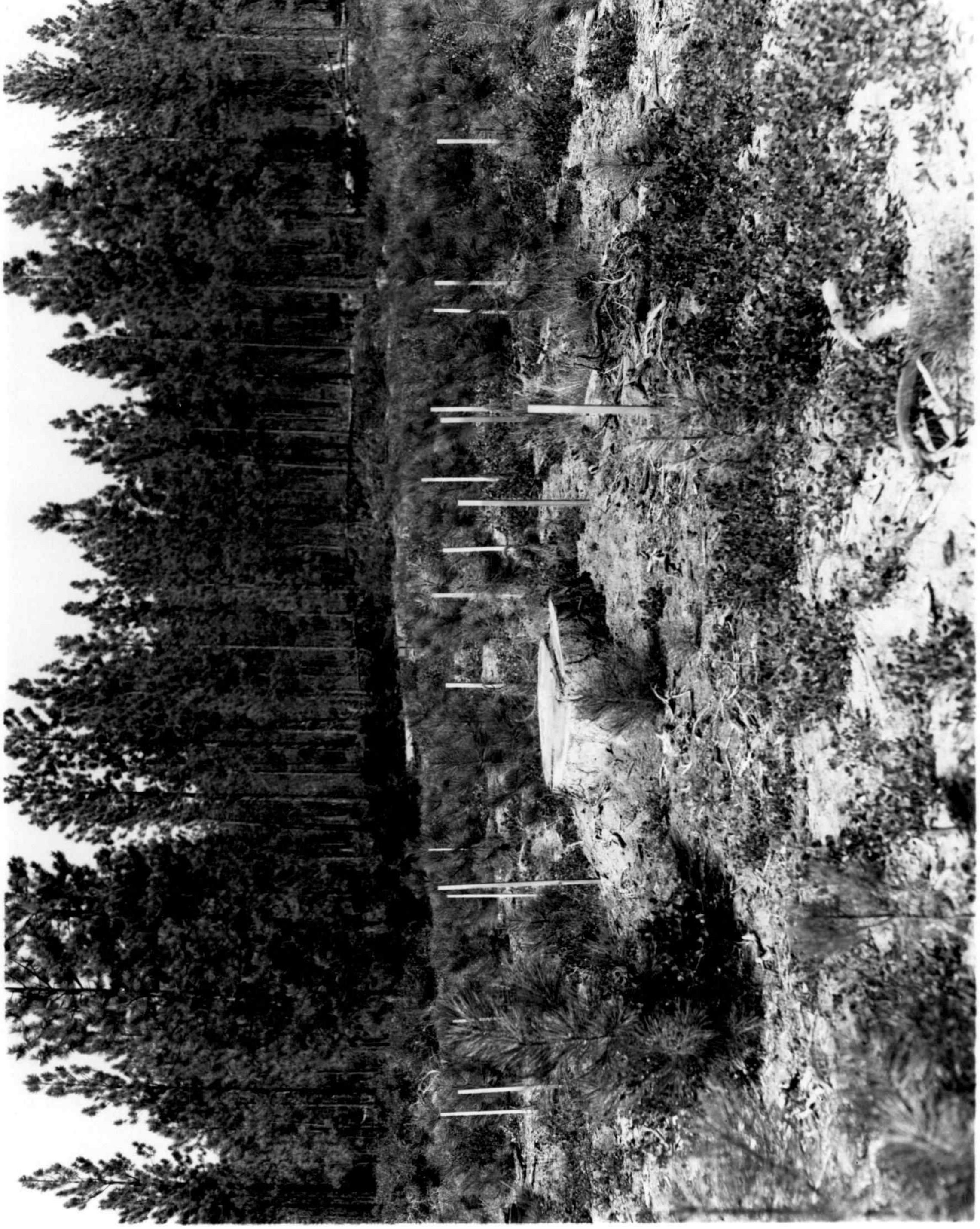
Excavated trees:

- ○ A. mellea free
- ◑ A. mellea attacked, pitching, living
- ● A. mellea attacked, pitched, dead (year died)

□ Unexcavated, healthy appearing PP

S Stump with diameter (inches)

Figure 6. Part of infection center 3 (Study Area II) with 48 inch diameter stump in foreground. Stakes mark site of armillaria-killed PP seedlings.



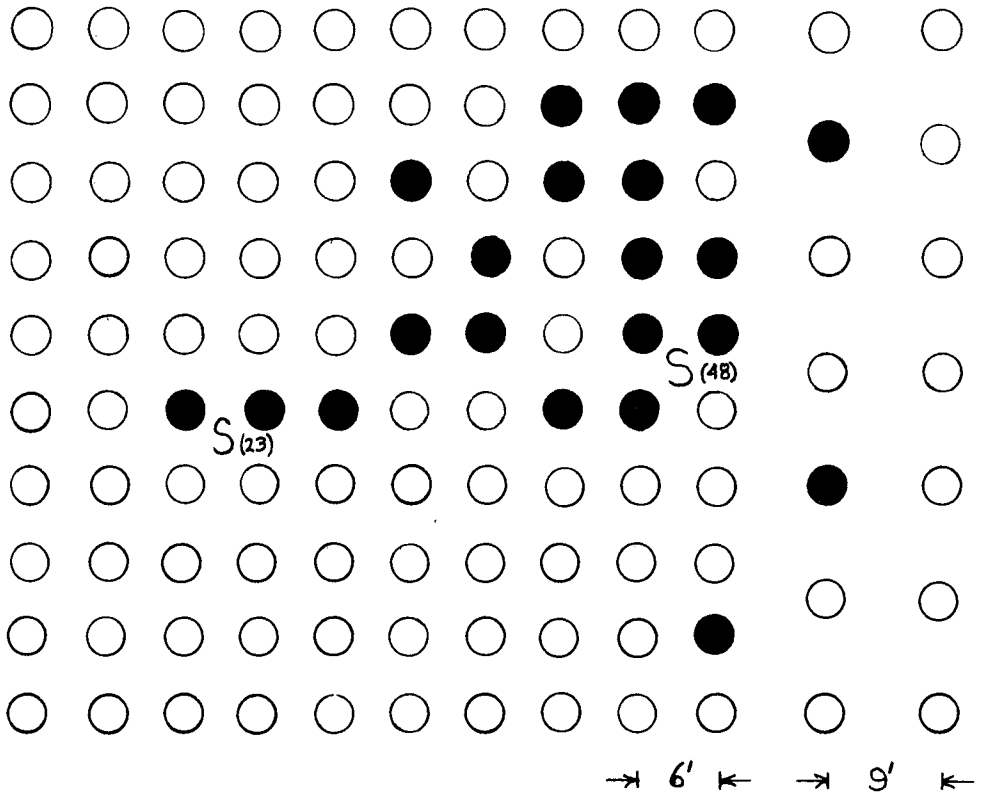


Figure 7. Plat of infection center 3 (Study Area II) in seedling PP planted in 1966.

2) PP, 90 mi N, and 3) incense cedar (Libocedrus decurrans L.), 85 mi N of Pringle Butte. Their accession numbers are 5, 10, and 9 respectively.

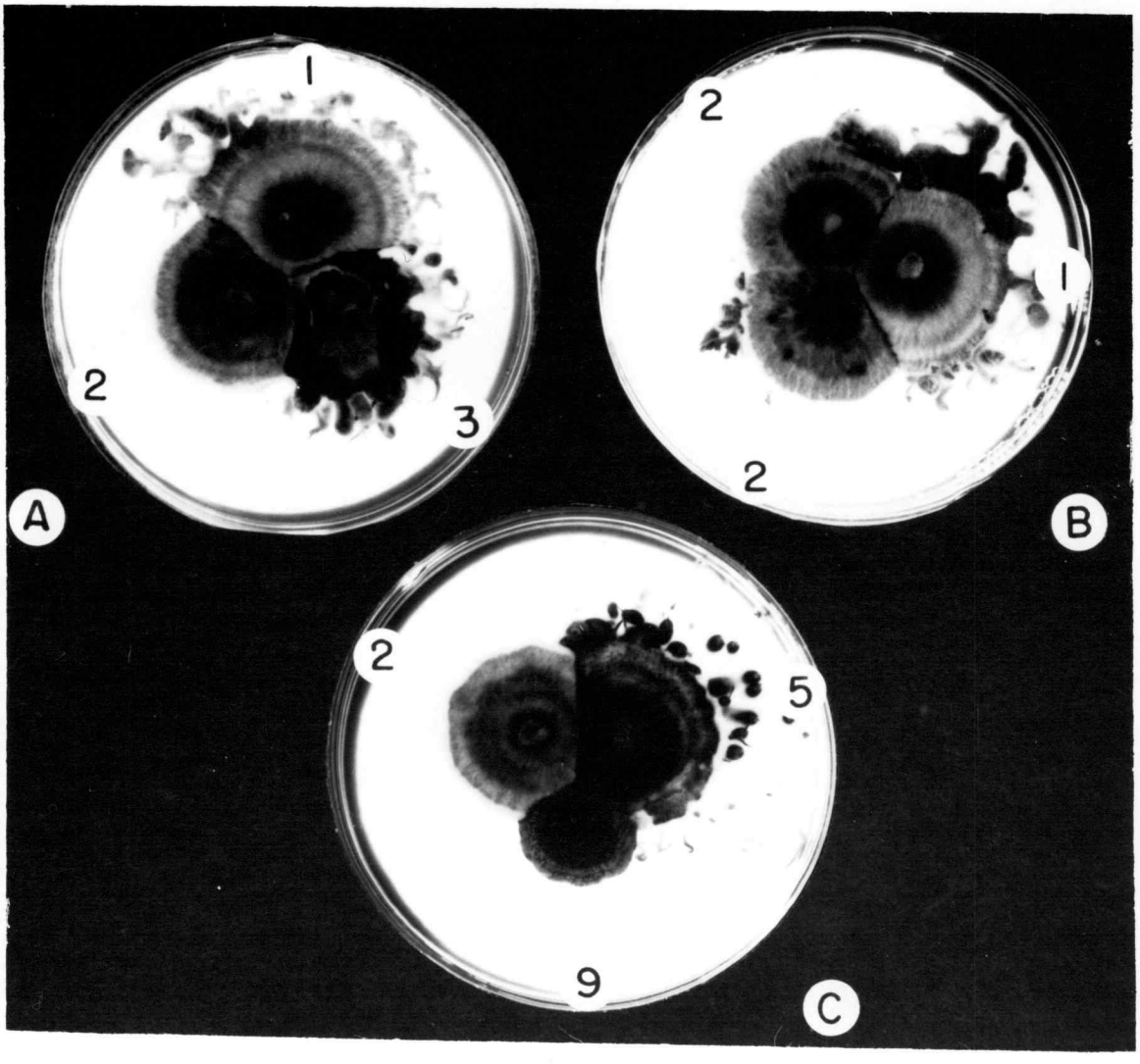
The laboratory methods brought together in paired culture all armillaria isolates from the Study Areas and peripheral plots on Pringle Butte to enable their separation into compatibility groups.

The results of each combination of paired isolates were evaluated according to the interaction between the colonies. Isolates that intermingled in paired culture were considered to be compatible and of the same parent mycelium; while isolates that did not intermingle and formed a dark line along the front of mycelial contact, were considered to be of dissimilar parentage (Figure 8, Adams and Roth, 1965; Childs, 1963; and Mounce, 1929). The MDP medium was used in all compatibility tests. Cultural interactions for each pairing combination were complete in 21 days. All colonies were dark-grown at 25 C.

Records were kept on the type of interaction (compatible vs. noncompatible) of all cultural pairings. Compatibility interactions among paired colonies were usually distinct of themselves alone, however, all the final relationships observed were also indirectly confirmed through compatible or noncompatible interactions among colonies belonging to the same or different compatibility groups.

Colony appearance was also a useful aid in identifying clonal

Figure 8. Paired colonies of A. mellea showing compatibility relationships among isolates representing groups A, B, and C. In this figure the compatibility groups are designated as follows: A = 1, B = 2, and C = 3. Colonies 5 and 9 represent other compatibility groups and were isolated away from the Pringle Butte locality. Demarcation lines are formed between 1, 2, and 3 (plate A). Colony 2 is compatible with itself and noncompatible with colony 1 (plate B). All colonies are noncompatible with each other in plate C.


1
2
3
A
2
2
1
B
2
5
9
C

lines. Among the more important features were relative abundance and morphology of rhizomorphs, color and morphology of surface mycelium, and the occurrence of mycelial exudations.

Excavation of PP in Infection Centers 1 and 2

Living and armillaria-killed pine saplings in infection centers 1 and 2 (Figure 4, 5) were excavated to allow examination of armillaria associations with the roots. Each root system examined involved one to several tap roots and was exposed to the D horizon. Lateral roots were exposed along a 1.5-2.0 foot radius centered on the root crown. Care was taken during excavation to achieve minimum disturbance of rhizomorphs, rhizomorph/root associations, and roots (living or dead) of all plants in the pit.

Sapling root systems, with associated rhizomorphs and roots from other plants, were sketched in two views: one, directly down on the entire root system, and the other a side view. Notes taken on the extent of armillaria attack included lesion development on roots of other trees in the pit, as well as on roots of the sapling. Rhizomorph/root associations were also included in the sketches.

Age data for PP was obtained through ring counts at ground level of excavated saplings, and indicated both total age of the trees and the number of years they have lived since being released in 1958.

Pathogenicity and Host Specificity of Isolates

Early in the study it was noticed that viable rhizomorphs were often found physically attached to roots and root crowns of the larger BB, SB, and M plants. The attachment was such that the rhizomorphs would split longitudinally when pulled from the roots. These shrubs appeared to be in good health and not suffering from the fungus.

The pathogenicity and host specificity of three armillaria isolates were tested on potted seedlings of PP, Douglas-fir, BB, and SB. Armillaria isolates recovered from PP, BB, and SB on Pringle Butte were used as inoculum. The isolates from PP and SB are identical genetically as judged from their compatibility reactions in paired culture although recovered from different hosts, and are genetically distinct from the BB isolate.

Ponderosa pine seedlings from the U. S. Forest Service nursery, Bend, Oregon; Douglas-fir seedlings from the state forestry nursery at Elkton, Oregon; and wildlings of BB and SB from Pringle Butte were potted in no. 10 tin cans. Plants endemic to the Pringle Butte area were potted in a soil mixture of 5 parts pumice soil to 2 parts greenhouse soil (a river-bottom sandy loam). Douglas-fir seedlings were potted in a 1:1:1 mixture of leaf mulch, garden soil (Willamette Valley), and greenhouse soil. A 1.8 × 15 cm test tube was buried upside down close to the tap root during potting to facilitate inoculation

and to avoid damaging the roots during the inoculation process.

All plants in tins were buried to root crown depth in a partially shaded location at the Pringle Falls field station in the fall of 1967. The plants were watered occasionally during the summers of 1968 and 1969.

