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RESEARCH ON PHYTOPHTHORA DISEASES OF AVOCADO

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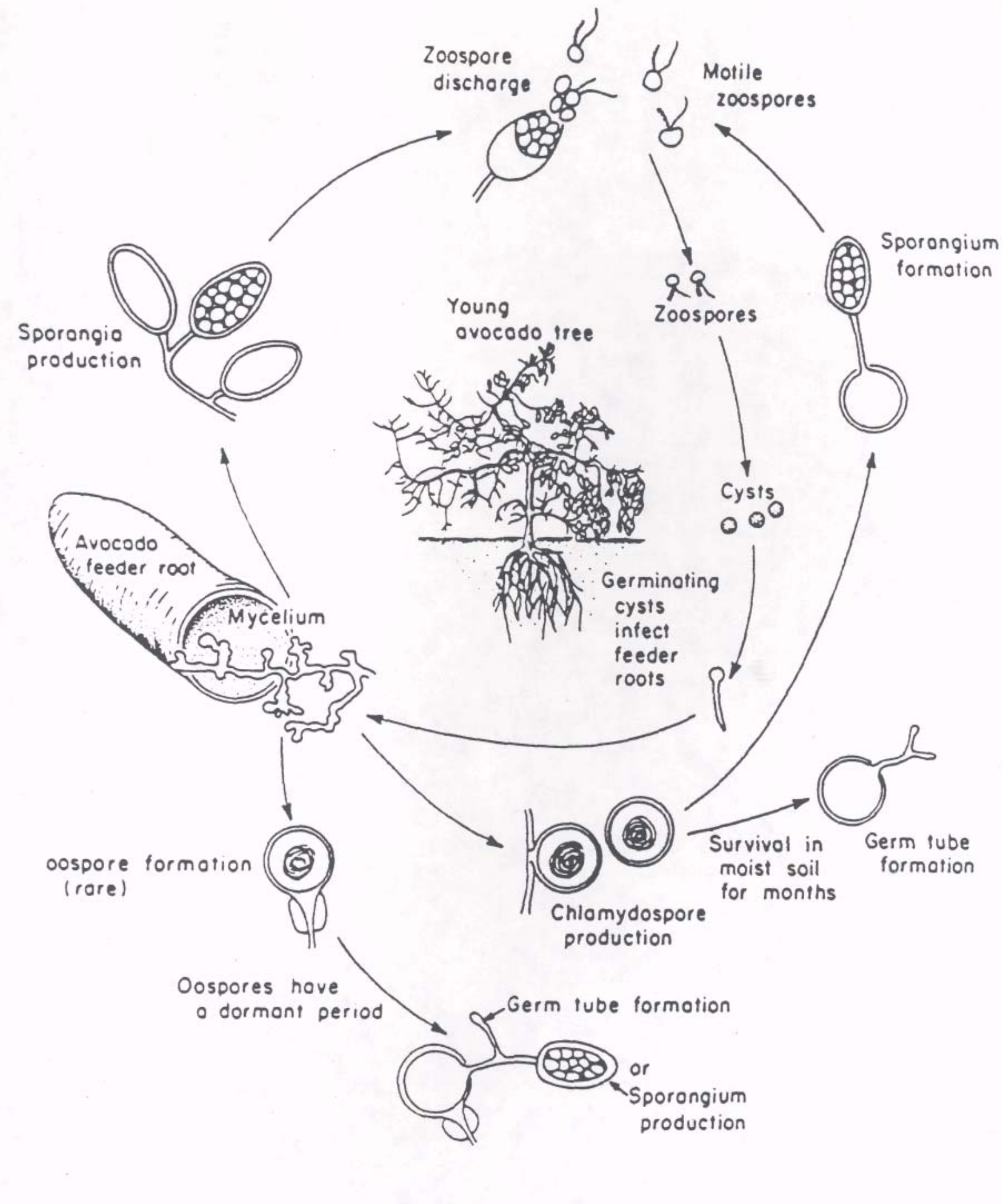
Phytophthora Root Rot

