# Seasonal Development of Armillaria Root Rot of Peach as Influenced by Fungal Isolates

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ABSTRACT

Four clones of *Armillaria mellea* isolated from plants in areas of California remote from one another were tested for pathogenicity on peach trees in the field at Riverside. Infection of trees from inoculum produced on fig or citrus wood was comparable. One year after inoculation, over 60% of the trees were killed by clone D-9; ca. 25% were killed by clones D-2 and D-86; and only 5% were killed by clone D-73. By the end of 3 years, the per cent killed was 100, 86, 71, and 60 for trees inoculated with clones D-9, D-2, D-86 and D-73, respectively. In 3 years, 86% of the 168 inoculated trees were dead. The per cent of those killed during the four seasons was approximately as follows: winter, 2; spring, 23; summer, 66; and fall, 10. After 3 years, infection had spread to 56 noninoculated trees as follows: dead, 14%; severely infected, 7%; moderately or slightly infected, 71%; and none visibly infected, 8%.

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*Additional key words:* California clones of *Armillaria*, pathogenicity of *Armillaria*, spread of *Armillaria*.

*Armillaria mellea* (Vahl) Quel. may be found on native vegetation in California, particularly on plants growing in dry stream beds, washes, and flood plains. It frequently is a problem when such areas are planted to woody crops such as citrus, prunes, peaches, or grapes. The fungus grows on the main roots and trunk. When the trunk is girdled, death results. Often infections may be restricted to roots for considerable periods, resulting in debilitated growth of the host. We isolated four clones from infected plants growing in drainage basins in areas remote from one another and tested them for virulence on peach trees in the field for 3 years.

The objectives of the study were to determine whether clones of *A. mellea* collected from areas in California remote from one another differed in pathogenicity in the field, and to determine whether there were differences in the rate of development of disease symptoms and spread of the fungus in the field.

MATERIALS AND METHODS.—*Isolates of Armillaria.*—The history of the clones from California is as follows: D-2, from citrus, Covina (Little Dalton Wash) (half of a 10-acre grove was killed by the fungus); D-9, from peach, Wheatland (Sacramento River) (peaches in this area have suffered continuing heavy losses due to *Armillaria*); D-73, from citrus, Bryn Mawr near Redlands (San Timoteo Wash) (in this area, many acres of citrus are out of production because of *Armillaria* infestation); D-86, from native oak near Fallbrook (the fungus had spread from oak and other native plants into an adjacent peach orchard).

We consider the isolates valid samples of an indigenous population of the organism because natural mixing of the strains by flooding or inadvertent mixing by man is unlikely due to the distances involved and to the differences in the various cultural operations.

The isolates were grown on five culture media to partially survey their growth habits. The media used were: cornmeal agar (CM); potato-dextrose agar (PDA); citrus agar (200 g citrus sucker growth ground and dried; 15 g agar, 20 g glucose, and water to make 1 liter), fig agar, and peach agar. Fig and peach agar are similar to citrus agar except that fig or peach wood is substituted for citrus wood.

*Preparation of inoculum and method of inoculation.*—Inoculum consisted of *Armillaria*-infested fig stem or citrus root pieces ca. 2.5 cm × 150 cm. Four to six pieces in 2-liter jars containing ca. 200 ml water were sterilized by autoclaving for 60 min at 121 C. The wood was inoculated with a 20-ml mixture of the fungus and associated culture medium. The mixture was prepared by chopping a petri dish culture of *Armillaria* along with the associated citrus agar medium in a Waring Blendor for 0.5 min. Subsequent experience demonstrated equal success and less contamination when intact cultures were used. The inoculated pieces of wood were held at 20 C for 20 months, at which time the wood was still firm, but thoroughly infested with the fungus.

Each peach tree was inoculated by removal of the soil from the root crown area and the placing of a piece of wood inoculum against a large root 20-30 cm deep in the soil. The soil was packed tightly to insure continued contact of the inoculum block with the crown root. Some slight wounding may have occurred to the roots and stems in the process of inoculation, but deliberate wounds were not made.

