AN ABSTRACT OF THE THESIS OF

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Title: DISTRIBUTION AND VARIATION OF ARMILLARIELLA MELLEA CLONES IN RELATION TO VEGETATIVE COVER AND SEVERITY OF ROOT ROT IN PONDEROSA PINE.

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Dr. Lewis F. Roth

Root rot due to Armillariella mellea (Vahl. ex Fr.) Karst. is unusually severe in ponderosa pine near Glenwood, Washington. Isolate relations, clonal variability, vegetation patterns, and climate were examined in relation to severity of disease in this area.

Ninety-one isolates from 70 infection centers were examined. Pairings of neighboring isolates showed that 52 clones exist in the area investigated. A survey of vegetation allowed each sampling site to be placed in one of three vegetation types of habitats (ponderosa pine, mixed species, or Douglas fir/grand fir). Examination of clones and vegetation patterns together revealed that some clones were continuous from one vegetation type to the next.

Mycelium growth rates, rhizomorph growth rates, and colony dry weights on MDP and malt extract media were measured for all isolates. Differences in these measures between clones and between isolates in different vegetation

types were statistically analyzed. Significant ($p < .001$) differences between clones and nonsignificant differences between vegetation groups were found for all measures. Significant ($p < .001$) differences were found between media for all measures, with MDP being superior for all isolates. Other morphological characteristics (color and density of aerial mycelium, presence of pseudosclerotium, tufts, exudations, and aerial rhizomorphs, and color and form of submerged rhizomorphs) did not differentiate between clones or vegetation groups.

These findings indicate that severity of Armillaria root rot in ponderosa pine in Glenwood may be due, not to morphologically or spatially unique clones in the ponderosa pine cover type, but to high pathogenicity of the biological species of A. mellea as a whole in Glenwood and/or to high susceptibility of ponderosa pine in this area.

Measurements of A. mellea clones involving more than one infection center showed that the average age of these clones was 466 years, based on a 1.0 m/yr. rate of spread. The age of the largest clone found was calculated to be 1,126 years.

Distribution and Variation of *Armillariella mellea* Clones in Relation to Vegetative Cover and Severity of Root Rot in Ponderosa Pine

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TABLE OF CONTENTS

* * * *

LIST OF FIGURES

Figure

LIST OF TABLES

Table

DISTRIBUTION AND VARIATION OF ARMILLARIELLA MELLEA CLONES IN RELATION TO VEGETATIVE COVER AND SEVERITY OF ROOT ROT IN PONDEROSA PINE

INTRODUCTION

This thesis examines one explanation for the distinctively severe local occurrence of Armillaria root rot in Pacific Northwest ponderosa pine. Armillariella mellea (Vahl ex Fr.) Karst., a root pathogen of many woody plants, is widely distributed through the Pacific Northwest. It parasitizes conifers on both east and west sides of the Cascade Mountains. Damage is generally insignificant in natural forests west of the Cascades, although some losses do occur in young Douglas-fir plantations. Both the amount of disease and disease severity increase in the Cascades and east of the Cascades for all tree species. In specific east side areas in Oregon and Washington, disease incidence and losses from Armillaria root rot are particularly high. One such place, previously described by Shaw (1975), is located in south central Washington on the eastern boundary of the Gifford-Pinchot National Forest near the town of Glenwood.

Armillaria root rot in the Glenwood area generally occurs as more or less circular patches of dead trees

(Fig.1A). Recently dead or dying trees occur about the perimeter of these "infection centers" and snags or fallen trees are found towards the interior. Infection centers originate from the stumps of large old trees harvested or otherwise killed at an earlier time. In large mature trees, infection apparently is effectively occluded within discrete areas on the root by pitch-impregnated barriers. When the infected tree is cut, defense barriers, accountable for the occlusion, break down and the stump and root system is rapidly colonized and saprophytically utilized. Within five years of the harvest date, young trees surrounding the stump may be infected and killed (Shaw & Roth, 1976). The outwards spread of disease from the stump through the usually dense understory occurs as the fungus invades the root systems of younger trees by root contacts or by rhizomorphs. Rhizomorphs are black fungal strands which resemble rootlets. They are capable of growth through the soil and of initiation of infection in roots they contact. Often, as infection centers enlarge into the understory stand away from the old stump, regeneration establishes in the middle of the infection center. For reasons not fully known these trees usually remain healthy above ground and a donut-shaped appearance of the center results (Fig. 1B). Another disease pattern occurs when several small infection centers merge to form a larger but less symmetrical

FIGURE 1

Armillariella mellea infection centers:

A) A park-like opening exists where snags and fallen trees are towards the middle and recently dead and dying trees are on the perimeter.

B) A donut-shaped infection center is formed when regeneration of conifers occurs in the "hole." Snags and dying trees are seen towards the perimeter.


A


B

opening.

Spread of the pathogen, A. mellea, in each of these centers appears to be solely by vegetative means, i.e., root contacts of rhizomorphs. Therefore all trees in each infection center are presumably colonized by the same fungal individual or "clone." Sporophores are produced in the fall, sometimes abundantly depending on suitable temperature and moisture (Fig. 2). Spread of the fungus by spores is not considered important in Glenwood (Shaw & Roth, 1976).

Damage by A. mellea in the Glenwood area is extensive and is intensified by mortality occurring in clumps rather than at random so that large non-productive openings are created. Wood volume loss from 1957 to 1971 was reported to be 24 cubic meters per hectare in this area (Shaw et al., 1976).

It is hypothesized that the unusual severity of disease in the Glenwood area is due to the uniqueness of those A. mellea clones present in the ponderosa pine cover type. The recent discovery of a very large (600 hectare) and very old ($\geq$ 460 years) A. mellea clone in Glenwood ponderosa pine (Shaw & Roth, 1976) suggests the existence of "ponderosa pine clones," "Douglas fir clones," etc. where each clone or set of clones is unique to each vegetative cover type.

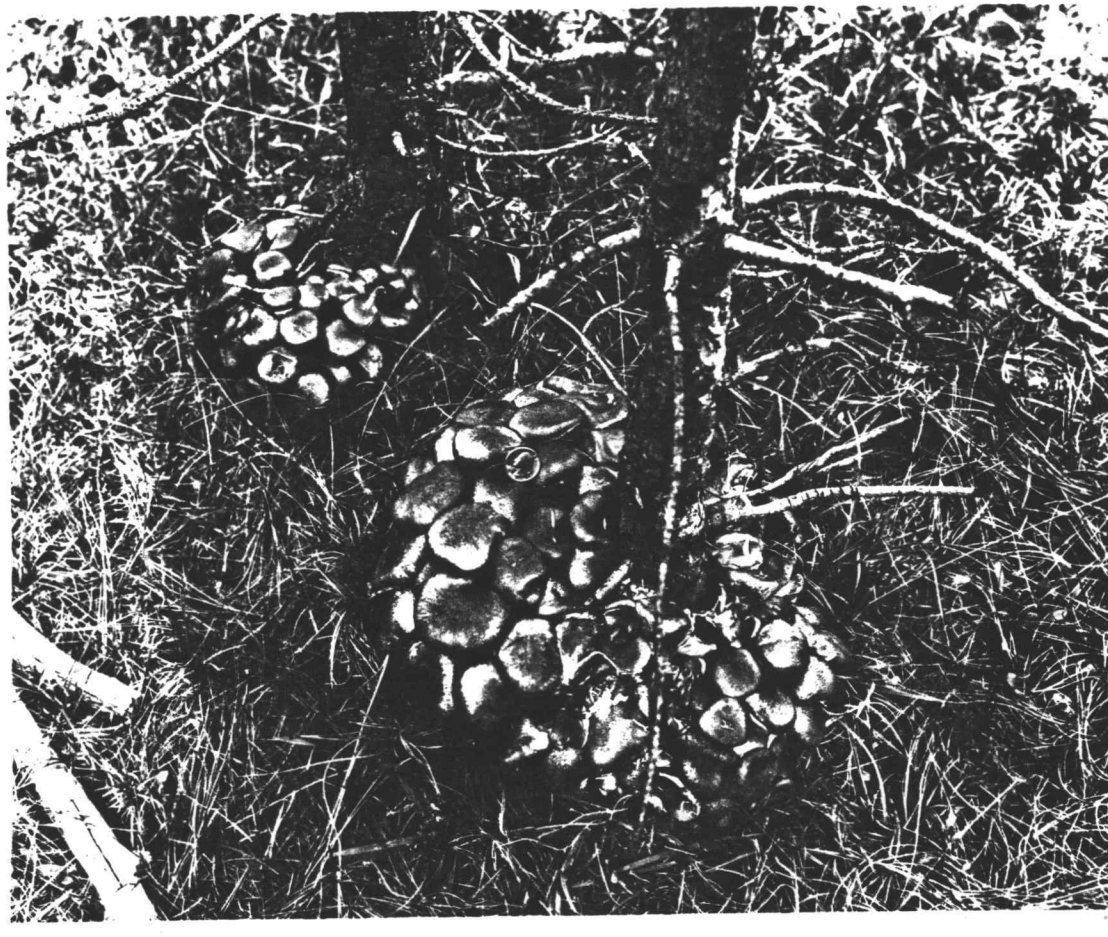
The primary objective of this study is to determine

the uniqueness of A. mellea in severely infected ponderosa pine stands by examination of relationships between A. mellea isolates from adjacent forest cover types to determine if spatial isolation or continuity of fungal colonies or clones occurs between different vegetation cover types and by examination of morphological variation in culture, or lack thereof, between isolates from different clones and different vegetative cover types. Secondary objectives are to determine the average size and age of A. mellea clones in Glenwood and to make further observations of isolate interactions when paired in culture for clonal determination.

FIGURE 2

Armillariella mellea sporophores clustered around the base of a dead ponderosa pine sapling.

(Photograph by L. F. Roth)



LITERATURE REVIEW

Armillariella mellea parasitizes over 600 plant species (Raabe, 1962, 1979), including shrubs, fruit trees and vines, ornamental plants, broadleaved and coniferous trees, some vegetables and weeds. The fungus is considered by many to be a facultative parasite (Gilmour, 1954; Leaphart, 1963; Sokolov, 1964; Singh & Richardson, 1973; Morrison, 1976). Apparently it may also exist in a mycorrhizal association with two species of orchid (Kusano, 1911; Raabe, 1962) and two Monotropa species (Campbell, 1971).

A. mellea is extensively distributed throughout the world. With the exception of deserts, uninhabitable mountains, and tundra, A. mellea occurs in a wide variety of habitats in the taiga, temperate and tropical forest zones (Sokolov, 1964). Serious damage or mortality caused by A. mellea in crop and timber trees has been reported in North America, England, Russia, Japan, Africa, New Zealand and Australia (see Table 1).

Due to its extremely wide host range and distribution, there is an extensive literature concerning A. mellea. This review covers primarily the English languages literature concerning the epidemiology, genetics, morphology in culture, and taxonomy of A. mellea. The

TABLE 1. Impact of Armillariella (Armillaria) mellea on various timber and crop trees throughout the world

LOCATION	*AUTHOR*	*HOST*	*IMPACT*
Pacific Northwest	Dolph & Hadfield (1971)	Conifers	A. mellea is the most common and widespread root disease in conifer plantations. Generally only a small number of trees are killed in each infected plantation. Armilleria root rot is expected to increase on poor sites and non-indigenous plantings.
Oregon, eastern	Johnson & Thompson (1976)	Ponderosa Pine	A comparison of mortality in thinned (4.8%) and non-thinned (8.9%) stands over 8 years was made. It was concluded that susceptibility to A. mellea was reduced by decreasing stress on the trees.
Pacific Northwest	Johnson (1976)	Douglas fir & ponderosa pine plantations	A. mellea is the most common and widely distributed root disease in the Pacific northwest but impact from Phellinus weirii is expected to be greater in the future than impact from A. mellea. A. mellea attacks weakened, low vigor trees.
Washington	Shaw et al. (1976)	Ponderosa pine	Persistent and increasing damage by A. mellea in south central Washington is reported. Wood volume loss of 24 cubic meters per hectare over 14 years was measured on 461 .004 hectare plots.
Oregon, southern	Filip (1977)	Mixed conifers	A. mellea is reported to be the principal or sole pathogen causing an epiphytotic. In the surveyed area 32% of the volume was infected. Most of the infected trees had diameters greater than 27 cm.

Idaho	Hubert (1950)	Western white pine	A. mellea is the most common root fungus on western white pine in Idaho. Fifty percent of the recently killed trees which were examined were infected with A. mellea. Infected trees were between 40 and 90 years old.
Idaho	Partridge & Miller (1971)	Conifers	A. mellea infection was found in 13.17% of all trees inspected; and 56.32% of all root-rotted trees were infected with A. mellea. A statistically significant association between bark beetles and A. mellea and other root rots exists particularly in Douglas fir, grand fir, and ponderosa pine.
California	Hendrickson (1925)	Orchard trees	A. mellea is of widespread economic importance in California. Tree vigor and age are thought to have no effect on resistance to the disease although young trees die more rapidly after infection occurs. Native oak trees were found to be the source of inoculum.
California	Schneider et al. (1945)	Stone fruit trees	A. mellea is considered to be the most serious disease of stone fruits in California. Of 79,000 acres of stone fruit, 10.7% were infected with A. mellea.
New Mexico	Weisse & Riflle (1971)	Ponderosa pine plantations	Serious mortality caused by A. mellea in one plantation of four was observed. Mortality was found to be inversely proportional to tree height. Trees of all vigor were attacked.
Montana	Bousfield & Carlson (1976)	Conifers	A. mellea root rot centers, 20 to 30 acres in size were found in western Montana. Three hundred to 400 acres of primarily Douglas fir, grand fir, subalpine fir and Englemann spruce were involved.

New York	Silverberg & Gilbertson (1961)	Northern white pine plantations	In 1/10 acre plat 38.3% of the trees were infected with A. mellea. Susceptibility was attributed to lowered vigor as the result of low soil nutrients.
Wisconsin	Pronos & Pattoh (1977)	Red pine	Losses of 12, 18, and 37% resulted from Armillaria root rot in 3 widely separated plantations. Mortality occurred within 10 years after planting. Pine mortality was correlated with the total number of dead oak stems/ha.
United States	Guillaumin (1977)	Apricot	A. mellea is the second most important disease in apricot. Disease development is reported to be more rapid in stressed or weakened trees.
Canada	Buckland (1953)	Douglas fir plantations	A. mellea attacks all vigor classes, but low vigor trees are attacked most frequently. Damage by the fungus is greatest in artificially planted sites.
Canada	Foster & Johnson (1963)	Douglas fir plantations	No significant reduction in future yield is expected due to mortality by A. mellea. Mortality may be beneficial in overstocked plantations.
Canada	Morrison (1976)	Douglas fir & other conifers	Damage by A. mellea is not significant in coastal forests and plantations. Some mortality occurs in stands up to 20-25 years, with attack incidence less than 2 to 3%. Mortality is greatest in stressed trees or those near an inoculum source. Significant damage occurs in some interior forests where all ages are killed. Mortality incidence is as high as 25%. Infection centers may occupy up to 3/4 acre.

Canada	Baranyay & Stevenson (1964)	Lodgepole	In 1962, 15.5% mortality was caused by A. mellea and stem rusts. Damage was most evident near decaying aspen stumps. Plots with the shortest trees were most severely affected.
Canada	Huntley et al. (1961)	Red and white pine plantations	An increase in disease by A. mellea occurred in plantations which were established on cutover land with stumps present.
Canada	Singh (1970)	Exotic conifer plantations	Mortality ranged from 5% on Larix sp. to 28% on Picea sitchensis. All species were attacked, especially those with low vigor. Mortality occurred in 6 to 10-year-old trees. High incidence of disease is attributed to low soil nutrients, low host vigor, high inoculum levels, and favorable soil pH for A. mellea.
Canada	Singh & Richardson (1975)	Conifer plantations	A. mellea is the most important disease affecting man-made forests in Newfoundland. Exotic tree species are more susceptible to A. mellea than are native species.
Canada	Singh (1978)	Conifers	A. mellea is the cause of root and butt rot leading to mortality or weakening. A. mellea is devastating in young plantations but may act as a natural thinning agent in dense stands. Major losses are due to mortality, windthrow, and growth reduction.
England	Hiley (1919)	Larch	A. mellea is reported to be responsible for more mortality of trees in Europe than any other parasitic agent. All conifer species and several broadleaved species are attacked.

England	Day (1927)	Conifers	A. mellea causes continual loss of forest trees throughout the world. In natural forests the fungus is confined to broadleaved or mixed broadleaved-conifer forests, rarely found in natural stands of pure conifer. It is extremely common in pure conifer plantations established on old broadleaved sites.
England	Greig (1962)	Conifers	Damage by A. mellea may be serious locally. Mortality increased up to the first thinning. A decrease in mortality is coincident with disintegration of hardwood stumps and roots.
England	Peace (1962)	Forest trees	Actual damage caused by A. mellea is small considering its prevalence throughout the world. With complete replacement of hardwoods with conifers, damage is absent or diminished in the second rotation.
France	Boullard (1972)	Exotic softwoods	Attacks on exotic softwoods by A. mellea and other root rot pathogens have increased in France due to extensive plantings of exotic species and to the use of unsuitable sites.
Finland	Hintikka (1974)	Spruce & pine plantations	A. mellea is considered a harmful parasite of forest trees in Scandanavia. It occurs most frequently on pine seedlings (10 - 15 years old) and also as a causal agent of heart rot of spruce. Economic loss in the latter instance is significant.
Yugoslavia	Uscuplic (1978)	Pinus strobus plantations	Mortality occurs within the first five years after planting. A. mellea is becoming much more severe in new conifer plantations which were established on previous oak and beech land.

Russia	Sokolov (1964)	Conifers	Armillaria root rot is severe in some areas of the USSR. A. mellea is more aggressive in artificially made, monospecies stands than in natural mixed stands. Infection may be chronic or may lead to mortality within a year.
New Zealand	Gilmour (1954)	Radiata pine	A. mellea is found in over 100,000 acres of radiata pine in New Zealand. A. mellea is not active as a parasite on P. radiata currently. It is thought to represent a potential danger as a parasite, however.
New Zealand	Shaw & Calderon (1977)	Radiata pine	Armillaria root rot is the most destructive disease in P. radiata established on cleared sites of indigenous trees. Mortality varies depending on the former species. Mortality (5 to 27% after 2 years) and growth reduction occur as the result of infection.
New Zealand	Shaw & Toes (1977)	Radiata pine	Annual loss in growth of 8 to 10-year-old radiata pine amounted to 14 - 24% as the result of infection by A. mellea. Growth loss in trees infected with A. mellea and Dothistroma pini was 53% an amount greater than the sum of loss from either agent alone.
Australia, western	Carne (1926)	Fruit trees	A. mellea is the most important root rot in western Australia in fruit trees. The roots and stumps of native eucalypts are considered to be the source of inoculum.
Australia, southern Tasmania	Bowling & McLeod (1968)	Eucalypt	The first incident of attack of native eucalypt by A. mellea is reported. Crown die-back of all classes occurred, especially in older second growth.

