## AVOCADO ROOT ROT

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The avocado root rot disease, caused by the soil fungus *Phytophthora cinnamomi*, still remains the number one problem facing the avocado industry in California as well as in most other avocado-producing areas. The same fungus is also causing tremendous losses in many other types of crops throughout the world — in eucalyptus forests in Western Australia, in various types of ornamental and coniferous nursery stock in California and in England; in camellias, rhododendrons, and azaleas in many areas; in pineapple in Hawaii, the Philippines and Queensland.

New records of diseases caused by *P. cinnamomi* are occurring in many countries. In none of these other countries have satisfactory control measures for the fungus been developed.

At the University of California, Riverside, our research on this fungus and the disease that it causes on avocado (*Persea americana*) has covered and continues to cover many aspects. This article presents a summary of many phases of our research but does not attempt to give a complete coverage of all of our research. We have established the following:

•Root rot is a disease, caused by the fungus *P. cinnamomi,* instead of merely asphyxiation or an excess water situation, as was believed for many years.

•Two factors are involved in the disease: the fungus, and excess soil moisture, usually brought about by restricted soil drainage.

•Three sport stages formed by the fungus are involved in the disease syndrome: motile zoospores, released from sporangia; chlamydospores; and thick-walled oospores. Zoospores and chlamydospores can infect roots, as can the vegetative phase of the fungus (mycelium); the role of oospores is not yet clear, though they are thick-walled resistant spores.

•The root rot fungus forms sporangia either in the presence of a non-sterile soil extract, or when young mycelia of the fungus are washed thoroughly in water to remove all nutrients and then placed in a solution containing salts such as calcium, magnesium and potassium. The bacteria and multitudes of other microorganisms in the non-sterile soil extract may really be accomplishing the same thing as does extensive washing - removing nutrients and essentially forcing the fungus into the sporulating stage.

•Two mating types of the fungus are now known; we first isolated the less common type  $(A^1)$  from cankers on macadamia trees in Hawaii. We have more recently found this  $A^1$  type on camellia in southern California, and in one instance on avocado roots from the Santa Barbara area. When both mating types  $(A^1 \text{ and } A^2)$  are present, the sexual stage

(oospore stage) is formed and there is increased opportunity for variation and development of more virulent strains of the fungus.

•The root rot fungus makes no growth at 50 °F, makes optimum growth at 70 to 80° and does not grow at 91 °F.

•In nutrition studies *P. cinnamomi* was found to utilize glucose better than sucrose, and to make slight growth on the 7-carbon avocado sugar (mannoketoheptulose).

•Our experiments have shown that *P. cinnamomi* is an efficient saprophyte. It can invade and live for some time on dead organic matter and can grow to a limited extent through the soil. It can survive in the soil, primarily in small dead roots, for up to six years, if the soil is kept in a moist condition.

•The root rot fungus forms resistant spore types — chlamydospores and oospores — in avocado roots. These spores undoubtedly are important in survival of the fungus for long periods.

•*P. cinnamomi* can be detected in the soil by using one of several methods that we have developed: selecting possible diseased roots from a suspect soil sample and planting these out on cornmeal or antibiotic agar in petri dishes in the laboratory, using an avocado fruit as a "trap" in the soil sample, or planting avocado seedlings or other susceptible plants in the soil.

•The avocado root rot fungus can be carried in the seed; if fruit falls on soil containing *P. cinnamomi,* the fungus can grow into the fruit and on into the seed. We have found that the fungus can be killed inside the seed, without damaging the seed, by heat treatment — using a hot-water dip for 30 minutes at 122 °F.

•In many tests we have not recovered the root rot fungus from native brush or from undisturbed, non-agricultural soil. It is very likely not a native fungus in California, and was probably brought in years ago from the tropics.