*Field plot design.*—Elberta peach (*Prunus persica* [L.] Batsch) scions previously budded onto Lovell peach root stock were planted as bare root stocks 0.9 m apart in rows 7.2 m apart in experimental fields at Riverside, Calif. In all, 224 trees were planted in 8 rows. The peaches were grown in a nursery free of *Armillaria*, and no evidence of infection was seen at planting. Infection from endemic sources was unlikely since *Armillaria* has never been found on the experiment station. In November 1962, half the trees were inoculated with fig wood inoculum and half with citrus wood inoculum. Three adjacent trees in a row were inoculated with the same culture of

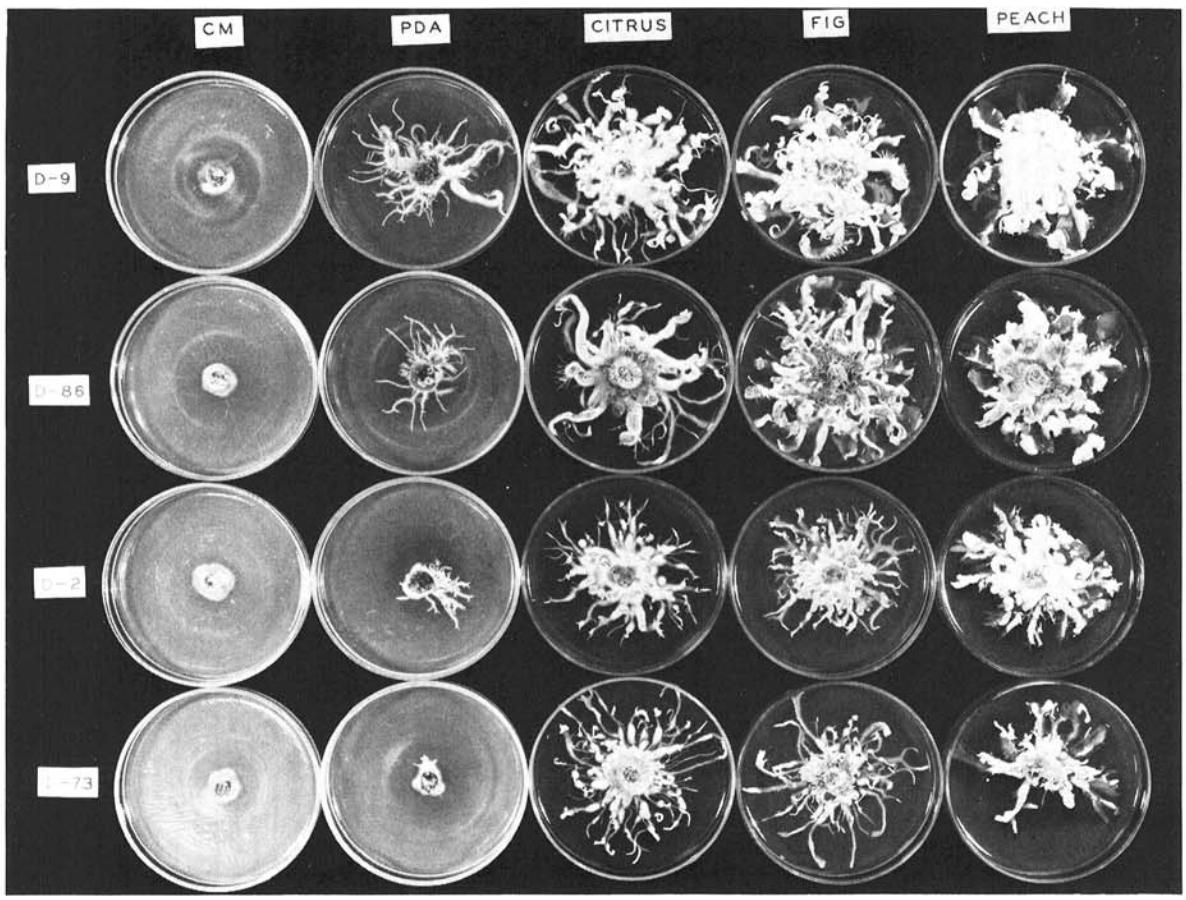


**Fig. 1.** Appearance of four clones of *Armillaria mellea* growing on five media, cornmeal agar (CM); potato-dextrose agar (PDA); citrus wood agar (citrus); fig wood agar (fig); and peach wood agar (peach). See text for origins of clones labeled in left-hand column of the figure.