Australia	Kile (1978)	Eucalypt	Losses in plantations of native hardwoods or exotic conifers from A. mellea may be locally severe but no serious wide-scale losses have occurred.
Japan	Ono (1965)	Larch plantations	Armillaria root rot is one of the most damaging diseases in larch plantations in Japan. Mortality is highest in the third and fourth years; no mortality occurs after 5 or 6 years.
Japan	Kawanda et al. (1965)	Larch plantations	A. mellea attacks 3 to 6-year-old and 30 to 40-year-old trees. Although only a small total area is affected, mortality is considered significant. Some correlations were found between severity of disease and soil conditions.
Africa, Belgian Congo	Wardlaw (1950)	Oil palm plantations	Disease by A. mellea is found in all soil types but death is most rapid in poor, sandy soils. Mortality occurs in 4 to 10-year-old trees. A. mellea originates from old, infected stumps of previous forest species.
Africa, Kenya	Gibson (1960)	Pine plantation	A. mellea is one of two diseases of any importance in exotic timber crops in Kenya. Infection by A. mellea is most severe in 5-year-old plantations. In 1959 29.8% of the plantations were infected. Disease is most frequent and severe on sites which were previously Montane rain forests.
Africa, Rhodesia	Swift (1968)	Conifer plantations	A. mellea was the cause of disease outbreaks in pine plantations in Rhodesia. A consistent mean mortality rate of 2 trees/year/plot was demonstrated for the 4 plots observed.
Africa, eastern	Olembo (1972)	Pinus patula, Cupressus lusitanica	A. mellea-free sites attributed to a biological or chemical factor present in the soil.

economic impact of A. mellea as a forest pathogen will be reviewed briefly at the beginning.

Impact in the Forest

The impact of Armillariella mellea on forest trees is reviewed briefly with regard to the kind of tree, plantation or stand that is most severely attacked and why. This is done in order to provide the reader with a picture of relative amounts and severity of Armillaria root rot throughout the world compared to that in Glenwood, Washington, with which we are particularly concerned.

Damage by A. mellea generally is through death rather than trunk decay. Decay is usually confined to the roots and root crown. Occasionally, however, serious decay of the lower bole does occur. Wardlow (1950) reported trunk rot in a plantation of oil palms; Morrison (1976) found small amounts of butt rot in older Douglas fir and other conifers in coastal British Columbia, Canada; and Kallio and Norokorpi (1972) and Hintikka (1974) found that A. mellea rotted the heart wood of spruce and aspen in Finland.

Armillaria root rot tends to be most damaging in young plantations and in non-indigenous plantings (Wardlow, 1950; Gilmour, 1954; Gibson, 1960; Greig, 1962; Peace, 1962; Ono, 1965; Singh, 1970; Dolph & Hadfield, 1971; Weisse & Riffle, 1971; Singh & Richardson, 1973; Singh,

1978; Uscuplic, 1978). Often when plantations are established on former hardwood sites, infection and mortality are high until the old hardwood stumps disintegrate. Several researchers (Gibson, 1960; Greig, 1962; Ono, 1965; Uscuplic, 1978) report little or no disease in the second conifer rotation on land previously supporting a natural hardwood forest. It is important to note, however, that size of foodbase may be more important than type. Disease, in the above cases, might have been just as or nearly as severe in the second rotation if the first rotation conifers were cut at an older age so that the stumps were large.

On the other hand, Filip (1977) found that A. mellea caused signicant damage in a natural mixed conifer forest in southern Oregon where most of the infected trees had diameters greater than 27 centimeters. Similarly, up to 25% mortality was found in some areas of interior British Columbia conifer forests where all ages of trees were killed (Morrison, 1976). Shaw et al. (1976), too, report a large and increasing amount of damage occurring in a ponderosa pine forest in south central Washington (Glenwood).

Despite evidence that A. mellea can attack both high and low vigor trees (Day, 1927; Hendrickson, 1925; Christensen, 1938; Buckland, 1953; Weisse & Riffle, 1971), disease caused by the fungus is often reported to be most

severe on sites where the trees are stressed or weakened by other agents. Poor site conditions such as low nutrients or excessive moisture (Day, 1929; Buckland, 1953; Silverborg & Gilbertson, 1961; Kawada et al., 1965; Ono, 1965; Dolph & Hadfield, 1971; Guillaumin, 1977; Manka, 1978), off-site conditions (Dolph & Hadfield, 1971; Singh & Richardson, 1973), high tree density (Pielou & Foster, 1962; Baranyay & Stevenson, 1964; Johnson & Thompson, 1975), defoliation by insects (Hudak & Singh, 1970; Singh & Bhure, 1974; Hudak & Wells, 1974; Wargo & Houston, 1974; Singh, 1975, 1978), animal browsing (Baranyay & Stevenson, 1964), and environmental damage (Dance & Lynn, 1963 in Singh, 1978; Hintikka, 1974) are some of the conditions reported to contribute to stress or weakening, and thus, predisposition to A. mellea. Since most reports of predisposition are based on observations rather than experiments comparing stressed and non-stressed trees, no definite conclusions can be drawn on the question of predisposition at this time.

On a world-wide scale, A. mellea damage is scattered and severe only locally. However, due to the pattern of disease spread, i.e., death of trees in small to large patches, the impact is much higher than if the same amount of disease occurred at random throughout the stand. Foster & Johnson (1963) state that morality by any agent affecting single trees at random could be beneficial

but that mortality in clumps could be extremely detrimental. Although Peace (1962) states that "the actual damage done by this fungus is surprisingly small considering its prevalence," his assessment of the impact of A. mellea on forests of the world may not be valid. High levels of disease and damage in natural forest may be more common than previously believed; with the greater utilization and increased surveys and inspection of forest lands, reports of A. mellea epidemics in these forests are increasing.

Epidemiology of Armillariella mellea

A review of certain aspects of the epidemiology of A. mellea is necessary since the study of clonal relationships is closely tied to the mode and rate of spread of the fungus. A discussion of the literature on both mode and rate of spread as well as a look at the question of hardwood substrate superiority will be made.

A. mellea can spread by means of rhizomorphs, root-to-root contacts, root grafts, and, perhaps, basidiospores. Spread by rhizomorphs and infection of healthy trees has been reported by many (Hartig, 1874; Hendrickson, 1925; Day, 1927; Thomas, 1934; Sokolov, 1964; Rishbeth, 1970; Hintikka, 1974; Guillaumin, 1977). Rhizomorphs originate and grow out from the mycelium within colonized stumps and roots of diseased trees. They may extend from a few millimeters to 10 to 15 meters from an

inoculum source (Sokolov, 1964).

Growth rates of rhizomorphs in both the laboratory and field have been measured by several researchers in the past few decades. Sokolov (1964) found that growth of rhizomorphs in culture was 16 millimeters per day at optimum temperatures (17.5° - 24.5° C.) and humidity (80 - 90%). Slightly lower rates were observed by others: 8.4 mm per day at 25° C. on 3% malt agar (average maxima of 12 isolates) (Rishbeth, 1968), about 9 mm per day at 25° - 27.5° C. on malt extract agar (Hintikka, 1974). Garrett (1956), measuring rhizomorph growth from woody inocula through tubes of soil, found maximal growth of 3.6 mm per day. Rishbeth, using similar methods, observed rates of 3.2, 5.7, and 9.1 mm per day for small, medium, and large rhizomorphs, respectively, at 25° C. In general, it was found that rhizomorph growth rates in the laboratory are dependent on temperature, humidity, CO_2 concentration, type of medium through which the rhizomorphs are growing, nutrient source, size of inocula, size of initial rhizomorphs, type of rhizomorph (terminal or lateral apex), and fungal isolate or strain used.

Rhizomorph extension in the field is generally comparable to growth rates in culture: 2 mm per day (Schmitz, 1843, in Hartig, 1874); .8 - 3.2 meters per year in New South Wales on peach (Kable, 1974); 50 cm per month in the summer near Leningrad (Sokolov, 1971, in Hintikka, 1974;

3.4 mm per day from July to October in Finland (Hintikka, 1974).

Spread of A. mellea from diseased to healthy trees by root contacts is also important and may be the primary mode of spread in particular situations or locations (Marsh, 1952; Molin & Rennerfelt, 1959; Greig, 1962; Redfern, 1970; Swift, 1968, 1972; Kable, 1974). In parts of central and west Africa few or no rhizomorphs are produced and spread of the fungus is entirely dependent upon root contacts or infection of stumps by basidiospores (Leach, 1939; Fox, 1964; Swift, 1972). Kable (1974) and Marsh (1952) found that progression of Armillaria root rot in a peach orchard and a black currant plantation, respectively, tended to be most rapid in the direction of root contacts, i.e., along rows rather than between rows.

The geographical coverage of one A. mellea isolate by vegetative means, by successive root-to-root or rhizomorph-to-root contacts, is often extensive. Shaw and Roth (1976) reported the existence of one A. mellea "clone" in a Washington ponderosa pine forest extending over 600 hectares, encompassing several infection centers. Enlargement of individual infection centers in this area occurred at the average rate of 1.0 meter per year. Childs (1963) similarly found extensive development of several Phellinus weirii (Murr.) Gilbertson root rot clones in a young Douglas fir forest, some which included

several infection centers separated by a 100 foot or wider zone of healthy forest.

Until fairly recently, the role of basidiospores in the infection and spread of A. mellea was poorly understood and considered unimportant by most. In 1919 Hiley suggested that basidiospores are the means by which stumps are infected and colonized by A. mellea. Leach in 1937 attempted unsuccessfully to inoculate stumps with basidiospores in Malawi. Limited success with spore inoculation of stumps was achieved by Molin & Rennerfelt (1958) in Sweden and Rishbeth (1964, 1971) in England. Rishbeth (1964) concluded that, despite a low incidence of infection by spores, particularly on conifer stumps, origin of infections by spores is important in establishment of new infection centers. Sokolov (1964), after successfully germinating spores on glass under natural conditions and on fresh aspen and spruce wood and bark in damp chambers, also concluded that infection of stumps with basidiospores does occur. Swift (1972), although achieving greater than 75% germination of spores on freshly cut wood discs under sterile conditions, was unable to get germination and colonization of stump surfaces in the field. He attributes his lack of success more to competing fungi than to unfavorable environment. Rishbeth (1970) conversely suggested that poor stump colonization may often be the result of the stump being too dry; he also suggests that low

temperatures may prohibit good mycelial growth into the stump if spore release and germination occur too late in the fall. Hintikka in 1973 found that different anatomical structures of different tree species at the cut surfaces were perhaps responsible for differences in the infection process and success of infection between conifer and deciduous tree species.

Although basidiocarps were rarely observed in some areas, Swift (1972) found that, in Rhodesia, viable spores were trapped during the rainy season, demonstrating that potential inocula may be present in areas devoid of fruiting bodies. Where basidiocarps grow profusely, large numbers of spores can be trapped during the fall; Rishbeth (1970) reports up to 1000 viable spores per square decimeter per minute in close vicinity to fruiting bodies from October to November in England.

Garrett (1956) established that the size and condition of the wood substrate acting as the inoculum source determines the success of infection. Larger volumes of wood, such as stumps or dead trees, have a higher infective ability than do smaller volumes, such as wood fragments or isolated root pieces. Wood which is dead is more readily colonized by A. mellea (Rishbeth, 1970).
For the above reasons, infections originating from a large colonized stump generally occur more frequently and consistently than do infections originating from the infected

roots of a living tree or from infected root fragments in the soil. In many cases natural and unmanaged forests have a much lower incidence of Armillaria root rot due, probably, to a lower volume of woody material which can be used as fungal substrate (Sokolov, 1964; Manka, 1978).

Both the distance from the substrate or "food base" and the type of food base are also important in epidemiology. Garrett (1956) found that the vigor of infection (% of tuber tissue infected) decreased with increasing distance between A. mellea inoculum (willow wood segments) and potato tubers. The decrease in vigor was caused by both an exhaustion of nutrients in the inoculum by fungal respiration and growth, and an increase in competition between rhizomorph growing apices for a constant supply of nutrients. It has generally been thought that conifer stumps tend to be unsuitable food bases even though they are as readily invaded by A. mellea as are hardwood stumps. Rishbeth (1972) found that pine stumps rapidly deteriorate and are more rapidly exhausted as food bases, with a concurrent decline in the infective capacity of the fungus. In hardwoods, slower rates of deterioration, superiority as a substrate, the ability to produce more infective "propagules" (rhizomorph growing tips), and the frequent delay of death by regrowth may contribute to hardwood superiority as a food base (Redfern, 1970, 1975).

There are numerous reports of disease outbreaks in

conifer plantations or fruit orchards which have originated from hardwood stumps of the previous indigenous hardwood or mixed hardwood-conifer forest (Hartig, 1874; Hendrickson, 1925; Zeller, 1926; Childs & Zeller, 1928; Leach, 1937; Gibson, 1960; Gibson & Goodchild, 1960; Greig, 1962; Sokolov, 1964; Rishbeth, 1972; Hintikka, 1974; Singh, 1975). On these sites, A. mellea often showed little or no parasitic action on the indigenous trees, behaving, instead, as a saprophyte. Shaw and Roth (1976), however, found Armillaria root rot in epidemic proportions in pure ponderosa pine forests where inoculum sources for new infection centers were large, old pine stumps. Similar observations have been made by others (Gibson, 1960; Weisse & Riffle, 1971; Adams, 1972; Swift, 1972; Redfern, 1975; Filip, 1977). Redfern (1975) concluded that coniferous food bases may be just as effective in perpetuating the disease if short rotations are adopted and thinning occurs frequently so that a constant supply of new food bases is available.

Cultural Characteristics of A. mellea

The growth characteristics and morphology of Armillariella mellea in culture are highly variable (Lisi, 1940; Benton & Erlich, 1941; MacLean, 1950;

Raabe, 1943, 1966, 1969; Gibson, 1961; Gibson & Corbett, 1964; Wilbur et al., 1972). Growth of A. mellea in culture is slow by comparison with other fungi (Edgecomb, 1941; Jennison et al., 1955; Snider, 1959) and is influenced by temperature, pH, light, proportion and form of carbon and nitrogen, and the presence of growth-promoting substances.

Reported pH values for optimal growth and rhizomorph production of A. mellea are variable (see Table 2).

Optimal temperatures for thallus or mycelial growth are reported to be between 21° and 29° C by various researchers. Benton and Erlich (1941) found growth to be optimal between 21° and 25° C ; Townsend (1954) and others (Reitsma, 1932, in Benton & Erlich, 1941; Wolpert, 1924, in Benton & Erlich, 1941) report best mycelial growth at 25° C. Gibson (1961), in testing over 100 isolates from various parts of the world, found that the temperatures for optimal growth varied among isolates; 54 grew best between 25° and 27° C , 46 grew best between 22° and 24° C , and 16 grew best between 27° and 29° C. However, no correlation between temperature optima and origin of isolate was found. Of 32 isolates tested by Raabe (1969), some grew best at 21° C and others at 25° C. Growth optima tended to vary depending on the medium used.

TABLE 2. Effect of pH on mycelial growth and rhizomorph development of Armillariella mellea as reported by various authors

	pH RANGE & OPTIMA			
	Mycelium		*Rhizomorphs*	
	pH Range	Optimal pH	pH Range	Optimal pH
Wolpert (1924)[1]	-	6.9 & 7.8[2]	-	-
Reitsma (1932)[3]	-	4.5 & 6.5	-	-
Lisi (1940)	-	6.0	-	-
Benton & Erlich (1941)	-	4.5 & 5.5	-	5.0
Townsend (1954)	3.1-8.8	-	3.6-8.0	5.6
Snider (1959)	-	-	4.0-7.0	-
Sokolov (1964)	2.5-8.5	-	-	-

[1]In Benton & Erlich (1941).

[2]Two optima are indicated.

[3]In Benton & Erlich (1941).

Temperature optima for rhizomorph growth were about the same or lower than those for mycelial growth. Benton and Erlich (1941) and Bliss (1946), respectively, reported greatest rhizomorph development at 21° C and 19.7 - 24° C. Based on total rhizomorph length, Townsend (1954) found no rhizomorphs produced at 10° C., 210 mm (average) per colony were produced at 20° C., 236 mm per colony were produced at 25° C., and very slow rhizomorph growth occurred at 30° C. Similarly Snider (1959) found that rhizomorph growth responded optimally at temperatures around 25° C. Gibson (1961) found, as with mycelial growth, that 48 of his isolates had optimal rhizomorph growth between 25° and 27° C., 26 between 22° and 24° C., and 12 between 27° and 29° C.

Light appears to be inhibitory to rhizomorph growth and elongation. Raabe (1953) and Snider (1959) found that rhizomorph growth was inhibited by bright white light. However, Townsend (1954) noted that although rhizomorph elongation was slowed in light, rhizomorph formation was not. He also reported that colony diameter was greater in the dark than in light.

There has been considerable research on nutrient requirements of A. mellea, especially carbon and nitrogen utilization and, more recently, growth-

promoting substances. Reitsma (1937, in Benton & Erlich, 1941) reported glucose to be the best source of carbon for growth of A. mellea in culture. Weinhold and Garraway (1966) showed that ethanol, as the sole carbon source, gave optimal rhizomorph growth and, in some media, unless ethanol was added, glucose, fructose, and sucrose were poor sources of carbon. Later, Garraway and Weinhold (1968) demonstrated that ethanol was necessary only for rhizomorph initiation and early development. Glucose could be used as the sole carbon source for subsequent growth. It is suggested that a similar situation may exist in the field where a growth-promoting substance such as ethanol is needed for rhizomorph initiation from undefined mycelium and "plaques" and, once established, utilization of carbohydrates from the food base can occur. Increased levels of carbon in the media result in increases of mycelial dry weight (Rykowski, 1976).

Peptone (Reitsma, 1932; Garrett, 1953; Azevedo, 1963) and L-asparagine (Rykowski, 1976) are shown to be superior sources of nitrogen for growth of A. mellea over other organic and inorganic forms of nitrogen. The addition of thiamine to media containing peptone was shown to greatly increase growth (Garrett, 1953; Azevedo, 1963). In contrast to the

effects of carbon, increased nitrogen tended to favor aggregation of mycelium into more organized forms such as rhizomorphs (Rykowski, 1976). Increases of nitrogen above a certain level, however, inhibited growth.