Inoculum employed in artificial inoculation of the test plants consisted of branch segments (1/2-3/4 × 2 1/2 in) of red alder (Alnus rubra Bong.) to serve as food bases. These segments were autoclaved for 1 hour at 17 pounds pressure, then buried upright in a mixture of 450 g each of Douglas-fir sawdust, Quaker oats (old-fashioned), and corn meal (Albers' yellow enriched and degerminated) in one gallon jam jars. All jars were moistened with one liter of 5 percent malt-extract then autoclaved at 17 pounds pressure for 2 hours. The jars, excepting the controls, were inoculated individually with one of the three isolates and incubated in the dark at room temperature for 4 months. Control jars were not inoculated.

In July 1968, the infected and control alder stem segments were removed from the sawdust-meal medium and inserted into the soil of each pot in place of the test tube. Pumice soil was firmed in over the inoculum segment in all pots.

The inoculated seedlings were examined after a period of 21 months. Eighty plants, representing half of the plants of each host-parasite combination were transferred to the greenhouse at Oregon

State University in November, 1968. The remainder were transferred in November, 1969. Roots and alder inoculum segments of all plants were examined for growth and development of A. mellea in April, 1970. Pitch and mycelium were looked for particularly in plants that died during the study.

Free soil was easily washed from the roots of plants in pumice soil. The heavy soil of the Douglas-fir pots was more difficult to remove without damaging armillaria/host relationships. This soil was thoroughly dried, then remoistened to wash the soil from the roots.

The alder inoculum segments were split longitudinally and rated for extent of decay. Extent of development of rhizomorphs in the soil and on host roots was noted. Seedling tissue in contact with rhizomorphs was examined for presence of parasitic attack by A. mellea.

Effect of Plant and Soil Extractives on Growth of A. mellea in Culture

Extracts from plants and soil of Pringle Butte were tested for their effect on growth of A. mellea in culture. The extracts were derived from plant tissue and soil collected in and around Study Area I. Growth measurements are based on mycelial dry weight, and rhizomorph production and elongation.

Fresh foliage and roots were collected from PP, SB, and BB in the spring and fall. Plant litter was collected in the spring from PP, SB, and BB, and in the fall from PP and SB only. Soil was collected in the fall only, from beneath the crowns of PP and SB, and as a control from an undisturbed, non-vegetated site.

The age of fresh foliage in spring collections varied with the plant source. Needles of PP were 2-5 years old. SB foliage was entirely of leaves formed the previous season, and foliage of the deciduous BB was of leaves formed in the current year.

Fresh foliage collected in the fall from PP consisted of the current years' growth plus needles from the past 2-4 years. Seasonal needle drop had been completed by the time of the fall collection. Fall collections of SB and BB foliage were of the current season's growth.

Fresh foliage collected from PP and SB was stripped from each plant in the field, sealed in plastic bags to avoid dehydration, and stored at -16 C within 8 hours of collection. BB foliage is small and difficult to remove from the plant in the field. A small sample of the foliage for dry weight determination was removed in the field and stored at -16 C within 8 hours of collection. However, the bulk collection of foliage for extraction was accomplished by oven-drying (50 C) small, foliated branches in paper bags. After 96 hours in the oven, the dried foliage could easily be shaken from the branches and

collected.

Living roots, 1/4 to 1/2 in. in diameter, free from A. mellea infections and rhizomorphs, were collected from PP, BB, and SB in the spring and fall. Root collections were stored at -16 C within 8 hours of recovery.

Both spring and fall litter from PP and SB were readily collected from the forest floor. Litter of BB was collected in the spring only and was separated from older material by screening over a one-eighth inch mesh screen. Spring litter collections represent foliage dropped the previous fall. Fall litter collections are of foliage dropped in the current season and collected before exposure to the fall rains.

Soil collecting points beneath the crowns of PP and SB are based on crown diameter. Circles were drawn on the ground at one-third and two-thirds the radial distance out from the plant center. Soil samples were taken from three points equally spaced around each of the two circles. These samples at ground level were from the A horizon. Similarly, soil samples were recovered from the AC horizon at points one foot deep directly beneath the A horizon sampling points. Thus, three soil samples represented each of four general sampling sites (Figure 9).

For each horizon, the three soil samples from three points on the same circle were mixed to yield a single composite collection

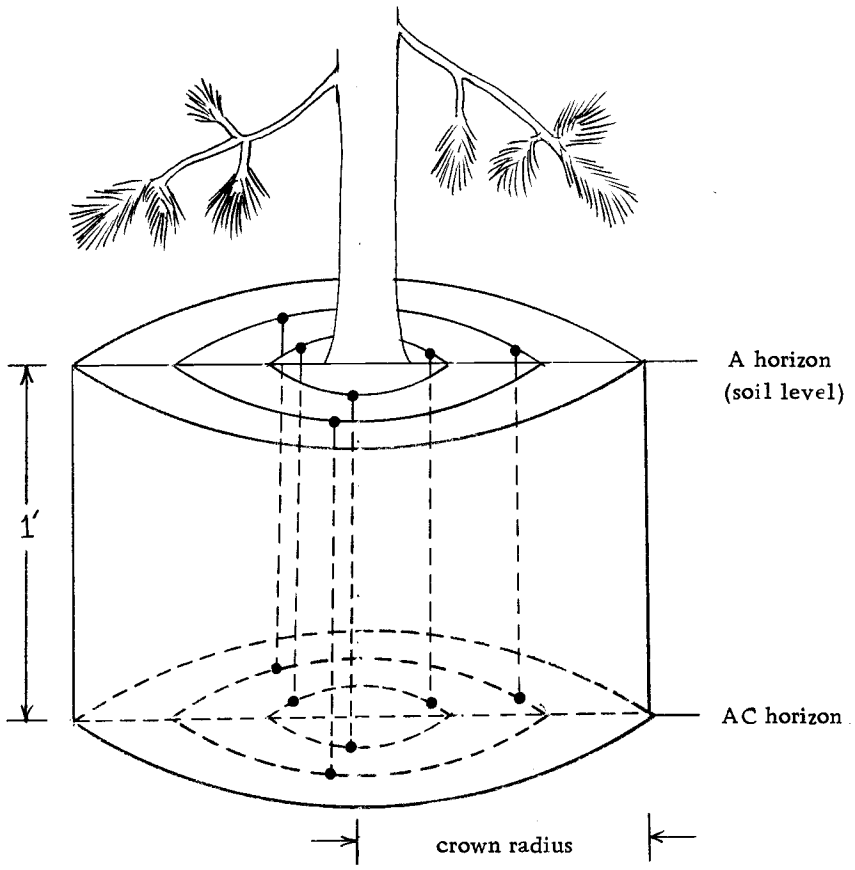


Figure 9. Diagram of soil sampling locations in A and AC horizons. The dots in each of the four circles mark sources of soil for the four composite samples. The vertical dimension in the sketch is exaggerated.

resulting in a total of four soil collections from under each plant.

Soil was collected for the control series from the A and AC horizons where natural openings occurred in the plant cover. Three samples from each horizon were mixed to provide a collection.

Plant tissue extractions were performed as follows. First, plant materials (foliage, litter and roots) were dried to a constant weight at 50 C and ground to pass through a 2 mm mesh screen. Extracts were prepared by mixing 50 g plant tissue (dry weight basis) with 250 ml distilled water and extracting without agitation at 5-8 C for 24 hr. After extraction, 150 ml distilled water were added to allow recovery of at least 250 ml extract. Solids and liquid were separated by centrifuging at 8,000 × G for 15 minutes. The extract was clarified by passage through ED 613 filter paper with suction on a Buchner funnel.

Extracts of soil material were prepared by mixing 90 g of the composite sample with 450 ml distilled water, and extracting without agitation at 5-8 C for 24 hours. The extract was clarified by passage through ED 613 filter paper under suction.

Preparation of growth media involved addition of the extracts to a basic medium prepared to give a final concentration of 1.0 percent malt-extract and one-half percent agar. This was supplemented with plant extract to give one of four final extract concentrations (1:1600, 1:168, 1:61, 1:40). A 1.0 percent malt-extract series

lacking plant extractive acted as a control for each test. An additional test series consisting of unsupplemented malt extract 1.0, 2.5, and 5.0 percent was included in the experiment. All media were adjusted to pH 5.2-5.3 and autoclaved for 10 minutes at 17 pounds pressure. While still liquid the media were pipetted into 4 oz. prescription bottles in 20 ml aliquots. The bottles were capped, and the medium reautoclaved for 10 minutes, after which they were laid on their flat sides to cool.

The various soil extracts were added to the basic medium as in preparation of media containing plant extractives. Soil extractives added gave one of three final extract concentrations (1:60, 1:30, and 1:15). An unsupplemented 1.0 percent malt extract series served as a control. All media were adjusted for pH and prepared as with the plant extractives.

Inoculum in this study of substrate and growth relationships consisted of two armillaria isolates recovered from PP and SB on Pringle Butte. The inoculum was grown centrally on a medium of 1.0 percent malt-extract and 2.0 percent agar in 9 cm Petri dishes for four weeks in the dark at 25 C.

Plugs (no. 2 cork borer) taken from the edge of the colonies were used to inoculate the various extract media and the controls in the prescription bottles. The inoculum plug was pushed into the semi-solid, one-half percent agar medium containing the extracts.

Three replicates were employed of each fungus/substrate combination. The cultures were incubated in the dark at 20-24 C for 2, 3, and 4 weeks, then evaluated for growth.

Colonies were rated for mycelial dry weight, and for production and elongation of rhizomorphs at the end of each growth period. No estimate was made of numbers of rhizomorph initials; only rhizomorphs that had elongated 2 mm or more were recorded.

To determine mycelial dry weight the prescription bottles and contents were autoclaved for 10 minutes to melt the agar prior to harvesting the mats. Mats were separated by filtering under suction in a Buchner funnel through tared ED 613 filter papers. Mycelial dry weights were taken after drying the mats and filter paper for 72 hr at 75 C.

Rhizomorph production was estimated through a rating system based on the numbers of rhizomorphs growing past an 18 mm diameter circle centered over the inoculation point. Rhizomorphs less than 18 mm in length were scored 1, while rhizomorphs of greater length were scored 2.

Rhizomorph elongation was expressed as the mean length in mm of the five longest rhizomorphs in the bottle.

RESULTS

Isolates of A. mellea recovered from the two Study Areas on Pringle Butte were found to belong to three culturally distinguishable, compatibility groups. Excavation of roots of healthy, diseased and dead PP saplings revealed infections of the root crown to be lethal, while infections of lateral roots were not related to killing of the host. Inoculation of potted conifer and shrub seedlings revealed differences in pathogenicity between A and C group isolates. Cold-water extracts of certain vegetation stimulated growth of A. mellea in culture.

Distribution of Armillaria mellea

Two hundred thirty-five isolates of A. mellea were recovered from Pringle Butte. Of these, 208 were from Study Area I, 16 from Study Area II, and 11 were from the peripheral plots (Table 1).

Three distinct groups of isolates (A, B, and C) as based on mycelial interactions in paired culture, were identifiable from Study Area I. All isolates of a group are intercompatible in paired culture, while paired isolates representing different groups are not compatible.

Isolates belonging to group A were found on killed PP saplings, stumps of the former overstory and living shrubs; group B isolates

Table 1. Frequency of recovery of 235 armillaria isolates tabulated by general recovery site and compatibility group.

Recovery site	Compatibility group			
	A	B	C	other[1]
	number			
Study Area I	49	12	147	-
Study Area II	16	-	-	-
Peripheral Plots	-	1	4	6

[1] Isolates not compatible with members of the A, B, or C groups.

were recovered from killed PP saplings and living shrubs, not from stumps; while group C isolates were found on stumps and living shrubs. Group "A" is comprised of 65 isolates, group "B" of 12 isolates, and group "C" of 147 isolates (Table 1).

Isolates of group A are quite similar in cultural appearance to those isolates comprising group B, yet a distinct dark line of demarcation (as viewed through the bottom of the Petri dish) is always formed in paired culture between colonies of these two groups. However, some differences in colony growth are apparent on examination of colony appearance. Group A isolates produce rhizomorphs that often reach a length of 30 mm in 3 weeks, while rhizomorphs from B group isolates seldom reach 15 mm in 3 weeks. Also, very short rhizomorphs are consistently formed on the surface of the inoculum piece in group A isolates and never in group B isolates. An additional feature of significance useful in separating the two groups can be noted in Figure 8, Plate B. Between colonies 1 (group A) and 2 (group B) is a line of dark-colored droplets of liquid that always forms on the mat surface along the contact zone in group A/B pairings.

Group C isolates produce rhizomorphs much more abundantly in culture than do group A isolates, and they develop relatively little aerial mycelium. In summary, cultural appearance among isolates of a group was quite constant on MDP medium and always supported

decisions based on the presence or absence of demarcation lines in paired culture in determining group affiliations.

Distribution of isolate groups in Study Area I are as follows. Group A isolates were recovered in mycelial form from armillaria-killed saplings and pine stumps, and in rhizomorph form from shrubs throughout Study Area I (Table 2).

Isolates of group B were recovered as mycelium from scattered killed pine saplings away from the infection centers, and in rhizomorph form from SB widely scattered in Study Area I (Table 2). This isolate was also recovered incidentally from a dead sugar pine pole (Pinus lambertiana Doug.) one-half mile northwest of the study area.

C group isolates were found in rhizomorph form primarily on roots of BB, M, and SB as living hosts in Study Area I (Table 2). Isolates of this group also occurred as rhizomorphs within hollow roots in the soil, and on the surface of root crowns of large stumps in infection centers 1 and 2. This isolate was not found on armillaria-killed PP seedlings or saplings.

Distribution of isolate groups in infection centers was as follows. Isolates of group A were recovered from infection center 1 in mycelial form from 14 armillaria-killed saplings (Figure 2, 4). Isolates were not recoverable from two seedlings (37-5a and b) killed about 1965. One stump only (22-80; cut in 1952) was examined in

Table 2. Frequency of occurrence of isolates of Armillaria in each of three compatibility groups as related to source of recovery in Study Area I.

Source	Compatibility group		
	A	B	C
	number		
Shrubs (BB, M, SB)	15(rh)[1]	5(rh)	134(4h)
Sapling pine:			
scattered trees	8(m)	7(m)	-
infection centers	23(m)	-	-
Stumps:			
infection centers	2(m)	-	7(rh)
Rhizomorphs in soil	-	-	6(rh)
Fruit body	1(fb)	-	-
Subtotals	15(rh) 33(m) 1(fb)	5(rh) 7(m)	147(rh) = 167(rh) = 40(m) = 1(fb)
Totals	49	12	147 = 208

[1] Fungal tissue from which rmillaria was isolated:
rh = rhizomorph, m = mycelium, fb = fruit body

this center with results as follows. Seven isolates of group A were recovered from wood of the roots of this stump, while C group isolates were recovered from rhizomorphs on the surface of four roots of this stump. Group C isolates were recovered in seven instances from rhizomorphs on roots of living BB and SB shrubs around a killed sapling (29-90), and in six instances were recovered from rhizomorphs pulled from hollow pine roots beneath the sapling. Members of group B were not found in this infection center.

