California Avocado Society 1969 Yearbook 53: 103-107

PRODUCTION OF SPORANGIA BY PHYTOPHTHORA CINNAMOMI IN PURE CULTURE

G. A. Zentmyer and Dah-wu Chen
Department of Plant Pathology, University of California, Riverside. Professor Chen has been a visiting Fulbright Scholar at Riverside for two years. His permanent address is: Department of Plant Pathology, Taiwan Provincial Chung-Hsing University, Taichung, Taiwan, Republic of China.

Considerable research in our laboratory at the University of California, Riverside, has concerned the avocado root rot fungus, *Phytoph-thora cinnamomi.* We have developed information on the various spore stages that the fungus produces and how these relate to disease development, on the many plants that the fungus attacks, on the effect of moisture in the soil on the development of the fungus, on the effect of many fungicides and fumigants, on resistance to the fungus, and other aspects (8, 9, 10, 12, 14, 15).

This paper reports a new development in relation to production of the principal spore stage that *P. cinnamomi* forms, the sporangial stage. This is the spore stage that produces the tremendous numbers of swimming spores (zoospores) that swim through water in the soil and are responsible for most of the root infection, so that any information relating to this stage is significant in relation to understanding and controlling avocado root rot.

We have reported in earlier publications (7, 8, 13) that the avocado root rot fungus does not produce sporangia in pure culture. In all previous studies we have had to use a non-sterile soil extract to develop this spore stage. This phenomenon was first reported by Mehrlich in Hawaii in 1935 (4), working with *P. cinnamomi* on pineapple. Other scientists in the United States and in New Zealand have also confirmed these findings (1, 3, 6). This situation has also been reported for other species of *Phytophthora,* causing many other types of plant disease.

In 1959 Zentmyer and Marshall (13) presented the theory that various microorganisms (bacteria and fungi) in the soil were responsible for this stimulation of production of sporangia in *P. cinnamomi,* as we found that the soil leachate lost its stimulatory property when it was autoclaved or filtered through filters that exclude bacteria.

Research in our laboratory, reported in 1965 (11), and in other laboratories indicated that bacteria in the soil were in some way involved in the stimulation of production of sporangia by the non-sterile soil extract. Several different types of bacteria were implicated in this process, including species in the genera *Chromobacterium* and *Pseudomonas.* The most logical supposition was that the bacteria were supplying some substance necessary for the fungus to produce sporangia, a substance which *P. cinnamomi* could not form by itself in culture.

Phytophthora cinnamomi was first reported from and described on cinnamon trees in

Sumatra by R. D. Rands in 1922 (5). At this time Dr. Rands reported that he obtained sporangia under sterile conditions by washing 8- to 10-day old cultures with sterile water several times. Many attempts have been made to repeat Rands' results, with no success over the past 35 years.

In 1967 one of the authors of this paper, Professor Dah-wu Chen, from Taiwan Provincial Chung-Hsing University in Taichung, Taiwan, came to our laboratory as a Fulbright Scholar. During this period he has been involved in research on *Phytophthora* and has developed the method which we are reporting here and which has been reported briefly in the journal Phytopathology (2).

In this study we grew the avocado root rot fungus on either pea broth or V8 juice broth. Pea broth was prepared by adding 500 ml of deionized water to 200 grams of frozen green peas and blending this mixture for 5 minutes, then centrifuging for 10 minutes, decanting the supernatant and adding deionized water to make 1 liter of broth. V8 broth was made by mixing 200 ml of V8 juice with 2 grams of calcium carbonate, centrifuging this mixture and diluting the supernatant with deionized water to one liter. The media was autoclaved. To produce sporangia pea broth was diluted to 1/8 and V8 broth to 1/10 with sterile deionized water.

First indications of production of sporangia in sterile culture occurred in some trials in the summer of 1968 when mycelial mats from young (one to two-day old) cultures were washed in mineral salt solutions. Within 24 hours some sporangia were observed, primarily on the mycelium at the margin of the colony.