Phytophthora cinnamomi is the cause of this devastating disease of the feeder roots of avocados. **Zoospores,** small motile spores with two flagella, are attracted to the actively growing feeder roots. They become attached to the root surface, round-up by forming a wall, and germ tubes develop which penetrate into the root tissue. Within about six hours the infected feeder root tissue is dead, and the fungus grows within this necrotic tissue for several days. When the food reserves of the root tissue are used up, the Phytophthora fungus forms thousands of spores: both sporangia and chlamydospores.

Sporangia can survive for days or even weeks under moist soil conditions. Under these wet soil conditions each sporangium can liberate up to 25 motile zoospores. The abundant zoospores have limited mobility (less than l/2 inch), but this can obviously allow dramatic disease development within the feeder root system of an infected avocado tree. Under moist, warm soil conditions there is an extremely rapid proliferation of zoospores within a tree root system. The main effect of the pathogen is on the entire feeder root system, which through its rapid destruction, destroys the ability of the avocado tree to take water and nutrients. Under the harsh semi-arid conditions found in California, this typically results in a rapid decline of the trees once sufficient feeder roots have been destroyed. Symptoms include die-back of the tree's branches, wilting and yellowing of the leaves, and ultimately complete defoliation. Frequently it is not realized that once foliar symptoms are expressed, this indicates that much of the feeder root system is already destroyed.

Chlamydospores serve the purpose of maintaining the pathogen apart from its avocado host. These spores contain large reserves of nutrients, both fats and carbohydrates, and can survive from months to years under moist soil conditions. However, they are sensitive to freezing temperatures and dry conditions within the soil. Chlamydospores germinate under warm moist conditions in response to an increase in root exudates. In the soil they usually germinate to form sporangia, which in turn liberate motile zoospores. The decaying pieces of avocado root contain many chlamydospores, and such debris constitutes a major source for further spread of this serious disease.

Oospores are only rarely found in avocado roots. They are not believed to be very important in the normal infection cycle. However, these spores are very thick-walled and much more resistant to cold and dry conditions than are chlamydospores. Thus, they may serve as an ultimate reservoir of survival under cultural conditions such as where the land is left fallow without irrigation or if fumigation is used to reduce the level of chlamydospores in the soil. Their long term ability for survival in avocado soils ensures that once P. cinnamomi has entered a grove it is essentially impossible to eradicate completely.



Disease cycle of avocado root rot caused by Phytophthora cinnamomi

Spread of the pathogen

Under extremely wet conditions, motile zoospores of the pathogen can be moved passively in run-off and irrigation water. As these spores can remain motile for over 24 hours such a passive movement of the pathogen can cause significant spread of the disease within an avocado grove. In addition, soil already contaminated with P.

cinnamomi contains decaying roots which hold both mycelium and chlamydospores, and these structures are highly infective. Physical disturbance and movement of wet soil can lead to the spread of the disease, both within a grove, and from property to property.

Ornamentals

P. cinnamomi is known to be parasitic on over 1000 different host plants, including many woody ornamentals such as azaleas, firs, pines and eucalyptus. It is also a cause of root rot on some other fruit trees such as peach, plum and almond. In addition, the fact that a particular plant is not a known host of P. cinnamomi is certainly not a safeguard against introduction of the disease. P. cinnamomi will cause limited infection of plant species which are not normally considered hosts. Since P. cinnamomi is now widespread (endemic) where avocados are grown, and as it can survive in soil apart from a host, there is no ornamental planting which does not pose a potential threat to avocado production.

Strategies for root rot control

There are four main factors to consider:

1. Certified nursery stock
2. Clonal rootstocks
3. Fungicides
4. Biological and cultural practices

1. Certified nursery stock. It is extremely important to raise avocado trees in an environment free from Phytophthora. In California, the voluntary certification scheme operated by the CDFA lays down the conditions necessary to **minimize** the possibility of infection of nursery trees by P. cinnamomi. It does not cover other Phytophthora species such as P. citricola which may also attack avocado.