Carbon-nitrogen ratios appear to be as important to growth of A. mellea as the type of each constituent. Townsend (1954) found that a ratio of three to five parts glucose to one part peptone or asparagine was most favorable to growth. Similarly Hamada (1940) observed optimum growth at a carbon-nitrogen ratio of four to one. He found that while formation of aerial mycelium was not dependent on the carbon-nitrogen ratio, rhizomorph and "brown membrane" (pseudosclerotium) formation was. Maximum luminescence and guttation occurred at ratios of carbon to nitrogen between 1:2 and 2:1. Substrate browning was dependent on the absolute amount of peptone and not on the carbon-nitrogen ratio since browning is the result of oxidation. As carbon concentrations increase, the optimum concentration of nitrogen for initiation and growth of rhizomorphs also increases (Garrett, 1953).

A number of substances have been reported to have growth-promoting effects on A. mellea: ethanol and other low molecular weight alcohols (Weinhold,

1963; Weinhold & Garraway, 1966; Pentland, 1967; Garraway & Weinhold, 1968; Sortkjaer & Allerman, 1972; Allermann & Sortkjaer, 1973); short- and long-chain fatty acids (Moody & Weinhold, 1972); indole-3-acetic acid (Garraway, 1970, 1975); auxins (Garraway, 1975); acetate (Weinhold, 1963; Sortkjaer & Allermann, 1972; Allerman & Sortkjaer, 1973); and chlorophenoxy acids (Pronos & Patton, 1978).

The morphology of the mycelium and rhizomorph have been extensively described by several researchers (Campbell, 1934; Cartwright & Findlay, 1946; Nobles, 1948; Gilmour, 1954; Townsend, 1954; Snider, 1959). Descriptions of the aerial mycelium of A. mellea tend to be confined to one of two types: a colorless, appressed and rather sparse mycelium (Benton & Erlich, 1941; Cartwright & Findley, 1946; Nobles, 1948; Gilmoure, 1954; Gibson, 1961) or a dense cottony, white luxuriant growth (Totten, 1917; Campbell, 1934; Benton & Erlich, 1941; MacLean, 1959; Gilmour, 1954; Gibson, 1961; Sokolov, 1964). Gibson (1961) describes four types of mycelial growth found in 140 isolates from various locations around the world: (1) aerial mycelium produced in small tufts only with the orange to brown tufts never exceeding 1.5 mm in height; (2) aerial mycelium produced in large tufts, 1.5 - 4 mm high, of

orange to brown color; (3) aerial mycelium sparse with no tufts; and (4) abundant, thick, nearly uniform, white, aerial mycelium with yellowish tints.

Many researchers have observed the development of a pseudosclerotium, a compact, dark-colored plate, which is formed over the aerial mycelium. Snider (1959) defines the pseudosclerotium as a "cylindrical or arched, melanin-impregnated sheath enveloping most of the lateral mycelia . . ." The pseudo-sclerotium is formed from thick-walled bladder- or dumbbell-shaped cells (Campbell, 1934; Gilmour, 1954) which arise from the aerial hyphae. The bladder cells are dark brown or yellowish-brown in color, 6 - 14μ (Campbell, 1934) to 25μ (Gilmour, 1954) in diameter.

Rhizomorphs originate from the center of the colony or around the edge of the inoculum plug and diverge radially (Campbell, 1934; Garrett, 1956; Snider, 1959; Sokolov, 1964). Both cylindrical and flat submerged rhizomorphs are formed in culture. Flattened rhizomorphs are generally white or cream-colored, although Gibson (1961) describes flat rhizomorphs which are dark brown to black in color. Sokolov (1964) writes that the "silky white" flattened rhizomorphs grow through the "entire thickness of the substrate in the form of fantastically twisted ribbons, toothed blades and glumes." Cylindrical rhizomorphs are dark and 1 - 2 mm to 5 mm in diameter (Gibson, 1961).

Submerged rhizomorph production among isolates varies considerably. Gibson (1961) describes three states of rhizomorph vigor: low, where rhizomorphs, if present at all, never exceed the radius of the aerial mycelium; moderate, where the radius of the rhizomorphs never exceeds two times that of the aerial mycelium; and high, where rhizomorph growth exceeds twice the radius of the aerial mycelium.

Aerial rhizomorphs, when formed, are always dark and cylindrical, giving rise to aerial hyphae where they emerge from the media (Campbell, 1934; Gibson, 1961; Sokolov, 1964). They may reach 1 - 1.5 cm above the substrate and grow to a diameter of 2 - 3 mm (Sokolov, 1964).

Snider (1959) describes five stages of thallus (mycelium and rhizomorph) development of monosporous isolates on potato glucose agar. The "pre-emergent" phase occurs during the first week of growth. Mycelium only is observed, with rhizomorphs initiating somewhat before the end of this stage. A brief "emergent" phase follows in which rhizomorph initials become visible; "microsclerotia," the site of rhizomorph origination at the media surface, become pigmented and enlarge; rhizomorph apices organize and the first few millimeters of growth occur. A short "lag" stage, where rhizomorph extension is accelerating but has not yet exceeded the radius of the central mycelium, precedes the "linear" phase. The linear stage is

characterized by a linear acceleration of rhizomorph growth five or more times faster than that of the mycelium. Rhizomorphs now extend beyond the mycelial radius, and numerical increase occurs by dichotomous or palmate branching at the apices. De-acceleration of growth (generally due to confinement in a petri plate), formation of hair zones (the zone of vegetative hyphae protruding through the mature rhizomorph epidermis which is analagous to a root hair zone of higher plants), lateral branching from the main rhizomorphs, and appearance of the pseudo-sclerotium occur in the "terminal" or final stage.

With a few exceptions (Sokolov, 1964; Raabe, 1969, 1972) A. mellea is not known to fruit in culture (Gilmour, 1954; Snider, 1959; Raper, 1966; Korhonen & Hintikka, 1974; Tommerup & Broadbent, 1975). Sokolov (1964) reported that in cultures greater than two months old, fruiting occasionally occurred as a group of tubercles on a common base; only one or two of a group of 14 - 22 immature caps usually developed fully. Raabe (1972) reported development of basidiocarps in culture from an isolate obtained from pear in California. Basidiocarp production in culture by Reitsma (1932) is cited by Raper (1966).

Genetics of Armillariella mellea

Although this study does not involve the investigation of the sexuality of A. mellea, it is useful and

clarifying when discussing pairing studies to be aware of some aspects of the nuclear life cycle, polarity, and morphological and nuclear changes which occur when single spore (haploid) isolates are mated.

Prior to 1973, the life cycle of Armillariella mellea was considered to be homothallic (Kneip, 1911), asexual (Raper, 1966), or homomictic (Burnett, 1956)[1] with no evidence of polarity or interaction between single spore isolates (Hintikka, 1973). Unlike most Hymenomycetes, the mycelium of A. mellea, for the most part, lacks clamp connections which, for those species possessing them, are a reliable indicator that sexual fusion has occurred and that the nuclear state of the mycelium is dikaryotic. Therefore, in A. mellea, any mating reactions which take place are not discernible using this criterion. Hintikka (1973) showed that a tetrapolar[2] pattern of sexuality was obtained when 120 single-spore isolates from one basidiocarp were paired in culture. The tetrapolar pattern was based upon consistent morphological changes in the combined mycelium of certain combinations of isolates. Single spore isolates were generally white with

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[1]Homothallic = asexual = homomictic. All are equivalent terms for a self-fertilizing mode of sexual reproduction.

[2]Tetrapolar sexuality refers to the situation where compatibility between monokaryons is based on interactions between two multigenic alleles; as opposed to bipolar sexuality where compatibility is determined by only one allele.

raised, aerial hyphae; in three-quarters of the combinations, isolate morphology did not change; in the other one quarter, the combined mycelium became dark and crustose, lacking aerial hyphae. Anderson & Ullrich looked at the mating reactions of monosporous isolates from 25 basiciocarps found on various host species throughout New England (1977) and from 72 basidiocarps from various locations in North America (1979). A tetrapolar pattern of sexuality was seen in all cases allowing the authors to ascribe to A. mellea universal sexuality.

The nuclear state of A. mellea is unique among hymenomycetous Basidiomycetes in that growing vegetative cells from non-monosporous material (i.e., not grown from a single spore) are uninucleate instead of dikaryotic. The monokaryotic state of tip cells was first observed by Kneip in 1911. Since then the lack of dikaryotic mycelium in rhizomorphs and in most tissue of the basidiocarp has been reported by several researchers (Singer, 1962; Berliner & Duff, 1965; Motta, 1969; Hintikka, 1973). Hintikka (1973) observed nuclear numbers in different types of mycelia: hyphal tip cells at the growing margin of the mycelium always contained only one nucleus; older cells, closer to the center of the colony, and the enlarged cells of the rhizomorphs and basidiocarp had between 10 and 15 nuclei per cell; cells at the base of the basidia regularly had two paired nuclei per cell (also observed by Singer,

1962). Several hypotheses are put forth by Hintikka to explain the monokaryotic conditions of the tip cells: nuclear migration may be occurring, plasmic factors may be involved, or the paired mycelium is diploid. Cytological and microspectrophotometrical evidence for a diploid nuclear state in A. mellea is presented by Korhonen & Hintikka (1974), and Tommerup & Broadbent (1975) and Peabody et al. (1978), respectively.

Reactions between isolates in culture are helpful in determining the degree of relatedness of the isolates. Matings or pairings can be made between two monosporous isolates, two isolates derived from plant tissue or basidiocarp tissue (diploid), or combinations thereof. The distinction between haploid-haploid and diploid-diploid mating reactions is not clear. Similar mechanisms seem to be involved so that in either type of pairing compatible matings result in hyphal fusions and incompatible matings result in little or no hyphal fusion.

Hintikka (1973) found that in A. mellea the combined mycelium in compatible pairings between two monosporous (haploid) isolates changed from the aerial, fluffy white mycelium, typical of monospore isolates, to a darker crustose mycelium with little or no aerial mycelium. Nuclear migration occurred in some of the combined mycelium, followed by a short dikaryotic phase where clamp connections were present. Somatic diploidization fol-

lowed (Korhonen & Hintikka, 1974).

Korhonen recently (1978) attempted to separate incompatible mating reactions between haploid isolates from the same basidiocarp on the basis of morphology and ascribe to each an allelic designation as was done with Schizophyllum commune Fr. In S. commune, a tetrapolar Basidiomycete, four reactions occur when a monokaryon is paired with each of the four incompatibility types derived from the same basidiocarp (e.g., A1B1 x A1B1, A1B2, A2B1, A2B2). When both alleles are different, a compatible reaction occurs and dikaryotic mycellum is formed. When both alleles are the same, a common-A-common-B mating, mycelia 'overlap' where the two isolates meet. When the A alleles are the same, a hemicompatible common-A mating, a region of thin and poorly developed hyphae occurs where the isolates meet ('flat' reaction). When the B alleles are the same, a hemicompatible common-B mating, a 'barrage' reaction occurs where the area between the two isolates is almost devoid of hyphae (Papazian, 1950).

Korhonen (1978) found, as with S. commune, that pairings between isolates with unlike A and B alleles resulted in a compatible mating and formation of crustose mycelium as described above. When both A and B alleles are the same (A=B=), an incompatible mating results where no interactions between the two mycelia occur, either macroscopically or microscopically. In hemicompatible

common-B matings (A≠B=), the reaction is generally identical to that of the incompatible mating. Hemicompatible common-A matings (A=B≠) usually result in the formation of a 'barrage zone' between the two mycelia where aerial mycelium is lacking or sparse. Sometimes a zone of crustose mycelium forms on the barrage line and it may spread to one or both isolates, making distinction between this reaction and a compatible reaction difficult.

Dikaryotic or diploid isolates of many Basidiomycetes which are genetically dissimilar react to one another when grown together in a petri dish; changes in morphology and substrate appearance result along or between the interface of the two colonies (Cayley, 1925; Schmitz, 1925; Mounce, 1929; Vandendries & Brodie, 1933; Childs, 1937; Verrall, 1937; Childs, 1963; Adams & Roth, 1967; Barrett & Uscuplic, 1970; Adams, 1974; Shaw & Roth, 1976). Several terms have been used to describe this reaction: 'line of demarkation' (Cayley, 1925; Schmitz, 1925; Mounce, 1929; Verrall, 1937; Adams & Roth, 1967; Adams & Roth, 1969; Adams, 1974; Shaw & Roth, 1976), 'mutual aversion' (Cayley, 1925), 'barrage' (Vandendries & Brodie, 1933), 'line of aversion' (Mounce, 1929), 'antagonism' (Verrall, 1937), 'sterility barrier' (Burnett, 1968), and 'interaction zone' (Barrett & Uscuplic, 1970). All of these terms seem to be describing the same or similar 'isolating mechanisms' (Burnett, 1975) for maintaining within

the species, separate or intersterile (Burnett, 1968) groups.

Descriptions of the morphological changes at the interface of two paired isolates of A. mellea have been minimal. Adams (1974) describes the line of demarcation in A. mellea as a dark line formed along the common interface, with no intermingling hyphae. Shaw (1975) states that the line of demarcation is descriptive of the "dark hyphae which appear along the area of meeting of two isolates paired in culture."

In paired cultures of Polyporous schweinitzii Fr. intermingling of hyphae takes place in the zone of contact. Further growth is restricted in this zone but aerial hyphae may build up on one or both sides of the zone. Discoloration along the interface occurs as the result of development of small knots and disruption of hyphae and the formation of dense chlamydospores in abnormally short-celled hyphae in this area (Barrett & Uscuplic, 1971).

The zone of antagonism between isolates of Fomes igniarius (L) Gill. is described by Verrall (1937) as a dark zone in the agar which separates the aerial mats; often the zone is free of mycelium except for invisible submerged hyphae which intermingle to some extent. Pairings of isolates of Fomes cajanderi Karst. result in the discoloration of the substrate or mycelium in the zone between the colonies (Adams & Roth, 1967). Mounce (1929)

describes the line of demarcation between isolates of Fomes pinicola (Sw.) Cke. as "a line formed by the dense growth on the part of one or, more frequently, both of the mycelia developed where the two mycelia met."

Cayley (1923) found that the growth of paired cultures of the ascomycete Diaporthe perniciosa Marchal. showing mutual aversion is arrested along the contact zone; submerged hyphae intermingle at first, but later disorganize and die. A thick-walled stromatic growth of mycelium forms at the edge of each colony along the contact zone which may or may not be accompanied by discoloration of the media.

The presence of an interaction zone or line of demarcation divides tested isolates into physiologically distinct groups. Various terms have been used to describe these groupings: 'strains' (Cayley, 1923; Schmitz, 1925; Barrett & Uscuplic, 1971); 'clones' (Childs, 1937 and 1963; Shaw, 1975; Shaw & Roth, 1976); 'mycelial types' (Rishbeth, 1978), and 'inter-sterile groups' (Burnett, 1968). The last term, however, is generally a designation restricted to monosporous isolate groupings.

In contrast to haploid or monosporous isolates, compatible diploid A. mellea isolates paired in culture do not undergo morphological changes; the two mycelia simply grow together (Adams, 1974; Shaw & Roth, 1976; Korhonen, 1978).

Taxonomy of Armillariella mellea

The fungus, Armillariella mellea (Vahl. ex Fr.) Karst., was first noted by Linnaeus in his work Flora Svecica in 1745 as Agaricus (Sokolov, 1964). Throughout the 19th century, the fungus was identified and given a number of names by different mycologists. Fries, in 1821, united Agaricus melleus and others (A. robustus, A. Persoonii, A. guttatus, A. bulbiger, A. constrictus, A. subcavus, A. mucidus, A. vagans, A. griseofuscus, A. denigratus, and A. rhagadiosus) into the tribe Armillaria. Later (1872) Quélet reduced Armillaria from tribal to generic status. The Armillaria group recognized by Fries and Quélet is characterized by white spores, a central fleshy stipe continuous with the trame of the pileus, attached gills, and an annulus which at first sheaths the stipe and then may be broken up into zones or scales (Kaufmann, 1922; Smith, 1949).

The genus Armillariella was established by Karsten in 1879 with Armillariella mellea (Vahl ex Fr.) Karst. as the type species. The species constituting the genus Armillariella are united on the basis of several morphological characters, including the presence of bilateral type of lamellar trama and the lack of amyloid spores (Singer, 1949). Although the transfer from Armillaria to Armillariella for those species previously in the former

group (e.g., A. mellea) has not been rapid, it has gained greater acceptance and usage since 1949 due to use by Singer in his major classification work, "The Agaricales in Modern Taxonomy."

A further note on the status of the species Armillariella mellea is in order in the light of recent mating studies by Korhonen (1978), Ullrich & Anderson (1977), and Anderson & Ullrich (1979). It appears that the root rot fungus identified in the field as Armillariella (Armillaria) mellea by forest pathologists may, in actuality, be a complex of Armillariella species. Korhonen found that approximately 500 A. mellea samples from several areas in Finland could be divided into three completely intersterile groups with different morphological and ecological features. One group was identified as A. mellea, another as A. bulbosa; the third was tentatively identified as A. ostoyae. Anderson & Ullrich (1979) were able to identify at least ten reproductively isolated groups, or species, of A. mellea in North America on the basis of monosporous mating interactions. The groups were not distinguishable on the basis of host or substrate origin nor by geographical origin. All isolates examined showed a tetrapolar pattern of sexuality.

Two Armillariella species, A. (Clitocybe) tabescens and A. saviczii, have been recognized for a number of years as being distinct from A. mellea but are often

grouped with A. mellea under the common name of the "honey fungus" (Sokolov, 1964). Armillariella tabescens is morphologically very similar to A. mellea, differentiated from A. mellea by lack of a scarious ring, dissimilarity of spores, no rhizomorphs or only a few poorly developed ones. Differences in cultural characteristics exist as well; A. tabescens grows more rapidly in culture, lacks luminescence, forms large numbers of fruiting bodies, grows best at higher temperatures, and can grow in a weakly alkaline medium (Sokolov, 1964; Rhoads, 1945). Armillariella saviczii is distinguished from A. mellea by its more restricted geographical distribution, smaller fruiting bodies, and thinner rhizomorphs (Sokolov, 1964).

METHODS

Site Description

Field work for this study was conducted in forests on the eastern flank of Mt. Adams in south central Washington, several miles west of the town of Glenwood. Sampling sites are located on state, private (St. Regis Paper Co.), and National Forest (Gifford-Pinchot) lands.