Group A isolates were recovered in infection center 2 as mycelium from nine armillaria-killed trees (Figure 2, 5). No attempt was made to recover armillaria isolates and establish their identity from two trees dying in 1970 as the disease progressed in this infection center. One group A isolate was recovered from the wood of one of seven stumps examined in the center (map location 55-60), and from a sporophore on the base of stump 55-18. This sporophore was the only mature armillaria fruit body found on Pringle Butte during this study. Eleven group C isolates were recovered from six of the seven stumps examined (49-81, 55-18, -56, -59, -60, 56-62) as rhizomorphs on the surface of the stump root crown. Only stump 63-1 failed to yield group C isolates. Members of group B were not found in this center.

Armillaria mellea was recovered in infection center 3 from 16 of 21 killed, seedling ponderosa pines (Figure 7). All isolates

were of the A group. Roots of the two large stumps were not examined, however, rhizomorphs were observed arising from a lateral root of the large stump adjacent to a dead seedling. The distance between stump root and seedling root crown was 4 inches.

Armillaria mellea was recovered in 10 of the 12 peripheral plots, plots one-half mile north, and one-half mile east of the center of Study Area I failed to yield the fungus. Group A isolates were not recovered from the peripheral plots. One B group isolate was obtained from a dying sugar pine sapling on a peripheral plot one-eighth mile west of Study Area I. Four representatives of group C were recovered. They were located one-eighth (BB) and one-half (BB, SB) mile south, and one-fourth (BB) mile east.

Armillaria isolates not belonging to the A, B, or C groups were also recovered from shrubs on the peripheral plots. One group of three culturally compatible isolates was recovered from widely scattered locations; SB, one-fourth mile north, and M, one-fourth, and one-half mile west of Study Area I. Finally, three individual isolates, not culturally compatible with each other nor with any of the other isolates recovered on Pringle Butte, were obtained from SB, one-eighth mile north; BB, one-eighth mile east; and SB, one-fourth mile south of Study Area I.

Excavation of PP in Infection Centers 1 and 2

A total of 33 seedling and sapling PP were excavated in infection centers 1 and 2 (Table 3, 4). An additional 12 trees listed in the Tables were examined in less detail in the absence of excavation. Living, dying, and armillaria-killed pine were included. Ages of these trees ranged from 8 to 91 years.

The root systems of all excavated pine were examined for armillaria-caused lesions (Figure 10, 11). Lesions were probably present on lateral roots extending beyond the excavated area, especially in trees over 20 years of age, but roots extending beyond the pit perimeter were not routinely examined.

Lesions were found on lateral roots of 6 of 8 excavated living trees over 20 years old, and on the root crowns of 5 of these trees. Mycelial development was not found on these trees between the lesions on lateral roots and the root crown.

Lesions were found on the lateral roots of 12 of the 20 trees (living and dead) over 20 years of age, and on the root crowns of 17 of these trees. The average number of lesions on lateral roots per tree for the 20 trees is 1.9, with a range of 0-7 lesions per tree. The average number of lesions per tree found on all excavated trees (Table 3, 4) is 1.4.

Copious production of pitch occurred on the root crown and

Table 3. Progress (through 1970) of mortality and resinosus resulting from attack by A. mellea on young PP remaining after overstory removal and thinning of the understory (1957-58). Infection center 1.

Map location (Fig. 4)	Number of years of tree life		Year of death [1]
	Before thinning	After thinning	
23-91	74	4	1961
37-14	7	5	1962
22-89	52	6	1963
23-51	71	7	1964
37-68[5]	56	7	1964
23-16[5]	61	8	1965
37-5a	0	8	1965
37-5b	0	8	1965
29-90	36	9	1966
30-72	18	9	1966
23-44[5]	64	9	1966
23-96	62	9	1966
23-53[5]	56	10	1967
29-19	65	11	1968
29-70	46	12	1969
23-65	61	12	1969
30-95	0	11[3]	2
23-32	73	13	2
22-39	78	13	2
22-57	65	13	2
23-94[5]	4	13	2
23-12[5]	4	13	2
23-37[5]	4	13	2

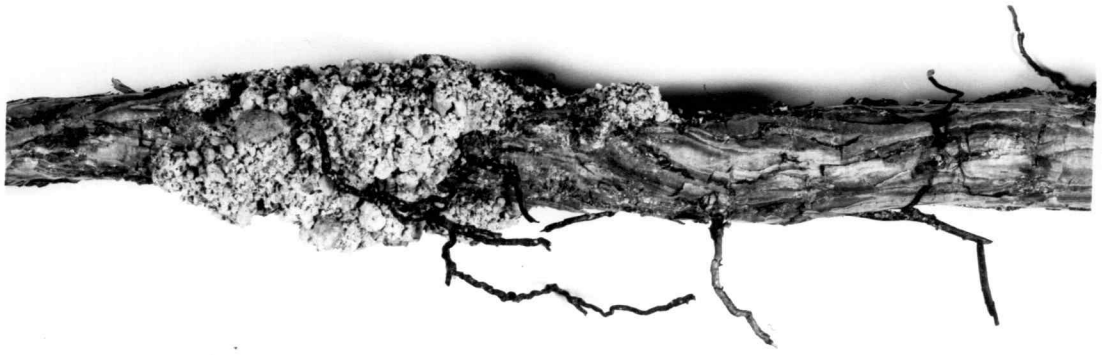
[1] Resinosus on root crown of all trees above dashed line.
[2] Tree living through 1970.
[3] Tree 11 years old in 1970.
[4] Data not collected.
[5] Tree not excavated.

Table 4. Progress (through 1970) of mortality and resinosus resulting from attack by A. mellea on young PP remaining after overstory removal and thinning of the understory (1957-58). Infection center 2.

Map location (Fig. 5)	Number of years of tree life		Year of death[1]
	Before thinning	After thinning	
56-91	9	7	1964
48-100	54	7	1964
56-51a	35	8	1965
62-9[5]	44	8	1965
55-29	0	9	1966
55-80	0	10	1967
55-88[5]	5	10	1967
56-83	55	10	1967
55-27	0	11	1968
56-65[5]	4	11	1968
55-5[5]	4	13	1970
55-97[5]	4	13	1970
56-51b	0	11[3]	2
56-44	2	13	2
56-81	26	13	2
56-93	38	13	2
56-62	55	13	2
56-2	1	13	2
55-48	2	13	2
56-42	5	13	2
56-24	7	13	2
56-11	13	13	2

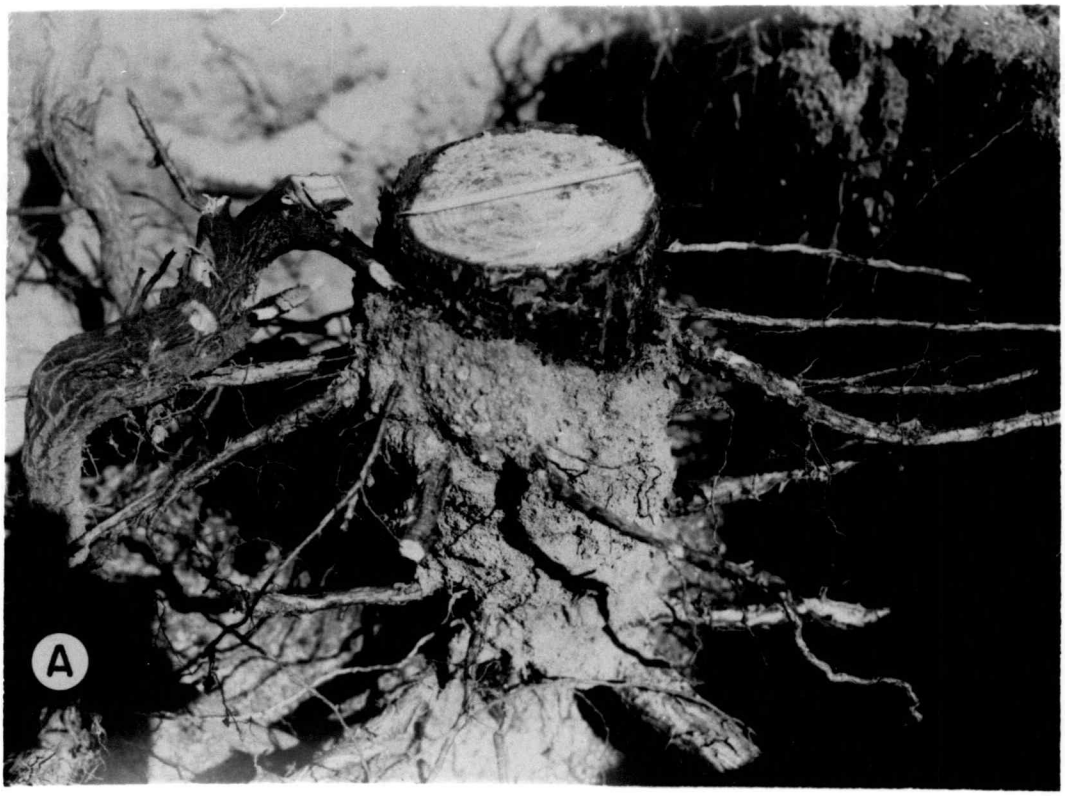
[1] Resinosus on root crown of all trees above dashed line.
[2] Tree living through 1970.
[3] Tree 11 years old in 1970.
[4] Data not collected.
[5] Tree not excavated.

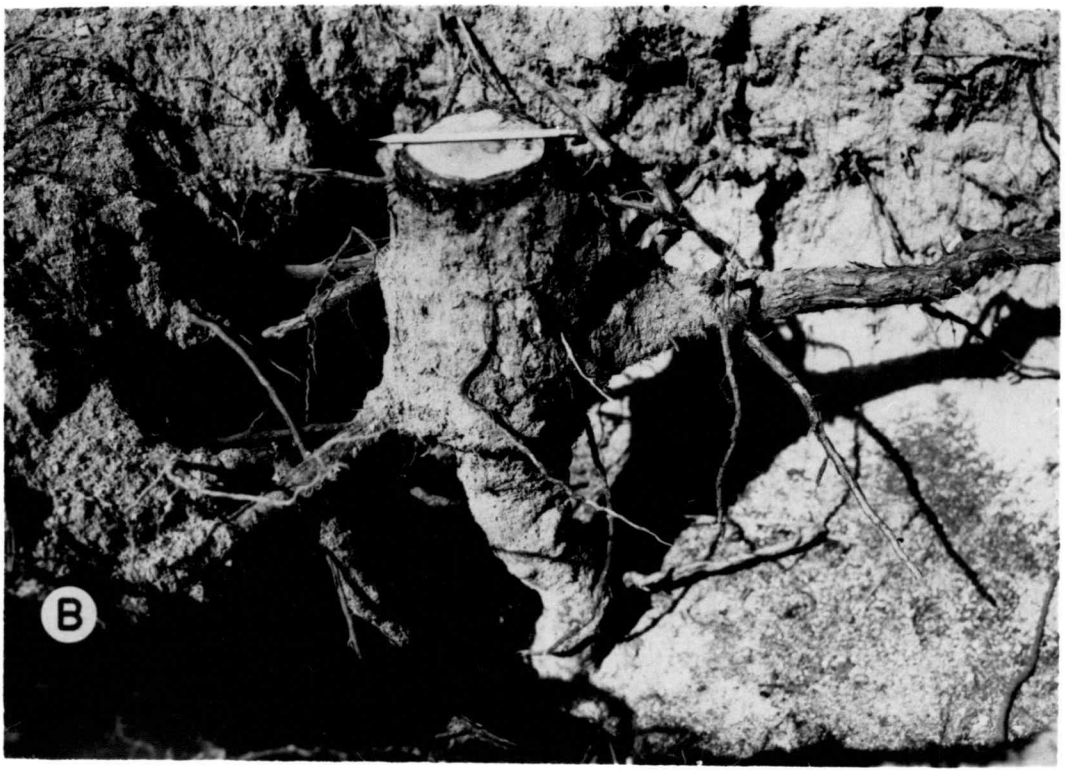
Figure 10. Armillaria-caused lesion on lateral root of pine sapling. The dark root-like rhizomorphs are clearly seen on the pitch impregnated pumice adhering to the wound site.




0 FULL SIZE-32 1 2 3 4 5

Figure 11. Excavated root systems of saplings 29-19PP (A), and 23-91PP (B), of infection center 1. Tree A appeared normal through 1968, but did not initiate growth in 1969. The crown was slightly off-color in early 1969 at the time of excavation. Tree B died in 1961. Note similarity in amount and location of pitching on root crown and upper tap root of both root systems.


A


B

upper tap root (Figure 11, 12) of all dead trees, and on 10 of 17 living trees examined (Table 3, 4). Mycelial development was either not present or limited to small pockets in the cambium of those trees with the earliest above-ground symptoms of armillaria attack, i.e., cessation of terminal growth and a barely perceptible dulling of the normally bright green foliage. Extensive mycelial development in the root system was found only in those trees that had been dead at least one year.

Growth of trees killed by A. mellea was not retarded prior to the time of death. Annual diameter increment and height growth did not reflect the duration of attack through growth reduction. On occasion of certain severe attacks the cambium was killed locally resulting in formation of partially complete rings. Portions of 2 to 3 rings would be affected prior to death indicating that the attack preceded death by 2 or more years.

The relative importance of root contacts as opposed to rhizomorphs in spread of A. mellea is of concern. Direct contact between armillaria-inhabited stump roots and roots of killed sapling pines was not found, suggesting that spread of infection by root contacts was of little or no significance here. Rhizomorphs were found in the soil around the roots of both living and dead sapling pine. These rhizomorphs were either free in the soil or in hollow decayed pine roots (Figure 13). Rhizomorphs could readily be found imbedded in the

Figure 12. Longitudinal section through root crown and upper tap root of saplings 29-19PP (A, B), and 23-91PP (C, D) of infection center 1. Compare amount of pitching between the two sectioned roots. Mycelial development of A. mellea between the bark and the wood was lacking in 29-19PP, and well developed in 23-91PP. Tree 29-19PP died the year prior to excavation, while 23-91PP died eight years prior to excavation.

