

STUDIES OF PHYTOPHTHORA CINNAMOMI

G. A. Zentmyer, L. J. Klure, F. B. Guillemet, E. O'Neal, O. K. Ribeiro, S. D. Campbell, and G. King

Research at the University of California, Riverside, augmented considerably by funds from the avocado growers, through the California Avocado Advisory Board, is continuing to provide further significant information on the life cycle and activities of the avocado root rot fungus, *Phytophthora cinnamomi*.

During 1977, an extensive laboratory and greenhouse experiment on artificial infestation of soil provided data on the most satisfactory methods for uniformly adding *P. cinnamomi* to soil for experiments, and on the numbers of fungal propagules (spores or other units) needed per gram of soil to initiate development of root rot. These methods can be extremely useful in future studies to more accurately assess the effect of different fungicidal treatments, the behavior of the fungus under various environmental stresses, the performance of resistant rootstocks, and the pathogenicity of different strains of the fungus.

Several different types of fungus inoculum were tested in the greenhouse, including hyphal fragments (blended mycelium without spores), sporangia, chlamydospores, and oospores. In addition, fragments of infested avocado roots and small infested seeds (millet) were used to simulate the behavior of the fungus when it has a "food base," or a piece of decaying organic matter from which it can spread.

Disease symptoms on *Persea indica* seedlings appeared earliest in treatments where the fungus had a food base. When infested roots and millet seeds were added to the soil, top symptoms (wilt, canker) appeared as early as five days after inoculation, and within two weeks almost all of the seedlings were showing moderate to severe symptoms. Seven weeks after inoculation the *P. indica* root systems were completely rotted, even at the lowest inoculum density studied, 0.5 inoculum pieces (root fragments or seeds) per gram of soil. These observations emphasize the importance of fumigation following the removal of diseased trees, indicating that comparatively few infested root pieces are needed to re-initiate the disease in a replant tree.

Infection also occurred when soil was infested with spores alone, without an added food base. The disease can be initiated by as few as one spore (propagule) per gram of soil, but symptoms are more severe and appear earlier when more propagules of the fungus are present. With chlamydospores, approximately 50% of the roots were rotted at 5 propagules per gram (ppg) of soil, and 98% root rot occurred at 20 ppg. With sporangia, approximately 50% root rot occurred at 10-20 ppg.

In this experiment also, artificial infestation with oospores (sexual stage) resulted in typical symptoms of root rot and cankers on *Persea indica* seedlings. Approximately

50% root rot occurred during seven weeks in soil containing 10-20 oospores per gram. This suggests that although it is difficult to germinate oospores in the laboratory, these spores do germinate in the soil and infest plants, and thus could play an important role in the disease cycle. Oospores have very thick walls, and thus would be expected to be more resistant to various adverse conditions (drying, fungicides, fumigation, etc.) than other spores such as chlamydospores or sporangia.

Methods for determining how many propagules of *P. cinnamomi* are present in soil are also being developed and tested. To date, these have included preparing soil dilution plates in the laboratory from field soil samples, using special media containing antibiotics that inhibit other soil microorganisms and permit *Phytophthora* to grow; sprinkling uniform small amounts of soil on the surface of these antibiotic agars; and placing *Persea indica* seedlings in soil traps made by successively diluting the test soil with sterile soil to reach a "dilution end point" which provides a measure of how much root rot fungus is in the soil.

When these methods of studying the population level of *P. cinnamomi* in the soil are further refined, they will be extremely useful in combination with the data from the artificial infestation experiments, in predicting the disease potential in various soils, and evaluating the effect of some of our treatments, such as the new soil fungicides, amendments, etc., in reducing the fungus population to a level that will not cause disease. These techniques are particularly important for correlating the results from different field plots.

Recent laboratory experiments have provided significant data on the effect of temperature on the formation and development of oospores. *Phytophthora cinnamomi* can form these thick-walled sexual spores between 12-30°C (53-86°F). The optimum temperatures for their production are between 15 and 24°C (59-75°F), lower than the best temperatures for growth of the fungus and for formation of sporangia and chlamydospores. This means that in California avocado areas the fungus can produce oospores year round in the well insulated soil environment (below the top few centimeters). The root systems of mature trees can extend below one meter (approx. 3 feet) deep; below 15 cm (6 inches) little annual fluctuation in soil temperature occurs in California avocado growing area. This is in contrast to some areas in the eastern United States and Europe, where the life cycle of the fungus can be interrupted by winter freezes.

With the increased interest in kiwi as an alternate crop in some of the avocado growing regions of California, a greenhouse experiment was conducted to determine the resistance of kiwi to *P. cinnamomi*.

Ten kiwi plants were grown in soil infested with the root rot fungus along with an equal number of susceptible avocado seedlings. After a period of nine months, the avocado seedlings were all dead while the kiwis made excellent growth and *P. cinnamomi* could not be recovered from the roots. In addition to this experiment, we grew two other groups of ten plants each in soil artificially infested with the A1 and A2 types of the fungus. After a nine-month period, the same results were observed as with the plants in naturally infested soil; there was no infection of the kiwi roots.

In a separate experiment, stems of 30 kiwi plants were inoculated with two A2 types and

an A1 type of *P. cinnamomi*. The plants were observed for three months, and there were no cankers or growth of the fungus.

Another plant in which there has been increased interest in southern California, is the jojoba plant. In an experiment conducted in the greenhouse, jojoba seedlings, provided by Dr. D. M. Yermanos, were grown in soil both naturally and artificially infested with root rot fungus. At the end of a six month period, the seedlings had made excellent growth and *P. cinnamomi* could not be recovered from their roots.