Persistence and Distribution of a Clone of Armillaria mellea in a Ponderosa Pine Forest

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ABSTRACT

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Clonal relationships of isolates of *Armillaria mellea* from a heavily diseased ponderosa pine forest were tested in culture. Members of unrelated clones formed dark lines of demarcation between paired isolates. Lines did not form between paired members of the same clone. All isolates from within a 600-hectare area, which included several root rot infection centers, were found to belong to a single clone. Continuity of the clone over the area suggested that fungal distribution had been by vegetative growth from a single point of origin rather than by spores. Measurements of twenty infection centers disclosed an average radial growth rate of 1.0 m/year. Calculations indicated that the clone had been spreading vegetatively for at least 460 years.

Additional key words: barrage reaction, mutual aversion, fungal genotypes, fungal growth rate, food base, epidemiology.

Despite extensive research (7, 8, 10, 12, 15), knowledge of *Armillaria mellea* (Vahl ex Fr.) Quél. is inadequate for good disease management in many situations. This report is concerned with the biology of the pathogen in a natural ponderosa pine (*Pinus ponderosa* Laws.) forest.

Adams (1, 2) demonstrated that *A. mellea,* like many other Hymenomycetes, forms dark lines of demarcation between cultured paired isolates of genetically distinct origin. No such lines form between isolates of the same genotype. Adams used line formation to test for the presence of fungal clones. His method appeared suitable for investigation of the historical development of Armillaria root rot in a ponderosa pine forest in south central Washington which was so severely diseased that hundreds of hectares have become nonproductive. Damage appears as expanding, circular, often discrete, infection centers (12, 13, 14). Centers reaching 0.5 hectare (ha) or more, made conspicuous by brown foliage of dead trees at their limits, apparently develop around the stumps of old-growth pines cut 10 to 30+ years ago.

MATERIALS AND METHODS

Isolates of *A. mellea* were obtained from infected pine roots in five root rot infection centers within 0.9 km of one another in a locality hereafter referred to as the Saddle Area. In one center (A) numerous isolates were collected along a 25-m radius outward from the presumed point of origin of the center, an old-growth pine stump. In another center (B), 400 m from A, seven isolates, excluding any from the stump, were similarly collected. At least one isolate was obtained from each of the three other centers. Twelve cultures, probably representing several centers, were isolated from infected pine roots and rhizomorphs in a 4-ha disease control plot near center B. In addition, cultures were obtained from: (i) infection centers in pine, 3 and 6.4 km distant, from the Saddle Area; (ii) a hazel shrub (*Corylus cornuta* Marsh.) 32 km away (designated H) and; (iii) a sporophore on a dead pine sapling 137 km distant in Deschutes County, Oregon (designated S).

Isolates were paired in various combinations in 90-mm diameter petri dishes on a standard growth medium (40 g malt extract, 20 g dextrose, 19 g agar, 5 g bacto-peptone, and 1,000 ml distilled water), or the standard medium plus 40 g/liter of pine wound resin (12). Resin was prepared by freezing crude resin with liquid nitrogen in a mortar, pulverizing the cake with a pestle, and sifting the powder through a 1.19-mm (16-mesh per inch) screen. Addition of resin to the standard medium significantly increased growth of *A. mellea* colonies in comparison to the standard medium (12).

Cultures were incubated in the dark at 25 C until colonies met along a broad face (approximately 3 weeks on the resin medium). Then they were examined by transmitted light for the presence of lines of demarcation. All isolates were paired at least twice.

Rates of growth of *A. mellea* in the forest were determined by measuring radii of the infection centers and dividing the distances by time passed since origin of the centers. The centers originated when the old-growth pines around which they subsequently developed were cut (12). The time of cutting was determined from examination of harvest records, appearance of the stumps with respect to felling characteristics, and extent of deterioration.

RESULTS AND DISCUSSION

Origin of infection centers.—The H/S pairing

regularly formed lines of demarcation (Fig. 1-A). Isolates obtained along the radius of an infection center did not form lines when the isolates were paired consecutively or out of order (Fig. 1-B). Similarly, lines were not formed upon pairing isolates among the five centers, nor upon pairing those from the centers with the scattered isolates from the 4-ha control plot. The latter formed no lines when paired among themselves but all formed lines with the H and S isolates. Isolates from the two infection centers outside of the Saddle Area formed lines of demarcation with each other and when either was paired with any isolate from the Saddle Area. All isolates from infection centers in pine showed distinct line formation when paired against either the H or S isolate.