•The root rot fungus can be easily spread by any means that moist soil is moved, or in drainage water from diseased areas. If the fungus gets into soil where nursery trees are growing it can be, and has been in a number of cases in the past, moved into a grove by means of the nursery stock. It can be carried on tools, or various types of equipment or shoes, which may carry soil containing the fungus.

•There is a close relationship between soil type and disease incidence, the disease being more severe and developing more rapidly on soils with restricted drainage. Much of tins survey work was done by R. M. Burns, and George Goodall in Santa Barbara County; we cooperated with them on the project.

•Low oxygen and soil saturation or presence of a water table can cause considerable damage to avocado roots, but much more damage is caused when the root rot fungus is present. If saturated conditions are not maintained the avocado can recover and make renewed growth.

•Optimum pH for disease development is 6.5, with less disease at very low pH (3.5 to 5), and less at high pH (8.0 or above).

•Optimum temperature for disease development is 75°F, though the disease develops

over a wide range of temperatures, from about 55°F to 90°F. No disease develops at 91°F and most isolates of the fungus do not grow at this temperature.

•Within practical limits no reduction in disease has been found in response to increasing or decreasing nitrogen, potassium, or phosphorus. At extremely high levels of N and K some reduction of root rot has occurred in our tests.

•The root rot fungus is very sensitive to drying the soil. When the moisture content of a sandy loam soil was reduced to approximately 1 percent the fungus could not be recovered after two months. Even when these soils were replanted to avocado seedlings and to susceptible *Persea indica,* no disease occurred, indicating that any spores that may have been present were also killed by the drying.

•The zoospores are attracted to avocado roots by substances that exude from the roots, the main chemicals in the root exudates that attract zoospores are glutamic acid and aspartic acid.

•The root rot fungus can invade avocado roots under conditions of very low oxygen tension (0.5 ppm oxygen), but is more active under conditions of good aeration.

•Some organic amendments, notably alfalfa meal, when mixed completely with diseased soil will control root rot. We have shown this effect in a number of experiments in the greenhouse, in which seedlings have grown very well in soil infested with the root rot fungus which was mixed with alfalfa meal before planting. This is apparently a combination of "biological control" brought about by the great increase in beneficial microorganisms in the soil, and chemical control. Alfalfa meal contains saponins, chemicals which are toxic to the root rot fungus.

•Avocado varieties, and particularly species of the genus of plants in which the avocado is classified (*Persea*), vary considerably in resistance to the root rot fungus.

•In the search for resistant rootstocks we have collected many species of *Persea* and many different avocado types in the native home of the avocado (Mexico, Central and South America), involving many thousands of seeds and also budwood collections. We have had cooperation in some of this work from pathologists in Mexico, and in several other Latin American countries.

•Resistance tests of these materials at Riverside have shown very high resistance in several of the small-fruited, non-edible species of *Persea* (which we hoped to use as rootstocks), including: *P. caerulea, P. chrysphylla, P. borbonia, P. skutchii,* and *P. Donnell-smithii.* None of these species unfortunately is compatible with the avocado. There are still over 60 species of *Persea* in Latin America that we have not collected. Tests in Mexico (under the direction of Dr. J. Galindo and Dr. A. Rodriguez) have shown that *P. pachypoda* and *P. cinerascens* also have high resistance. Our tests confirm this.

•By many tests, in greenhouse and in the field, we have found that there is some resistance to root rot in several avocado varieties and in miscellaneous un-named avocado seedlings. One variety that has consistently shown some evidence if resistance is the Duke, a Mexican type first brought into California in 1912.

•Duke seedlings vary considerably in resistance; we have made several selections of individual seedlings that have shown better resistance than others, and are propagating

these as clones, by rooting cuttings. Duke 6 and Duke 7, for example, are Duke seedlings that showed better resistance than other Duke seedlings in resistance tests. They are now being propagated as cuttings for trial as rootstock material. If they would produce large numbers of seed there would be a possibility of using seedlings of these for rootstocks, though the seedlings would undoubtedly vary more in resistance than do the cuttings.