Since determination of relationships between *Armillariella mellea* isolates from adjacent forest types was desired, sampling was made in an east-west direction perpendicular to the general geographic trend of vegetation types, running through each type. The range of sampling sites extended from the pure ponderosa pine forest type in the east to a mixed conifer type to the west. The dimensions of this east-west transect were enlarged by sampling to the north and to the south off of the main transect at several points. Because of variations in land use and timber types, these north and south extensions are neither consistent in length nor frequency along the transect.

Timber at the eastern end of the sampling transect (Fig. 4) is predominantly ponderosa pine (*Pinus ponderosa* Dougl.) with a small percentage of Oregon white oak (*Quercus garryana* Dougl.), Douglas fir (*Pseudotsuga menzesii* (Mirb.) Franco), and grand fir (*Abies grandis* (Dougl.)

FIGURE 3

Vegetation typical of the eastern end of the sampling transect. Ponderosa pine, ceanothus, bitterbrush and bracken fern are the predominating timber and brush species.

Lindl.). Bitter brush (Purshia tridentata (Pursh) D.C.), snow brush (Ceanothus sp.), rabbit brush (Haplopappus sp.), snowberry (Symphoricarpos sp.), bracken fern (Pteridium aquilinum (L.) Kuhn), pine grass (Calamogrostis sp.), and elk sedge (Carex geyeri Boot.) are the major understory species. Douglas fir and grand fir become more abundant as one moves west with more or less equal parts of ponderosa pine, Douglas fir, and grand fir in the overstory. A change in brush species from the Ceanothus-Purshia-Pteridium complex to predominantly western hazel (Corylus cornuta Marsh), ocean spray (Holodiscus discolor (Pursh) Maxim.), and snowberry occurs. At the western end of the transect, grand fir and Douglas fir are the major tree species with a small proportion of ponderosa pine and scattered larch (Larix occidentalis Nutt.), cottonwood (Populus trichocarpa T. & G.), aspen (Populus tremuloides Michx.), and willow (Salix sp.) (Fig. 5). More species occur in the understory: hazel, ocean spray, bracken fern, vine maple (Acer circinatum Pursh), wild rose (Rosa gymnocarpa Nutt.), thimbleberry (Rubus parviflorus Nutt.), blackberry (Rubus ursinus Cham. & Schlecht.), Oregon grape (Berberis sp.), and others.

Glenwood and the study area to the west fall into Franklin and Dyrness' (1973) Pinus ponderosa and Pseudotsuga menzesii-Abies grandis zones in their Washington Cascades Province. Soils are of volcanic origin, derived

FIGURE 4

Vegetation typical of the western end of the sampling transect. Douglas fir, grand fir, and numerous shrubs predominate.

from andesite, sandstone or glacial till.[3] The mean annual precipitation varies from 80 centimeters in the east to 160 centimeters to the west, much of this falling as winter snow. Summers are usually hot and dry, particularly in the Pinus ponderosa zone. Diurnal fluctuations are wide, with hot days and cold nights. Cooler, more mesic conditions exist in the Pseudotsuga menzesii-Abies grandis zone. Mean maximum temperature in July falls between 24 and 27 degrees Centigrade and mean minimum temperature in January varies between -9 and -7 degrees Centigrade. Elevation ranges between 600 and 1200 meters.

Field Sampling Procedure

For convenient access to infection centers, the transect followed a small logging road running east and west. The road itself is narrow and for most of its length rarely used. Disturbance by vehicles to vegetation near the road was slight. Fungus samples were taken from roots of diseased trees or from sporophores. Selection of sampling sites was strongly influenced by visibility of symptomatic trees (dead or dying trees with fading, dead, or no foliage, either singly or in groups) and ease of accessibility from the road. Before samples were taken from a site, confirmation of A. mellea was made by examin-

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[3]Information in this paragraph is taken from Franklin & Dyrness, 1973.

ing suspect trees for white mycelial fans beneath the bark of roots or the root crown (Figs. 6A&B) for excessive pitching, or for the presence of rhizomorphs on or attached to roots. The presence of mycelial fans was the most reliable indicator as rhizomorphs were hard to detect or absent and pitch-impregnated wood rarely yielded A. mellea when cultured in the laboratory. Dead or dying trees adjacent to the road with no signs of A. mellea were not included as a sample nor were those dead or dying trees with large scars or girdling caused by climatic or animal agents.

The intensity of sampling in the overall study area varied depending on particular circumstances. In those locations where A. mellea damage was extensive, sampling sites were located at more or less prescribed intervals of .2 to .5 miles along the transect. Where damage was more widely scattered, samples were taken where an infection center or single infected tree could be observed.

The relative abundance of major tree species and the presence or absence of shrub species was tabulated at each sampling site. A five-point scale was used: 0 = 0%, tr. < 1%, 1 = 1%-9%, 2 = 10%-49% and 3 = 50%-100%.

A. mellea was recovered primarily from root and basal crown wood of infected trees and secondarily from sporophores collected from various sites in the fall. In order to obtain root samples, some excavation of the root

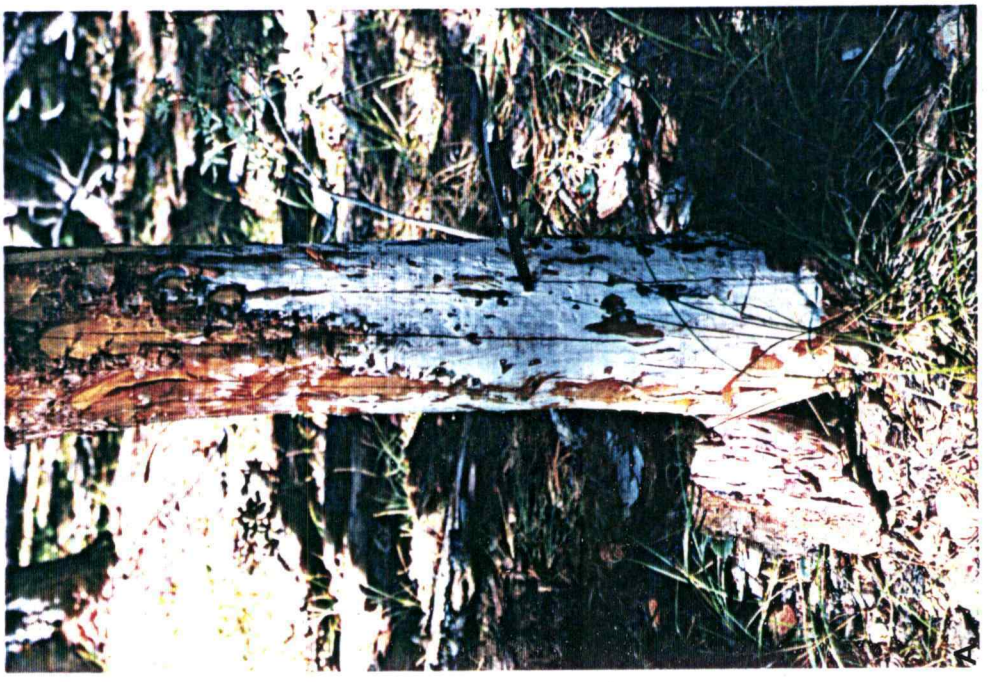
FIGURE 5

Mycelial fans of Armillariella mellea on ponderosa pine:

A) Bark from the lower bole of ponderosa pine has been removed to reveal extensive fan development in the cambial region.

B) A fragment of a fan is being stretched to show thickness and elasticity.

(Photographs by E. Knudtson)



system was necessary (Fig. 7). Several wood fragments or root portions were taken from each suspect tree if possible.

Laboratory Recovery

Wood chips (approximately 5 mm x 10 mm), mycelial fan fragments, and sporophore stipe tissue were aseptically plated onto media as soon as possible after recovery of infected material from the field. Wood or root portions were washed under running tap water to remove dirt and debris before isolations were made. One basic medium was used for all isolations: 30 gm malt extract, 20 gm dextrose, 5 gm bacto-peptone, 16 gm flake agar, 994 ml distilled water, 6 ml ortho-phenyl-phenol (OPP) (Russell, 1956), and 2 ppm streptomycin sulfate. Plates were incubated in the dark at room temperature. Growth of A. mellea out of a wood chip was seen after six to eight weeks of incubation. Growth from stipe tissue was more rapid, generally four weeks or less. Subcultures were made until the culture was free of contamination; a small amount of the colony was then placed in a screw-capped test tube containing malt agar (Difco) for semi-permanent storage. After the isolate was established in the tube, it was stored at 5° C.

FIGURE 6

Excavation of upper root system of ponderosa pine. Excavation facilitated removal of wood chip from infected root for later isolation in laboratory.

Identification of Clones

Isolates were identified as to relationship to one another by growing them together on the stardard isolation media described above. Inoculum pieces (about 1 cm by 1 cm) from two isolates were placed two to three centimeters apart near the center of a petri plate. The plates were incubated at 25° C. in the dark. Examination of the paired colonies for interaction was made when the two colonies met along a common interface. All pairings were replicated four times.

When more than one isolate from a sampling site or infection center was recovered, each isolate from that site was plated with every other isolate from that site. This resulted in two pairings when two isolates were recovered, three pairings with three isolates, and six pairings with four isolates. Plating procedures were as given above. Several sets of pairings were subsequently carried out between all isolates from an infection center and an unrelated isolate (e.g., 11A x 85A, 11B x 85A, 11C x 85A, 11D x 85A) to determine if all isolates from one infection center show consistent behavior. This was confirmed in all cases so that only one isolate from each multi-isolate infection center was used throughout the remainder of the pairings.

All isolates were plated with neighboring isolates.

When more than one pair of isolates in a row showed compatibility, pairings were made between isolates on either end of the compatible group (e.g., if isolate A is compatible with isolate B, and B is compatible with C, then A is paired with C to determine if A and C are compatible). Random pairings were also made between various isolates along the transect, and between transect and non-transect isolates.

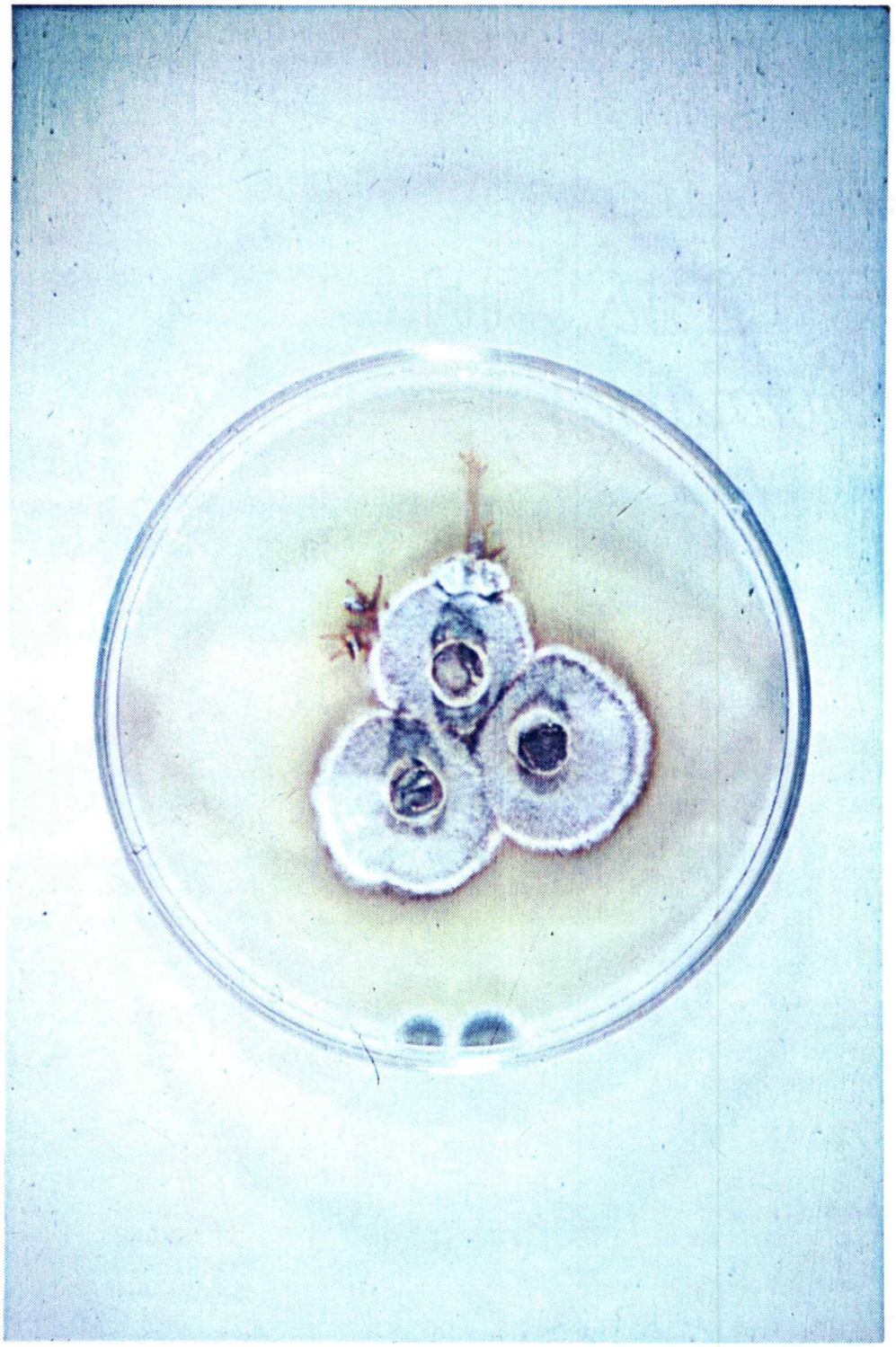
Twelve three-way pairings were carried out on neighboring isolates to determine if compatibility relations between two isolates might change after contact with a third isolate (Fig. 8). Possible exchange of nuclear material or secretion of inhibitory substances into the media could influence compatibility reactions. For the three-way pairings, a small piece of each mycelium was placed two to three centimeters from the center of the plate, equidistant from the other two pieces.

Isolate Variation

Isolates from 60 sampling sites along the transect were plated on 20 milliliters of malt-dextrose-peptone (MDP) (standard isolation media minus the addition of OPP and streptomycin sulfate) and incubated at 23° to 25° C. in the dark. When radial growth of the majority of isolates covered more than one half of the plate (about four weeks), eight subcultures were taken, five on MDP - 20 ml.

FIGURE 7

Three-way pairing of Armillariella mellea isolates on MDP media.



plates and three on 1.5% malt extract - 20 ml. plates. Inoculum pieces were removed from just inside the growing edge of the colonies with a six mm. diameter cork borer and placed in the center of the plate. All plates were then incubated at 23° to 25° C. in the dark. Observations of the following characteristics were made after one and two weeks: diameter of the mycelium, formation of rhizomorphs, length of the five longest rhizomorphs, and emergence of rhizomorphs beyond the radius of the mycelium. Observations and measurements of the following characteristics were made after three weeks: presence or absence of exudation, mycelial tufts, and pseudosclerotium; color and form of rhizomorphs; color and character of the mycelium; diameter of the mycelium; length of the five longest rhizomorphs; and mycelial dry weight. Dry weights were determined after the media in the plates was melted, the colony extracted, and then rinsed under tap water. Each was placed on weighing paper and dried at 75° C. for 24 hours.

Analyses of variance were carried out to compare mycelium growth rates (mm/day), rhizomorph growth rates (mm/day), and colony dry weights (gm) between isclates in different clones, and between isolates in different vegetation types.

RESULTS

Clone-Vegetation Relations

Ninety-one A. mellea isolates were collected along the transect (including north-south side branches off the main east-west transect) from a total of 70 infection centers. Table 3 lists transect isolates, type of material from which A. mellea was isolated, host species, if applicable, and the distance between isolates.

Isolates collected along the transect show, for the most part, incompatible reactions when paired in culture with isolates from neighboring infection centers. However, isolates from ten groups of infection centers did show compatibility. Fifty-two clones were shown to exist among the isolates examined. Figure 10, a map of the study area, diagrams spatial relations between sampling sites and designates clones (common numbers among circles) based on compatibility reactions between neighboring isolates.

All isolates from the same infection center usually showed cultural compatibility. Of twenty-one multi-isolate infection centers examined along the transect and elsewhere, only two showed questionable or incompatible reactions with the other isolates in the center.

TABLE 3. Field Data for Collected A. mellea Isolates

ISOLATE NUMBER	SOURCE *Tissue*[b]	HOST *Species*[c]	DISTANCE[a] *Miles*
1	W	P	-
2	W	P	.2
3	W	P	.2
L26	SP	P	.2
5	W	P	.2
6	W	P	.2
7	W	P	.3
9	W	P	.5
10	W	P	.3
10.5	W	P	.3
11	W	P	.2
12	W	P	.2
13	W	P	.2
85	W	P	.4
86	W	P	.1
87[d]	W	P	.2
88	W	P	.2
89	W	P	.3
92	W	P	.7
95	W	P	.5
79	W	P	.2
82	W	DF	.3
100	W	P	1.4
101	W	DF	0
102	W	P	.2
103	W	P	.2
104	W	P	.2
15	W	P	.2
L2	SP	P	.4
17	W	P	.2
L28	SP	P(?)	.3
L19	SP	?	.6
L18	SP	?	.3
L23	SP	?	.7
L22	SP	?	.6
N18	W	P	.4
19	W	P	.4
N20	W	P	.3
23	W	P	.7
24	W	P	.2
N25	W	P	.2
28	W	P	.5
N29	W	GF	.3
L8	SP	?	.5
L17	SP	?	.1
L11	SP	DF	.2
L9	SP	GF	1.0
32	W	P	.2
L12	SP	P	.1
33	W	DF	.2
34	W	P	.3
L16	SP	DF	0
A	W	DF	.3
L	W	P	.5
N	W	P	.4
O	W	GF	.3
L5	SP	P	1.4
C	W	GF	.6
L15	SP	DF	.2
D	W	GF	.4
E	W	GF	.6
H	W	P	.1
L10	SP	GF	.1
I	W	P	.4
J	W	P	.2
L6	SP	GF	.4
F	W	GF	.2
L7	SP	GF	.5
G	W	DF	0
L14	SP	GF	.3

[a]Distance between isolate and the one preceding it.

[b]A. mellea was isolated from wood (W) or sporophore (SP) tissue.

[c]Hosts sampled were ponderosa pine (P), grand fir (GF), and Douglas fir (DF).

[d]Indented isolates indicate side branches off of main transect; e.g., the distance between isolates 86 and 15 is .2 miles.