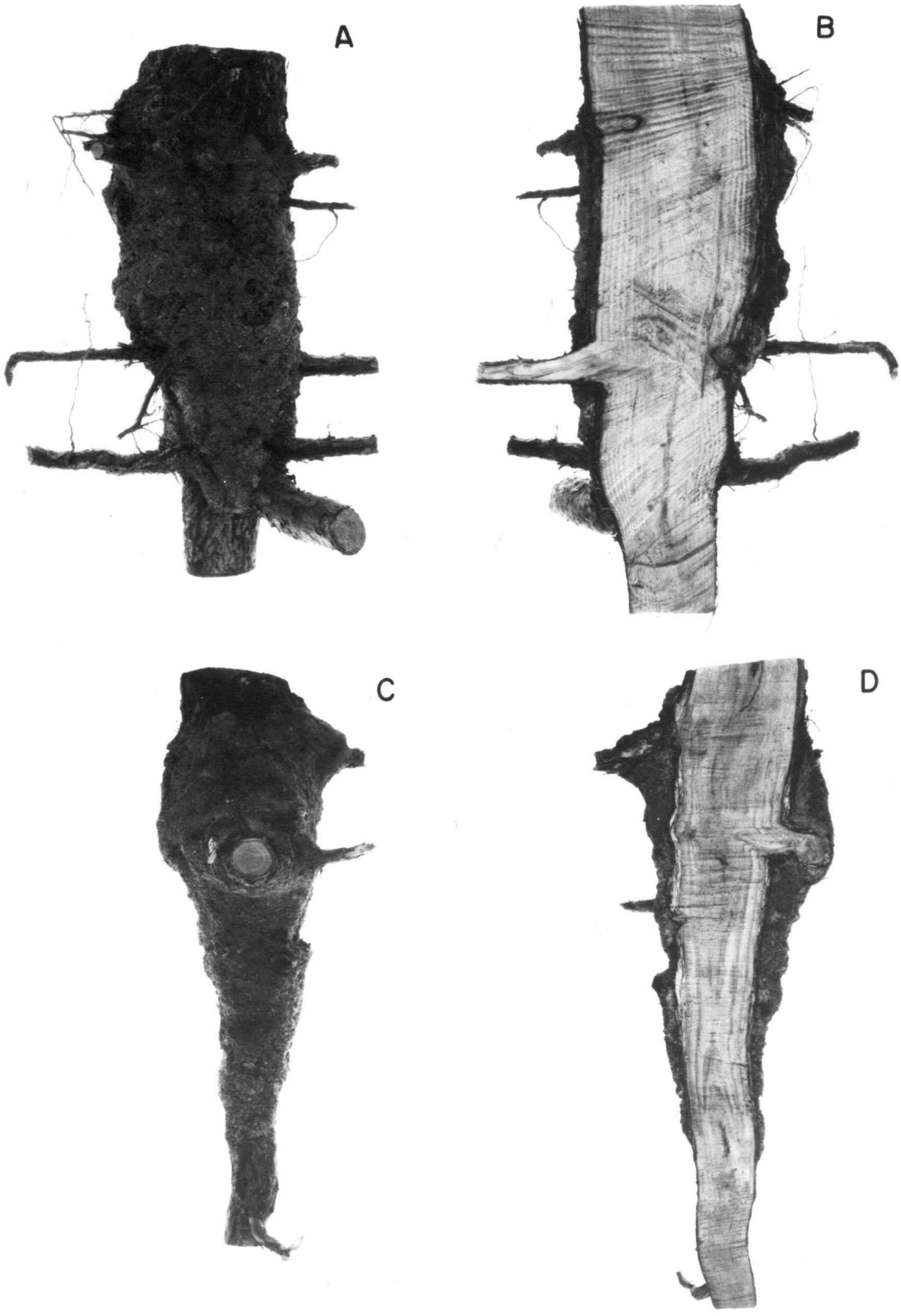

A
B
C
D

Figure 13. Rhizomorph development in rotten ponderosa pine roots from stumps of former overstory trees. Top: a rhizomorph restricted in further elongation following considerable branching in the soil upon emerging from the old root. Middle: a sparsely branched rhizomorph in a small, intact root. Bottom: moderately branched rhizomorphs in a larger root of one inch diameter.

pitch and pumice mass covering lesions on lateral roots and the root crown-upper tap root area of all dead trees and trees producing abundant pitch.

No attempt was made to trace individual rhizomorphs through the soil from food base to diseased pine sapling. In the course of excavating pine root systems, rhizomorphs were looked for as they occurred in the pits. It was noted that large and relatively unbranched rhizomorphs were found only in hollow roots of stumps of the former overstory. Smaller and more branched rhizomorphs were found free in the soil at the ends of intact hollow rootlets or associated with damaged hollow roots. Rhizomorphs in hollow roots were found at various depths within the confines of the excavation pits (Figure 14, 15) through the C horizon.

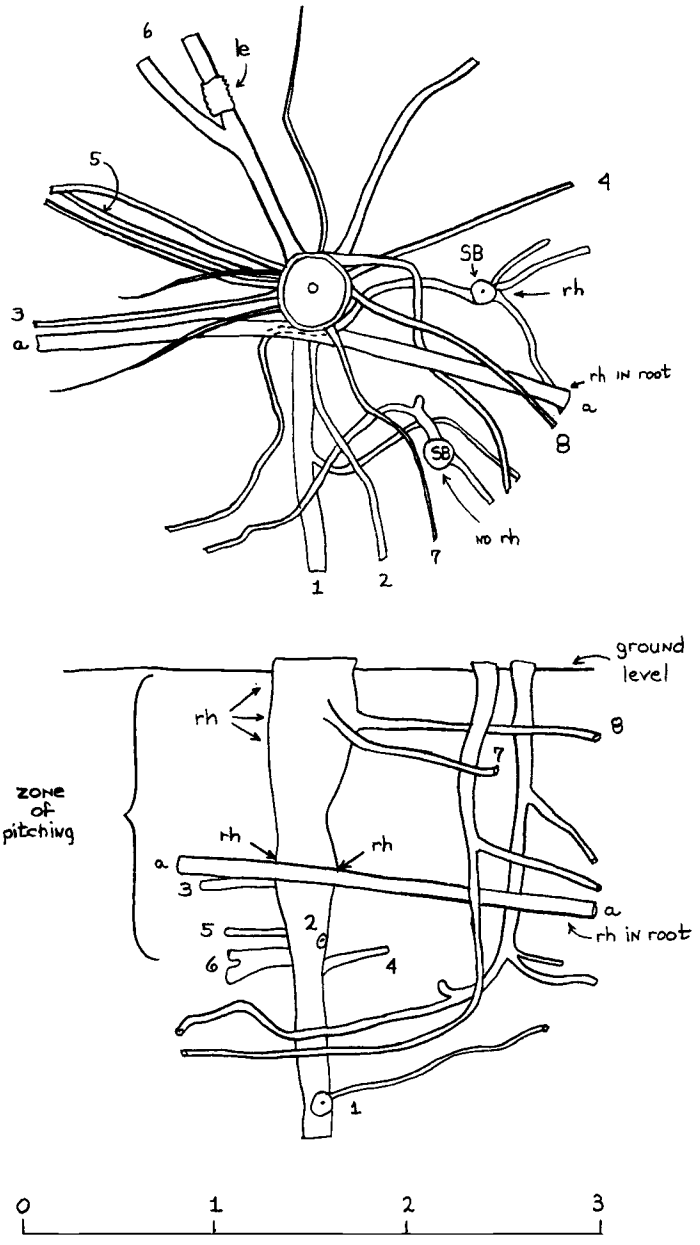
Pathogenicity and Host Specificity of Isolates

To test for specificity of pathogenicity, armillaria isolates of compatibility groups A and C were used to individually inoculate BB, SB, PP, and Douglas-fir seedlings.

Rhizomorphs of both the A and C groups were found on all the seedling species in pots (excepting controls) at the close of the study. Conifer seedlings were killed by the A group isolate and not by the C group isolate; shrub seedlings were not killed by either isolate.

Test plants were not killed in either the control series

Figure 14. Diagram of the root system of sapling 29-19PP, infection center 1, as viewed from above and one side. Position is shown of lateral and tap roots in relation to living and dead major roots of nearby vegetation.



scale in feet

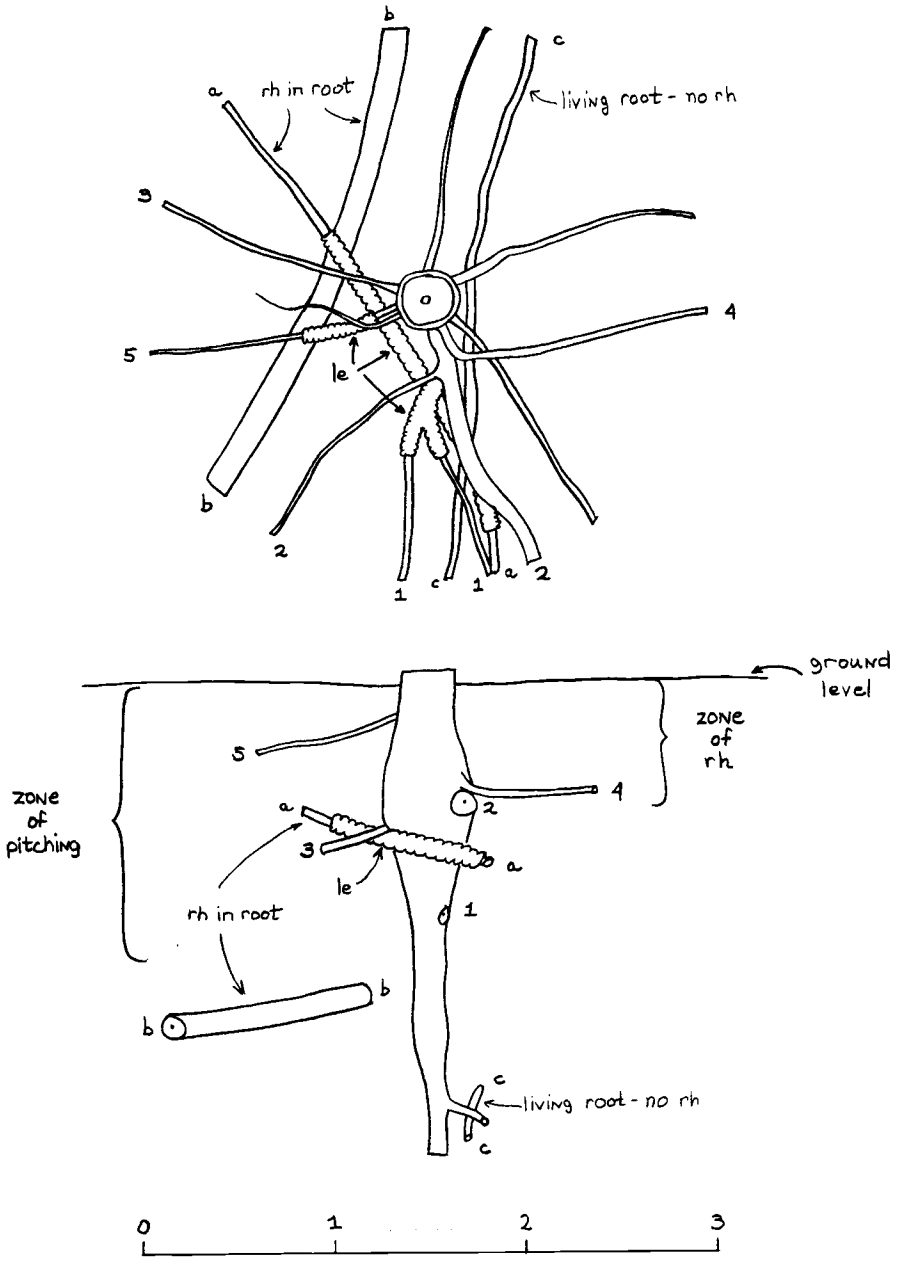
Tree:

Height	16.5'
D.B.H.	4.4"
Age	76 years

Legend:

rh	rhizomorph
le	lesion
a-a	continuous roots of tree outside pit area
3	comparative roots on above and side views
SB	snowbrush

Figure 15. Diagram of the root system of sapling 23-91PP, infection center 1, as viewed from above and one side. Position is shown of lateral and tap roots in relation to living and dead major roots of nearby vegetation.



Tree:

Height	13.7'
D. B. H	2.7"
Age	78 years

Legend:

rh	rhizomorph
le	lesion
a-a, b-b, c-c	continuous roots of tree outside pit area
3	comparative roots on above and side views

(non-inoculated alder branch segments), or the C group isolate series, however, viable rhizomorphs of the C group isolate were found on the root crowns and upper tap roots of the four test plant species, as in SB (Figure 16).

Ponderosa pine and Douglas-fir were susceptible to attack by the A group isolate, with 70 and 45 percent respectively, of the test plants being killed. Though the upper roots of the shrubs were physically encircled with living rhizomorphs of the A group isolate, these plants were not further attacked (Table 5).

Effect of Plant Tissue Extractives on Growth of *A. mellea* in Culture

Figures 17-25 showing the effect of plant extractives on growth of *A. mellea* in culture appear in the Appendix. The results were as follows. Foliage extractives stimulated growth of *A. mellea* in culture, while extracts from roots and litter generally did not stimulate growth. Soil extracts did not stimulate growth.

All foliage extracts supported growth of *A. mellea*. Greatest mycelial growth (mg dry weight basis) was obtained from cold-water extracts of fresh-dried foliage of PP and SB, while extracts of BB supported lesser mycelial growth (Figure 17). Growth of *A. mellea* on foliage extract of PP and SB, over the range of the four concentrations used, compared favorably with growth supported by the 1.0-5.0 percent malt extract controls. Spring foliage appears to give better mycelial growth than fall foliage for PP and SB, and

Figure 16. Inoculum piece, an alder branch segment (left) and rhizomorphs which developed during 21 months in the soil. Stem and root section of living SB seedling (right) with rhizomorphs attached to root crown. This physical attachment of rhizomorphs with roots of living SB, BB, and M is very common on older plants on Pringle Butte.

Table 5. Pathogenicity and host specificity of two armillaria isolates on SB, BB, PP, and DF (Douglas-fir). Armillaria isolates are from the A and C compatibility groups. Data recorded 21 months after inoculation.

Test plant	Isolate	Test plants	Plant response		Condition of inoculum		Test plants with rhizomorphs [1]
			Living	Dead	Living	Living and rhizomorphs	
			Number				
BB	Control	10	10	-	nc [2]	-	-
	C	10	10	-	10 [3]	10	5
	A	20	20	-	19	19	14
SB	Control	10	10	-	nc	-	-
	C	10	10	-	8	8	5
	A	20	20	-	16	16	16
PP	Control	10	10	-	nc	-	-
	C	10	10	-	10	10	6
	A	20	6	14	17	15	15
DF	Control	10	10	-	nc	-	-
	C	10	10	-	10	10	10
	A	20	11	9	20	20	14

[1] Rhizomorph physically attached to host root.

[2] Noncolonized control alder branch segments.

[3] Number of alder branch segments sustaining A. mellea throughout study.

generally equivalent growth for BB during the two seasons.

Extracts of roots and litter generally did not support mycelial growth equivalent to that obtained from foliage extracts (Figure 18, 19). No seasonal differences could be detected among the root and litter collections.

