

Peter Oudemans and Michael D. Coffey—\*

***Phytophthora citricola* - Advances in our Understanding of the Disease**

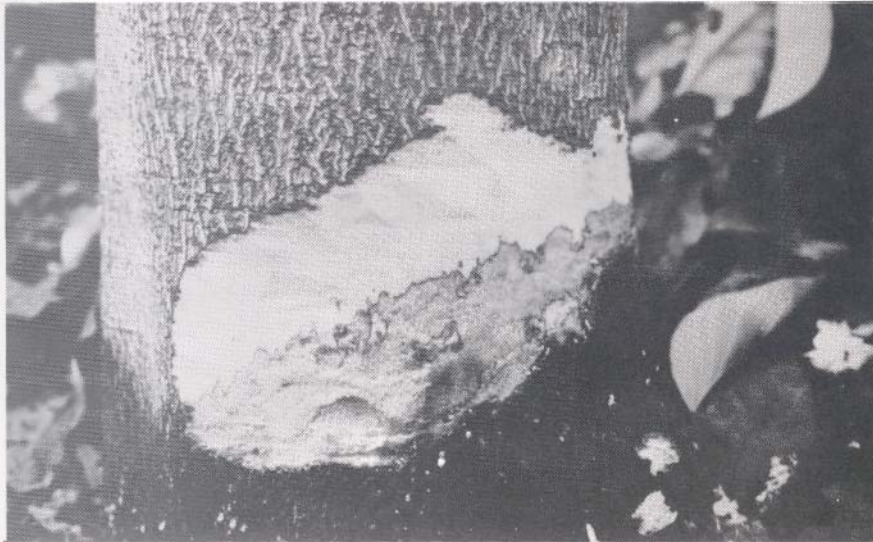
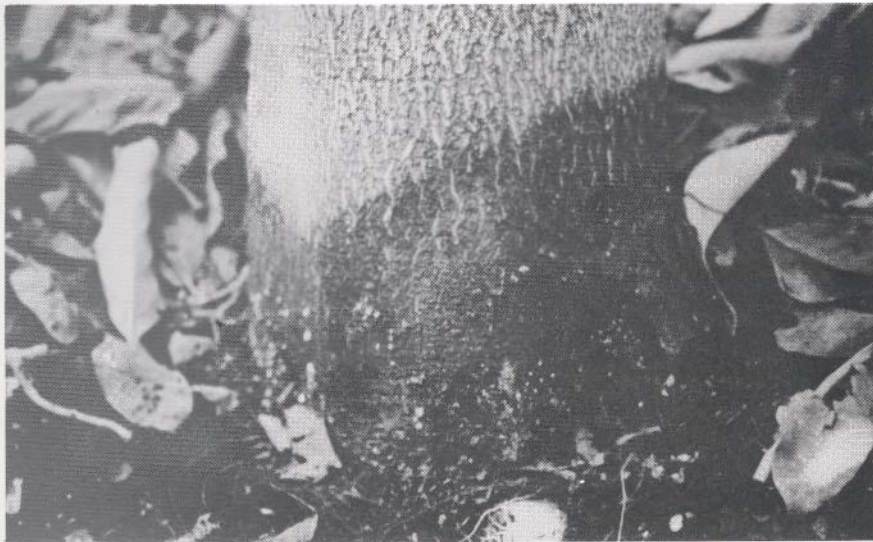
*Phytophthora* has plagued avocado growers since the early days of the industry. Until recently, *P. cinnamomi* was the most serious disease threat facing growers. However, as the industry expands, new disease problems will arise. This article deals with another *Phytophthora* species, *P. citricola*, which is emerging as a major threat to the avocado industry.

Fawcett (1918) and Home (1934) independently reported isolating *Phytophthora* species from trunk cankers of avocado trees. The first definitive identification of *P. citricola* on avocado was accomplished by Zentmyer, et al. (1973,1974). At that time, experimental evidence based on limited tests of pathogenicity and known distribution of infested groves led researchers to believe that *P. citricola* posed little immediate threat to the avocado (Zentmyer, et al. 1973, 1974). More recently, there has been a substantial expansion in the distribution of *P. citricola* among the avocado growing areas in California (Coffey and Cohen, 1984). We now believe that *P. citricola* presents an increasing threat to avocado production in California.