Compatibility between isolates was not confined to isolates derived from the same source; A. mellea isolated from root tissue was found to be compatible, in some cases, with isolates grown from sporophore stipe tissue. Similarly, among transect and non-transect isolates examined, some derived from ponderosa pine were, in a few cases, compatible with isolates derived from Douglas fir or grand fir.

When compatible reactions between isolates from different infection centers were obtained, clone size was measured. Size of clones varied between .2 and 1.0 miles in length along the transect. The mean length of all clones involving more than one infection center was .58 miles. The largest clone found in the Glenwood area (involving non-transect isolates) extended 1.4 miles in length. A difference in the number and size of multi-infection center clones in ponderosa pine and mixed conifer stands (ponderosa pine/Douglas fir/grand fir or Douglas fir/grand fir) is seen. Four multi-infection center clones were found in ponderosa pine and six were found in mixed stands. The mean length along the transect of clones incorporating more than one infection center in ponderosa pine is .53 miles; the mean length in mixed stands is .62 miles.

The relative abundance scale referred to in the "Methods" section was developed in order to place vege-

tation at each sampling site into one of three forest types: ponderosa pine, ponderosa pine/Douglas fir and/or grand fir, Douglas fir/grand fir (Table 4). When the relative abundance of one of the above three tree species rose above one, the site in which it was found was placed in the category naming it as the sole or joint species. As Figure 9 depicts, ponderosa pine sites are found only on the east end of the transect, mixed sites (having pine as one of the components) are found throughout most of the transect although predominating in the middle, and fir (grand or Douglas) sites are found exclusively on the western end of the transect, particularly at higher elevations (roads extending north from the main transect general rise in elevation). It is apparent from Figure 9 that there is no sharp division between vegetation types in this area; instead, much interdigitation and mixing of pine and other species occurs along a broad band several miles wide, running north and south.

When vegetation and clonal designations at each sampling site are mapped together (superimposed), as in Figure 9, continuity or discontinuity of clones between different forest types can be easily seen. Five of the ten multi-infection center clones (clones 4, 23, 30, 31, and 40) extend over more than one vegetation type; the other five (clones 2, 3, 6, 9, and 52) are confined to one type of vegetation. Therefore, for half of the multi-

TABLE 4. Major Tree and Shrub Species at Sampling Locations

	TREE SPECIES						*PREDOMINANT SHRUBS & LESSER VEGETATION*											
Sampling Location[a]	Pinus ponderosa	Pseudotsuga menzesii	Abies grandis	Quercus garryana	Populus trichocarpa	Populus tremuloides	Salix sp.	Acer circinatum	Corylus cornuta	Pursha tridentata	Ceanothus sp.	Haplopappus sp.	Symphoricarpos sp.	Pteridium equilinum	Holodiscus discolor	Rosa gymnocarpa	Rubus parviflorus	Rubus ursinus
1	3[b]			tr						x[c]	x			x				
2	3	tr								x	x	x		x				
3	3	tr		tr						x	x			x				
L26	3	tr	tr							x	x	x	x	x				
5	3	tr		1						x	x			x				
6	3	tr		2						x	x	x	x	x				
7	3	tr		2						x	x			x				
9	3	1		2						x	x	x	x	x				
10	2	2		2							x		x					
10.5	2	2		2							x		x					
11	3	1	2	2							x	x	x	x				
12	3	1		2				x			x		x	x				
13	3	tr	tr	2					x		x	x	x	x		x		
86	3			tr					x			x						
87	2	2		tr	tr				x				x	x	x		x	
88	2	2		2	tr			x	x				x		x	x	x	
89	1	3	2	2					x		x		x			x		
92	2	2	tr	1					x		x	x				x		
95	2	2		2	tr						x			x			x	
79	3	2	1				x	x	x				x					
82	2	3	1	1	tr		x		x		x		x			x	x	
100	3	2		1					x	x	x	x		x				
102	2	2		2	tr				x		x		x	x				
103	2	3		1	tr				x		x		x	x	x	x	x	x
104	2	2		2	tr				x		x		x	x	x			
15	3	tr		tr								x						
L2	3	2		tr					x			x	x	x		x		
17	3	tr		tr					x			x	x			x		
L19	3	2	tr	tr				x				x				x		
L18	2	3	2	tr					x				x	x		x	x	
L23	3	2	2	tr				x	x			x	x	x	x			
L22	3	1	1	tr					x		x			x		x		
N18	3	1	1									x						
19	3		1	tr	tr				x	x	x	x	x					
N20	3	1		1			x		x			x	x		x			

(Cont.)

Sampling Location	TREE SPECIES						PREDOMINANT SHRUBS & LESSER VEGETATION										
	Pinus ponderosa	Pseudotsuga menzesii	Abies grandis	Quercus garryana	Populus trichocarpa	Populus tremuloides	Salix sp.	Acer circinatum	Corylus cornuta	Pursha tridentata	Ceanothus sp.	Haplopappus sp.	Symphoricarpos sp.	Pteridum equilinum	Holodiscus discolor	Rosa gymnocarpa	Rubus parviflorus	Rubus ursinus
23	2	3	1	2			x		x				x			x		
24	2	3		1	tr		x		x				x		x	x		
N25	2	2	tr	1			x		x				x		x	x		
28	1	3	2	tr	tr													
N29	1	3	1		tr		x		x				x		x		x	
L8	3	2	tr	1			x		x			x	x		x			
L17	3	2		2	tr				x			x	x					
L11	1	3	1	1				x	x				x		x		x	x
L9	tr	tr	3										x		x		x	
32	2	2	2	tr				x	x			x	x		x		x	
33	2	2	2				x	x	x				x		x	x		
34	2	2	2						x				x					
A	1	3	2		tr		x	x	x				x	x	x		x	
L	1	3	2					x	x				x		x		x	
N	3	2	1	1				x	x				x	x	x	x	x	x
O	2	3	1				x	x	x		x		x	x	x	x	x	x
L5	3	2	1															
C	3	2	2				x					x	x					
L15	3	2	2									x	x		x	x		
D	tr	2	2					x	x				x	x	x	x		
E	1	2	3		tr			x	x			x	x		x	x	x	x
H	1	2	3			tr		x	x				x		x	x		x
L10	tr	1	3					x					x				x	
I	tr	2	2					x	x				x		x	x	x	x
J	1	2	3					x	x				x	x		x	x	
F	1	2	2					x	x				x		x	x	x	
L7	3	1	2			1						x	x		x			
G	1	2	3					x					x		x	x	x	
L14		2	3			tr		x								x	x	

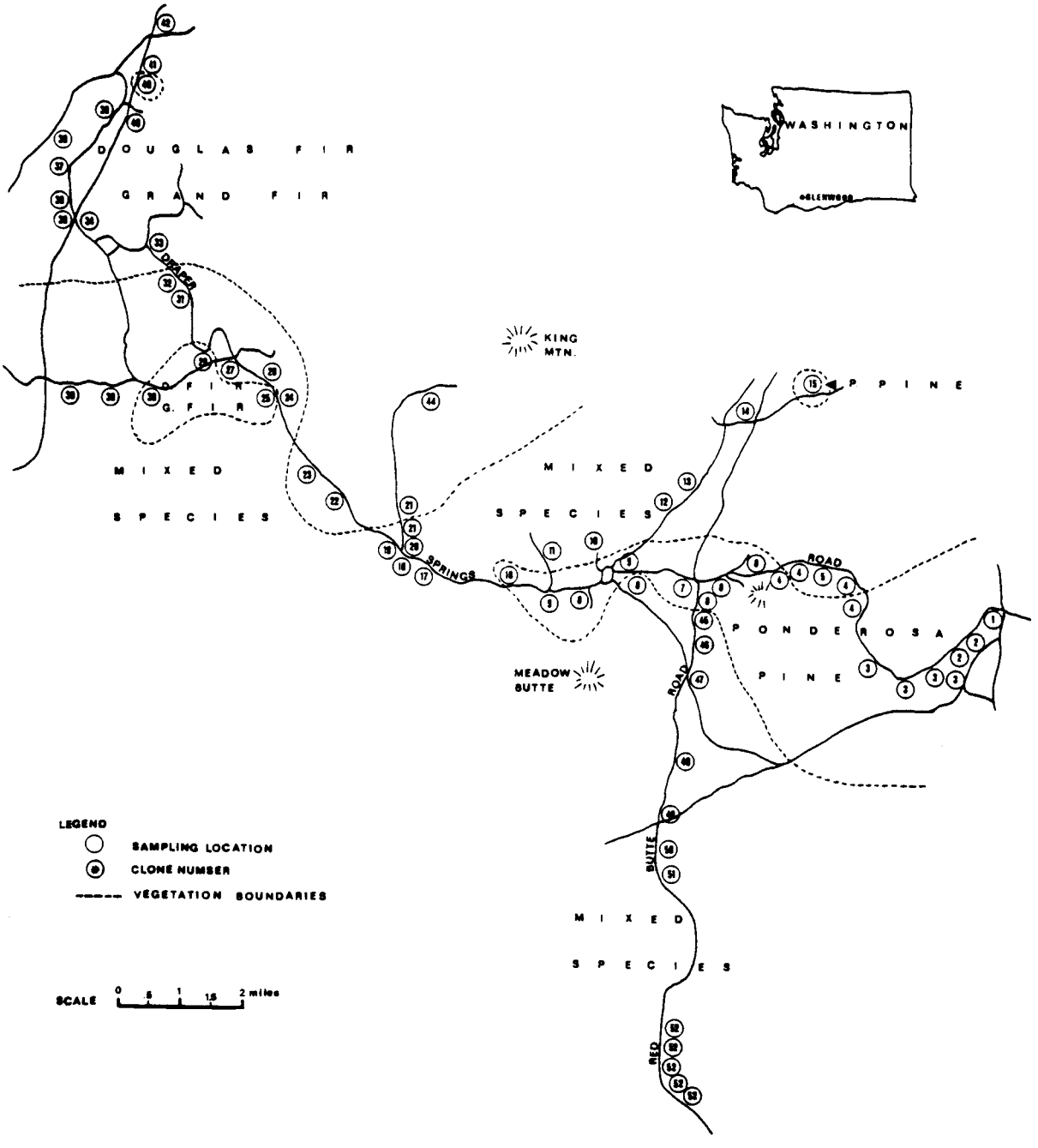
[a]Sampling locations are identified by the same designation as the isolate(s) which was collected there.

[b]Numbers represent relative abundance of each species at each sampling location: 3=>50%; 2=11-50%; 1=1-10%; tr=<1%.

[c]"x" designates the presence of a particular species at that site.

FIGURE 8

Area map of sampling sites, clonal designations, and vegetation types.


WASHINGTON
DOUGLAS FIR
GRAND FIR
DOUGLAS FIR
G. FIR
MIXED SPECIES
MIXED SPECIES
MIXED SPECIES
KING MTN.
MEADOW BUTTE
SPRINGS
ROAD
ROAD
BUTTE
RED
PONDEROSA PINE
P PINE
LEGEND
SAMPLING LOCATION
CLONE NUMBER
VEGETATION BOUNDARIES
SCALE 0 .5 1 1.5 2 miles

infection center clones examined, continuity of fungal colonies over different vegetative covers does occur.

Compatibility Relations in Culture

Compatibility or incompatibility in culture between two isolates was determined by several factors. Incompatibility was concluded if one or more of the following events occurred: discolored or dead hyphae at the interface, strong pigmentation of the media at the interface (Figure 10), formation of a sclerotial wall within the media by one or both of the isolates near the contact zone (Figure 11), different morphology of the two isolates (Figure 12), or the consistent formation of a zone devoid of hyphae between the two isolates. Compatible isolates showed none of the above characteristics when paired in culture; mycelia grew together with the resultant thallus resembling one which originated from a single inoculum (Figure 13).

Relations between isolates previously paired with one another were not altered in three-way pairings.

Cultural Variation and Morphology

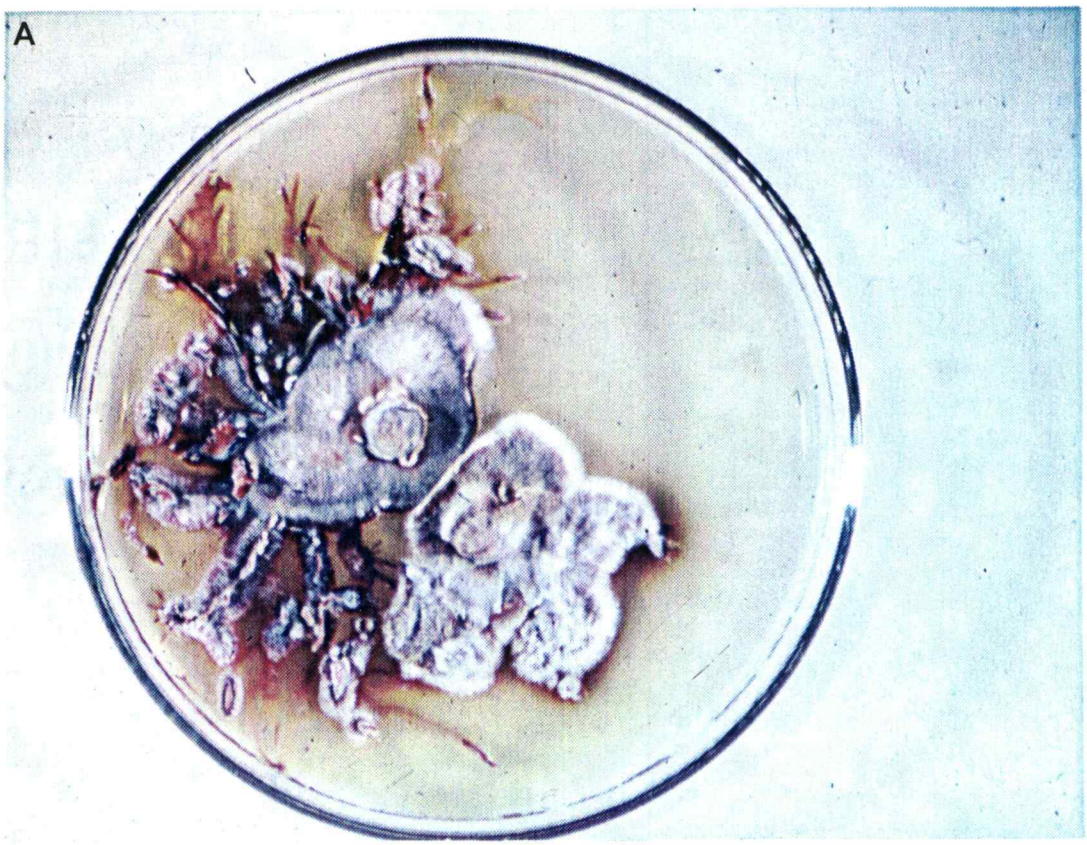
Rhizomorph and mycelium growth rates were recorded on 60 isolates over a three week period. Colony dry weights were taken at the end of the three week period. These measurements were made in order to determine if

FIGURE 9

Incompatible isolates of Armillariella mellea paired in culture. Strong pigmentation occurs within the media at the interface and beneath both colonies:

A) Surface or top view of colonies.

B) Petri plate inverted to show bottom side of colonies.


A

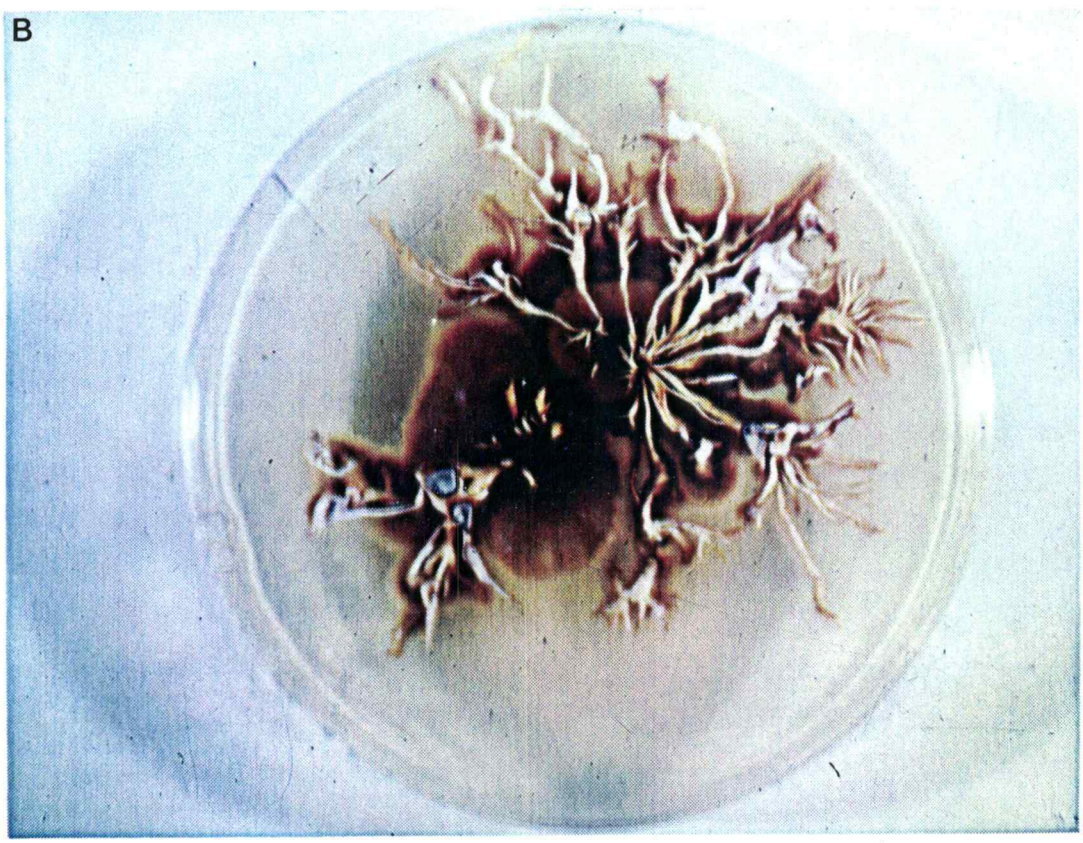
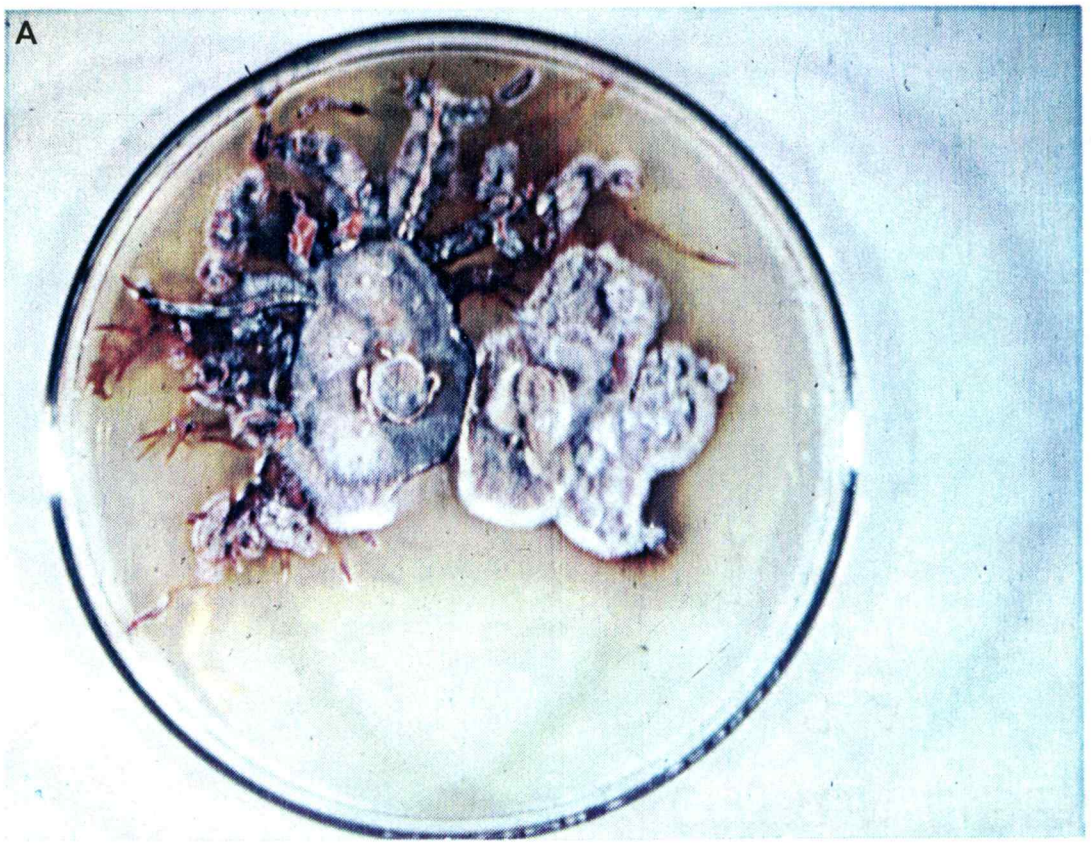

B

FIGURE 10

Incompatible isolates of Armillariella mellea paired in culture. A dark sclerotial wall (see arrows) at the interface within the media is formed by one isolate. Wall formation is greatest and most rapid where contact with the other colony occurs.