Rhizomorph initiation was stimulated most with the cold-water extracts of fresh-dried foliage (Figure 20), with little stimulation from extracts of roots and litter (Figure 21, 22). Rhizomorph production was greatest among the two highest extract concentrations (1:40 and 1:61) of PP and SB foliage. Comparative data for BB foliage extracts is not available. Rhizomorph production was poor in the control series as compared to production on foliage extracts. Few rhizomorphs were produced in the 2.5 and 5.0 percent malt-extract control series, and none in the 1.0 percent control.

Greatest rhizomorph elongation occurred on extracts of fresh-dried foliage, either spring- or fall-collected. Spring-collected foliage from PP and SB generally stimulated rhizomorph elongation to a greater degree than fall-collected foliage; the opposite was found for BB foliage (Figure 23).

Low levels of rhizomorph elongation were observed in the root and litter series, as well as in the control series (Figure 24, 25).

Additional studies were made testing the stimulatory effect of chopped rather than milled tissue; also, a duplicate series of studies

was run using an armillaria isolate from SB. The results of these studies did not differ from those reported above.

Data from assay of soil extracts are not presented. Soil extractives gave no stimulation over the 1 percent malt-extract control series with respect to mycelial mat weight, and rhizomorph production and elongation.

DISCUSSION

A Concept: Armillaria mellea as a Native Forest Plant

An important question not often considered regarding "sudden outbreaks" of A. mellea on native or plantation vegetation is one which inquires into the duration of the fungus on the site prior to disease incidence. Singh (1970) notes armillaria killing of exotic and native conifers in a plantation established in 1958 in Newfoundland. His observations suggest "that the fungus was established early in the history of the plantation." It is more likely, however, that the fungus was native to the pre-plantation forest and most obviously expressed its presence amongst the highly susceptible young-growth exotics planted there.

The condition in Newfoundland is perhaps analogous to the situation in infection center 3 (Study Area II) of this study. Here, A. mellea is undoubtedly native to the study area, and although the fungus can be found (as rhizomorphs) on M and SB throughout the plantation (about 30 acres), PP seedlings are under attack only around one of a number of large pine stumps present. It is likely that the fungus became locally established in the roots of this overstory tree prior to its harvest in 1966. A. mellea developed quickly

within the moribund root system after removal of the tree, subsequently killing the first of the seedling pines within two years of planting. Similar events would appear to account for the presence of infection centers 1 and 2 (Study Area I) in the PP saplings there.

At the present late date it is impossible to say whether A. mellea was actively killing PP in the study areas prior to logging and to understory thinning. Trees previously killed by A. mellea would have been removed at the time of thinning and thus not now available for observation.

Even though A. mellea is found on roots of SB everywhere on Pringle Butte, it is unlikely that infection centers as they are now developed existed in the understory of the mature stand. The stumps now acting as food bases, were then living and even though infected to some degree were vitally restraining significant development of the fungus. Once the overstory trees were felled all restraint against A. mellea was removed and the massive stump and root substrate was quickly exploited by the fungus.

The concept presented here that the fungus is as "native" to the forest in which it "suddenly appears" as are the trees of that forest is important in understanding the ecology of A. mellea as a pathogen in plantations. This concept has often been overlooked or ignored in the establishment of plantations on forest sites as well as in the reporting of armillaria "outbreaks."

Age of *Armillaria mellea* Colonies

It is significant that armillaria isolates of group A in this study were found seven-eighths of a mile apart and that distances between isolates of group B as great as three-fourths of a mile occurred. How is it possible that we should isolate members of the same clone so far apart? The answer must lie in the great importance of rhizomorphs in local movement of the fungus from substrate to substrate, and in the fact that the colonies of *A. mellea* in many cases, perhaps in most, are very old.

Estimates of age of these colonies as they occur on Pringle Butte must necessarily be based on incompletely understood parameters and are therefore quite speculative. Nevertheless, it appears instructive to estimate colony age as it bears on the expressed concept that *A. mellea* is a native and long-lived member of the forest community.

In temperate climates, fungi, like higher plants, can be thought of as "annuals" or "perennials," separation being based on whether the vegetative body (thallus) "overwinters." The life span of perennial fungi, such as those forming fairy-rings and the wood-rot fungi, can be measured in terms of many years. It has been shown for example, that the mating-type factors of mycelia of fairy-ring fungi have remained unchanged for a period estimated

to be between 100 and 150 years (Burnett and Evans, 1966) thereby indicating mycelial age and genetic stability of the mating-type factors. Wood decay fungi are limited in the number of years the thallus may survive by their confinement within a definite quantity of organic substrate. But this may be very long (several hundred years) in the case of some heart-rot fungi.

Because of the almost infinite food base the perennial root inhabiting fungi, particularly A. mellea, that combine both parasitic and saprophytic abilities are among the potentially most long-lived of fungi. The parasitic abilities of A. mellea allow the fungus to become established as a primary invader of woody substrates where it may persist for many years in chronic development on roots of its host. Following host decline or death the fungus grows more rapidly within the root tissue. The fungus may persist here without outward movement for many years, depending in part on the mass of the root system and in part on the proximity of new substrate. In any event during this saprophytic period food reserves apparently are accumulated in the thallus to provide the energy needed for development of the highly evolved rhizomorphs that are so important in local growth of the fungus from the existing food base to new substrate.

Estimates of age of the giant colonies of A. mellea found in this study must be based on knowledge of the rate of movement of

the fungus between components of the vegetation. Unfortunately, long-term studies of rhizomorph growth from large food bases buried in the soil, i.e., stumps, have not been made, so direct estimates are not possible. In theory, however, there should be few limiting factors impinging upon the longevity of an armillaria colony once it becomes well established under forest conditions such as those of the ponderosa pine forest type on Pringle Butte.

Unlike a tree that is intrinsically bound to its site of origin, successful colonies of A. mellea enlarge from their point of origin to colonize new substrate. Thus, over the "lifespan" of a colony it enlarges at least radially to occupy new substrates while declining on the older food base materials. In effect, this form of growth represents a continuous "repropagation" of the parent colony on new substrates under locally varying environmental conditions to most optimally assure survival of the original genotype on the site. Therefore, in my estimation, it is entirely possible that some armillaria colonies on Pringle Butte may have their origin in the development of the pine forest regenerating after the eruption of Mt. Mazama 6500 years ago, or earlier.

The use of the term "colony" here is not meant to imply the presence of physical continuity among all mycelia of the same compatibility group no matter how widespread. A giant colony is probably represented by many independent entities living on the various

substrates offered by the vegetation of the site. These entities in their turn are independently carrying on the biological investments of the original "parent" colony.

Shrub Root Systems and *Armillaria mellea*

Age and type of root system developed by each of the three major shrubs on Pringle Butte seems to affect the incidence of association of their roots with rhizomorphs of *A. mellea*. Age of the root systems is an important factor in exposure to armillaria attack, i.e., rhizomorphs of *A. mellea* were rarely found on roots of seedling shrubs, but were common on the older, well-established SB and BB. Also, the shrubs differ markedly in rooting habits and this difference is reflected in the consistency with which *A. mellea* could be found on the roots of large specimens of each species.

Snowbrush ceanothus (SB) has a large and well-developed tap root and on old plants a root crown that may reach 10 inches in diameter. With an important exception as noted at the end of this section rhizomorphs were found encircling the roots and root crown of large SB everywhere observations were made on the slopes of Pringle Butte.

Antelope bitterbrush (BB) typically develops a slender, deep penetrating tap root, with a few small lateral roots arising at irregular intervals along the tap root. The plant is sensitive to competition

from other, larger vegetation and is found most often as a solitary plant or in very small clumps generating from rodent buried seeds. Armillaria mellea was rarely found on young BB and not always on older plants. The infrequent association of A. mellea with BB probably is due to escape through space arrangements and rooting habits of the plant.

Manzanita (M) is rarely found with a deeply penetrating root system. The shrub layers to form large clumps about a common origin. In time the original taprooted progenitor dies and the layered progeny are maintained through their non-taprooted, multi-lateral root system. Rhizomorphs of A. mellea seem not to easily associate with this type of root system; consequently, the fungus was found only infrequently on roots of manzanita.

Day (1927) describes A. mellea attack of conifers as a two stage process. In the first stage ". . . the rhizomorph becomes attached to the outside of the host, and this attachment takes place by one side of the rhizomorph definitely growing into and forming in the dead outer cork cells . . ." He further describes the attachment of the first stage as being very firm and requiring force to remove the rhizomorph from its attachment point. This description well fits association of A. mellea with all shrubs on Pringle Butte. The second stage, penetration of the host, was not found on the shrubs here.

Snowbrush ceanothus (SB) may have some value as an indicator host for detecting the presence of A. mellea where overt signs of armillaria attack are not present. Early detection of A. mellea in preplantation sites could be important in reducing the severity of loss of young pine due to root disease. In areas like Pringle Butte where fruiting of A. mellea is rare, identification of the fungus through rhizomorphs is an acceptable method. With but one exception rhizomorphs were found without fail on root crowns of large SB on Pringle Butte: the scattered SB along the fringe of its vegetational zone adjacent to the Pinus/Purshia/Festuca community lacked rhizomorphs of A. mellea. Elsewhere in eastern Oregon and northern California rhizomorphs of A. mellea have been consistently found on SB. Conversely, where SB is absent, as in the Pinus/Purshia/Festuca community of low-lying areas, A. mellea is also absent. Armillaria mellea may not be universally present throughout the range of SB, but if the fungus is present on the site examination of SB roots would probably reveal the presence of the fungus.

The Importance and Advantage of Stumps in Armillaria Infection

Armillaria infection centers on Pringle Butte are always associated with large stumps which are acting as food bases for the fungus in its attack on pine reproduction. In the sapling root excavation pits,

rhizomorphs were commonly found inside decayed and hollow roots of these large stumps in the pit (Figure 14, 15). These rhizomorphs are presumed to have originated from the fungal body decomposing the large roots and stump mass, and to have extended outwardly within the decaying roots. Bark of the roots decays more slowly than the wood resulting in a network of "tubes" distributed in the form of the former root system. Rhizomorphs of both A and C groups of A. mellea were found growing through these "tubes".

In addition to proximity to the massive food base other advantages can be postulated for the growth of rhizomorphs within decayed and hollow roots rather than free in the soil. An advantage of primary importance would be utilization of less energy for rhizomorph growth than would be required for growth through even the light pumice soils of the area. Garrett (1951) found ". . . that the maximum radius of rhizomorph spread from a food base is always greater than the maximum radius for successful infection." Thus, the maximum radius for both rhizomorph extension and infection may be increased by growth along the path of low resistance offered through decayed or hollow roots.

Further advantage for rhizomorph growth through decaying roots may result from moderation of the environment to be found in the root during the growth period. Though rhizomorphs are perennial and can withstand extremes of temperature and moisture, greater

growth occurs under a more moderate, constant environment. Decayed roots of large stumps may provide such an environment.

Life Histories of Armillaria mellea on Pringle Butte

Historically, through an assumed role as food bases, hardwoods have been linked to armillaria attack of conifers. In the literature softwoods are generally considered to be of minor significance as food base material subtending armillaria infection centers. Other than the broad leafed shrubs, which appear not to act as food bases in infection of the young pine noted in this study, hardwoods are not found on Pringle Butte. Here, the large pine stumps and root systems of the former old-growth overstory offer a massive substrate for armillaria development.

These large stumps, now acting as food bases, were probably infected prior to the time of overstory removal in the Study Area in 1957. Lateral roots of large stumps exposed during recovery of A. mellea from these stump masses were found often with considerable pitch impregnated pumice surrounding lesion areas; this pitching seems likely to have occurred only while the tree was living. However, extensive development of A. mellea from these incipient infections in roots of mature trees probably was delayed until felling of the overstory. Rhizomorphs growing from the food base distribute the fungus locally leading to infection and often death of young PP

saplings and to more enduring relations with living shrubs.

The shrubs may act in turn as "reservoir" hosts in passively perpetuating A. mellea within the stand. Later, over a period of many years, older, now less susceptible PP which earlier escaped damage are attacked leading to the characteristic incipient infections on the roots.

The foregoing is a description of the suspected life history of members of infection group A, members of which can be found at this time on the hosts mentioned. Of these hosts, the seedling and sapling pines are the most transitory in time with regard to survival of A. mellea. The truly important hosts with regard to long term survival of A. mellea are the shrubs, especially SB, for they passively support the fungus for many years in close proximity to present and potential food bases from which once again the attack of young pine can occur.

The large stumps serve effectively in the food base capacity from which other pines are attacked and infection re-established on the shrubs, but are relatively short-lived participants in the long term role of perpetuation of A. mellea in the forest. Stumps of trees cut in 1952 are badly decayed and will not support A. mellea in attack of young pine and shrubs much longer. Armillaria mellea could be recovered from moist, woody tissues of the large stumps, but could be recovered only from tissue in the cambial region of

young, dead pine.

Sapling pine (root crown diameter of 6 to 8 inches) killed by A. mellea in the early 1960's has failed to act in turn as a food base. Further, it was difficult to recover the fungus from these earliest of the killed young pine. The fungus was not found decaying internal sapwood tissue of any of the young pine trees excavated. The sapwood of these trees killed by A. mellea seems to dry out quickly and as such will not support growth of the fungus.

The large stumps are important in the pathological history of A group members in the young-growth stand; isolates of this group were recovered from the deeper tissues of these stumps. However, B group members were not recovered from stump tissue and little can be said of their affiliations with stumps. Members of the C group follow much the same distribution pattern as those of the A group, but unlike members of the A group, those of the C group are not pathogenic on young pine.

Rhizomorphs of C group members may be found on the surfaces of roots of pine stumps and living pine, however, C group members were not recovered from the deeper tissues of the stumps. Perhaps significantly, C group isolates were recovered from 88.6 percent of the shrubs bearing rhizomorphs in Study Area I, while A and B group isolates together were recovered from the remaining 11.4 percent. Thus, shrubs would appear to be the preferred host for

the C group members. The preceding host/fungus associations disclose two biologically distinct fungal groups within the same species, each occupying its distinct ecological nitch, but sharing the shrubs in common as reservoir hosts.

Attack and Early Development of *Armillaria mellea* on Ponderosa Pine

Lesions, representing armillaria attack sites were found on lateral roots, tap roots and root crowns of dead, dying and living PP saplings. Lesions caused by *A. mellea* were easily identified through the characteristic mass of pumice soil cemented in pitch around the attack site. Rhizomorphs were often embedded in the mass.

Armillaria attack of lateral roots leads to pitching and death of the affected tissue locally and often to death and colonization of the distal portion of the root by the fungus. Inactive lesions on lateral roots of living PP indicate the potential of healthy roots to sometimes occlude the fungus upon attack. Some roots with lesions had been girdled by pitch at the infection site, while others had a more localized pitching from lesions not severe enough to kill the root distal to the lesion. *Armillaria mellea* was never found invading living tissue proximal to the attack site on lateral roots even when adjacent tissue had been killed by fungal action. Consequently, in this study, *A. mellea* was never found to have first colonized a

lateral root and then subsequently spread through the root to attack the entire root system.