There is unfortunately no absolute guarantee of not introducing root rot into a nursery, but stringent hygiene can significantly reduce the possibility. The planting of trees obtained from careless nursery operations which have failed to guard against P. cinnamomi, has been a major factor in the spread of the disease in California.

Good nursery practices include:

(a) The use of well-drained fumigated soil mixes.

(b) The propagation of all trees on benches to reduce the possibility of spread of infection involving P. cinnamomi.

(c) The provision of adequate drainage within the nursery to reduce the risks of introduction and spread of P. cinnamomi by flooding.

(d) The periodic testing of roots for the presence of P. cinnamomi during the 18-month production period required for a clonal rootstock. The most sensitive method available involves the trapping of P. cinnamomi using highly susceptible seedlings of Persea indica.

(e) The minimal use of fungicides. Fungicides and especially systemic fungicide such as Ridomil® should not be used in the nursery practice of raising avocados, since such compounds would actively suppress, but definitely not eradicate p. cinnamomi, thus making its early detection virtually impossible.

2. Clonal rootstocks. Clonal rootstocks such as Duke 7 and G6 have moderate field resistance or tolerance to P. cinnamomi. Providing they are planted in adequately drained soils and a fungicide such as Ridomil® is used at recommended rates they can be established successfully in the presence of P. cinnamomi. However, they do not present an answer to root rot in situations where the soils are poorly drained and where cultural care is less than perfect.

Any young avocado tree, whether it be on a seedling or a clonal rootstock, requires proper planting conditions. Irrigation practice is critical in an environment such as southern California where temperatures may reach 100 to 105° F literally within days of planting. Excessive dryness or excessive wetness can be more damaging than root rot to the root systems of young avocado trees. Based on our own experiences the key to good irrigation is frequency, at least twice a week, and very careful management.

Currently our rootstock program is undergoing a major expansion and we are in the process of critically testing new potentially resistant selections. The most promising of these selections which are available to growers are the G755A, G755B and G755C selections, which appear to be hybrids of avocado and a near relative, Persea schiedeana. P. schiedeana is known in Guatemala as **chucte** or **coyou.** It is an edible fruit similar to avocado. Rootstocks of pure P. schiedeana have been used in Central America (Honduras) for fifty years. They are often extremely vigorous in their growth, withstanding waterlogged conditions and are graft compatible with avocado. Until the discovery of the G755 selections from Guatemala in late 1975, no resistance to root rot had been found in P. schiedeana.

G755 has been tested in the laboratory, greenhouse and field. It is the first rootstock selection found to possess true resistance to invasion by P. cinnamomi. The older rootstocks such as Duke 7 and G6 do not possess this type of resistance, but rather depend on their ability to regenerate new feeder roots at a pace faster than those destroyed by root rot. G6 and Duke 7 might be considered **TOLERANT** rootstocks, rather than resistant. Most probably G755 also possesses very high **TOLERANCE** and will be found to regenerate roots at a fast rate. In the field, G755 selections are superior to G6 and Duke 7, where root rot is severe.

Ultimately our research is aimed at the development of rootstocks with sufficient resistance to P. cinnamomi to allow little or no fungicide usage. The current aims of our program are to develop such rootstocks.

How close is G755 to such a performance? Fungicides such as Ridomil® or Aliette*, used in the first two years, give a 30-50% improvement in performance based on measurements of the increase in tree trunk diameter. Obviously G755 is not the complete answer, but in my opinion it is at least the first significant step forward in the eleven years since the Duke 7 was released for grower use. The G755 selections were released in October 1984, nine years after Duke 7, and represent the beginning of a new era in rootstock research.