**DIAGNOSIS**

Trunk cankers caused by *P. citricola* can be tentatively diagnosed on the basis of host symptoms in the field; however, isolation and a positive identification should be made before remedial action is taken. Initial infections are invisible, since they occur below the soil surface. Once trunk cankers become visible on the tree, the disease is usually at an advanced stage. These cankers appear as blackened areas on the bark at or near the soil line. Once the bark is cut away, the canker can be seen as a deep seated lesion characterized by the darkened appearance of the wood (see Figure 1). To identify *P. citricola*, it must first be isolated in pure culture. The isolation procedure is facilitated by the use of a selective agar medium such as PARPH-cornmeal. This medium contains several antibiotics which prevent growth of bacteria and fungi other than *Phytophthora*. *P. citricola* can be isolated from three sources: bark pieces, feeder roots, and in some cases from soil. The bark samples (2 in.) are taken from the edge of an active canker and surface sterilized in a 70% alcohol solution for approximately two minutes. They are then rinsed in sterile distilled water and cut into small (0.1 in.) sections and plated directly on to the

\* Department of Plant Pathology, University of California, Riverside, California 92521.



*Figure 1: A canker at the base of an avocado trunk caused by P. citricola; a) appearance on bark surface, b) darkened appearance of wood below the bark surface.*

PARPH-cornmeal agar medium. Feeder root samples are collected in a circular pattern around the base of the tree. Generally, four samples are collected at a distance of four feet from the base of the tree and four more are collected at up to eight feet from the base. These are combined to make up one sample. Feeder roots are removed from the well mixed sample, washed, and surface sterilized for five minutes in a 5% solution of common household bleach. Feeder roots are then rinsed well in sterile distilled water and plated

on PARPH. Plates are incubated at 25°C. With both bark and feeder root samples, growth of *Phytophthora* isolates can be detected in 2 - 3 days. The isolates are then plated on V8C (V8 juice, calcium carbonate and water) agar plates in prelude to identification. An example of the rate of recovery from these two types of tissue sample is given in Table 1. Isolation from soil has been much less successful. Use of baiting techniques, such as the *Persea indica* seedlings trap, generally yield inconsistent results. Isolation from feeder roots is especially important in determining the presence of the pathogen before visible symptoms are expressed.

TABLE 1: A comparison of the number of isolates of *Phytophthora citricola* obtained when isolations were made from either feeder roots or bark samples in infested avocado groves.

Site	# of Trees	Feeder Roots	Bark	No Recovery
1	4	2	2	1
2	3	0	3	0
3	8	6	4	2
4	1	NT	1	0
5	8	4	2	4
6	8	4	6	0

## DISEASE CYCLE

A schematic representation of a possible disease cycle is presented in Figure 2. It is assumed that the roots are attacked initially, with no above ground symptoms being expressed. Following infection and colonization of the feeder roots, sporangia are produced. Swimming zoospores are released from the sporangia when sufficient free water is present. The zoospores may then spread to infect other neighboring healthy feeder roots and thereby increase the population of *P. citricola* in the root zone. Spread of the pathogen between trees may also be via the zoospore stage.

Another spore structure produced by *P. citricola* is the oospore. These thick walled spores are produced as a result of sexual fusion. *P. citricola* produces abundant oospores. This is unlike the case with *P. cinnamomi*, where two different strains, an A1 and an A2 type, must be present for abundant oospore formation to take place. The A1 strain of *P. cinnamomi* is rarely found in avocado root rot situations, thus reducing the importance of this structure in that disease cycle. In contrast, with *P. citricola* as has been shown with other homothallic species of *Phytophthora*, it is believed the oospore is capable of infecting the tree host and may play an important