Many different variations of this method were tested, and the following method was found to result in consistently large numbers of sporangia. Many young colonies of *P. Cinnamomi* were formed on a V8 agar plate following placing 10 or more inoculum pieces on the plate; thus the fungus developed over the entire plate in 2 to 3 days. The resulting mat of mycelium was chopped with sterile forceps into very small pieces and over 200 of these pieces were placed in a petri dish containing pea broth or V8 broth. These inoculum pieces developed rapidly into small colonies which nearly merged in 18 to 24 hours to form a large mycelial mat. With this method the entire mat contained uniform, vigorous young hyphae of the fungus; this condition of abundant young hyphae was found to be essential for production of sporangia by *P. cinnamomi.*

The young mycelial mat was then carefully washed with a sterile mineral salt solution, containing calcium nitrate, potassium nitrate, magnesium sulphate, and iron (in the form of a chelated iron solution, FeEDTA). Four successive washings at one-hour intervals with 15 to 20 ml of the salt solution were usually enough to induce formation of sporangia. Shorter intervals of washing were also successful. After washing the petri dishes were placed under fluorescent lights at 24°C; sporangia usually began to form within 8 hours after the beginning of washing, and the numbers reached a maximum in 24 to 36 hours.

Most of these tests were conducted with our standard isolate of the avocado root rot fungus, SB-216-1. The original culture was isolated from avocado roots in Santa Barbara County; this is a single zoospore culture from the original. In addition to this isolate of the fungus we tested over 20 other isolates of *P. cinnamomi* from a number of different plants and from different countries. AH isolates produced abundant sporangia

in pure culture following washing with the mineral salt solution as described above.

The sporangia were tested to determine if they would release zoo-spores normally, by washing the cultures with sterile deionized water and placing them in an incubator at approximately 5°C for 15 to 20 minutes, then returning the cultures to room temperature of 24°C. Normal zoospores were released within one hour after the chilling treatment. The zoospores infected sterile roots of *Persea indica,* the susceptible plant related to the avocado which can be grown in test tubes in the laboratory, thus confirming that they were normal, mature zoospores.

We also tested the mycelium of the fungus and the wash solutions noted above to determine if they were sterile at the time of formation of sporangia, by placing mycelium in tubes of several media on which bacteria and other microorganisms grow readily: Difco nutrient broth, Eugon broth, and trypticase soy broth. No bacteria or other contaminants were found in any of the broth cultures, confirming that the sporangia were produced on mycelium of *P. cinnamomi* free from other microorganisms.

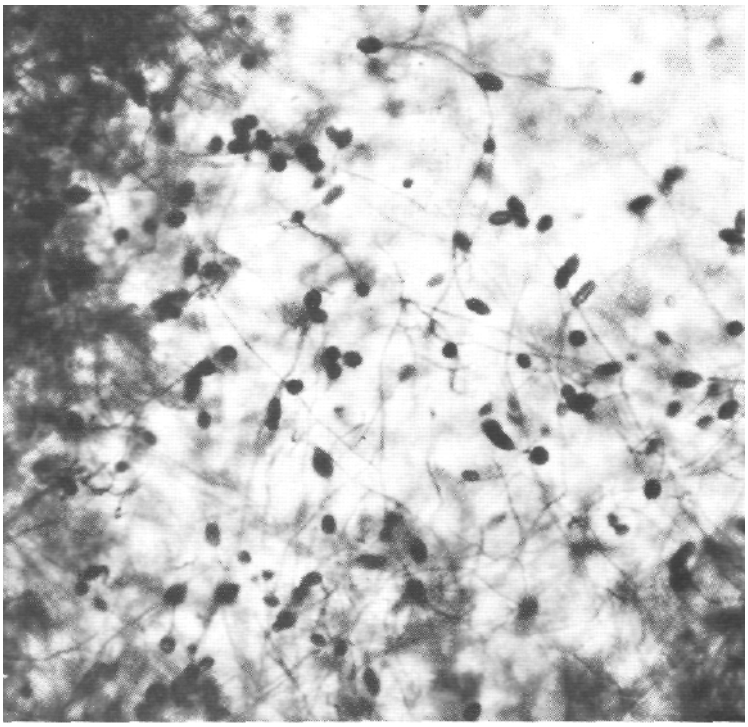

Figure 1. Sporangia produced by PHYTOPHTHORA CINNAMOMI in pure culture.