A) Top view of colonies.

B) Petri plate inverted to show bottom side of colonies.


A

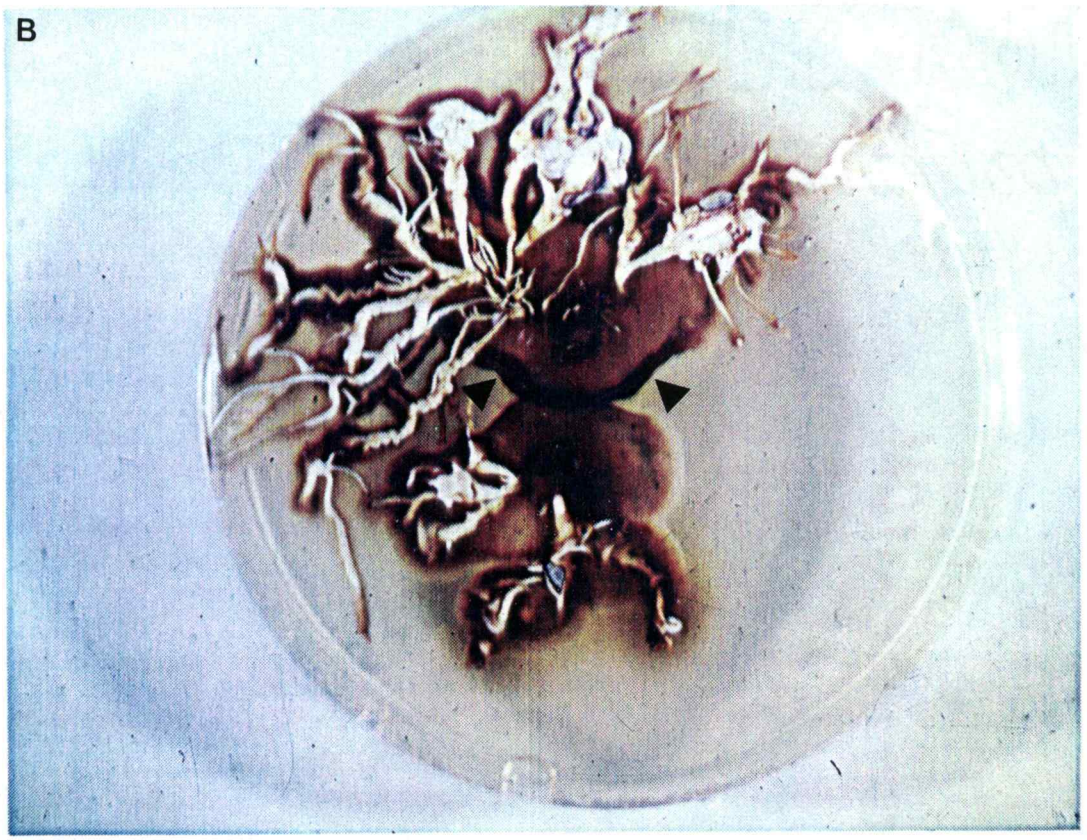
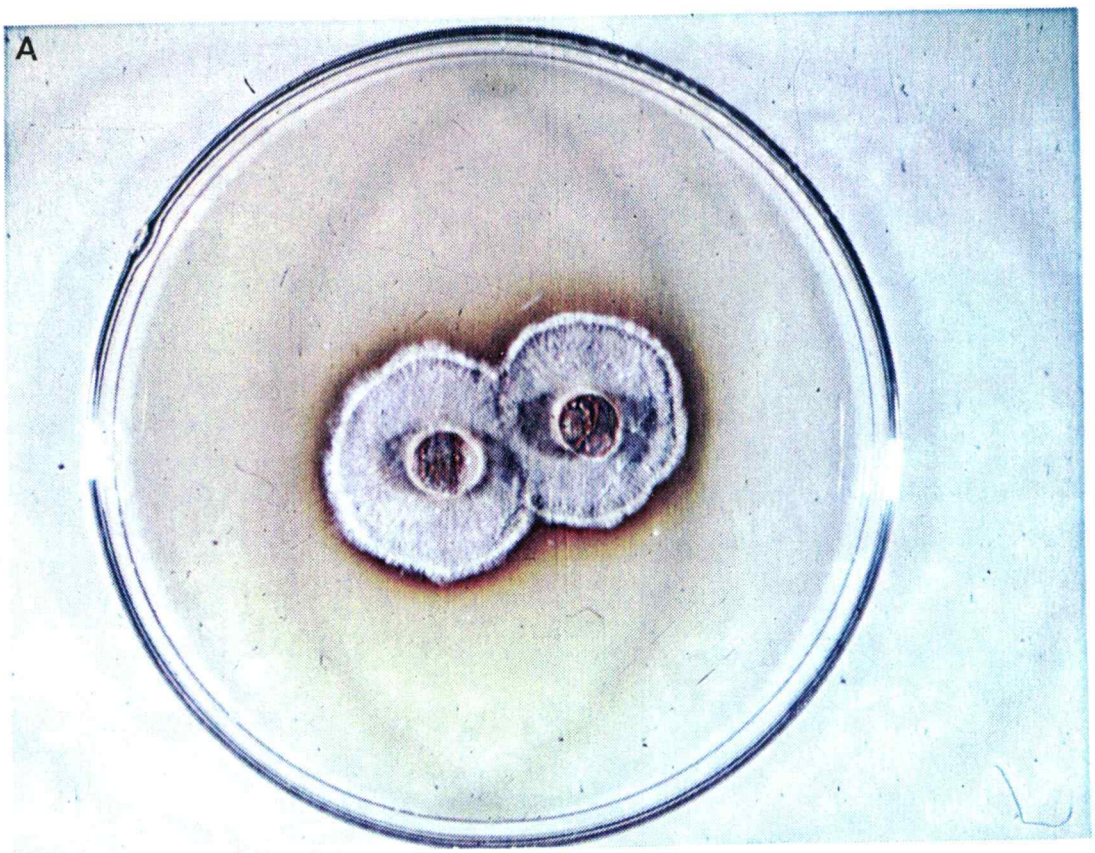

B

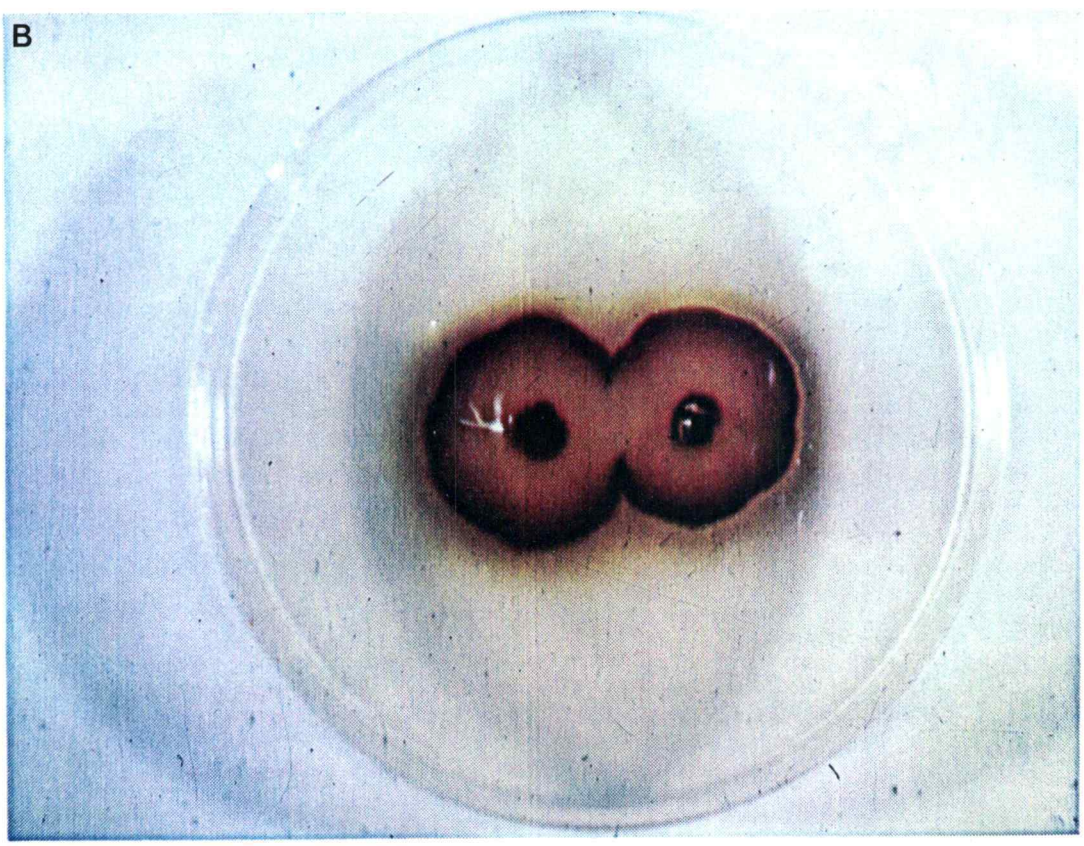
FIGURE 11

Incompatible isolates of Armillariella mellea paired in culture. The two isolates are morphologically dissimilar. Color and amount of aerial mycelium, quantity of rhizomorphs, aerial rhizomorphs, and diameter of colonies differ.

FIGURE 12

Compatible isolates of Armillariella mellea paired in culture. Fusion of aerial mycelium is seen in A. Fusion of the pseudosclerotium is best observed from the underside of the colonies (B).


A


B

differences between isolates belonging to different clones or vegetative habitats could be discerned through measurable cultural characteristics.

One-way analyses of variance showed that highly significant differences exist between clones for all three variables measured (mycelial growth rate, rhizomorph growth rate, and colony dry weight) on both malt extract and MDP media. Insignificant differences were found between isolates in different vegetation habitats. Means and $\underline{F}$ values are shown in Tables 5 and 6.

In addition, two-way analyses were carried out to examine if differences between clones and vegetation groups (isolates belonging to one of the different vegetation habitats: ponderosa pine, mixed species, or grand fir-Douglas fir) remained significant when both media types were analyzed together. These analyses also allowed for the examination of media by clone and media by vegetation group interactions. As shown in Table 7, clone differences remained significant and vegetation group differences remained non-significant. These analyses also indicated that differences between media were highly significant for all measures (means and standard deviations are included in Tables 5 and 6). Growth rates and dry weights were greater in all cases on MDP media.

The interactions between vegetation groups and media types were non-significant. The interactions

TABLE 5. Means & F Values for Analyses of Variance of Clone Differences within Media

	MDP Media		*Differences Between Clones Within MDP Media*			*Malt Extract Media*		*Differences Between Clones within ME Media*		
	Mean[a]	*SD*	*F*	*df*	*Sig.*	*Mean*[a]	*SD*	*F*	*df*	*Sig.*
CLONES										
Mycelium Growth Rate	2.04	.60	9.92	42/212	$p < .001$	1.64	.32	4.31	42/118	$p < .001$
Rhizomorph Growth Rate	1.05	.92	5.50	42/210	$p < .001$	.07	.18	17.17	41/117	$p < .001$
Colony Dry Weight	.74	.18	4.93	42/212	$p < .001$	.48	.03	1.81	42/118	$p < .01$

TABLE 6. Means & F Values for Analyses of Variance of Vegetation Group Differences within Media

VEGETATION										
Mycelium Growth Rate	2.03	.59	2.50	2/252	NS	1.63	.31	2.17	2/158	NS
Rhizomorph Growth Rate	1.07	.92	.75	2/250	NS	.06	.18	2.57	2/156	NS
Colony Dry Weight	.75	.19	.47	2/252	NS	.48	.03	.42	2/158	NS

[a]These are grand means. Individual means are included in Tables 15 and 16 in Appendix B.

TABLE 7. F Values for Two-way Analyses of Variance: Media Interactions

	CLONE X MEDIA		
	Mycelium Growth Rate		
	F Value	df	Sig.
Clone main effect	7.45	42/330	p < .001
Media main effect	154.56	1/330	p < .001
Clone X Media effect	4.47	42/330	p < .001
	Rhizomorph Growth Rate		
	F Value	df	Sig.
Clone main effect	4.18	42/327	p < .001
Media main effect	269.49	1/327	p < .001
Clone X Media effect	2.87	42/327	p < .001
	Colony Dry Weight		
	F Value	df	Sig.
Clone main effect	2.90	42/330	p < .001
Media main effect	464.76	1/330	p < .001
Clone X Media effect	2.50	42/330	p < .001

	VEGETATION TYPE X MEDIA		
	Mycelium Growth Rate		
	F Value	df	Sig.
Vegetation main effect	3.50	2/410	NS
Media main effect	61.34	1/410	p < .001
Vegetation X Media effect	.28	2/410	NS
	Rhizomorph Growth Rate		
	F Value	df	Sig.
Vegetation main effect	.16	2/406	NS
Media main effect	164.73	1/406	p < .001
Vegetation X Media effect	.92	2/406	NS
	Colony Dry Weight		
	F Value	df	Sig.
Vegetation main effect	.227	2/410	NS
Media main effect	308.42	1/410	p < .001
Vegetation X Media effect	.348	2/410	NS

between clones and media types were significant for all measures. These significant interactions suggest that, although all clones showed higher growth rates and dry weights on MDP media, the differential effectiveness of these media varied considerably between clones. Results of all analyses of variance are included in Appendix A.

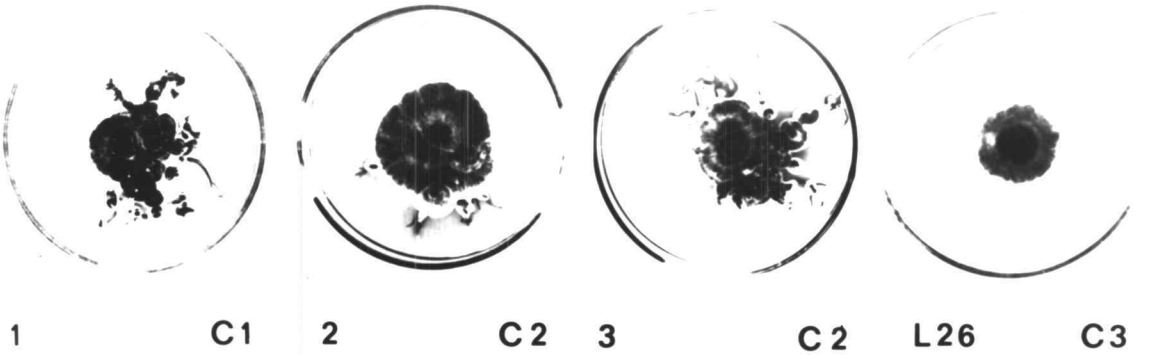
Colony appearance (Fig. 14) was noted for each isolate but no statistical analysis was carried out for any of the characteristics other than those referred to above. Largest differences in morphology were seen in isolates on different media.

All isolates except two (H and L7) growing on MDP media showed extensive pseudosclerotial development after 21 days. The pseudosclerotium, a tough, red-brown to purple-black thickened layer of cells, was formed over the entire colony surface in most cases. A layer of mycelium, varying in density and length generally covered the pseudosclerotium. Density and length of the mycelium over the pseudosclerotium was not consistent among sub-cultures of the same isolate. One isolate (H), however, showing limited pseudosclerotial development, also showed consistent lack of hyphae over the pseudosclerotium tended to be greatest towards the center of the colony and least about the perimeter.

