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Phytophthora citricola — Advances in our Understanding of the Disease

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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 Phvtophthora 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. 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^2) sections and plated directly on to the 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.

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 Al and an A2 type, must be present for abundant oospore formation to take place. The Al 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 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 Phvtophthora 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 appears 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 to 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 hopefully 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.

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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

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

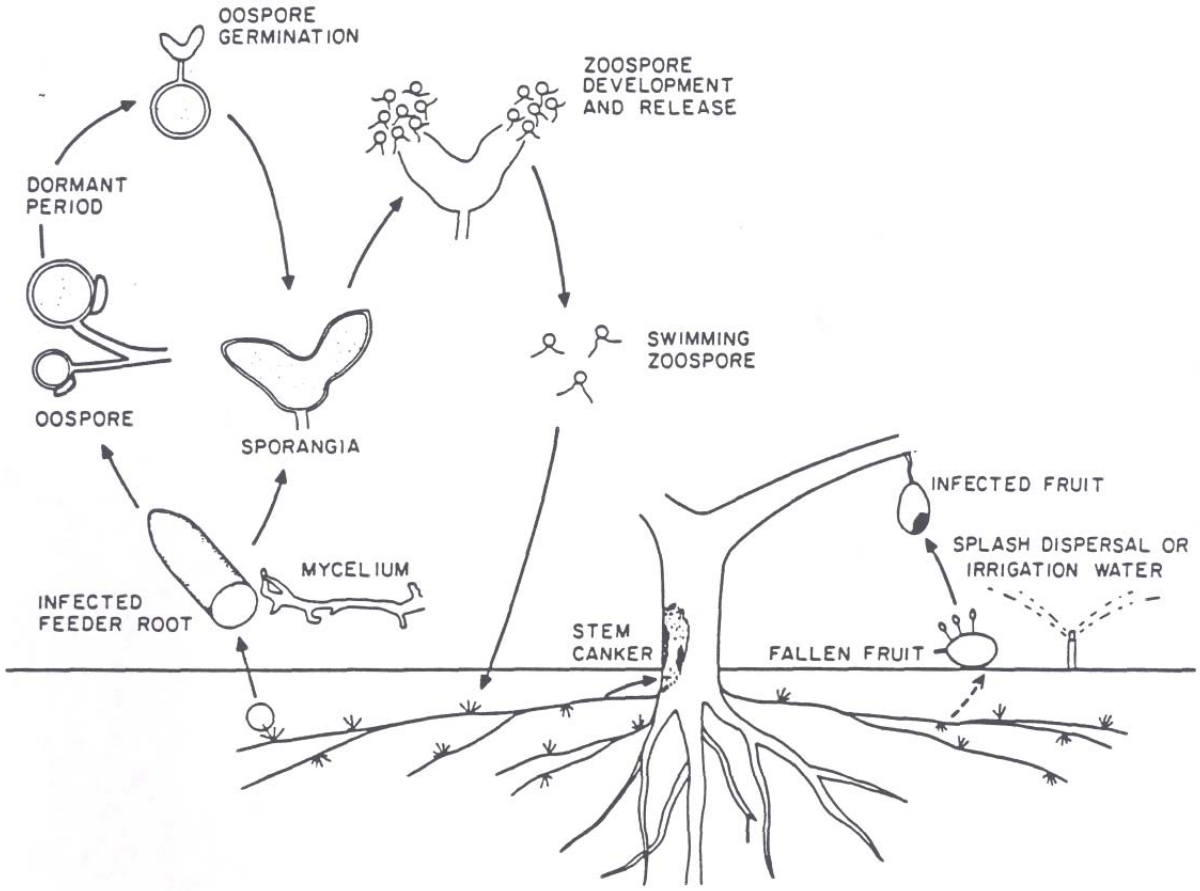
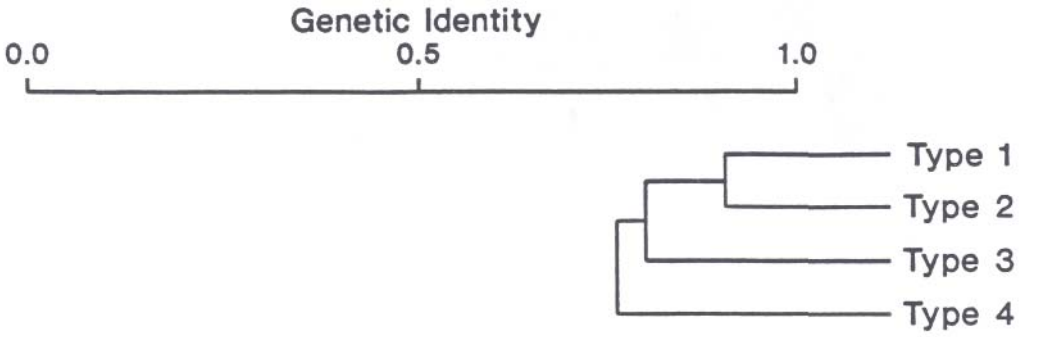


Figure 3: Isoenzyme groups within *Phytophthora citricola*



Type 1: Avocado, CA.

Type 2: Raspberry CA.
Pine, CA.
Various Hosts, S.A.

Type 3: Various Hosts, S.A.

Type 4: Tree Crops, CA.
Citrus, Australia