The lack of line formation among cultures from the Saddle Area shows that the visibly discrete infection centers there arose predominately, if not exclusively, from a single *A. mellea* genotype. The results indicate that distribution of the infection centers reflects vegetative growth, interrupted to be sure, over many years from a single point of origin, rather than intermittent origin from spores. Raabe (6) showed that single-spore isolates of *A. mellea* from a single basidiocarp exhibited as much variation in cultural characteristics as did an array of isolates from various hosts obtained from widely separated localities in California. If this variation is indicative of the genetic diversity of the fungus, including compatibility reaction, then lines of demarcation should have resulted between some paired isolates from closely associated infection centers if these centers arose from spores.

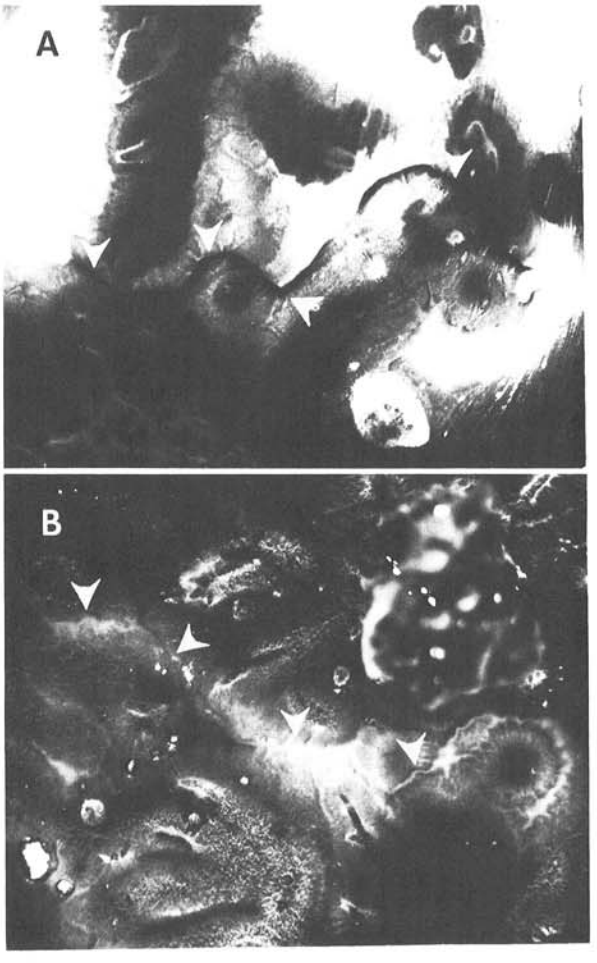

Fig. 1. Parts of paired cultures of *Armillaria mellea* enlarged approximately × 2.6 in A and × 2.1 in B. **A)** shows formation of the dark line (arrows) when members of differing clones meet. The dark line is absent between members of the same clone in B.

Rate of enlargement of infection centers.—If fungal spread through the forest is by vegetative growth, the rate becomes of particular interest. Estimation of growth rate was somewhat complicated because of the need to correct for the "park effect" (11), and for time required after felling for the formerly quiescent *A. mellea* to colonize the root system of the old-growth stump around which the infection center developed. These corrections were used because it is not known if rate of colonization of the old roots and subsequent spread among the young trees, which requires repeated infection, are the same.

In the forest that was studied, widely spaced old-growth trees typically stand in park-like openings, with few small trees occurring beneath their crowns, although forbs and shrubs may be abundant (Fig. 2). Young-growth pine stands irregularly occupy the land between the parks.

The radius of the park, the distance from the old-growth tree to surrounding young trees, as measured from six or seven widely spaced old trees 70-130 cm diameter at breast height, on each of three *Armillaria*-free sites (20 trees in all), was 9.1 m (range 6.1 m to 11.9 m). No differences were apparent among locations or in relation to tree diameter.

In areas with diseased trees, roots of young trees at the edge of the park are the first to contact infected roots of the old tree or to be infected by rhizomorphs from them. These trees die, the fungus colonizes their roots and spreads by root contact or rhizomorphs to trees still farther out, enlarging the infection center.