*Armillaria* growing on one or the other of the substrates. Every fourth tree was not inoculated, and served as a control. In total, 168 trees were inoculated; 42 for each of four cultures of *Armillaria*.

Water was supplied by furrow irrigation, the plots were cultivated, and the trees were pruned each fall, according to commercial practice.

*Method of observing disease.*—The plots were observed weekly the 1st year after inoculation but less frequently thereafter as the incidence of new infections decreased. A tree was considered dead when the leaves wilted and dropped off. The observations were continued for 36 months, when 86% of the inoculated trees were killed by the fungus. The mean trunk circumference of surviving trees was 40 cm. The surviving plants were dug up, and the root crowns carefully examined for infection by *Armillaria*. The examinations of trees rated as dead confirmed that the trunks were girdled by *Armillaria*. Occasionally, even though the tops of some plants wilted, one or two surface roots escaped infection and maintained some top sucker growth through the rest of the season.

RESULTS.—In earlier experiments (*unpublished data*), we tested the pathogenicity of 22 clones of *Armillaria* isolated from various hosts in California, of 11 clones from 8 other states in the USA and of 1 from Ontario, Canada. Considerable variation in pathogenicity was observed. One culture from Maine and one from Wisconsin were not pathogenic to peach in the field, but they were pathogenic to *Pelargonium hortorum* in the glasshouse. With the California isolates, 10 were equally pathogenic on peach and geranium, and 9 were more pathogenic on peach than on geranium. None of the California isolates was more pathogenic on geranium than on peach. As a corollary to these experiments, the field test on peach herein described, involving four distinct California isolates, was made.

*Cultural variation of the four isolates.*—The differences in cultural appearance of the four isolates is apparent in Fig. 1. The differences were quantitative, varying in growth rate and in the formation of various morphological structures. Clone D-9 grew more vigorously than the others on all media. None of the clones grew well on CM agar, and only D-9 grew reasonably well on PDA. Each clone produced rhizomorphs on all media.

*Effect of pathogenicity of clones growing on fig wood compared to isolates growing on citrus wood.*—The type of wood used as substrate for the inoculum may have affected the rate of infection initially, but the differences were not obvious after the 1st year. The initial differences varied with the clone of *Armillaria*; in one case, fig wood was best, in another, citrus wood was best, and in two cases, there were no differences in pathogenicity related to the kind of wood used for inoculum. After the 1st year, the differences were even less distinct. Apparently, both woods provided ample sources of energy for *Armillaria* to invade and establish itself on the peach roots.

*Comparison of the effect of clones on the rate of development of disease.*—Since there were no significant differences in pathogenicity between trees inoculated with fig or citrus wood inoculum, the data were bulked and are presented in Fig. 2. The most obvious differences were in the rate of kill in 1961, the first year after inoculation. Plants were inoculated in November 1960, and by November 1961 over 60% of the peach trees were killed by clone D-9; ca. 25% were killed by D-2 and D-86; and only 5% were killed by D-73. After 21 months, 100% of the plants inoculated with D-9 were dead. However, the rate of kill of trees by the other clones increased with time, so that by the end of 3 years, the per cent killed was 85.7, 71.4, and 59.5 for clones D-2, D-86, and D-73, respectively. During the 3 years, 86% of the 186 trees inoculated were killed. The number of dead trees was closely related to the seasons of the year. The percentage of those killed during the four seasons was approximately as follows: December, January, and February, 2%; March, April, and May, 23%; June, July, and August, 66%; and September, October, and November, 10%. Although there are insufficient data from these experiments to explain the results, various probabilities will be discussed below.

Culture D-9 was the most pathogenic of the isolates, and D-73 the least pathogenic. All isolates were very pathogenic, however, since by the end of the 3rd year, ca. 72% of the trees inoculated with isolate D-73 were killed.

At the termination of the experiment, all surviving trees were pulled and carefully examined for signs of infection by *Armillaria*. Only three of the inoculated trees had no discernible infection, showing that all the cultures were highly pathogenic to peach.

