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# Identification of Clones of *Armillaria mellea* in Young-Growth Ponderosa Pine[1]

**Abstract**

Isolates of *Armillaria mellea* recovered within a study plot were divisible into three clones on the basis of paired culture tests. Members of two clones were recovered from *Armillaria*-killed Ponderosa pine as well as other vegetation of the study plot, while members of the third clone were not found on the pine. Some factors affecting distribution of *A. mellea* are discussed.

## Introduction

Ponderosa pine (*Pinus ponderosa* Laws.) is the predominant and most important conifer on the east slope of the Cascade Range in Oregon and Washington. The honey fungus, *Armillaria mellea* (Vahl) Quél. (also called *Armillariella mellea* Karst.), can be a significant pathogenic factor during regeneration of pine on sites that formerly sustained old-growth pine.

*Armillaria mellea* is endemic to forests of both temperate and tropical climates (Cartwright and Findlay, 1958). Thus, the fungus is often established in managed forests and plantations well before disease "outbreaks" occur. Realistic management of young Ponderosa pine must take into account any long-term disease conditions that may be inherent to the site. Therefore, the development and distribution of *A. mellea* within a managed forest is of considerable interest to the forest manager (Weiss and Riffle, 1971).

Successful establishment of *A. mellea* in a conifer forest usually leads to an increase in colony dimensions. In time this colony may form a part of a mosaic with other *Armillaria* colonies already present (Pielou, 1965). Identification of these individual colonies as they occur can be extremely important in long-term management planning. Early detection of pathogenic colonies of *Armillaria* within the site could lead to delimitation and control of potential infection centers.

Identifying individual clones among isolates of a species has been attempted with success for several forest fungi (Adams and Roth, 1969; Barrett and Uscuplic, 1971; Childs, 1963, 1970; Mounce, 1929), but does not include work with *A. mellea*. The identification technique is based on an incompatibility reaction which occurs between isolates of different ancestry (of the same species) in competition for a common substrate in paired culture, while isolates of common ancestry are compatible under identical circumstances. These reports prompted an investigation into the usefulness of the incompatibility reaction as a means of identifying individual *A. mellea* colonies as they occur in a forest situation. Throughout this report isolates of *A. mellea* representing the same colony in the field will be referred to as members of the same clone in culture.

[1] Based on a Ph.D. thesis submitted to Oregon State University.

**Study Area**

Field studies reported here were conducted on the east and south intermediate slopes of Pringle Butte in the Pringle Falls Experimental Forest, Deschutes County, Oregon.