•Topa Topa seedlings are very susceptible to root rot; Mexicola seedlings are similarly susceptible, as are most other Mexican seedlings and most Guatemalan and West Indian seedlings tested.

•There is some evidence, based largely on field plantings, that non-topworked seedlings or cuttings are more resistant to root rot than similar seedlings or cuttings with Fuerte or Hass scions. This is being studied further.

•Resistance in Duke, or in a few other recent selections, is not nearly as high as in the small-fruited, non-graft-compatible species of *Persea*. Under very severe disease situations in the field, trees on Duke seedlings or cuttings will develop severe root rot. There are indications that under less severe disease situations, the trees on Duke seedling or cuttings will grow moderately well — they will grow better and become affected with root rot less rapidly than standard trees on Topa Topa or other similar Mexican rootstocks.

•In a field planting in Ventura county, trees on Duke seedling and Duke cutting rootstocks produced more fruit than those on Topa Topa rootstock, and did not develop as severe root rot symptoms in the first six years of the plot as did trees on Topa Topa rootstocks; the plot was then damaged by a freeze and by a fire, and is just recently back in production.

•The root rot fungus can be killed by high dosages of several soil fumigants. Our research in the laboratory and greenhouse showed many years ago that *P. cinnamomi* could be killed by Vapam, D-D, Telone, methyl bromide, and chloropicrin. Ethylene dibromide and carbon disulfide were less effective.

•*P. cinnamomi* can be killed or prevented from growing by many other types of chemicals — including chemicals containing copper, many new organic fungicides, chlorine etc. We have tested several hundred chemicals in the laboratory for control of the avocado root rot fungus; many of them are effective in laboratory tests, but very few of them are similarly effective when we test them in the soil for actual control of the disease, as they are absorbed or inactivated rapidly by contact with soil. This is particularly true for copper which is readily complexed by organic matter in the soil.

•The most effective material that we have found in our search for a chemical that could be applied in the irrigation water to living trees to cure root rot or prevent them from becoming diseased is Dexon (d-di-methylamino benzenediazosodium sulfonate). It has given outstanding results in the greenhouse when applied regularly to seedlings planted in soil heavily invested with *P. cinnamomi*.

•Based on many laboratory tests Dexon apparently is effective in controlling *Phytophthora* because very low dosages prevent the fungus from forming sporangia and swimming spores.

•*P. cinnamomi* affects hundreds of plants in addition to the avocado, in a wide variety of plant families. There are over 400 species of plants affected. Some of the more common ones include camellia, heather, rhododendron, azalea, several pines, and many other conifers, pineapple, eucalyptus, peach, grape, and many other types of ornamental nursery stock.

The information developed in our many experiments and tests with the fungus and with the disease, as outlined above, provides an essential basis for developing control measures for this devastating disease. The more we understand about the fungus and about the fungus-plant relationship, the better are the chances of developing effective control measures.

Based on some of the findings noted above, we have tried the following experiments in relation to controlling the disease:

•Adjusted the soil pH to a very low level (pH 3-3.5) in greenhouse tests and in field plots — no control was obtained. In general the trees made poor growth at this low pH level.

•Studied the effect of irrigation on disease development. Reducing the amount of water applied to diseased trees can definitely retard the development of root rot; the vital problem is in trying to adjust the irrigation amount and frequency to a fine balance between the amount that the tree needs to maintain growth and productivity and the amount that will encourage root rot.

•Tested materials showing some resistance in greenhouse tests in the field. Field tests, involving several thousand trees, but until the past year with only relatively small numbers of any particular clone, have tended to confirm the greenhouse results — replanted trees on Tena Tona rootstock or Topa Topa seedlings die out rapidly from root rot, while trees on Duke seedlings have some resistance, trees on Duke cuttings have higher resistance, and the small-fruited non-graft-compatible species of *Persea* have very high resistance.