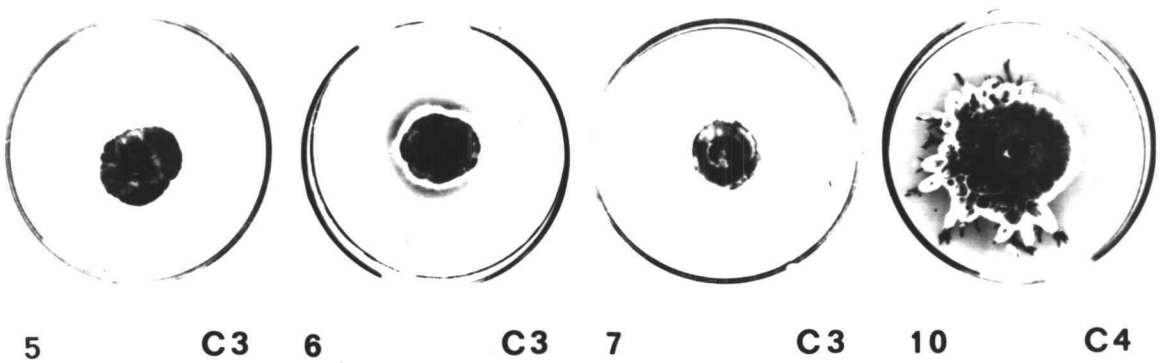
Pseudosclerotium development was much more limited

FIGURE 13

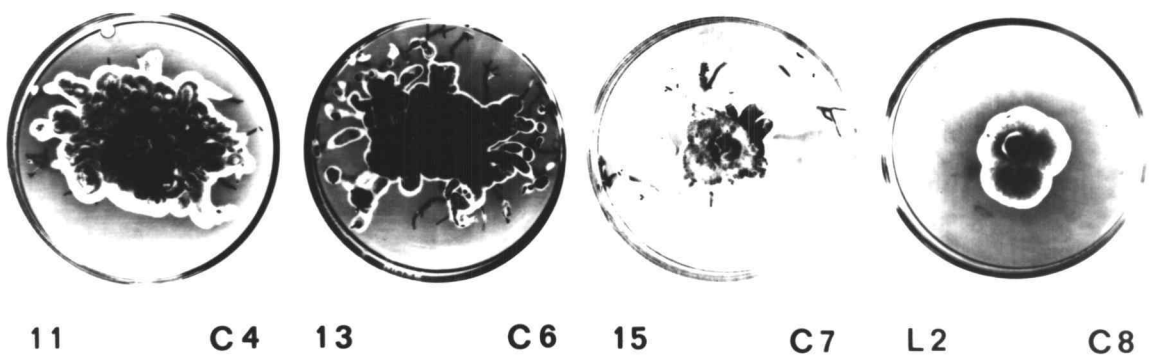
Armillariella mellea isolates used to examine variability of A. mellea in Glenwood. Isolates growing on MDP media at 23° C. for 21 days.



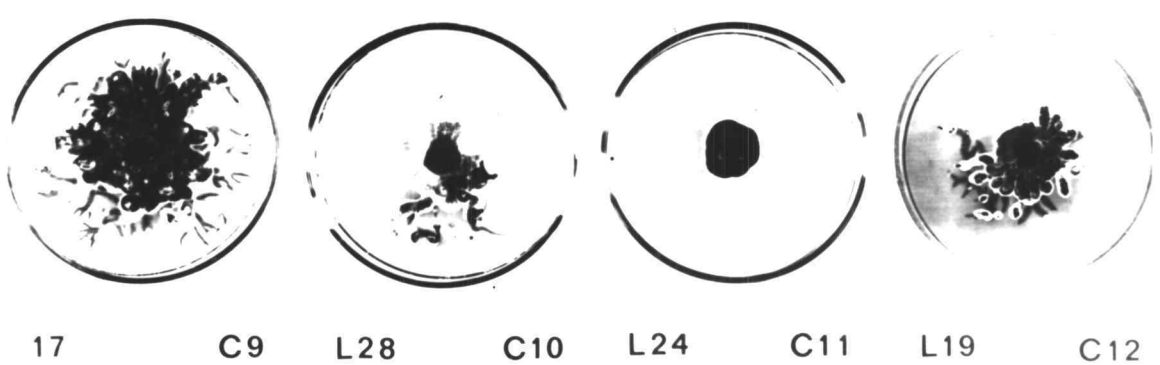
1 C1 2 C2 3 C2 L26 C3



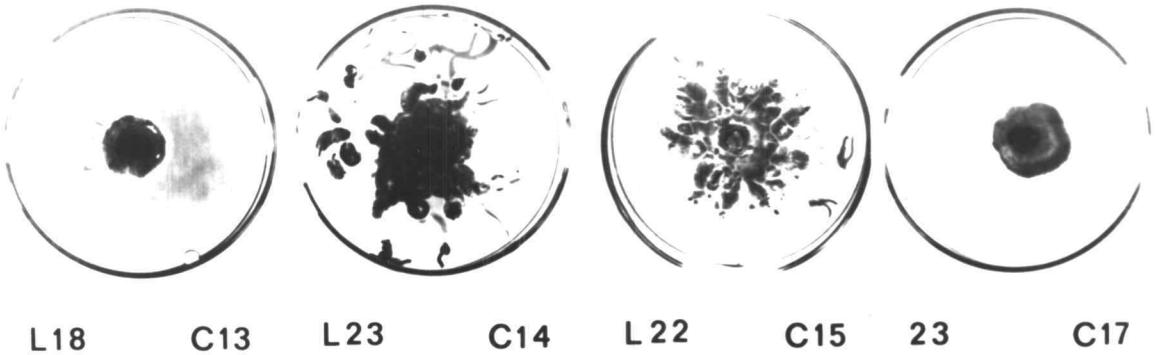
5 C3 6 C3 7 C3 10 C4



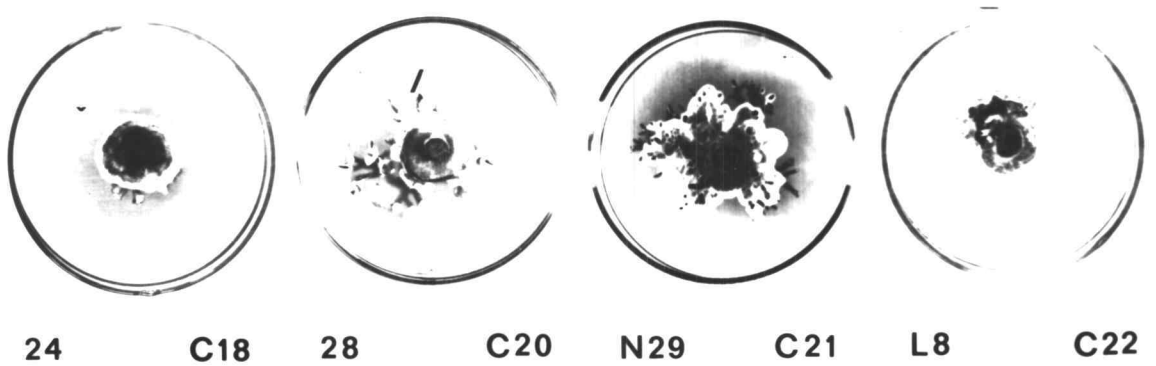
11 C4 13 C6 15 C7 L2 C8



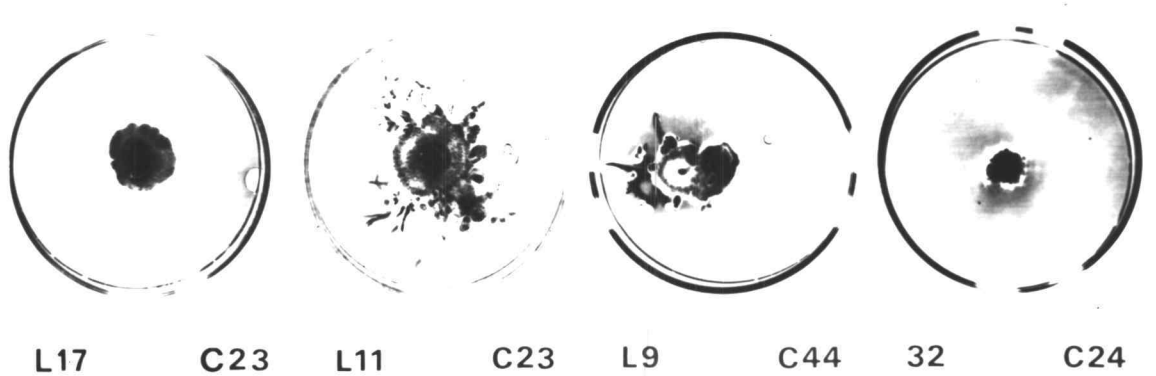
17 C9 L28 C10 L24 C11 L19 C12



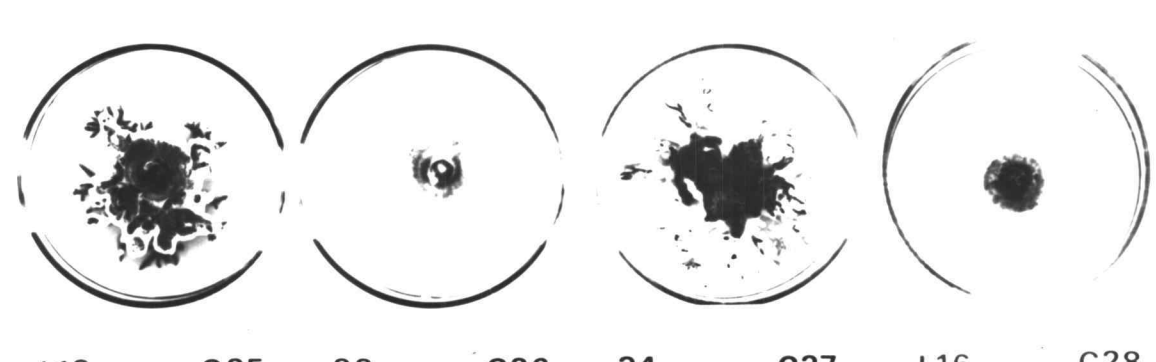
L18 C13 L23 C14 L22 C15 23 C17



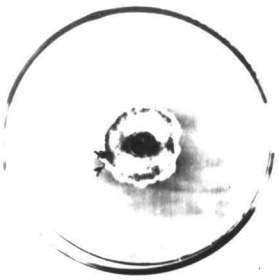
24 C18 28 C20 N29 C21 L8 C22



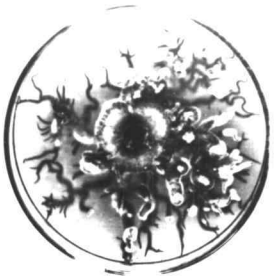
L17 C23 L11 C23 L9 C44 32 C24



L12 C25 33 C26 34 C27 L16 C28



L C30



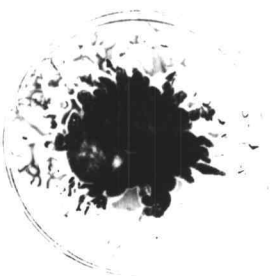
N C30

O C30

L5 C43

C C31

D C31



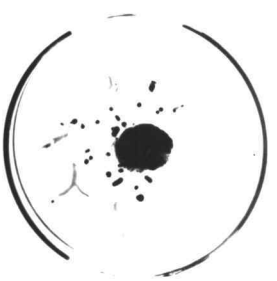
L15 C32

E C34

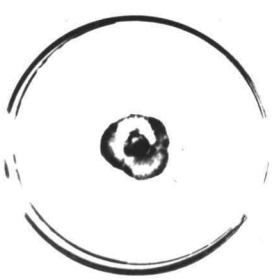
H C35

L10 C36

I C37



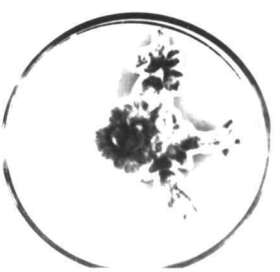
J C38



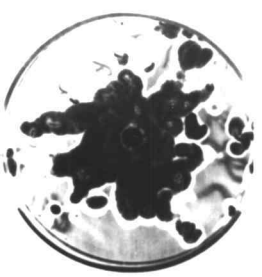
F C40

L7 C40

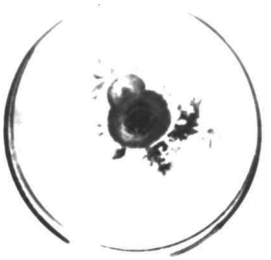
L14 C42



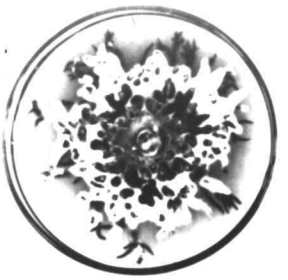
87 C45



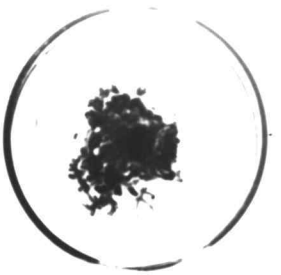
88 C46



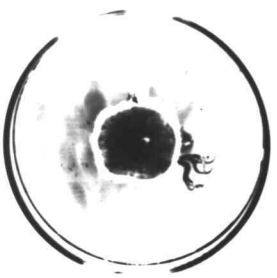
92 C48



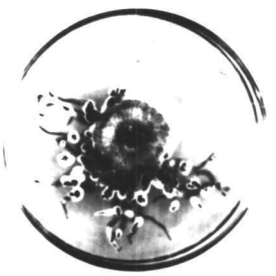
95 C49



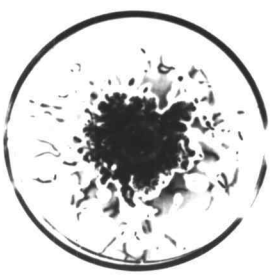
79 C50



82 C51

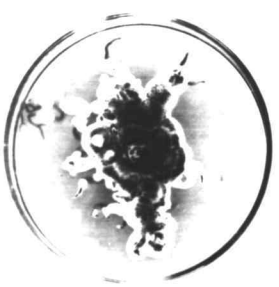


100 C52

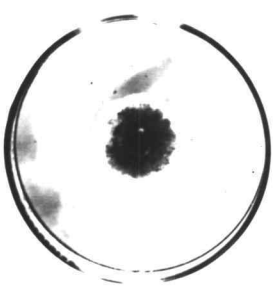


101 C52

102 C52



103 C52



104 C52

when the isolates were grown on 1.5% malt extract media. While 50% of all malt extract cultures showed good pseudosclerotial development, 46% showed pseudosclerotia forming only in small patches or sectors or a few millimeters around the initial inoculum plug, and 4% showed no development at all. Mycelium development in these cultures always preceded pseudosclerotial development by a large margin both in time and space as compared to more rapid and extensive development on MDP media. Mycelium formation over the pseudosclerotium on malt extract media tended to be sparse or absent.

Mycelium, for all isolates, was hyaline when growing through the media. Aerial hyphae, including that over the pseudosclerotia, varied from white to tan, often with brownish or yellowish areas. Small upright tufts of hyphae were often formed on the inoculum plug and sometimes on the colony surface. Tuft color ranged from pure white to tan to dark brown.

Rhizomorphs were produced much more abundantly on MDP than on malt extract media. When produced, rhizomorphs were white to cream-colored and flattened. As they grew in length, dichotomous or palmate branching occurred. Lateral branches appeared generally only after growth had extended to the sides of the petri plate. Rhizomorph growth in MDP is more rapid and often may exceed the radius of the mycelium, whereas rhizomorph growth

on malt extract media rarely exceeds the radius.

Aerial rhizomorphs were formed in A. mellea colonies on MDP primarily, particularly when rhizomorph growth exceeded mycelium growth. Morphology of aerial rhizomorphs are brown and cylindrical and generally less than one or two cm long. Where submerged rhizomorphs emerged onto the media surface, new mycelium formed about them and ultimately fused with the original colony, creating a lobed appearance.

DISCUSSION

The distribution and variation of Armillariella mellea isolates and their relationship to vegetation patterns and to one another was examined in order to find an explanation for the unusual severity of Armillaria root rot on ponderosa pine in this area. The hypothesis that severity of disease was due to the uniqueness of A. mellea clones in ponderosa pine cover type was investigated. Accumulated evidence from this study shows that the A. mellea clones present in the ponderosa pine cover type are neither unique spatially nor morphologically. Disease severity must therefore be attributed to factors other than those investigated. The impact of climatic changes on host susceptibility and the pathogenicity of A. mellea are discussed in relation to severity of disease in Glenwood. The identification and origin of clones in Glenwood is also discussed.

Severity of Disease as Influenced by Vegetation Changes

The geographical area investigated cannot be separated into two or three distinctly different areas based on major tree species. Instead, a large amount of mixing and interdigitation of species occurs, due to variation in topography and accompanying climatic and geological dif-

ferences. Within the area of investigation climate and site limit the reproduction and growth of Douglas fir and grand fir to the east and ponderosa pine to the west. Individuals existing on such climatic and geographical boundaries are naturally more vulnerable to small climatic changes which threaten their existence. Frequent shifts in climate over the millenia[1] may have prevented long-term establishment of ponderosa pine in this area and thus prevented or slowed the selection and reproduction of those individuals resistant to A. mellea. Recession to the east during drier periods would create a break in the selection process since the area to the east of Glenwood appears to have very little Armillaria root rot. Therefore, in addition to or accompanying the lack of opportunity to adapt, the existing gene pool of ponderosa pine in Glenwood may not include or may have lost genes for resistance to A. mellea.

The continuity of clones from one vegetation type to another as seen in a portion of the isolates examined may, indeed, reflect an above-ground shift of vegetation over a stable below-ground fungal colony. Conversely, however, continuity of clones from one type of vegetation to another may be the result of outward fungal growth beneath a stable

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[1] See Appendix C for discussion of past climatic and vegetation changes in the Pacific Northwest.

above-ground tree community. The latter situation, although possible, is not supported by the past and present evidence of vegetation changes. Computation of clonal age using a growth rate of 1.0 meter/year (Shaw and Roth, 1976) indicates that some of the biggest clones are between 800 and 1,200 years old. Replacement of one tree species with another could conceivably take place several times over the life-time of these fungal colonies.

From these observations gathered in Glenwood, perhaps we can expect more root disease problems in tree communities which exist on similar species boundaries or in other types of unstable vegetation communities.

Severity of Disease as Influenced by Isolate Variation

Significant differences between clones were found when data from three separate sets of measurements (mycelial growth rates, rhizomorph growth rates, and dry weights) were analyzed. However, it is likely that all clones examined in the Glenwood area belong to the same biological species. General morphological features were consistent over all isolates examined. Anderson and Ullrich (1979) and Korhonen (1978) have been separating out biological species of Armillaria (Armillariella) mellea on the basis of monosporous pairings which delineate intersterile groups. Anderson and Ullrich found that specimens collected from one local area usually belonged to only one bio-

logical species although mating type alleles often were different. They also found that biological species of A. mellea are not confined to one host species. In Glenwood, several clones were found to encompass isolates obtained from more than one host species.