The loss of some portion of one or several lateral roots of the PP saplings appears not to affect host vigor to the point of influencing armillaria attack. However, attack in the root crown region leads to extreme pitching and death of the affected root crown tissue, and to decline of the entire tree. Subsequently, the entire root system is rapidly colonized through the cambial tissue by mycelial growth of A. mellea.

Extensive development of A. mellea in PP saplings is not concurrent with earliest signs of attack of the host. Mycelial development in cambial tissue was found only in those trees either moribund or dead, but even then not until active pitch production had ended. In trees showing the earliest signs of stress, i.e., slight dulling of the normally bright green foliage, and cessation of growth, mycelial development was found only occasionally and then limited to small (1-2 inch diameter) pockets localized in the cambium of the root crown.

As noted in the Results section saplings attacked by A. mellea maintained annual ring widths consistent with previous years' up to the time of death. This indicates sudden death of the host rather than gradual decline over a period of several years as would occur if death were due solely to slow, progressive development of

A. mellea within the host tissue.

The term "death" as used here is for convenience only. Above-ground symptoms as described above signifies "death" of the tree, but does not allow for a period in which the roots are declining, yet are still living. How long the roots remain alive after the apparent "death" of the tree is a moot question. Nevertheless, it is probably sometime during the period when the roots are moribund that A. mellea begins extensive development in the cambial tissue leading to the ultimate and true "death" of the tree.

In summary, the young pine of this study appear to be killed by A. mellea only through attack at the root crown; attack of lateral roots is not lethal. The following sequence of host and fungus interaction is believed to take place in the killing of ponderosa pine saplings by A. mellea:

1) rhizomorphs penetrate host tissue at the root crown,
2) the host responds through copious pitching generally over the root crown region--this represents a hypersensitive-type reaction (see following section),
3) host tissue in the root crown region is killed by the pitch and pitching stops,
4) host root vigor declines and the roots become less resistive to mycelial development of the fungus,

5) mycelia of A. mellea spreads rapidly through the moribund and dead roots.

The Hypersensitive Reaction in Ponderosa Pine Under Attack by Armillaria mellea

The question as to whether ponderosa pine shows resistance to armillaria attack is important in view of recent trends toward establishment of plantations on cutover pine forest land. My data indicates an affirmative answer to this question.

The interaction between sapling PP and A. mellea observed in this study finds a close analogy in the hypersensitive reaction (HR) first described in connection with the rust fungi. The HR is broadly defined as an involvement between a resistant plant and an infectious, biological agent that leads to localized killing of host tissue with the exclusion of the pathogen.

The analogy, however, does not compare directly with the usual theme in the historical sense. In the first place in the "typical" HR, the host as an entire organism is rarely, if ever, killed as a direct response to one or a few infections. Armillaria attack of lateral roots, within limits, does not directly harm the host, but direct attack upon the root crown is always lethal. Secondly, while pathogens described in reference to the HR are typically obligate parasites, A. mellea can act both saprophytically and parasitically.

And, finally, unlike the single propagule of HR-inducing organisms which is permanently excluded from colonizing the host tissue, A. mellea remains in physiological contact with its food base through which it may ultimately realize infection of the attacked tree.

Although fungi evoking the HR are typically parasites of green plant parts, there is no reason why a root pathogen should not elicit a similar host response upon attack of a responsive host. The basic sequence in the HR is the same, the pathogen attacks the host which responds through a local destruction of tissue about the point of attack. There are no definite limitations placed upon what the form of host response may be as long as the pathogen is excluded (however temporarily) from further colonization of the host. The significant feature of the HR is the exclusion of the pathogen through a physiological host response.

This is what is happening in armillaria attack of roots of PP saplings on Pringle Butte. Armillaria attack of any part of the root system stimulates a host response in the form of resin exudation about the infection site. Small lateral roots of sapling pine may be killed locally by resin permeation of living tissue. The fungus may later enter the root, but will colonize only the distal portion if entry is made. Larger lateral roots are not often girdled locally through resin permeation during armillaria attack.

Armillaria attack of the root crown region in general, evokes

an extremely strong response on the part of the host. The response, in terms of resin exudation, was strong enough to girdle the root crown on all attacked saplings examined. The root crown is the "heart" of the tree, once living tissue here dies the roots immediately decline in vigor becoming highly liable to armillaria attack and colonization.

The Importance of the Food Base in Artificial Inoculation Studies

According to Patton and Riker (1959) artificial inoculation studies with A. mellea by a number of workers have given inconsistent results. Often, infection of normally susceptible hosts was not achieved leading to confusion between field observations and experimental data.

Artificial infection of any host by A. mellea is highly, if not completely, dependent on prior colonization of an intermediary substrate, commonly called a "food base". If inoculations are attempted with inoculum that is inadequate as a food base, results of infection of plants under test will be misleading. Food base requirements were ideally met in the study reported here.

The following data is included in this section of the thesis to emphasize its significance in evaluating results of inoculation studies with A. mellea. Ninety-two percent of the alder branch inoculum

segments (the food base) maintained viable armillaria mycelium throughout the duration of the study of 21 months (Table 5). Rhizomorphs had developed from 98 percent of these inoculum pieces, becoming attached to the outside of the host roots of 78 percent of the remaining 110 test plants. Non-inoculated controls are excepted from these figures.

The question of infection/no infection is valid only after contact of inoculum and suscept has been assured. The discussion can then logically proceed to comparisons of pathogenicity of isolates or susceptibility of hosts. In this study, rhizomorphs of both the A and C groups were firmly in contact with shrub roots, yet no infection was found that might lead to death of the root. The rhizomorphs were attached superficially, yet firm enough to cause tearing of the rhizomorph when removed (described by Day, 1927). This kind of attachment to the roots was strikingly similar to that found naturally occurring on shrubs (BB, M, SB) on Pringle Butte and elsewhere in the ponderosa pine forest, yet all seedlings remained healthy.

While the two armillaria isolates acted alike in failing to attack the shrubs, they reacted independently toward conifer seedlings of two species. Rhizomorphs of the A group isolate were found on 15 of 20 PP; 14 of these 15 trees had died by the conclusion of the study. Similarly, 9 of 14 infected Douglas-fir, from a group of 20, had died. Rhizomorphs of the C group isolate were found on 6 of 10 inoculated

PP, and 10 of 10 Douglas-fir, none of which had died.

These results show similarities and differences between the A and C group isolates. Members of both groups interact similarly with the three shrub hosts, but differentially on the two conifers. These highly successful inoculations emphasize the importance of thoroughly establishing A. mellea in an adequate food base substrate in order to achieve effective inoculations.

Physiological Strains of Armillaria mellea

Consideration of results of the inoculation study, in which distinct separation of the pathogenic A group and the non-pathogenic C group confirmed field observations of differences in pathogenicity of these two groups, raises the subject of strain differences. Little is known concerning degrees of virulence among isolates of A. mellea, however, the fungus is usually regarded as potentially parasitic wherever found. This is so, perhaps, because being a root inhabiting fungus its parasitic activities are much more prominent than are its saprophytic ventures.

Are there "physiologic" strains as suggested by Childs and Zeller (1929) from observations of parasitic "oak" and saprophytic "fir" strains of A. mellea recovered in western Oregon, or is there merely variation in virulence and pathogenicity as believed by Raabe (1955, 1967)?

Data presented here would support the concept of Childs and Zeller (1929). Group A (perhaps also group B) isolates represent physiological strains of A. mellea capable of killing young pine, but not shrubs. Group C isolates represent a strain that is non-pathogenic on either conifers or shrubs.

Interpretation of the physiological relationship of the A group isolates to the woody shrubs, and the C group isolates to both the woody shrubs and the conifers is a matter of terminology. Raabe (1967) would call the relationship in which rhizomorphs develop on roots of healthy, but susceptible hosts, weak parasitism. Childs and Zeller (1929) treated the situation in terms of parasitic (killing of host) or saprophytic (no killing of host), the latter even though A. mellea was "solidly attached to the bark of the trees at ground line."

Raabe (1955, 1967) presented evidence of variation in pathogenicity and virulence in A. mellea. Under his scoring system both the A and C group isolates of this study would be rated high in pathogenicity, while the A group isolates would have a high virulence rating on PP and Douglas-fir and a zero rating on BB and SB. The C group isolates would have a zero virulence rating on all hosts. If Raabe's isolates were all recovered from dead and dying hosts (he does not indicate under what host circumstances his isolates were collected), then he probably has not collected isolates that

compare reasonably well with Childs and Zeller's saprophytic "fir" strain, or the group C isolate of this thesis.

Growth of *Armillaria mellea* on Plant Extractives

At the outset of this study, a relationship was hypothesized between the distribution of the major vegetation on Pringle Butte and occurrence of *A. mellea*.

Extracts from roots, litter, and soil had no influence on growth of *A. mellea* in culture. These results would appear to indicate that these materials are not effective in influencing distribution of the fungus in the soil. Foliage extracts greatly stimulated mycelial and rhizomorph growth and could have use as substrates in the laboratory media.

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APPENDIX

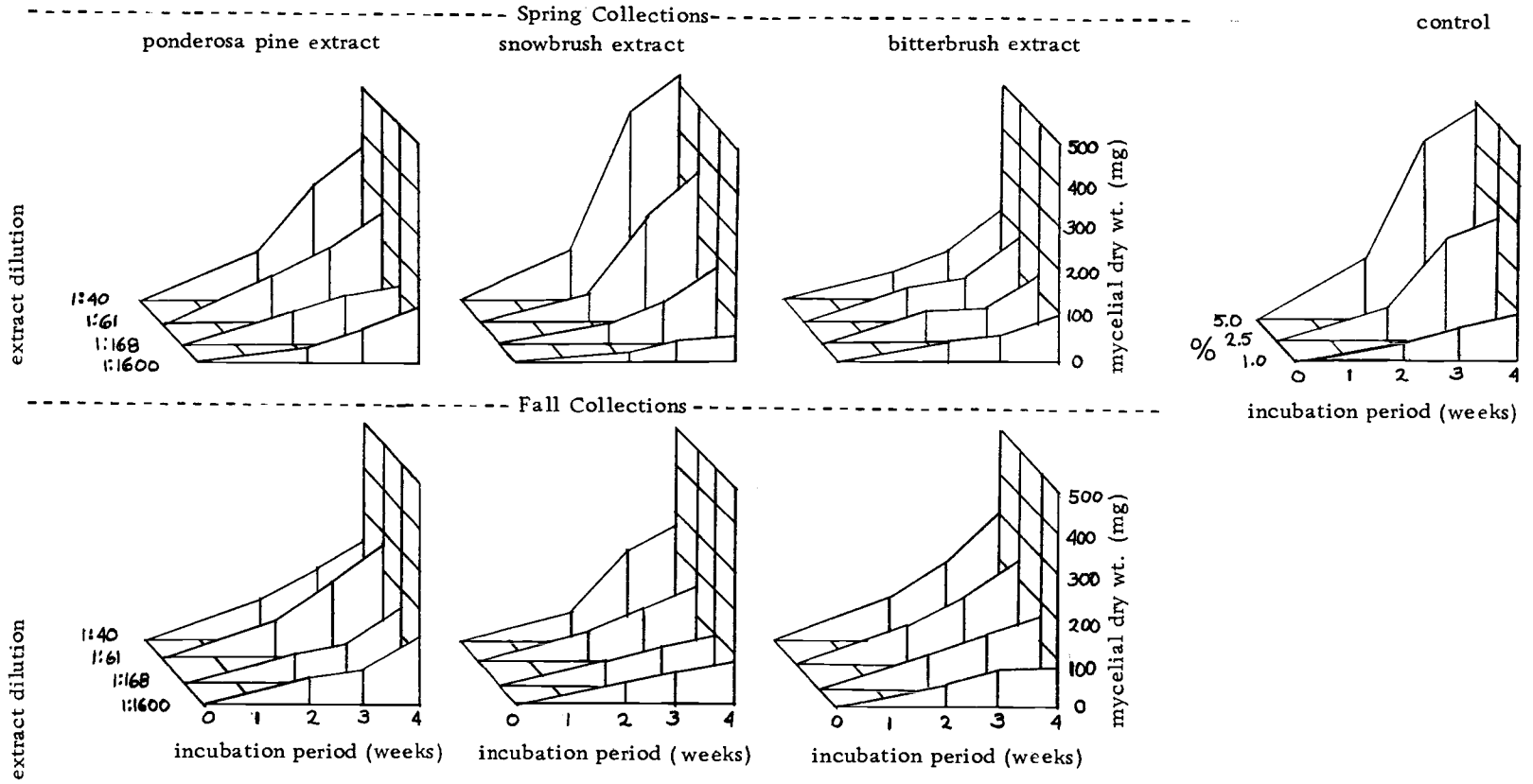

Figure 17. Mycelial growth of A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of foliage from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt.) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt extract is included.