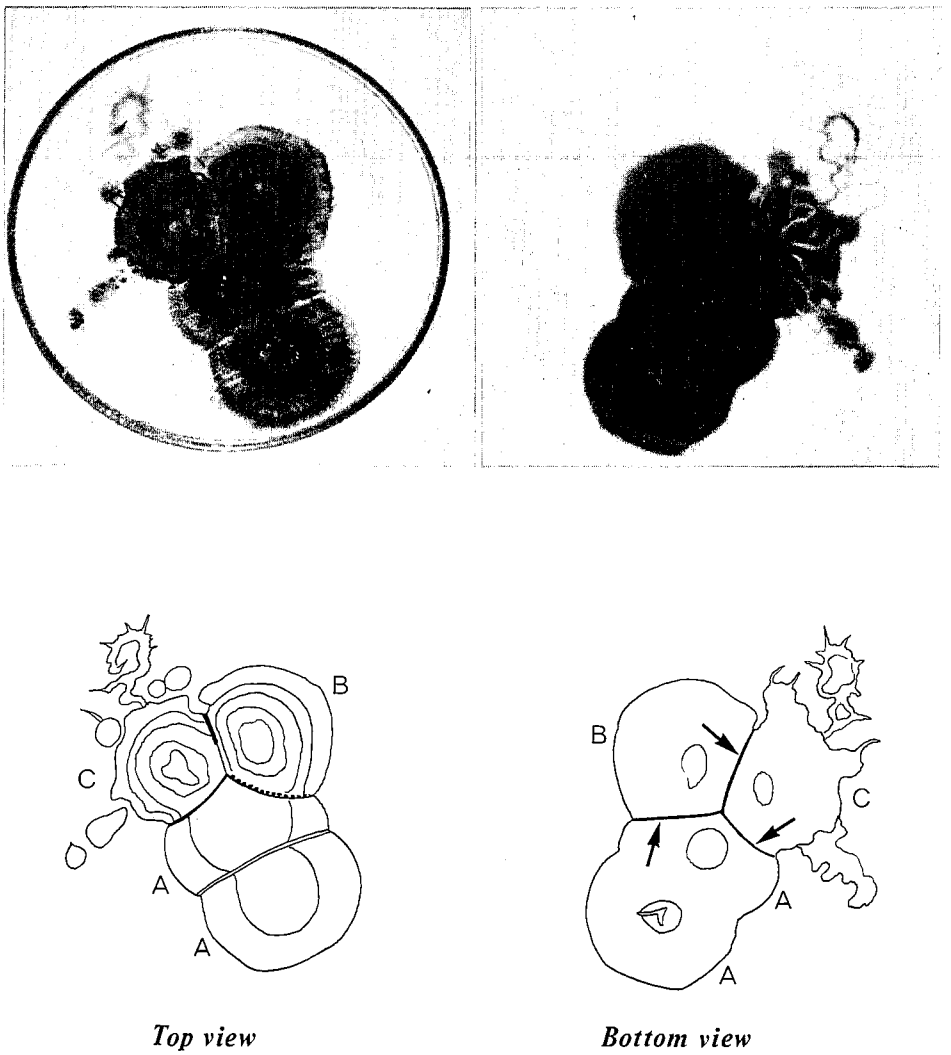


Figure 1. Top (left side) and bottom (right side) views of the same paired isolates are illustrated in photo and drawing. Noncompatible interactions between Clones A, B and C, and a compatible Clone A pairing are shown. Incompatibility between paired isolates is most clearly shown in bottom view as a dark line of demarcation (3 arrows) along the common interfaces of the three center colonies.

The forest is a facility of the Pacific Northwest Forest and Range Experiment Station headquartered in Portland, Oregon.

Pringle Butte vegetation corresponds closely to the *Pinus/Ceanothus-Purshia* community of Dyrness and Youngberg (1966). Ponderosa pine is found in nearly pure stands throughout the study area, with shrub vegetation consisting of snowbrush ceanothus (*Ceanothus velutinus* Dougl.), antelope bitterbrush (*Purshia tridentata* (Pursh.) DC.), and pine manzanita (*Arctosaphylos parrayana* var. *pinetorium* (Rollins) Wiesl. and Schreib.). *Armillaria mellea* is present throughout the study area as rhizomorphs on the roots and root crown of the shrubs.

Except for two light sanitation cuts, the east side of Pringle Butte had no logging history prior to 1957. In that year 140 acres of old-growth Ponderosa pine was logged under intensive care to minimize damage to understory saplings (Barrett, 1960). These released saplings were thinned in early 1958 to a 13 by 13 foot spacing. The Study Plot and Infection Centers 1 and 2 of this report are located within this released stand.

Infection Center 3 is located within an 8-acre pine growth study plantation on the south slope of Pringle Butte. Site vegetation consisted mainly of old-growth Ponderosa pine and shrubs. All stumps of the felled pine were left in place, while most of the shrubs roots were removed in clearing the shrubs from the site. Two-year-old pine seedlings were planted in pairs throughout the site on 6-foot centers. The infection center of this report is located in the pine transplants surrounding a 48-inch diameter stump.

#### Study Plot and Infection Centers

*Armillaria* isolates were recovered in the field from one study plot and three infection centers on Pringle Butte. Two of the infection centers are within the study plot, while the third in the seedling plantation on the south slope was described under Study Area.

1. Study Plot

The study plot is a 250 by 450 foot rectangle on the east slope of Pringle Butte. Roots of all vegetation were examined for *A. mellea* along six 10-foot wide transects through the plot. These transects established on 50-foot centers parallel to the long axis of the plot enabled a systematic sampling of sapling Ponderosa pine and shrub roots in the plot. The study plot is about 2.6 acres in area and 20 percent of the plot was traversed by the transects.

2. Infection Centers 1 and 2

Each infection center is composed of at least one large stump, intermixed living, dying and dead sapling pine, and numerous shrubs. Physical dimensions of each infection center are estimated by the number of young pine under attack by *A. mellea* at the time of study. The apparent diameter of each of these infection centers in 1970 was 50-75 feet.

The average age of *Armillaria*-attacked saplings in Infection Centers 1 and 2 is: 61 years (range: 36-78 years) and 47 years (range: 35-55 years), respectively.