•Fumigated many plots in the field to attempt to eliminate the fungus, using chemicals found effective in laboratory and greenhouse. Field trials have shown several fumigants to be effective in drastically reducing the root rot fungus population in the soil, and in the case of several plots involving a small number of trees, actually eliminating the fungus. Very high dosages must be used for effectiveness in the field. The most effective materials have been methyl bromide, D-D or Telone, vapam, and mylone.

•Added fungicides to the irrigation water or applied them around living trees in the field to control or prevent root rot. In some of our field plots the fungicide Dexon, which gives very good results in the greenhouse, has given good control of root rot; in some others it has not been effective. The lack of persistence and consequent necessity for reapplying the material six or eight times a year makes it very expensive. Other materials are being tested and we are provided with many experimental chemicals by the agricultural chemical companies that produce various types of fungicides.

•Applied amendments such as alfalfa meal to diseased trees and trees just becoming diseased, based on good results with this material in the greenhouse. We have treated several hundred large avocado trees in the field by working alfalfa meal into the surface soil; in a few cases some temporary control of root rot occurred, but generally the

treatment was not successful — probably because it was not possible or practical to mix the alfalfa meal with all of the soil in which the roots were growing in the field as could be done in containers in the greenhouse. In our greenhouse tests on alfalfa meal mulch applied to the surface was not effective whereas mixing the meal with the soil was effective.

•Treated barrier zones around diseased areas with soil fumigante. Making use of our results on soil fumigants effective against the root rot fungus it has been possible to prevent or retard spread of the root rot fungus in some cases by establishing chemical barriers — in which a strip of soil 5 feet wide is fumigated every six months with Vapam or D-D or Telone or Mylone, The situation has to be a special one, with no root rot fungus above the area to be protected that might wash down into the healthy area, in order for a barrier to have a chance of success. The exact location of the fungus in the grove must be determined by taking samples from trees around the obviously diseased area.

•Developed a program by which nursery stock can be grown and certified free from any *P. cinnamomi* — using the information that we have developed on spread of the fungus and on hot-water treatment of seed.

•Tested the effect of various amendments including fertilizers, vitamins, growth substances, minor elements etc. on root rot on large trees in the field. None of the treatments has shown any response to date.

•Developed methods of reducing spread of the fungus, in the grove, or from diseased to healthy groves. This involves directing drainage water so it does not run from a diseased to healthy area, preventing movement of moist soil on tools, shoes etc. and by other means.

Some of our research in the laboratory has developed information that gives us good leads for further experiments in controlling the disease. For example:

•Some chemicals we know are translocated to and exuded from the roots when applied to the leaves of plants. This use of downward moving systemic chemicals has seemed to me an excellent possibility for controlling root rot, so we have been looking for effective materials for some time. We have tested several materials that have been reported to move down into the roots, with no success so far in controlling root rot. New materials are being developed continually, so there are still possibilities here.

•The root rot fungus produces sporangia in response to a reduction of its food supply and the presence of calcium, potassium and magnesium salts. By adjusting nutrient conditions in the soil it may be possible to keep the fungus in the vegetative state, so that it does not produce swimming spores which spread and intensify the disease so rapidly.

•Certain chemicals such as the fungicide Dexon act as "antisporants" and prevent the root rot fungus from forming spores. Other types of chemicals may be even more effective and may persist longer in the soil; we are looking for anti-sporulants or chemicals that are effective by other means. If we can find out more about what is involved, in the way of chemicals, enzymes, growth substances etc. in production of spores, we will know better how to prevent their formation.

•In other tree crops it is known that the scion affects the susceptibility of the rootstock to disease. There is some indication of this with avocado, indicating that grafting trees to Fuerte or Hass increase their susceptibility to root rot. If some scions increase susceptibility, there is a possibility that others may decrease susceptibility.