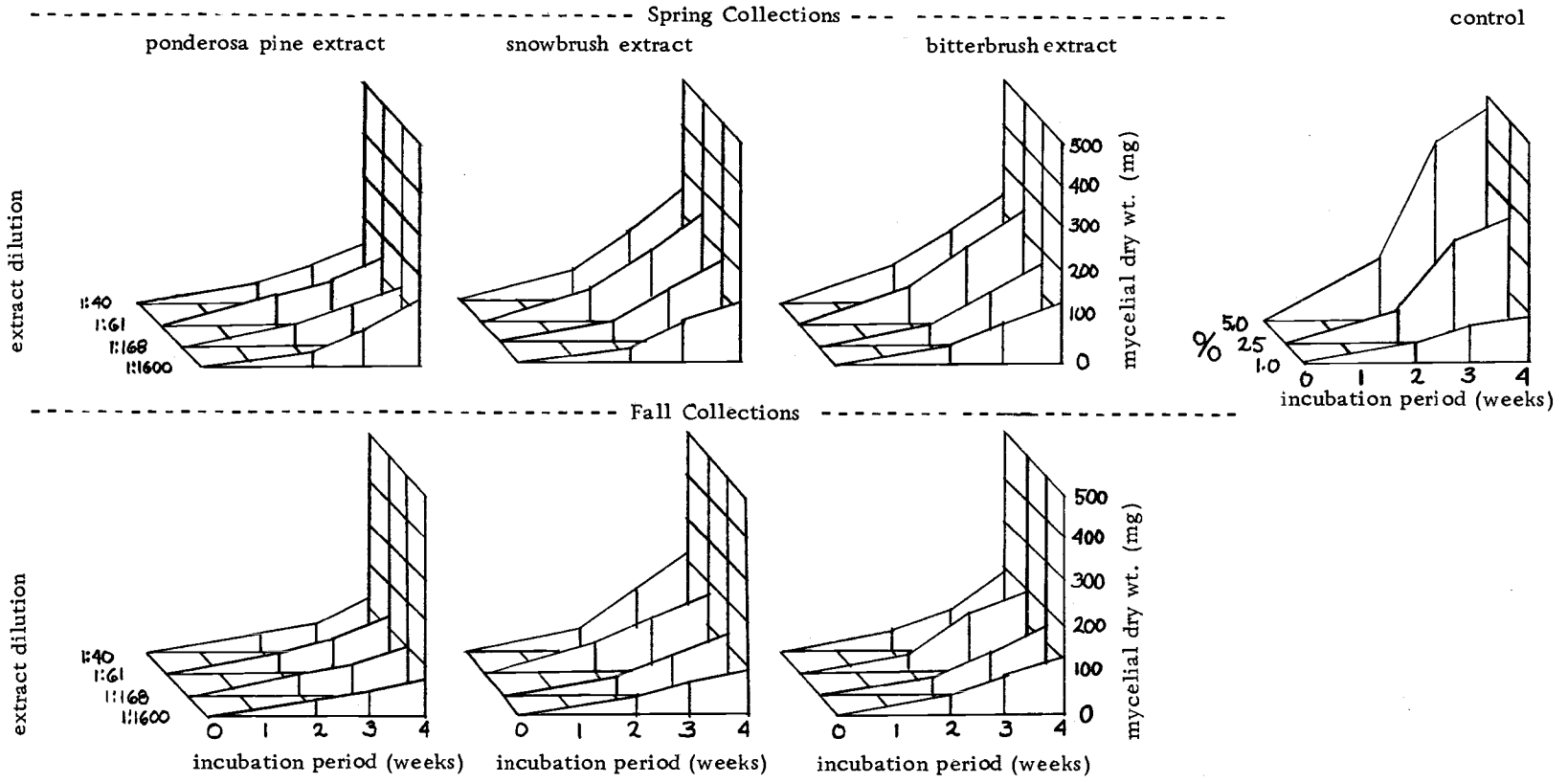

Figure 18. Mycelial growth of A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of roots from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

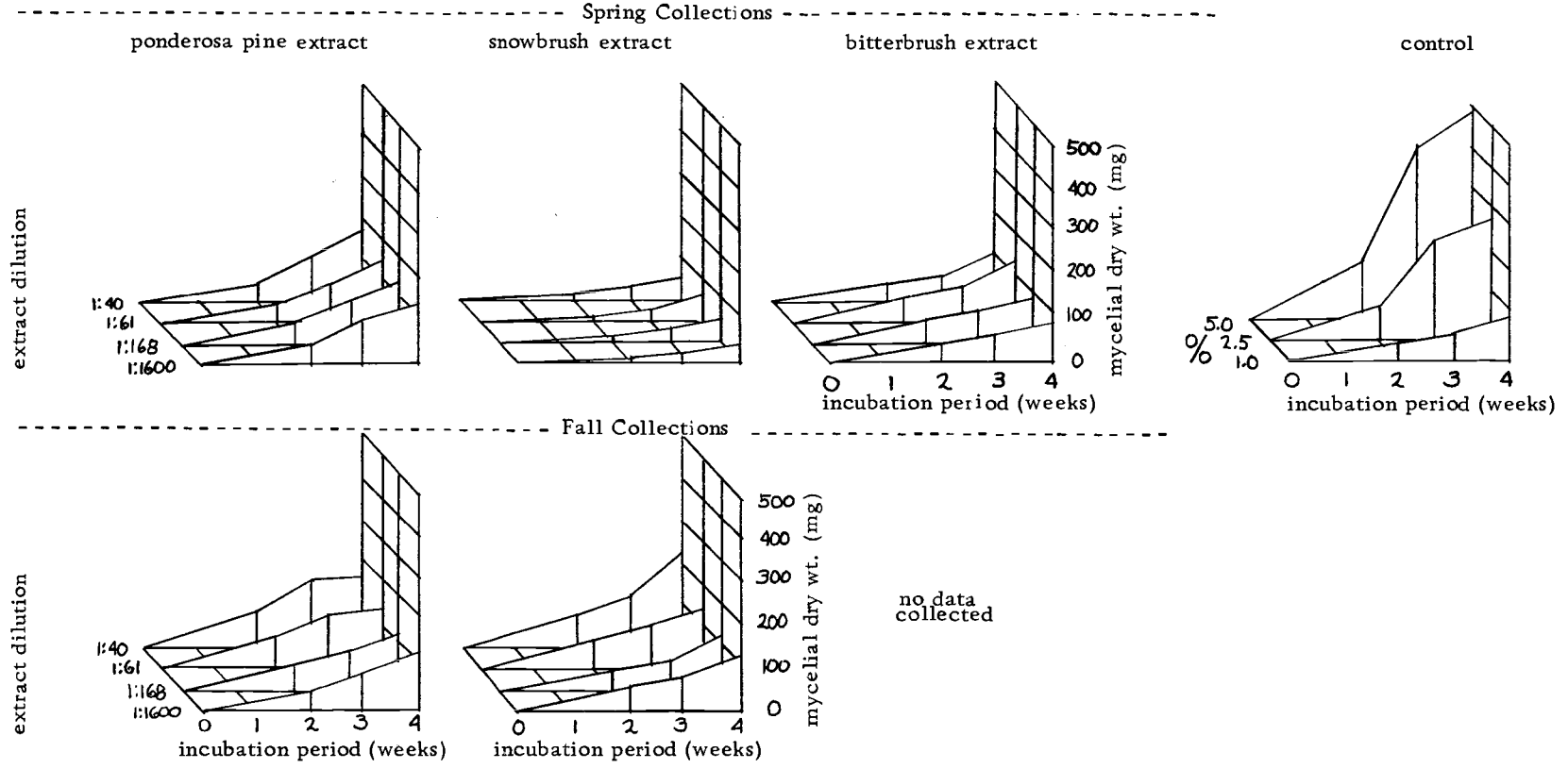

Figure 19. Mycelial growth of A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of litter from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

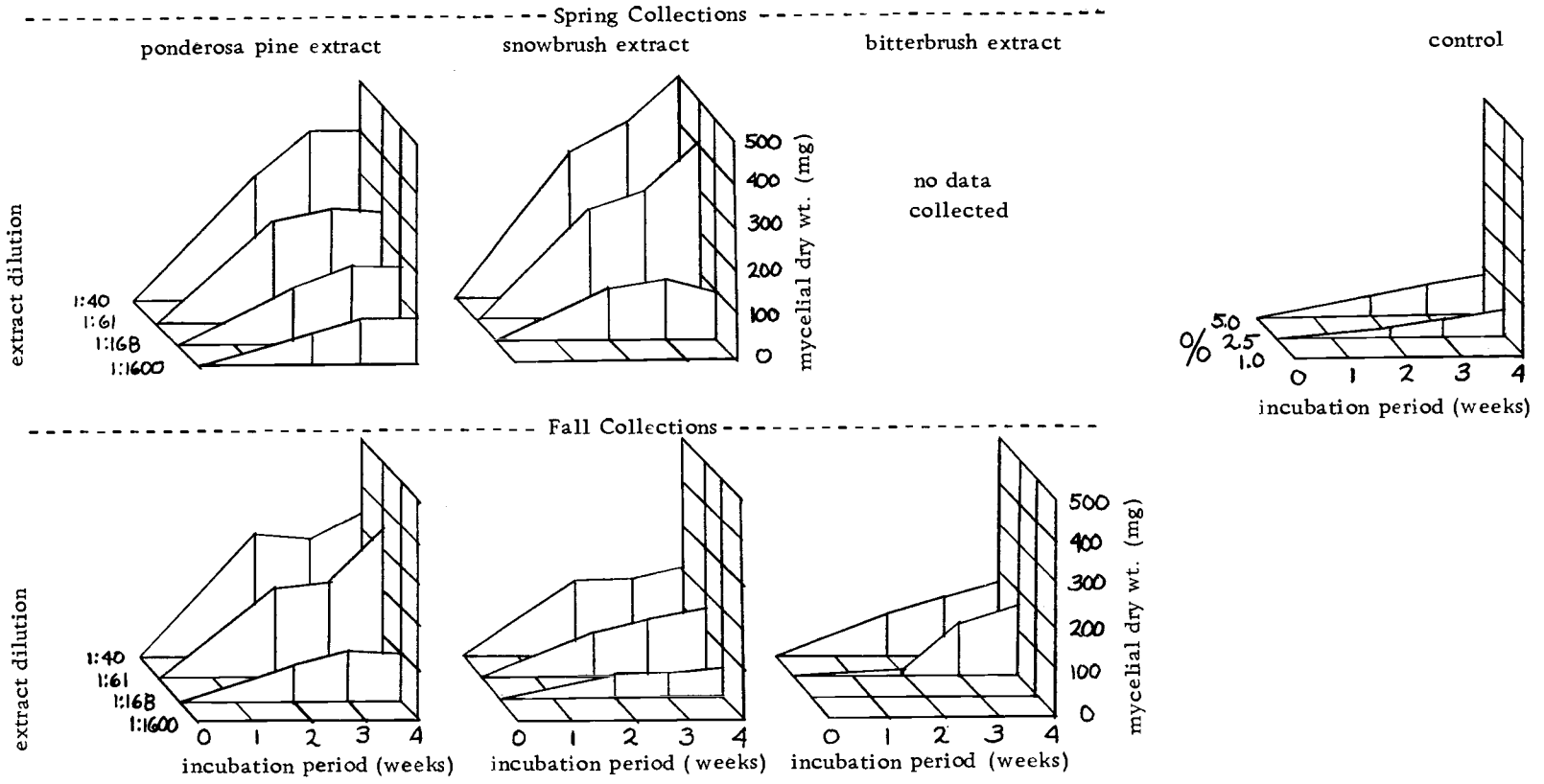

Figure 20. Rhizomorph production by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of foliage of PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

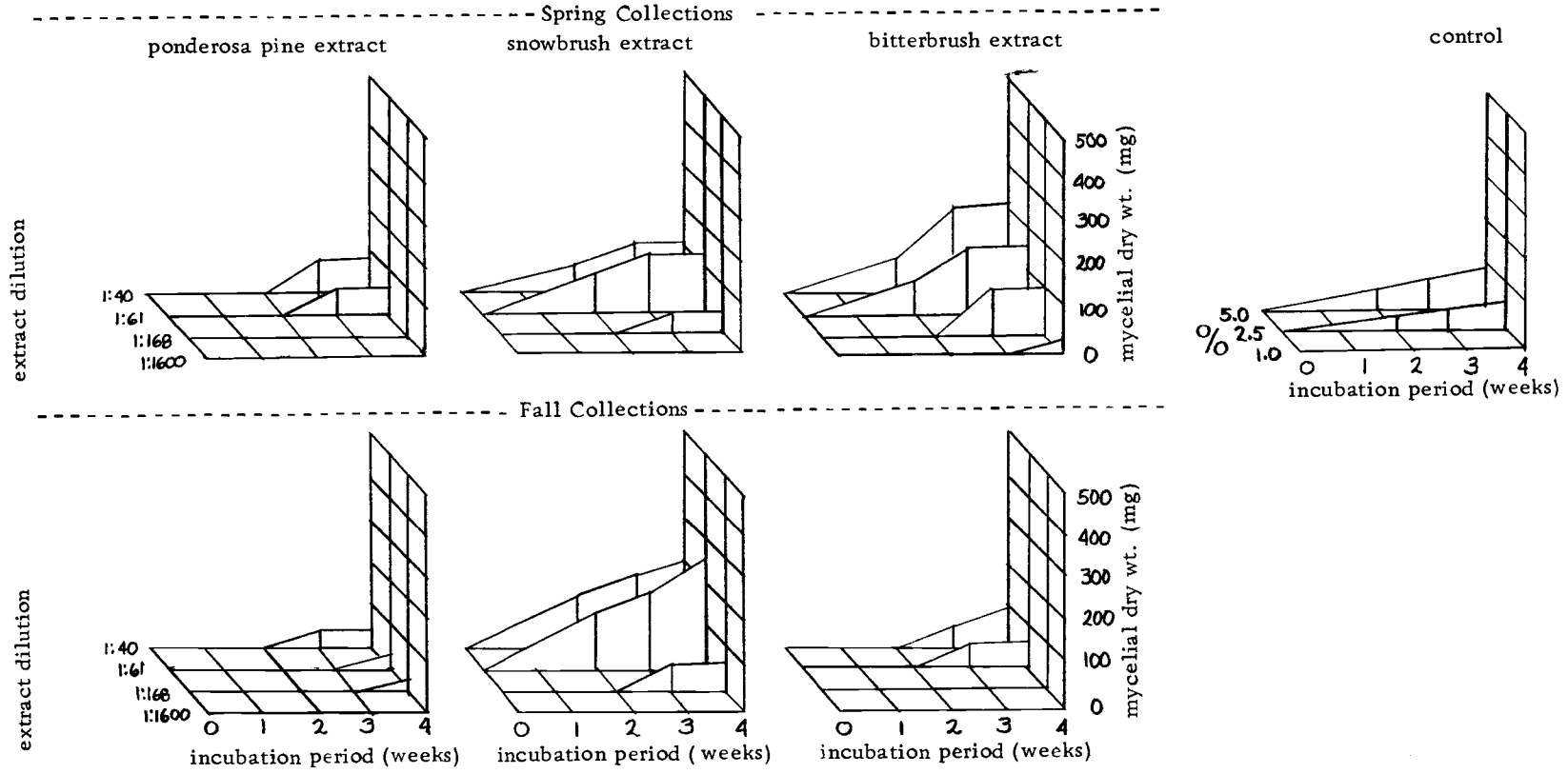

Figure 21. Rhizomorph production by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of roots from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