Measurements of 20 infection centers, from the central old-growth stump to the outermost dead tree in the young stand were obtained from stumps cut in 1941, 1953, 1958, 1961, and 1965. Nine meters was subtracted from each measured radius to correct for the radius of the park.

Time since cutting was determined by subtracting the date of cutting from the year of measurement (1974). Five more years was subtracted to account for time required for the fungus to colonize roots of the old tree and for the encounter with trees on the edge of the park. Five years were employed because: (i) J. S. Hunt had earlier noted in this locality that infection centers were first evident approximately 5 years after the initial cutting in 1941 (Hunt, *unpublished);* and (ii) we observed that infection centers associated with the 1965 stumps first became evident around 1970.

With the above adjustments for distance and time, rates of spread were calculated by dividing the distance by the time. Average rates (m/year), ranges (m), and number of data points for stumps of the three felling periods 1941, 1953-1958, and 1961-1965 were: 0.87, 0.61 to 1.04, 11; 1.21, 0.64 to 1.98, 5; 1.26, 0.76 to 1.80, 4, respectively. The average rate of radial increase of the 20 centers was 1.0

m/year. As might be expected there was less variability in the rates for the older centers with more data points (1941) than for the more recent centers.

Even though these calculations of rates of enlargement of infection centers are the only ones known to the authors that are based on data from natural (nonplantation) forests, rates of spread have been reported for *A. mellea* in other situations. Marsh (5) figured the rates of spread in apple orchards and a black currant plantation in England, to be 1.5 and 1.8 m/year, respectively. Rishbeth (9) calculated the rate of spread along roots of European ash in a Cambridge, England, garden at 1.5 m/year, along roots of inoculated Norway spruce at 1.6 m/year, and in a Douglas-fir plantation at 1.5 m/year. Swift (16) observed a rate of 5 m/year over 3 years in a slash pine plantation in Rhodesia. Kable (4) reported rates in a peach orchard in Australia to vary from 0.8 to 3.2 m/year with the majority between 0.8 and 1.3 m/year.

Factors including soil temperatures and other soil conditions; tree species, size, and spacing; and fungus strain may influence the rate of spread. Even so, most of the rates previously reported are within a range comparable to our values. The highest rate, 5 m/year (16), was based on data from a young, rapidly growing, plantation of a highly susceptible species (3) in a region with high soil temperatures. On the basis of soil temperatures alone a slower rate of spread than those previously reported would be expected at our Washington State site where the ground usually is covered with snow from November to May, and soil temperatures are lower (1974 August mean at 30 cm varied from 7-10 C; G. Filip, *personal communication*) than in any of the other areas for which rates of spread are reported.

If in time past *A. mellea* grew through the stand at the calculated rate of 0.87 m/year, then the fungus in the Saddle Area has been spreading vegetatively for at least 460 years. This is the time required to cross half of the greatest distance (0.8 km) between sources of isolates not forming demarcation lines. Four-hundred and sixty years is an unrealistically conservative estimate of tenancy because the sampling was too limited to locate the outer limits of distribution of the clone, and spread was most likely slower in the undistrubed old-growth forest than in the present young stands. Nevertheless, 460 years establishes the presence of the fungus, at least somewhere in the natural stand, prior to any forest modification by man, in fact, prior to establishment of the recently logged

Fig. 2. An old-growth ponderosa pine showing the park effect. The man in the left background is halfway between the old-growth tree and the regeneration stand beyond the park. On some sites ground cover in the park is bitter brush (*Pershia* sp.) rather than grass and sedge.

old-growth stand. If Adams' ideas (1, 2), which these results support, on *A. mellea* colony development and expansion are correct, then the fungus could have been present on this site for thousands of years.

These observations and hypotheses are noteworthy in several respects: (i) The old-growth pine forest that formerly occupied the study site developed in the presence of wide-spread *A. mellea* and the present serious outbreak in the replacement forest repeatedly has been triggered by timber harvest. (ii) *Armillaria mellea* occupies its site largely by vegetative means; thus epidemiological models associated with the profuse sporulation common to the Hymenomycetes are inappropriate for this fungus. (iii) Depending perhaps on the extent of continuity among infection centers, a fungus colony of great age and size is described. (iv) Timber managers engaged in removing *Armillaria* from their land by physical means can proceed with confidence that reinfection by spores is unlikely.

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