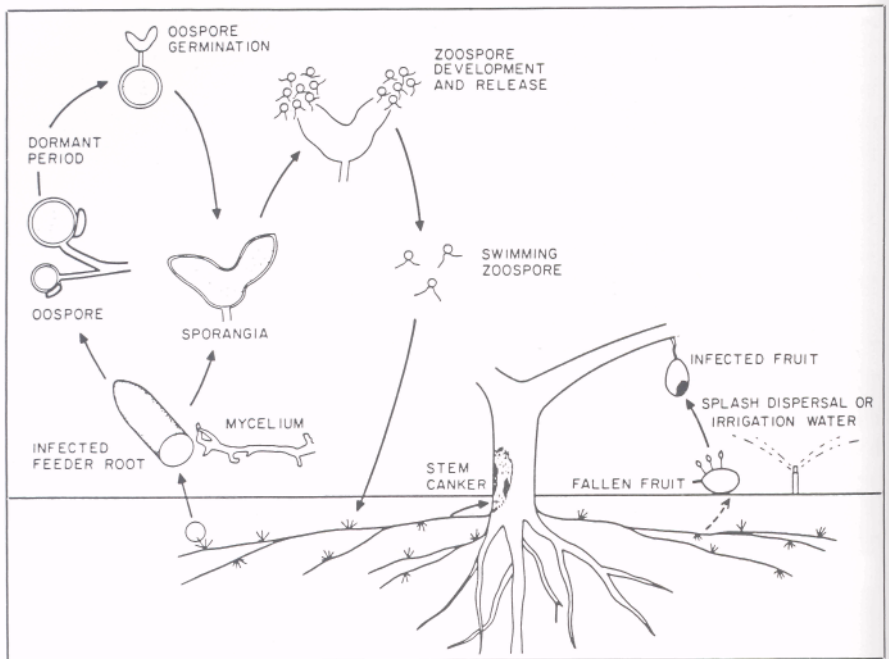


Figure 2: A proposed disease cycle for *Phytophthora citricola* attacking avocado.

role in the disease cycle (Mitchell and Mitchell, 1983). Perhaps the most significant feature of the *Phytophthora* oospore is its ability to persist for long periods in the soil apart from any host (Weste, 1983).

We are unsure of how *P. citricola* first enters an avocado grove. Presumably, it may either be present already and over time has become disseminated or, alternatively, it may enter as an infection on trees brought directly from the nursery. Recently, we have made positive isolations of *P. citricola* from some nursery stock suggesting this as a likely mechanism. At this time, we are unable to explain why the most severe disease situations are seen in groves upwards of 10 years old. Clearly, more work is required to develop a better understanding of the factors which influence the introduction, development, and spread of this increasingly important disease.

Apart from the trunk canker phase, a fruit rotting phase has also been observed recently in California in one grove (Koike, et. al. 1987). Infected fruit were observed on the lower branches of the avocado tree. The inoculum was probably derived from the ground and splashed up on to the fruit.

### **ISOENZYMES AND PHYTOPHTHORA IDENTIFICATION**

Due to their inherent morphological simplicity, many *Phytophthora*

species are difficult to identify. Our objective has been to develop a new technology for accurate and rapid identification of *Phytophthora* species. We have chosen to use horizontal gel electrophoresis of isoenzymes to achieve this objective. Initial results with six species have demonstrated that this is a rapid and reliable method for *Phytophthora* diagnosis (Oudemans and Coffey, 1987). It was an initial concern that the scenario in avocado groves could parallel the situation in northern California with cherry and walnut trees (Mircetich and Matheron, 1976 and Mircetich and Matheron, 1983). In this case, root and crown rot of cherry was shown to be a disease complex involving three species of *Phytophthora*. Six known species, in addition to four unknown ones, were implicated in root and crown rot of walnut. To determine if this story might be repeated in avocado, we sampled groves for the presence of *Phytophthora* and subsequently identified the isolates using isoenzyme analysis. Our results have established that only two *Phytophthora* species, *P. citricola* and *P. cinnamomi*, are present in root or crown rot situations at the present time.

Further studies have compared isolates of *P. citricola* from a wide range of hosts and geographic origins using isoenzyme analysis. The results, illustrated in Figure 3, indicate that isolates from avocado are unique and are easily differentiated from isolates derived from other hosts. In addition, the "avocado type" has never been isolated from any other host. Preliminary results suggest that while isolates of the avocado type are pathogenic on avocado fruit, isolates derived from other hosts, such as citrus, are not (Ouimette, unpublished). These data suggest that, although *P. citricola* is present in California on other hosts, it is unlikely that these "types" spread on to avocado. In further host range experiments, we will attempt to determine if this trend will hold up for the rest of the isoenzyme groups. Most important are the isolates seen in group 2 (Figure 3) which

