California Avocado Society 1959 Yearbook 43: 110-112

SPORE STAGES OF THE AVOCADO ROOT ROT FUNGUS, PHYTOPHTHORA CINNAMOMI

George A. Zentmyer
Plant Pathologist, Citrus Experiment Station, University of California, Riverside.

Phytophthora cinnamomi, the fungus that causes avocado root rot, has been known for some time to produce three different types of spores: zoospores, oospores, and chlamydospores. Zoospores are small swimming spores, produced in sporangia, which are believed to be the primary agent in rapid intensification of the disease. Oospores are resistant spores whose function is not well understood in this disease, though they probably enable the fungus to live through unfavorable periods. Chlamydospores are larger, thicker-walled spores than the zoospores; they germinate readily and may serve primarily to increase the fungus population in the soil.

Recently a method has been developed in our laboratory for producing large numbers of sporangia consistently. This makes it possible to study more accurately the effect of various factors on production of this important spore-bearing structure.

The method of obtaining sporangial production consists of preparing a leachate from a non-sterile soil, using 10 grams of soil per 100 ml. of water, and filtering this extract through filter paper. When disks cut from a laboratory culture of the fungus growing on potato dextrose agar or V-8 agar are placed in this extract in a petri dish, sporangia are formed in abundance in two to three days. Normally **Phytophthora cinnamomi** does not produce this spore stage in laboratory culture.

A highly significant point has been discovered about this soil extract which stimulates production of sporangia. If this extract is sterilized, either by steam or by filtering through bacteriological filters, the extract loses its property of stimulating the sporangia to form. Further research is being conducted with this extract, to try to determine just what is involved in this phenomenon, for, if we can find what it is in various soils that encourage the fungus to produce these spores this will provide a better basis for knowing how to prevent their formation.

Using this method, a number of things have been discovered about production of these sporangia. The effect of temperature on formation of these structures was studied, and it was found that the optimum temperature is 24° C. (75.2° F.), with no sporangia produced at 33° C. (91.4° F.), and none at 9° C. (48.2° F.). The following table shows the relative production of this spore stage at various temperatures.

PRODUCTION OF SPORANGIA BY PHYTOPHTHORA CINNAMOMI AT VARIOUS TEMPERATURES IN SOIL EXTRACT

Temperature (degrees Fahrenheit)	Average Number of Sporangia Produced per Square Millimeter
48° F.	0
54° F.	0.01
59° F.	1.7
64° F.	3.2
70° F.	10.1
75° F.	17.5
81° F.	15.1
86° F.	9.9
91° F.	0

It was found that when the soil extract is diluted up to one million times it still is capable of stimulating the production of sporangia. Data on this phase are presented in the following table.

Dilution of Soil Extract	Average Number of Sporangia per Square Millimeter
0	24.1
1 to 10	15.4
1 to 100	8.6
1 to 1,000	1.1
1 to 10,000	4.4
1 to 100,000	2.7
1 to 1,000,000	4.0

Cultures incubated at 24° C.

The effect of light on production of sporangia by **Phytophthora cinnamomi** was also studied. In the presence of the soil extract, cultures of the fungus were exposed to continuous artificial light, to continuous darkness, and to alternating periods of light and dark. Abundant sporangia were produced under all three conditions, but production was significantly greater in continuous darkness than in continuous light.

The normal pH of the soil extract used in these experiments was approximately 7.2. Reducing the pH to an acid condition (pH 4.5) did not affect production of this spore stage.

Copper has been known for a long time to be toxic to the zoospores and the mycelium of various species of Phytophthora. Little evidence has been presented on toxicity of copper or other fungicides in relation to formation of sporangia. Using the soil extract method it was determined that as little as 1 part per million of copper ion completely prevented the formation of sporangia. Reducing the copper concentration to 0.2 parts per million resulted in formation of some sporangia but production was not normal even with this low amount of copper. Tests with copper fungicides to control avocado root rot in the greenhouse and field have not given very encouraging results unless the copper can be mixed with the infested soil. The present forms of copper are fixed quite readily in the upper layers of soil when the material is applied to the soil surface as a drench. Other chemicals are under investigation in this regard.

The oospore stage of this fungus is another form that is difficult to produce in the laboratory; it has been rarely produced except occasionally in old cultures, according to all past reports on **P. cinnamomi**. In the laboratory at Riverside we found that this stage is readily produced if an aqueous extract made of small avocado feeder roots is inoculated with the fungus. Further work is continuing on this phase in order to determine what this stimulatory substance is, and to find out what role these thick-walled oospores play in avocado root rot. These spores are difficult to work with; to date they have not been germinated. Research on the nature of the substance stimulating the production of this oospore stage will facilitate producing these spores for research purposes, as well as providing clues on how to prevent formation of the spores, and furnishing information of basic scientific interest.

The third spore stage, the chlamydospore stage, is readily produced by the fungus particularly if it is growing on an aqueous medium. Certain agar media also are more favorable for production of these spores than others; agar made from V-8 juice is a particularly good medium for chlamydospore production. These spores germinate readily in water, sending out many germ tubes and increasing the fungus population rapidly.

In cooperation with Dr. L. J. Klotz of the Department of Plant Pathology at Riverside, the effect of several chemicals on germination of the zoospores of **P. cinnamomi** was studied. Copper and chlorine were found to be very toxic to these swimming spores; germination was inhibited by 0.5 parts per million of copper and by 0.125 parts per million of chlorine. Ammonia was not so toxic to the spores, preventing their germination only when 25 ppm of NH_3 was used. Zoospores were produced from sporangia in the soil extract medium noted above by lowering the temperature of the extract slightly. When spores were actively swimming drops of the spore suspension were pipetted into depression slides and the toxicant added. Results of these tests with copper and chlorine are presented in the following table.

TOXICITY OF COPPER AND CHLORINE TO ZOOSPORES OF PHYTOPHTHORA CINNAMOMI

Treatment	Dosage ppm	Per Cent Germination
Chlorine	0.5	0
	0.25	0
	0.125	3.5
	0.0625	49
	0.03125	87
	0.0156	80
	0.0078	88
Copper	3.125	0
	1.56	0
	0.78	13
	0.39	55
	0.195	89
	0.0975	86
None	-------	89
None	-------	93

This great sensitivity of **P. cinnamomi** to copper and to chlorine indicates that these materials should be of use in preventing possible spread of the fungus in irrigation waters by treating reservoirs and other water sources.

The technical assistance of Miss Lee Ann Marshall in much of this work is gratefully acknowledged.