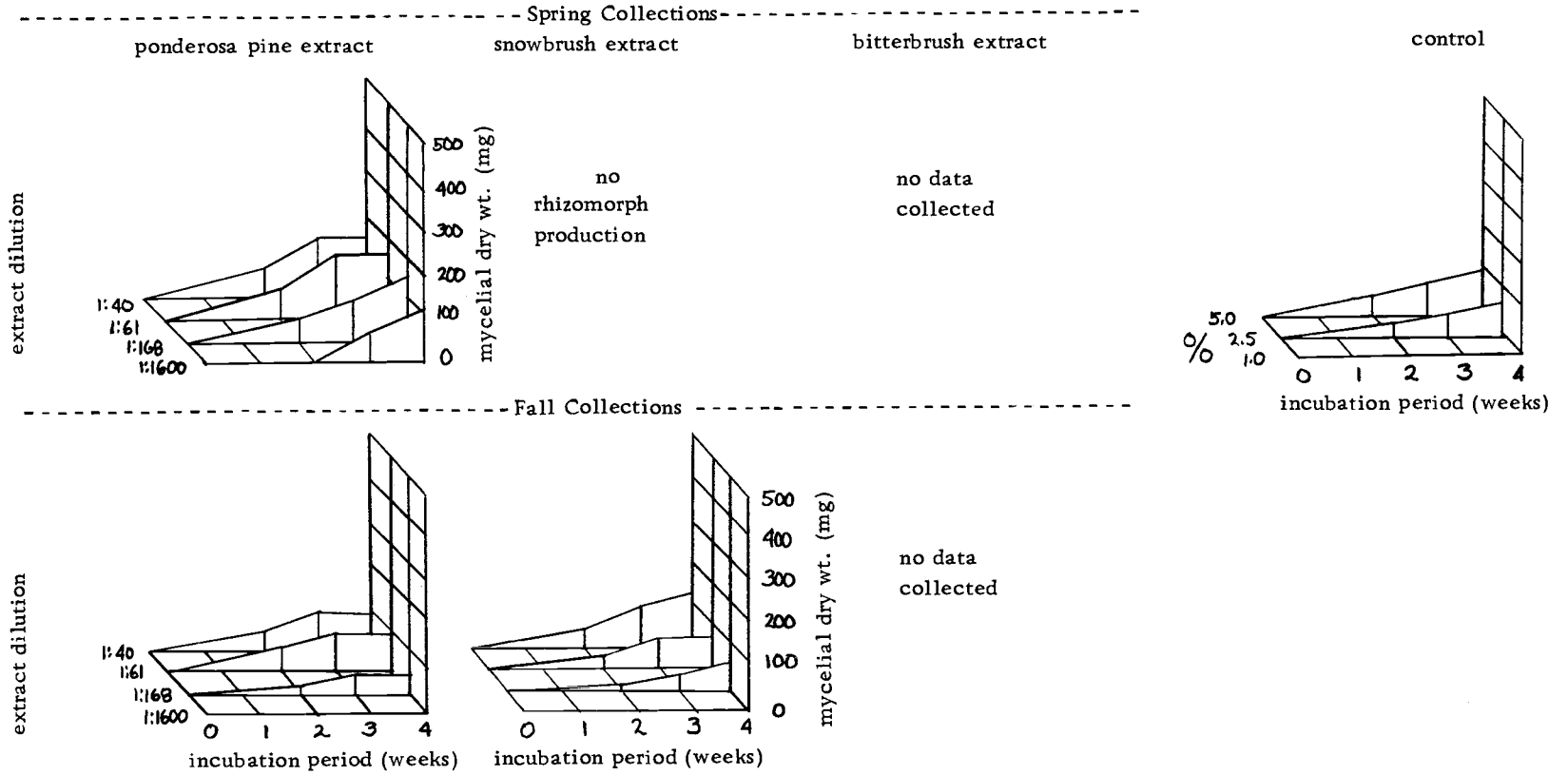

Figure 22. Rhizomorph production by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of litter from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

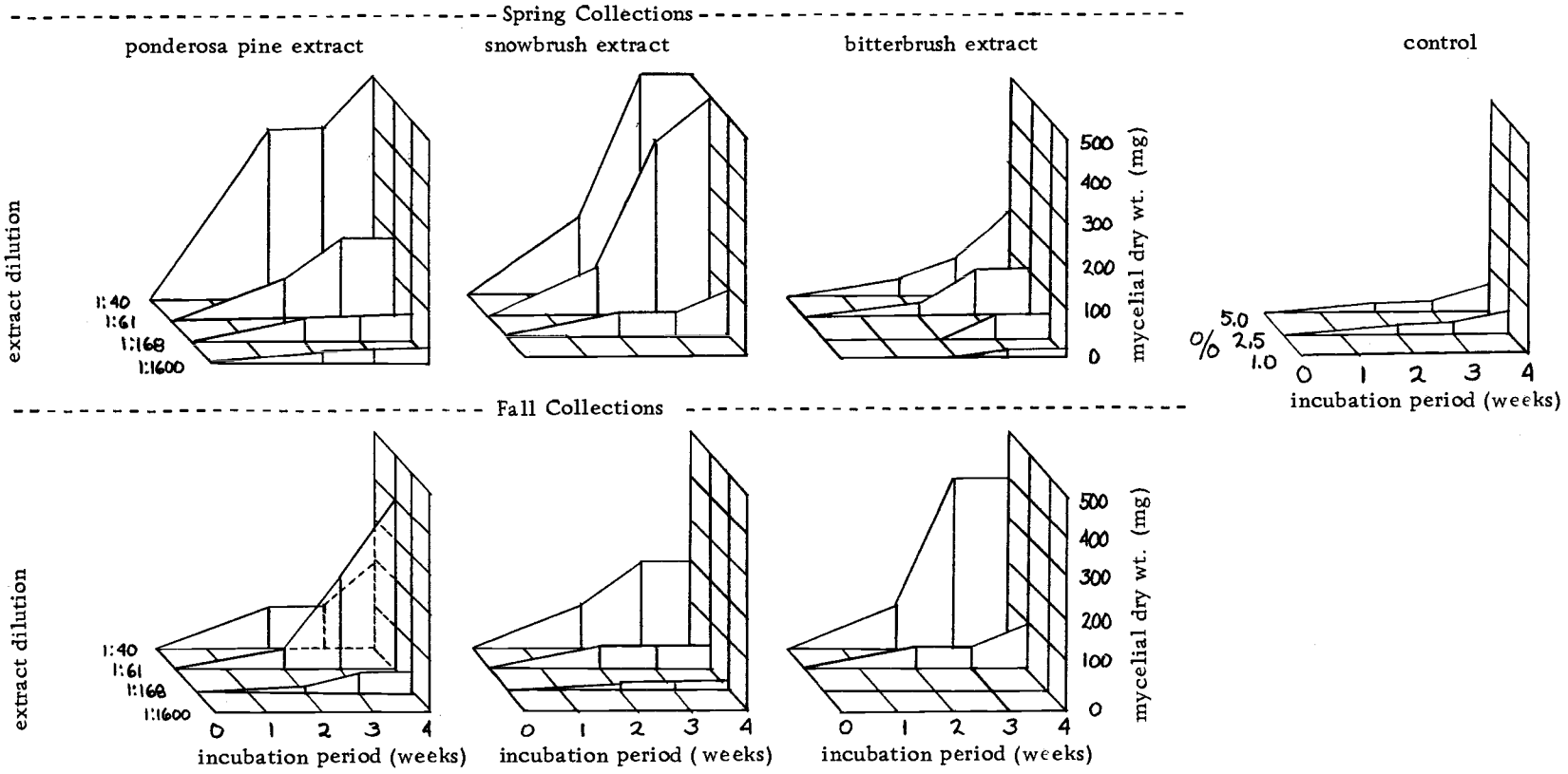

Figure 23. Rhizomorph elongation by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of foliage from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.

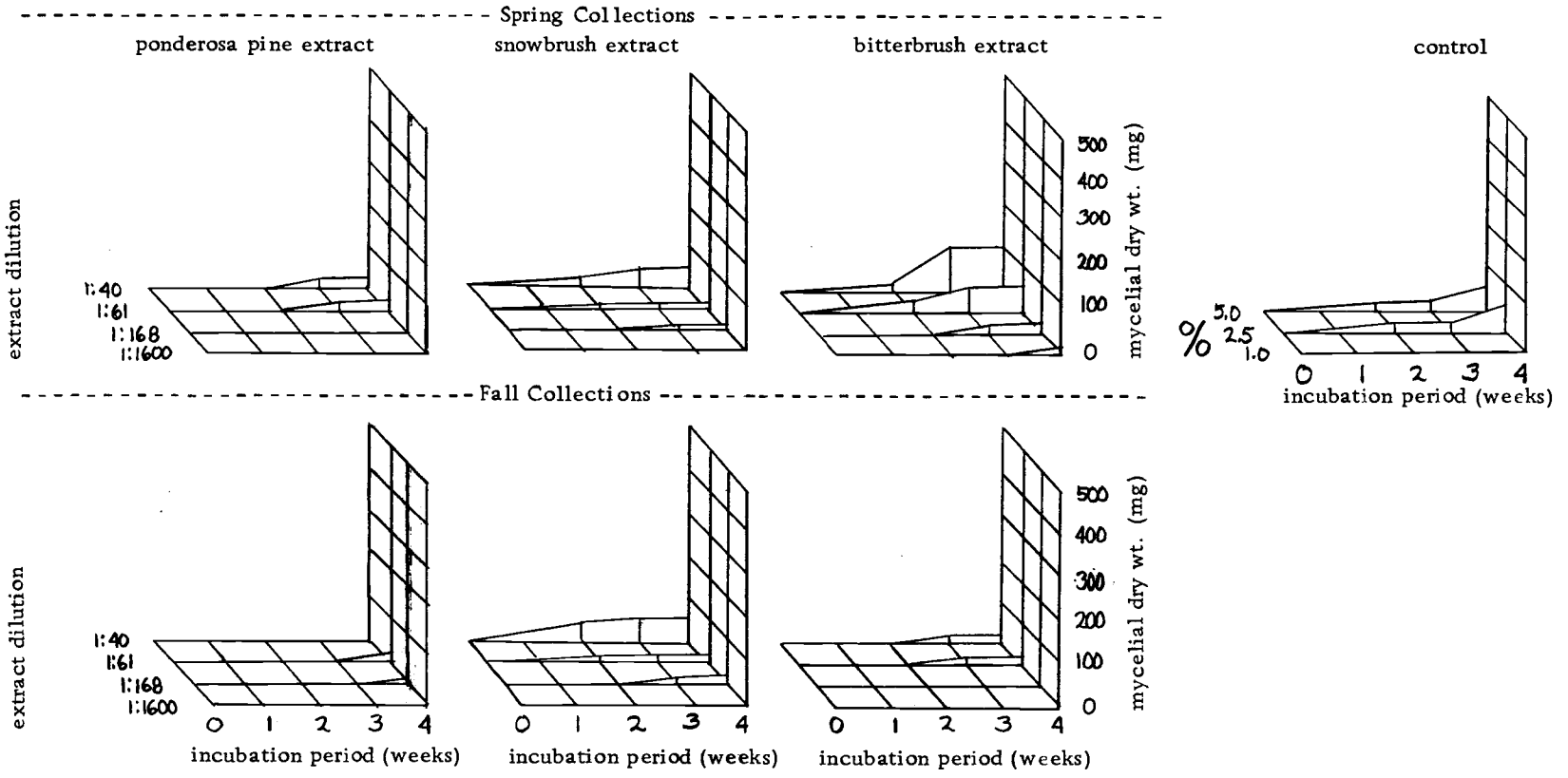

Figure 24. Rhizomorph elongation by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of roots from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concenrations (in percent) of malt-extract is included.

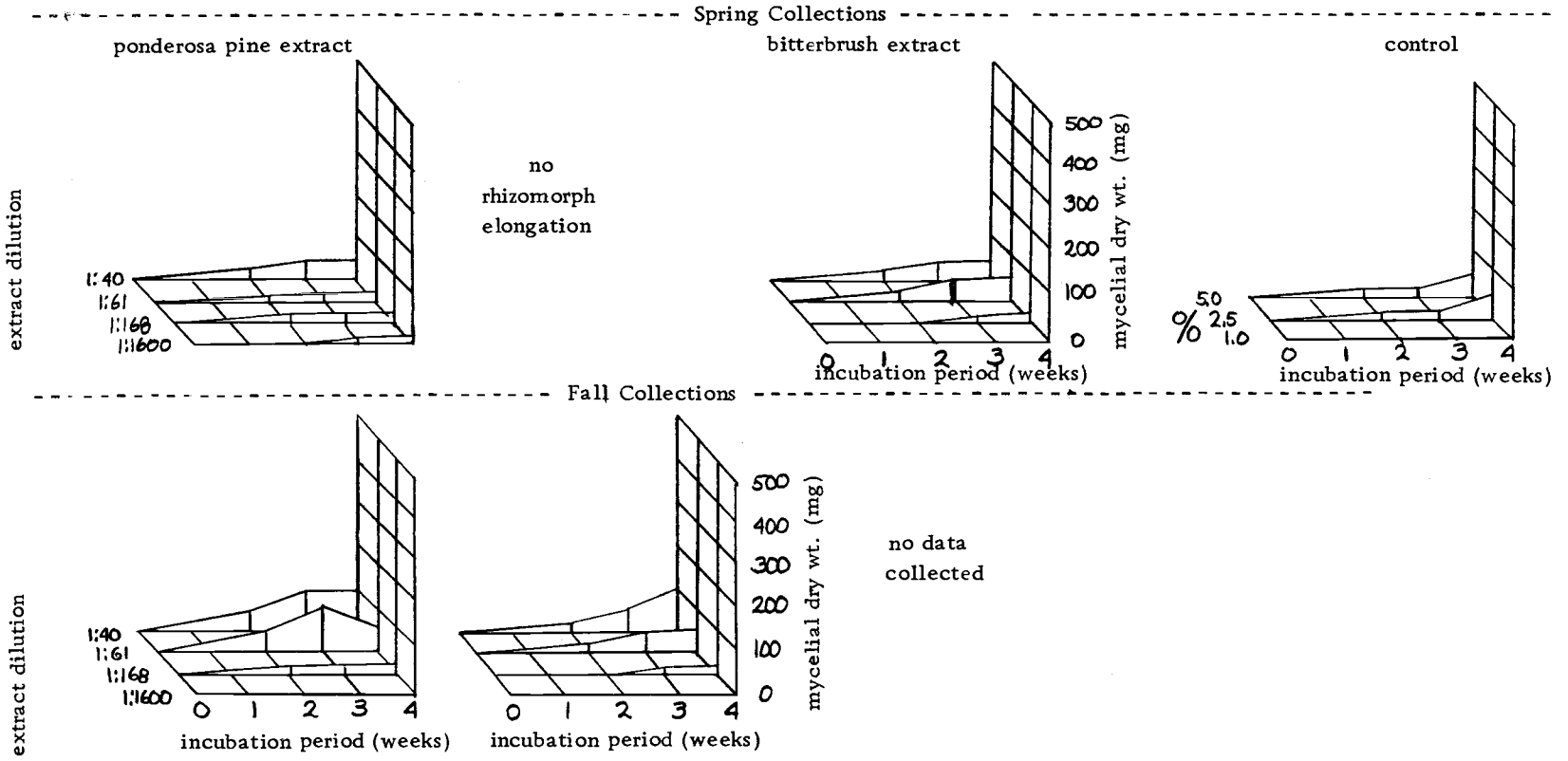

Figure 25. Rhizomorph elongation by A. mellea on 1.0 percent malt-extract medium supplemented with four dilutions of cold-water extract of litter from PP, SB, and BB. Spring and fall collections are represented. Growth measurements (mg dry wt) were taken on the second, third, and fourth weeks. A control series of three unsupplemented concentrations (in percent) of malt-extract is included.