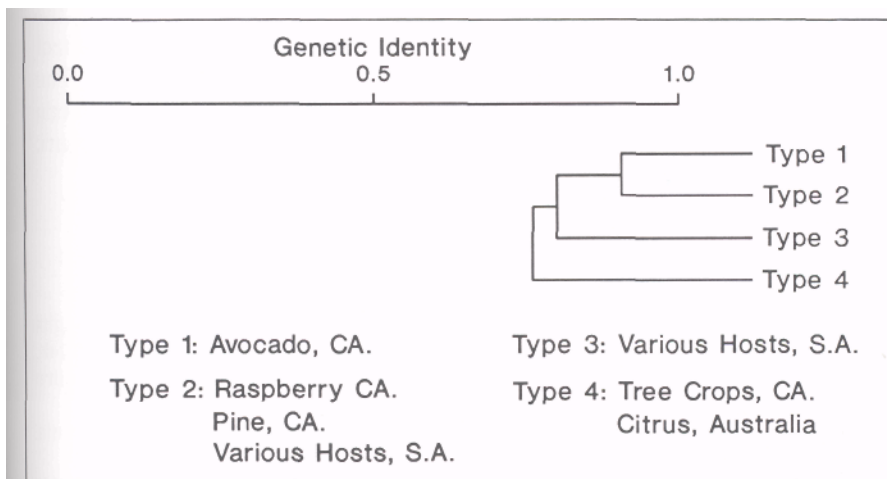


Figure 3: Isoenzyme groups within *Phytophthora citricola*.

appear to be the most widespread in California and are also present on the largest diversity of hosts.

## CHEMICAL CONTROL

In the past, chemical control trials in infested avocado groves were largely unsuccessful. At this point, we believe that, for effective control of *P. citricola*, timing of the first chemical application will be very critical. Unlike root rot, in our experiences the decline due to *P. citricola* cannot be easily checked once advanced stages are reached. To gain effective chemical control, applications should be made before the trees begin to decline.

We are currently testing the fungicide Aliette® to evaluate its potential as a trunk injection for controlling the disease. In laboratory studies, *P. citricola* proved to be very sensitive to the active ingredient of Aliette®: phosphorous acid (Coffey and Bower, 1984). Aliette® is presently being tested in two separate field trials. At this point, both trials are at the end of the first year of treatment, and it is too early to predict the outcome.

## DISCUSSION

*P. citricola* now appears to present a serious threat to future avocado production and should not be allowed to reach epidemic proportions as has occurred with *P. cinnamomi*. Some important questions remain to be answered. How is the pathogen disseminated? Also, what factors affect expression of the disease in the avocado grove, and over what time period does the disease process operate? We are continuing to develop methods for early detection and identification of the pathogen, coupled with attempting to develop suitable chemical treatment strategies which can help in control of this disease in the field. Further research should concentrate on both the development of resistant rootstocks and studies of biological control methods which may also help to reduce the impact of the disease. The ultimate aim is deployment of sound integrated control methods which can provide both practical and economic solutions to the *P. citricola* problem.

## REFERENCES

- Coffey, M.D., and L.A. Bower. 1984. In vitro variability among isolates of eight *Phytophthora* species in response to phosphorous acid. *Phytopathology* 74: 738-742.
- Coffey, M.D., and Y. Cohen 1984. Crown and collar rot of avocado: a need for more research. *California Avocado Society Yearbook* 69-74.
- Fawcett, H.S. 1916. A bark disease of avocado trees. *Annual Report of the California Avocado Society* 152-154.
- Home, W.T., L.J. Klotz, and M.B. Rounds. 1941. Avocado trunk cankers. *Annual Report of the California Avocado Society* 46-47.

Koike, S.T., D. Ouimette, and M.D. Coffey. 1987. First report of avocado fruit rot caused by *Phytophthora citricola* in California. *Plant Disease* 71: 1045. Mitchell, D.J., and M.E. Kannwischer-Mitchell. 1983. Relationship of inoculum density of *Phytophthora* species to disease incidence in various hosts. In: *Phytophthora, Its biology, taxonomy, ecology and pathology*. Editors D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao. Mircetich, S.M., and M.E. Matheron. 1976. *Phytophthora* root and crown rot of cherry trees. *Phytopathology* 66: 549-558. Mircetich, S.M., and M.E. Matheron. 1983. *Phytophthora* root and crown rot of walnut trees. *Phytopathology* 73: 1481-1488. Oudemans, P.V., and M.D. Coffey. 1987. Use of isoenzymes in the taxonomy of *Phytophthora* species. *Canadian Journal of Plant Pathology* 9 (3): 884. Weste, G. 1983. Population dynamics and survival of *Phytophthora*. In: *Phytophthora, Its biology, taxonomy, ecology and pathology*. Editors D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao. Zentmyer, G.A., L. Jefferson, and C.J. Hickman. 1973. Another species of *Phytophthora* on avocado in California. *California Avocado Society Yearbook* 125-129. Zentmyer, G.A., L. Jefferson, C.J. Hickman, and Y.C. Ko. 1974. Studies of *Phytophthora citricola* isolated from *Persea americana*. *Mycologia* 66(5): 830-845.