**Methods**

1. Recovery of *A. mellea* in Study Plot and Infection Centers

Shrubs uprooted during this study and *Armillaria*-killed sapling pine were the principal sources of *A. mellea* collections along the transects of the study plot. Rhizomorphs were found on the shrub roots, while the root cambium of *Armillaria*-killed sapling pine yielded mycelial collections of *A. mellea.*

*Armillaria* was recovered in the infection centers primarily from shrubs, *Armillaria*-killed seedling and sapling pine, and large stumps (3-4 foot diameter) of the former overstory. Isolates of *A. mellea* collected here came basically from three sources: 1) mycelium in the wood of stumps and their roots; 2) mycelium of mycelial fans in the cambium of the stump-root systems, and *Armillaria*-killed seedling and sapling pine; 3) rhizomorphs. Rhizomorphs were found: a) within hollow roots of pine; b) on the root surfaces of stump roots, young pine, and living shrubs; c) in the soil away from roots.

2. Laboratory Recovery of *A. mellea*

Rhizomorphs and mycelia of *A. mellea* collected on Pringle Butte were placed onto media in the laboratory to recover the fungus. A basic medium of 3 percent malt extract, 2 percent dextrose, 0.5 percent peptone, and 2 percent agar was used for all cultural work. The pH was adjusted to 5.0-5.3 with dilute HCl or NaOH. Orthophenylphenol (OPP) was added (0.006%) to the basic medium (Russell, 1956) for initial recovery of *A. mellea.*

Rhizomorphs were prepared for recovery of *A. mellea* with a vigorous washing in cold, running water for 6-12 hours, followed by surface sterilization in 20 percent Clorox for 10-15 minutes. After a brief rinse in sterile water, the rhizomorphs were asceptically cut into 10-15 mm lengths and placed onto the basic medium plus OPP.

Fragments of mycelia from mycelial fans, and *Armillaria*-infested wood from large stumps, were placed directly onto the basic medium for recovery of the fungus. These cultures were incubated in the dark at 25° C.

3. Isolate Identification

Separation of all isolates recovered within the study plot and infection centers into clones required observation of isolate interactions in paired culture. Isolates that freely intermingled when paired (Fig. 1) were considered to be compatible, i.e., possess the same compatibility-genotype (Adams and Roth, 1967), and therefore were of the same parent mycelium. Conversely, an isolate pairing in which a dark line formed along the common interface (Fig. 1) was considered to be a pairing between isolates of dissimilar parentage. The dark line is here referred to as a line of demarcation.

Cultural interactions for each pairing combination on the basic medium were completed in 14-21 days. Paired isolates were incubated in the dark at 25° C.

Growth habit of an isolate aided in identifying members of clones, but was not by itself considered definitive. Among the more useful of these features were relative abundance and morphology of rhizomorphs, color and morphology of surface mycelium, and the occurrence of mycelial exudations.

*Armillaria mellea* isolates from Ponderosa pine and incense cedar (*Libocedrus decurrans* L.), recovered in central Oregon outside the Pringle Butte area, were tested in paired culture against members of the clones identified on Pringle Butte. Incom-

patibility was found among all pairings between Pringle Butte isolates and those isolates recovered elsewhere in central Oregon.

**Results**

1. Identification of Clones

A total of 243 isolates of *A. mellea* were recovered in the study plot and three infection centers on Pringle Butte. Based on mycelial interactions in paired culture, all the isolates were separated into three clones (A, B, and C). Clone A is represented by 83 isolates, Clone B by 11 isolates, and Clone C by 149 isolates. All isolates of a clone are intercompatible in paired culture, while isolates of different clones are not compatible in paired culture. Isolate appearance remained constant within each clone during the study.

2. Distribution of Clones

*Armillaria* isolates recovered in the study plot, which does not include the locality of Infection Center 3, are grouped in Table 1 according to clone and recovery source. Collections along the transects were primarily from shrubs.

TABLE 1. Frequency of recovery of *A. mellea* as related to clone and recovery source in the study plot.

| | Clone | | |
|---|---|---|---|
| Recovery Source | A | B | C |
| Sapling pine[1] | 9(m)[2] | 7(m) | — |
| Shrubs[3] | 15(rh) | 4(rh) | 124 (rh) |
| Rhizomorphs[4] | 1 | — | 5 |

[1] *Armillaria*-attacked, above-ground symptoms apparent.
[2] *Armillaria* tissue collected: m = mycelium, rh = rhizomorph.
[3] Snowbrush, bitterbrush, manzanita.
[4] Rhizomorphs collected away from living plant roots.