The tests reported here have shown that it is possible to produce large numbers of sporangia of the avocado root rot fungus under sterile conditions by thorough washing of 16 to 36-hour old cultures with a mineral salt solution. Further studies of the salt solution indicate that calcium and magnesium are the most important cations involved in the differentiation of the sporangia. The anions involved with these cations do not appear to be important. Also, a very small quantity of chelated iron greatly increased the number of sporangia produced.

Thus the two most important factors involved in the process of sporulation by *P. cinnamomi* are: a sudden reduction of the food supply which forces the fungus into the sporulation stage instead of the vegetative stage, and the cation effect exerted by the salt solution used in the process of washing and incubation during sporangial formation.

We do not yet know the relation between the stimulation of sporangium production by bacteria in the non-sterile soil extract and the stimulation of sporangium production by the nutrient depletion process described here. Perhaps the bacteria are merely contributing also to depletion of nutrients in the solution in which *P. cinnamomi* is growing, and thus not providing some substance required for production of sporangia but instead removing nutrients and thus initiating the cycle of spore formation.

This discovery is of great value in research on the avocado root rot fungus, as it will greatly facilitate studies of the fungus in the laboratory, and also gives us some significant leads as to factors involved in production of this important spore stage.

LITERATURE CITED

1. Chee, K. H., and F. J. Newhook. I960. Relationship of microorganisms to sporulation of **Phytophthora cinnamomi.** New Zealand Jour. Agric. Res. 9:32-43.
2. Chen, D. W., and G. A. Zentmyer. 1969. Axenic production of sporangia by **Phytophthora cinnamomi.** Phytopathology 59:1021.
3. Marx, D. H., and F. A. Haasis. 1905. Induction of aseptic sporangial formation in **Phytophthora cinnamomi** by metabolic diffusates of soil microorganisms. Nature 206:673-074.
4. Mehrlich, F. P. 1935. Non-sterile soil leachate stimulating to zoosporangia production by **Phytophthora** sp. Phytopathology 25:432-434.
5. Rands, R. D. 1922. Streepkanker van Kaneel, veroorzaakt door **Phy tophthora cinnamomi** n. sp. Meded. Inst. voor Plantenziekt No. 54, pp. 1-54.
6. Royle, D. J., and C. J. Hickman, 1963. **Phytophthora cinnamomi** on highbush blueberry. Plant Disease Reporter 47:266-268.
7. Zentmyer, G. A. 1955. Diseases of avocado. Calif. Avocado Soc. Yearbook 39:44-58.
8. Zentmyer, G. A. 1959. Spore stages of the avocado root rot fungus, **Phytophthora cinnamomi.** Calif. Avocado Soc. Yearbook 43:110-112.
9. Zentmyer, G. A. 1901. Chemotaxis of zoospores for root exudates. Science 133:1595-1596.
10. Zentmyer, G. A. 1961. Resistance to Phytophthora root rot of avocado. Proc. Caribbean Region for Hort, Sci. 5:85-89.
11. Zentmyer, G. A. 1965. Bacterial stimulation of sporangial production in **Phytophthora cinnamomi.** Science 150: 1178-1179.
12. Zentmyer, G. A., S, Hashimoto, J. D. Gilpatrick, and R. M. Burns, 1964. Progress of chemical control of Phytophthora root rot. Calif. Avocado Soc. Yearbook 48:86-89.

13. Zentmyer G. A., and L. A. Marshall. 1959. Factors affecting sporangial production by **Phytophthora cinnamomi** (Abst.) Phytopathology 49:556.

14. Zentmyer, G. A., A. O. Paulus, and R, M. Burns. *1967.* Avocado root rot, Calif. Agric. Exp. Sta. Circular 511: 16 pp.

15. Zentmyer, G. A., and W. A. Thorn. 1967. Hosts of **Phytophthora cinnamomi.** California Avocado Society Yearbook, pp. 177-186.