*Rate of spread to noninoculated adjacent trees.*—After 3 years, the spread of infection to noninoculated trees was pronounced. Of the 56 noninoculated trees, 7 died, and by the following year, others had symptoms of a predictable death. When the trees were dug and examined carefully, 14% were dead, 7% were severely infected, 71% were moderately to slightly infected, and 8% were not visibly infected.

The data were examined to determine whether the infestations were related to the flow of irrigation water, but no correlation was found. Infection to a number of healthy trees spread through interlocking roots from trees inoculated with the various clones as follows: from D-2, 7; from D-9, 6; from D-73, 4; and from D-86, 3. Thus, the spread was more rapid from the virulent clones, D-2 and D-9, than from the less virulent clones, D-73 and D-86.

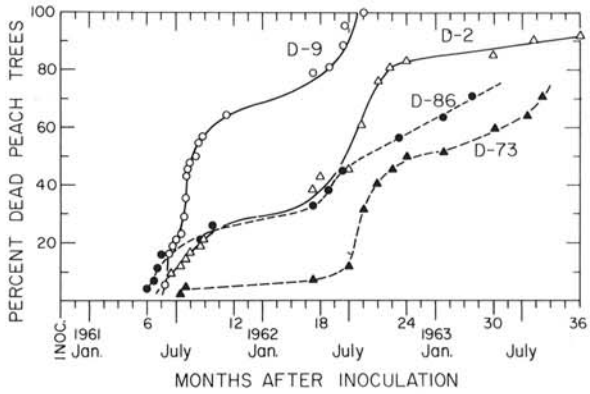


**Fig. 2.** Seasonal development of disease on peach inoculated with four clones of *Armillaria mellea*.

*Sporophore production.*—Only the culture obtained from the southern-most part of California, D-86, produced sporophores on infected trees. Sporophores were produced in each of the 3 winter months of the experiments. Although these observations have no direct relation to the work reported herein, they are reported because of their potential value in studies of the life cycle of the fungus.

DISCUSSION.—These experiments indicate that considerable differences may exist in the degree of pathogenicity of geographic isolates of *Armillaria mellea*. Raabe (2) has succinctly reviewed the pertinent literature concerning the pathogenicity of *Armillaria mellea* in discussing his work on variation in pathogenicity and virulence in *Armillaria*, so it need not be done here. He showed that there were differences in virulence and pathogenicity among isolates of *Armillaria* when Monterrey pine, dahlia, or peach growing in pots or containers were inoculated with the fungus. Our experiments extend those of Raabe by showing that differences in the degree of virulence of geographic isolates of *Armillaria* may readily be demonstrated in the field as well. Also, we have pointed out the sharp seasonal development of the disease as it exists in the field in southern California.

In one respect, our experience with *Armillaria* has differed from Raabe's. In 20 years, we have isolated only one clone from the field that did not produce rhizomorphs in culture, whereas Raabe (2) reported that 3 of 10 isolates of *Armillaria* did not produce rhizomorphs on PDA. Of the three, one was highly virulent, one moderately virulent, and one weakly virulent. In our experiences, field strains differ greatly from those derived from single spores. In the latter case, lack of well developed rhizomorphs is common, and such cultures are nonpathogenic.

The rapidity with which clone D-9 attacked and killed peach trees was surprising, although it was the

fastest growing clone in culture. The rate of kill was correlated with the season of the year, being highest in the summer and lowest in the winter. The least kill occurred during the cool winter months, which coincide with the dormant period of the host and the sporulation period of the fungus. Maximum disease development occurred in late summer and early fall. Probably infection was maximum in late spring and early summer when temperatures were lower, since Bliss (1) has shown that the optimum temperature for infection by *Armillaria* on Lovell peach is between 17 and 24 C continuous soil temperature. Also, top growth of peach was optimum at a higher soil temperature. In our experiments, infections probably were rapid in late spring and early summer when soil temperatures were moderately low. In late summer and early fall, air temperatures at Riverside usually exceed 35 C and often exceed 40 C. We have recorded soil temperatures at the 6-inch depth of 37 C during these periods. Under these conditions, the growth of *Armillaria* probably is checked, but the stress for water in a weakened infected plant is so great that it may die. Presumably, this could account for the large number of dead plants occurring at this time.

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