*Armillaria* was collected in the three infection centers primarily from the dead and dying pine. Isolates were also recovered from some shrubs in Infection Center 1, and from stumps in Infection Centers 1 and 2. The results of these findings are summarized in Table 2. Isolates of Clone B were not found in the three infection centers.

TABLE 2. Frequency of recovery of *A. mellea* as related to clone and recovery source in the three infection centers.

| | Number of Recovery Sources | Clone | |
|---|---|---|---|
| Recovery Source | | A | C |
| *Infection Center 1* | | | |
| sapling pine | 14 | 14(m)[1] | – |
| pine stump | 1 | 1(m) | 1(rh) |
| shrubs[2] | 7 | – | 7(rh) |
| rhizomorphs[3] | 6 | – | 6 |
| *Infection Center 2* | | | |
| sapling pine | 10 | 10(m) | – |
| pine stumps | 6 | 1(m) | 6(rh) |
| *Infection Center 3* | | | |
| seedling pine | 16 | 16(m) | |

[1] *Armillaria* tissue collected: m = mycelium, rh = rhizomorph.
[2] Snowbrush, bitterbrush, manzanita.
[3] Rhizomorphs collected away from living plant roots.

## Discussion

1. Identification of Clones

Isolates of *A. mellea* recovered in the study plot and three infection centers on Pringle Butte belonged to three readily distinguishable clones. Separation of isolates into clones is based primarily on compatibility relationships in paired culture, with secondary emphasis on similarity of isolate morphology.

These studies with *A. mellea* have demonstrated that formation of a line of demarcation between paired isolates is indicative of basic differences between those isolates. In all noncompatible pairings, the line of demarcation was noticed as a distinct (though not always intense) discoloration along the common interface in paired culture. Compatible isolates mingled in paired culture without distinctive interaction.

Morphology of isolate growth has been observed by several workers during compatibility studies with basidiomycetous fungi. Both Verrall (1937), and Barrett and Uscuplic (1971) noted the similarity among members of compatible lines of *Fomes igniarius* and *Polyporus schweinitzii,* respectively. Conversely, dissimilar appearance within compatible lines were found among isolates of *Fomes cajanderi* (Adams and Roth, 1967).

Major characteristics of isolate morphology remained constant within each *Armillaria* clone identified during this study. This agrees with observations of *Armillaria* growth habit made by Gibson (1961) and Raabe (1967). Cultures of Clone A are similar in gross morphology to cultures of Clone B, however, characteristic differences are present. Clone A isolates produce rhizomorphs that reach 30 mm in three weeks, while rhizomorphs of Clone B seldom reach 15 mm in the same time. Also, very slow growing rhizomorphs form on the top of the inoculum piece in Clone A cultures and not in Clone B cultures. A distinct line of demarcation, with droplets of dark liquid, always forms in Clone A/B pairings. Isolates of Clone C produce rhizomorphs abundantly, develop little aerial mycelium, and form a line of demarcation with the other clones in paired culture.

Usage of "line of demarcation" in this report follows that of Mounce (1929), who first used the term to indicate distinctive zones that formed between certain isolates of *Fomes pinicola* in paired culture. Use of this term connoted the presence of some kind of mycelial interaction beyond the inconspicuous meeting of compatible hyphae in paired culture. As Barrett and Uscuplic commented (1971), usage of specific terminology to describe the cause rather than the effect of the incompatibility reaction is to be avoided. "Line of demarcation" is a descriptive term used here to describe the appearance of discolored hyphae which are observed visually as a dark line, and does not attempt to indicate the physiological basis of this phenomenon.

2. Distribution of *Armillaria* on Pringle Butte

*Armillaria mellea* was found to be generally distributed over the slopes of Pringle Butte, but was not found anywhere off the slopes in the surrounding lodgepole pine-bitterbrush forest type. Members of the three clones identified in this report alone were found in the study plot and infection centers. Isolates of four other clones were recovered within a half mile of the study plot center.

An *Armillaria* colony enlarging about its point of origin may, over a period of several thousand years, become very large. Such a colony on Pringle Butte is undoubtedly fragmented into many independent entities each utilizing some substrate

offered by the site. These entities in their turn are carrying on the biological role of the colony when first established. The term "colony" as used here does not imply a physiological continuity between all entities of the colony in the field, but rather notices a fundamental relationship in which all entities of the colony have developed from a common origin. Colony members, represented together as a clone, are identified through compatibility tests in paired culture.