•Dr. Bartnicki in our Department at Riverside has found a soil microorganism, *Streptomyces,* which can attack the cell walls of *Phytophthora.* This microorganism may be useful in control; we are planning to test it to see if it can be increased in the soil and will reduce *Phytophthora.* 

•We know, from some of our chemotaxis work, that the swimming spores of *P. cinnamomi* are attracted to amino acids and some other chemicals. We are testing some of these materials in the soil to see if they will attract the zoospores at random, so that the zoospores will not then be attracted to the avocado roots.

•Research by Dr, W. A. Ayers of the U.S.D.A. in Beltsville, begun when he spent a sabbatical leave in my laboratory at Riverside, is giving very significant information on chemicals produced in the soil by bacteria that stimulate *P. cinnamomi* to produce sporangia. This is exciting new information on the life cycle of the root rot fungus. If we know the definite structure of these chemical stimulants we would have a good chance of developing other chemicals that would counteract them.

If additions of materials such as alfalfa meal can provide at least temporary changes in the soil microflora and reduce the effects of root rot, perhaps there are some soils that naturally contain more beneficial microorganisms and in which root rot development is retarded. This form of "biological control" may explain why root rot develops more slowly in some soils than in others, in some cases where restricted drainage is not involved.

•Information in relation to taxonomic relationships of species of *Persea* is giving us a better basis for additional collections in Latin America and should contribute a great deal toward finding the best resistant and graft-compatible rootstock. Also Dr. W. B. Storey in the Plant Science Department is developing information on the chromosome number in resistant species of *Persea* as compared to avocado; this will give more basis for determining how closely these other species are related and whether hybrids may be possible.

•We are beginning combination treatments of various types of approaches which show some promise in reducing disease effects — for instance, combining soil fumigation or soil chemical treatment with use of resistant rootstocks, or using combinations of fungicides, fertilizers and cutting back the trees, etc.

•Dr. T. Murashige in the Plant Science Department is planning to see if irradiation of avocado and *Persea* seeds and seedlings will result in material that may have more resistance or increased compatibility.

•We plan to try to determine the chemical or structural basis for resistance of the smallfruited species of *Persea — P. borbonia, P. caerulea* etc. This should provide information that will give us a better basis for control. There is some indication that a chemical is involved in resistance.

## Outlook —

What are the possibilities of actually controlling Phytophthora root rot? A few points are worth comment here. We have worked at this for many years, and other pathologists in other areas have been involved in research on this problem for some time. Is control really feasible? I think that it is.

One aspect of disease control is prevention. We have made much progress in this area as outlined in this paper, by finding out how the fungus is spread, then developing methods to provide nursery stock free of the fungus. We have also developed methods for eliminating early infections, or drastically reducing the fungus population, and retarding spread within a grove, or from a diseased to a healthy grove.

Resistant rootstocks still seem to be the best means to control such a disease. We are finding more indications of some resistance, and with the expanded collecting program will have a much better chance of finding a resistant and compatible stock, or an intermediate type that could be used as a "sandwich" between resistant, non-compatible rootstocks and susceptible scions.

Also there are some exciting new possibilities in other aspects of control besides resistance, as noted earlier in this paper — new types of soil fungicides, systemic fungicides to apply to the foliage, new approaches based on combinations of treatments. With increasing technological developments in pathology, chemistry, biochemistry, and physiology, prospects look good for better control.

Increased information on the fungus — specific factors involved in spore production, in spore germination, in chemotaxis, toxin production etc. will provide leads that could be the basis for effective control.

With a disease of this type which is greatly favored by excess soil moisture, approaches to control which aim at weak points in the fungus life cycle are important. This approach might be a new development in irrigation — such as drip irrigation which we are testing in relation to root rot — or some simple chemical or nutrient shift that would prevent the fungus from forming spores, or some new chemical that would prevent the spores from swimming and finding the avocado roots.

There are still many promising avenues to explore in relation to solving this difficult and most serious root rot problem.

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