When variation between isolates in different vegetation habitats was examined, no significant differences were seen between ponderosa pine isolates, mixed species isolates, and fir isolates. Differences seen in severity of disease between ponderosa pine and other tree species appears to be due, not to differences in A. mellea from one vegetation type to the next, but to the high pathogenicity of the biological species as a whole as well as the high susceptibility of ponderosa pine in Glenwood. It is possible, of course, that certain characteristics peculiar to pathogenicity or virulence may vary between isolates in different vegetation types while those cultural characteristics which were measured do not. It would be interesting to see if other isolates collected throughout the world, belonging to the same intersterile group as the Glenwood isolates (see paper by Anderson and Ullrich, 1978), possess similar traits of pathogenicity in similar sites on similar hosts.

Identification and Origin of Clones

The term "clone," defined as a group of organisms derived from a common ancestor by the process of mitosis, has

been used extensively to describe vegetative communities of fungi in the field. A new term, "mycelial type," was recently coined by Rishbeth (1978) to describe a similar vegetative grouping of A. mellea. Use of "mycelial type" rather than "clone" was deemed appropriate by Rishbeth because of the complexity and uncertain genetic status of rhizomorph systems in the oak infection centers he studied in England (personal communication). His centers often yielded more than one mycelial type due, perhaps, to multiple spore infection of stumps or anastomosing rhizomorph systems. Use of clone in this paper is considered appropriate for several reasons: infection by spores is considered rare and has not been proved otherwise, spread of A. mellea in Glenwood is thought to be primarily by vegetative means (Shaw and Roth, 1976), and all multi-isolate infection centers examined, with the exception of two, contained isolates compatible with one another.

Clones were identified by compatible or incompatible reactions which occurred when (diploid) isolates were paired in culture. The 'line of demarcation' formed by A. mellea, as referred to in the literature (Adams, 1974; Shaw & Roth, 1976) was never obvious in the pairings carried out in this study. Incompatible isolates were identified primarily by the lack of fusion of mycelium rather than by the presence of one characteristic such as a line of demarcation. A brown line of pigmentation was observed by both

Korhonen (1978) and Ullrich and Anderson (1978) in interspecific pairings but not in intraspecific pairings. Adams, and Shaw and Roth, however, reported pigmentation of the demarcation line of geographically close isolates in Deschutes County, Oregon, in the former, and Glenwood, Washington, in the latter. Different kinds of media on which the isolates were paired (malt extract, Korhonen, Anderson and Ullrich; pitch media, Shaw and Roth; MDP plus OPP, Adams; MDP, present author) may make comparisons employing this indicator difficult. Pigmentation of media, possibly due to oxidation of peptone, under or around one or both isolates in incompatible matings in these studies, was not equated with the brown line phenomena described by the above researchers. There is the possibility that some of the isolates examined in this study originated from the same parent mycelium but showed incompatible reactions when paired together due to mutation or other mechanisms of genetic changes in part of the mycelium. Thus, clone size and age may be actually greater than estimations based on compatibility reactions would show them to be.

Although spread of A. mellea by spores is not significant locally in Glenwood, infection by spores may be very important in the origin of new infection centers over long periods of time. The large number of clones found in the relatively small sampling area tend to support this idea. The numerous incompatible isolates have

arisen either from individual spores (or matings thereof) or, less likely, from genetic changes accompanied by separation of large infection centers into several small ones by reinvasion of vegetation into them.

An average age of 466 years for multi-isolate clones (assuming a growth rate of 1.0 meter/year [Shaw and Roth, 1976]) is comparable to that found by Shaw and Roth (1976) in the "saddle region" of Glenwood. The calculated age of the largest clones examined (extending 1.4 miles in length) is 1,126 years, which is considerably larger and older than any reported previously.

CONCLUSIONS

One explanation for severe Armillaria root rot in Glenwood, Washington was examined. The hypothesis that severity of disease may be due to uniqueness of clones in the ponderosa pine cover type was not supported by examination of isolate relations, clonal variability, and vegetation patterns.

The study of genetic relationships between Glenwood isolates, examination of pathogenicity of individual clones, and comparisons of susceptibility of Glenwood ponderosa pine to that of non-Glenwood ponderosa pine are some aspects of research needed to better understand this unique disease situation.

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APPENDICES

APPENDIX A

TABLE 8. Means and Standard Deviations of Clones on MDP and Malt Extract Media

CLONE #	MDP MEDIA						MALT EXTRACT MEDIA					
	Mycelium Growth Rate		*Rhizomorph Growth Rate*		*Colony Dry Weight*		*Mycelium Growth Rate*		*Rhizomorph Growth Rate*		*Colony Dry Weight*	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	1.7775	.5035	1.1400	.7400	.6558	.1085	1.2233	.1922	..0200	.0346	.4657	.0074
2	2.9425	.4551	1.9200	.7967	.8205	.1391	2.0600	.0938	.0350	.0412	.4768	.0318
3	1.7950	.2321	.0237	.0342	.5991	.0284	1.6300	.1943	.0017	.0058	.4718	.0227
4	2.4475	.5187	1.4383	.6855	.8283	.0965	2.2662	.2975	.0913	.0564	.5111	.0289
6	3.1440	.1960	2.3800	0	1.0124	.0409	1.6200	.1706	.1567	.0513	.4827	.0085
7	2.2840	.2736	1.6840	.2029	.8128	.0694	1.5733	.2065	.0367	.0321	.4637	.0072
8	2.0100	.4361	.9360	.9904	.8056	.2680	1.4767	.1266	0	0	.4737	.0042
9	2.1544	.4328	1.7700	.7065	.8762	.1782	1.6517	.6009	.0983	.1095	.4607	.0271
10	2.2380	.4948	.9580	.9342	.6618	.1205	1.7767	.3235	.3300	.2762	.4623	.0284
11	1.3420	.1527	.6020	.8535	.6300	.1320	1.0933	.1266	.0300	.0265	.4757	.0120
12	1.6075	.2254	1.5075	.3655	.8460	.1604	1.4550	.2333	0	0	.4640	.0113
13	1.6520	.4100	.7420	.9330	.6228	.1399	1.6133	.2532	.0167	.0289	.4613	.0085
14	2.2250	.8267	1.4775	1.0638	.7380	.1248	1.6967	.2413	0	0	.4737	.0021
15	3.1000	0	2.2150	.0354	.8050	.0014	1.9333	.0577	.0900	.0265	.4610	.0078
17	1.5820	.1517	.0480	.0319	.6104	.0508	1.5567	.1550	0	0	.4750	.0122
18	1.4300	.1927	.6225	.2241	.6108	.0410	1.2367	.0808	--	--	.4593	.0081
20	1.8080	.2217	1.2820	.5783	.8342	.1266	1.7267	.1922	.1367	.0551	.4943	.0163
21	1.6200	.3087	1.2300	.5106	.6142	.1064	1.4600	.3843	.0833	.0764	.4777	.0263
22	1.6900	.1568	.6850	.5034	.6085	.0535	1.4300	0	.0533	.0503	.4280	.0170
23	1.6825	.2133	.2712	.4017	.6027	.1179	1.5567	.1988	.0133	.0216	.4808	.0141
24	1.4650	.1446	.2525	.4659	.6118	.0305	1.4600	.0520	0	0	.4713	.0021
25	1.5420	.2653	.5200	.4462	.5786	.0446	1.5567	.1963	.1400	.1000	.4557	.0078
26	2.6075	.8728	1.7500	1.1058	1.0085	.2952	1.6650	.2051	.1000	0	.4695	.0389
27	1.5040	.1216	.3040	.3607	.6044	.0366	1.4933	.1550	.0300	.0520	.4973	.0210
28	2.0760	.5700	.7320	.7772	.7168	.1415	2.1267	.2603	.0100	.0173	.4720	.0010
30	2.0285	.5472	1.2377	1.1221	.7430	.2184	1.5500	.1930	.0313	.0323	.4745	.0557
31	1.7270	.2024	.9367	.8607	.7547	.2019	1.5417	.1237	.0267	.0468	.4803	.0143
32	3.0000	.4630	2.3800	0	.8252	.1001	1.5800	.3732	.0867	.1415	.4613	.0160

(Cont.)

34	1.7320	.2363	.0240	.0182	.6404	.0505	1.7133	.0451	.0067	.0115	.4773	.0060
35	1.9200	.0447	.9100	.5216	.6602	.0524	1.6333	.2868	0	0	.4620	.0087
36	2.1050	.5442	1.6275	.9374	.9708	.2366	1.9050	.3323	.1300	.1838	.4875	.0064
37	2.3800	.6848	.8040	.7087	.8108	.2089	1.5700	.1706	0	0	.4777	.0057
38	1.8075	.1382	1.0250	.1859	.7183	.0596	1.5250	.1344	0	0	.4780	.0071
40	2.3140	.3765	1.4060	.2515	.8296	.0716	1.6200	.0707	.0250	.0354	.4740	.0085
42	3.8980	.3175	2.3800	0	1.0712	.0598	1.0700	.1697	1.4750	.4738	.4820	.0014
43	1.9040	.3317	.4880	1.0580	.6956	.2028	1.3367	.0808	0	0	.4590	.0044
44	2.5700	.6622	1.6560	.9057	.7574	.1350	2.0267	.1206	.0633	.0929	.5073	.0381
45	1.6675	.0660	.0300	.0294	.5755	.0168	1.6367	.1922	.0567	.0379	.4807	.0110
46	1.6200	.1393	.8525	.6121	.7093	.1373	1.3033	.0709	.0333	.0577	.4823	.0101
48	2.6860	.5098	1.8300	.5990	.8058	.0947	1.4767	.0808	.0667	.1155	.4540	.0132
50	2.1467	.4236	2.3800	0	1.1657	.1438	1.5200	0	.0900	0	.5020	0
51	1.7200	.1163	.3550	.5570	.6575	.0370	1.4133	.1159	0	0	.4600	.0056
52	1.8913	.2299	1.2191	.9605	.7920	.1832	1.7940	.2368	.0600	.0730	.4997	.0254

TABLE 9. Means and Standard Deviations of Vegetation Groups on MDP and Malt Extract Media

	MDP MEDIA						MALT EXTRACT MEDIA					
	Mycelium Growth Rate		*Rhizomorph Growth Rate*		*Colony Dry Weight*		*Mycelium Growth Rate*		*Rhizomorph Growth Rate*		*Colony Dry Weight*	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Ponderosa Pine	2.1730	.5550	1.0728	.9814	.7563	.1629	1.7164	.3593	.0438	.0669	.4752	.0273
Mixed Species	1.9614	.5555	1.1365	.9602	.7626	.1974	1.5949	.3126	.0416	.0600	.4780	.0240
Douglas fir	2.0249	.6678	.9695	.7983	.7364	.1867	1.6021	.2550	.1125	.3171	.4804	.0276

APPENDIX B

TABLE 10. Analyses of Variance of Mycelium Growth Rates on MDP Media

Clones

Source	df	MS	F	Sig.
Clones	42	1.441	9.915	$p < .001$
Residual	212	.145		
Total	254	.359		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.872	2.501	N.S.
Residual	252	.349		
Total	254	.353		

TABLE 11. Analyses of Variance of Rhizomorph Growth Rates on MDP Media

Clones

Source	df	MS	F	Sig.
Clones	42	2.639	5.500	$p < .001$
Residual	210	.480		
Total	252	.840		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.638	.752	N.S.
Residual	250	.849		
Total	252	.848		

TABLE 12. Analyses of Variance of Colony Dry Weight on MDP Media

Clones

Source	df	MS	F	Sig.
Clones	42	.098	4.928	$p < .001$
Residual	212	.020		
Total	254	.033		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.016	.466	N.S.
Residual	252	.035		
Total	254	.035		

TABLE 13. Analyses of Variance of Mycelium Growth Rates on Malt Extract Media

Clones

Source	df	MS	F	Sig.
Clones	42	.239	4.309	$p < .001$
Residual	118	.056		
Total	160	.104		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.210	2.173	N.S.
Residual	158	.097		
Total	160	.098		

TABLE 14. Analyses of Variance of Rhizomorph Growth Rates on Malt Extract Media

Clones

Source	df	MS	F	Sig.
Clones	41	.110	17.172	$p < .001$
Residual	117	.006		
Total	158	.033		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.078	2.566	N.S.
Residual	156	.031		
Total	158	.031		

TABLE 15. Analysis of Variance of Colony Dry Weights on Malt Extract Media

Clones

Source	df	MS	F	Sig.
Clones	42	.001	1.810	$p < .01$
Residual	118	.001		
Total	160	.001		

Vegetation

Source	df	MS	F	Sig.
Vegetation	2	.000	.422	N.S.
Residual	158	.001		
Total	160	.001		

TABLE 16. Analyses of Variance of Clone and Media Effects

MYCELIUM GROWTH RATE

Source	df	MS	F	Sig.
Clones	42	.843	7.447	p < .001
Media	1	17.498	154.561	p < .001
C x M	42	.506	4.472	p < .001
Residual	330	.113		
Total	415			

RHIZOMORPH GROWTH RATE

Source	df	MS	F	Sig.
Clones	42	1.297	4.179	p < .001
Media	1	83.647	269.488	p < .001
C x M	41	.891	2.871	p < .001
Residual	327	.310		
Total	411	.759		

COLONY DRY WEIGHT

Source	df	MS	F	Sig.
Clones	42	.037	2.989	p < .001
Media	1	6.001	464.762	p < .001
C x M	42	.032	2.500	p < .001
Residual	330	.013		
Total	415	.037		

TABLE 17. Analyses of Variance of Vegetation and Media Effects

MYCELIUM GROWTH RATE

Source	df	MS	F	Sig.
Vegetation	2	.881	3.501	N.S.
Media	1	15.435	61.341	$p < .001$
V x M	2	.071	.281	N.S.
Residual	410	.252		
Total	415	.292		

RHIZOMORPH GROWTH RATE

Source	df	MS	F	Sig.
Vegetation	2	.086	.161	N.S.
Media	1	88.071	164.729	$p < .001$
V x M	2	.494	.924	N.S.
Residual	406	.535		
Total	411	.775		

COLONY DRY WEIGHT

Source	df	MS	F	Sig.
Vegetation	2	.005	.227	N.S.
Media	1	6.713	308.422	$p < .001$
V x M	2	.008	.348	N.S.
Residual	410	.022		
Total	415	.040		

APPENDIX C

PAST CLIMATE AND VEGETATION CHANGES IN THE PACIFIC NORTHWEST

Considerable evidence (geological and other) indicates that climatic changes have occurred throughout the Pacific Northwest over the millennia. Changes in vegetation have accompanied or followed climatic fluctuations. Some evidence exists for a warming, drying trend, occurring in the last few centuries in the Pacific Northwest and most parts of the world.

Maximum glaciation in the Pacific Northwest occurred about 25,000 years ago (Hansen, 1947). Postglacial time extends from approximately 11,000 years to present. Several major climatic trends on the north Pacific Coast (as well as throughout most of the world) have been described and documented (Hansen, 1947; Heusser, 1960) since the last major glaciation:

1) 19,000 to 15,000 B.P. (before present)--climate moist and cool
2) 15,000 to 8,000 B.P.--increasing warmth and dryness
3) 8,000 to 3,000 B.P.--maximum warmth and dryness ("Hypsithermal")
4) 3,500 B.P. to present--return to cooler and moister climate.

Within this last cool and moist climatic regime, a series of fluctuations has occurred. Recent climatic trends are documented by pollen and fossil deposits, dendrochronology (the study of past climate through tree ring analysis), human records, archeology, the study of existing glaciers, and geological evidence of past glaciers. World-wide climatic changes, occurring over the last 3000 years, are divided into three categories by Lamb (1969). Following the climatic optimum (Hypsithermal) which terminated about 4000 years ago, a colder climate occurred in the early Iron Age, culminating between 900 and 450 B.C. (2900 to 2400 B.P.). A secondary or "little" optimum prevailed during the Middle Ages and ended between 1000 and 1200 A.D. (800 to 1000 B.P.). This warming trend was followed by a "little" ice age occurring between 1430 and 1850 A.D. (550 to 130 B.P.), particularly in the northern hemisphere.

Several facets of evidence show that during the past few centuries the climate in the Pacific Northwest and throughout much of the world has shown a warming/drying trend. There has been a general recession of glaciers in Washington, Oregon, Alaska, Montana, and elsewhere over the last few centuries (Hansen, 1947; Porter & Denton, 1967; Sigafoos & Hendricks, 1972; Scott, 1974) indicating warming and drying. Chronology of recent glacial moraines formed by several glaciers on Mt. Rainier (Washington) indicates the termination of modern glacial advances between

1525 and 1910 (Sigafoos & Hendricks, 1972). Temperature throughout the world has increased 0.5 to 1.0° C. over the last 100 years; in England, lowest values are thought to have occurred in the 1690's with increasing values from thereon (Lamb, 1969). Lamb (1969) describes changes in sea surface temperature, sea level, sea ice, rainfall, air circulation patterns, and other phenomena over the last 500 years which provide evidence for a general warming trend in the last few centuries.

The relation between climatic changes and corresponding vegetation changes has been investigated in recent years in several western North American locations (Hansen, 1944, 1947; Heusser, 1965; LaMarche & Mooney, 1972; Baker, 1976; Ritchie, 1976; Mack et al., 1976; Mack et al., 1978). Mack et al. (1978), examining pollen sequences from Big Meadow in northeast Washington found diploxylon pines (e.g., ponderosa pine) associated with warm, dry periods (9700 to 3300 B.P.) and Picea, Abies, and Tsuga species associated with cooler, moister periods (3300 B.P. to present). Ritchie (1976) describes changes in western interior Canada in the early post-glacial period from spruce-dominated forests to spruce-pine-birch-poplar or to grassland, coinciding with warmer and drier growing seasons.

While vegetation changes over large periods of climatic change and in rapidly changing areas, such as glacial edges, have been examined, the effect of relatively small

climatic changes, such as those occurring within the last 500 years, upon tree species composition within vegetation transition zones (such as that found in the Glenwood area) is unknown. In the study area, a prolonged warming, drying trend hypothetically could push Abies, Pseudotsuga, Picea, and other more mesic-loving species towards the west, upwards in elevation to cooler, moister habitats with the coincident westward movement of Pinus species from the east. The presence today of Douglas fir and grand fir on the cooler and damper northern aspects of the pine forest around Glenwood and the presence of very old and very large relic Douglas firs (Fig. 14) in essentially pure ponderosa pine stands support this idea.

FIGURE 14

Large, relic Douglas fir in ponderosa pine forest.

(Photograph by L. F. Roth)