The number and distribution of *Armillaria* colonies on Pringle Butte is not known, but the distribution of some colonies may be extensive. Members of Clone A were recovered in the study plot and in Infection Center 3, seven-eighths of a mile southwest of the study plot. Similarly, a member of Clone B was recovered from a dying sugar pine (*Pinus lambertiana* Doug.) pole one-half mile northwest of the study plot. Four members of Clone C were recovered within a distance of one-half mile south and east of the study plot.

Actual colony size may be quite large and the colony very old, while an infection center, obvious though it may be, represents only an "instantaneous view" in time of the colony presence. Infection centers restricted to a single foodbase (the stump), as they appear to be on Pringle Butte, remain relatively small and short-lived. Over a period of years separate infection centers may coalesce to form a larger center, but longevity of activity in these centers depends upon the effective lifespan of the foodbase. Shrubs within these centers may in turn act as reservoir hosts in perpetuation of *Armillaria* in close proximity to susceptible vegetation.

Obviously, age of *Armillaria* colonies identified in this study cannot be adequately estimated. Based on extreme distance between recovery sites for members of each clone, it is of interest to consider that some *Armillaria* colonies on Pringle Butte must be very old. Once *Armillaria* becomes established on Pringle Butte, there appears to be little that interferes with colony survival. Certainly, the three colonies identified in the study plot should be considered as native to Pringle Butte as is the more readily apparent vegetation of the site. Since asexual spores are not known in *A. mellea,* rhizomorphs (as an extension of the main body of the fungus) are most probably responsible for the wide dispersal of individual colonies over Pringle Butte.

Of special interest is the observation that members of Clone C were not found to be a primary agent responsible for the killing of young pine on Pringle Butte. Only members of Clones A and B were found invading roots of dead and dying young pine and the wood of the large stumps.

**Acknowledgments**

Grateful acknowledgment is given to Dr. L. F. Roth for advice and encouragement throughout my graduate studies under his guidance. The author thanks James Barrett and Walter Dahms of the Pine Silviculture Laboratory, USFS, Bend, Oregon, for providing the opportunity to collect the data of this paper and for support throughout the study.

**Literature Cited**

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

————. 1969. Intraspecific competition among genotypes of *Fomes cajanderi* decaying young-growth Douglas-fir. Forest Science 15(3): 327-331.

Barrett, D. K., and M. Uscuplic. 1971. The field distribution of interacting strains of *Polyporus schweinitzii* and their origin. New Phytol. 70: 581-598.

Barrett, J. W. 1960. Intensive control in logging Ponderosa pine. Iowa State J. of Science 34(4): 603-608.
Cartwright, K. St. G., and W. P. K. Findlay. 1958. Decay of timber and its prevention. 2nd ed. Her Majesty's Stationery Office, London. 332 p.
Childs, T. W. 1963. *Poria weirii* root rot. *In*: Symposium on root diseases of forest trees. Phytopathology 53(10): 1124-1127.
————. 1970. Laminated root rot of Douglas-fir in western Oregon and Washington. Research Paper PNW-102. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland. 27 p.
Dyrness, C. T., and C. T. Youngberg. 1966. Soil-vegetation relationships within the Ponderosa pine type in the central Oregon pumice region. Ecology 47(1): 122-138.
Gibson, I. A. S. 1961. A note on variation between isolates of *Armillaria mellea* (Vahl ex Fr.) Kummer. Trans. Brit. Mycol. Soc. 44: 123-128.
Mounce, Irene. 1929. Studies in forest pathology. II. The biology of *Fomes pinicola* (Sw.) Cke. Canada Department of Agriculture Bulletin No. 111, Ottawa. 75 p.
Pielou, E. C. 1965. The spread of disease in patchily-infected forest stands. Forest Science 11: 18-26.
Raabe, R. D. 1967. Variation in pathogenecity and virulence in *Armillaria mellea.* Phytopathology 57: 73-75.
Russell, P. 1956. A selective medium for the isolation of basidiomycetes. Nature 177: 1038-1039.
Verrall, A. F. 1937. Variation in *Fomes igniarius* (L) Gill. Minnesota Agricultural Experimental Station, Technical Bull. 117, Duluth. 41 p.
Weiss, M. J., and J. W. Riffle. 1971. *Armillaria* root rot in a Ponderosa pine plantation in New Mexico. Plant Dis. Rep. 55: 823-824.

*Received May 7, 1973.*
*Accepted for publication October 17, 1973.*