Currently under advanced testing are sixteen new rootstock selections. The more promising ones are planted in large numbers, in various trials throughout the state. In a large 8-acre planting at Embarcadero Ranch in Goleta we have 60 to 90 trees each of the more promising selections. In total we have over 4,000 trees under field observation in California, the majority of these are in their second or third year of planting.

Some of the more promising selections are **Thomas** and Barr Duke. **Thomas** is a Mexican selection. It is a rootstock recovered from an extremely vigorous Fuerte growing in a root rot area in Escondido. All other trees in that grove were in poor shape; this one was superb. We have tested it in root rot situations without fungicides and it has done well. We are hopeful that it will prove to have a performance at least equal to G755. **Barr Duke** is a third generation Duke line, selected as an extremely vigorous seedling of Duke 6, and growing in a root rot area in Fallbrook. It also looks very promising at this early date.

At this point I would like to make a plea for help from growers. If you do have an outstanding tree in a root rot area, one that has been surviving for at least 5 years, where all others have failed, let us know. It could be another Thomas or Barr Duke. We would attempt to recover the rootstock.

I believe the next fifteen years will see major progress in the field of rootstock of research. The major obstacle to advancing at a more rapid rate is a lack of sufficient research funding. Substantial research support is vital to allow collection and testing of the coyou, Mexican and Guatemalan types which form the basis of our rootstock program. Critical testing requires skilled technicians; field evaluations require large-scale plantings and long-term measurements of tree growth and fruit production in root rot areas. Before we can release new rootstocks, we need 10-15 years for a critical study. There is no suitable alternative to such an approach.

However the substantial Investments necessary to allow this program to be successful are surely at the center of the future health of the Industry. Avocado root rot 1s here to stay and growers must learn to live with the problem. Our program 1s designed to make that possible.

3. <u>Fungicides.</u> There was a major breakthrough in the mid-to-late 1970s when fungicides highly active against <u>Phytophthora</u> were discovered in Switzerland by Ciba-Geigy and in France by Rhone-Poulenc. The first compound to be tested in our program was **metalaxyl,** produced by Ciba-Geigy and known under the trade name of **Ridomil®.** It is extremely active against P. cinnamomi, as little as one-tenth of a part per million (w/v) being highly inhibitory. If you consider that Ridomil® contains about 250,000 parts per million of metalaxyl, you get a clear idea of its potency.

Ridomil® works by interfering with the genetic expression of the root rot organism. It does not kill the fungus, but prevents its normal growth and development. In particular, it stops the processes of formation of chlamydospores and sporangia. Thus it effectively prevents further build up of the <u>Phytophthora</u> on avocado roots. Once Ridomil® is sufficiently diluted (less than 1/10 part per million), it no longer works as effectively. As the residue of the fungicide drops to about 1/100 part per million, the inhibition of the fungus no longer takes place. Under normal usage, following label recommendations carefully, a single application of the fungicide should be effective for about 8 weeks.

Ridomil® is extremely mobile in soils and very soluble in water. With three well-timed applications there should be good protection for a whole growing season.

Since compounds like Ridomil® also have vulnerabilities it is ultimately important to have alternative options when planning good fungicide strategies for control of avocado root rot. One of the more obvious vulnerabilities of a systemic fungicide such as Ridomil® is the possibility that strains of Phytophthora resistant to the product can develop. Another potential factor undermining fungicide efficacy is one well-known with some herbicides: repeated use of the product may create a soil with a much greater microbial capacity to degrade the compound. Since product failure, whatever the cause, may result in poor rootstock performance, or even failure in a severe root rot situation, it is important to develop less vulnerable control strategies.

Of significant assistance in planning such control strategies may be the discovery that another fungicide fosetyl-aluminum, produced by Rhone-Poulenc in France under the trade name Aliette®, also has good efficacy against avocado root rot. It is the only fungicide that can be applied to the foliage and will move down into the roots. In a fungicide trial in Fall brook, we demonstrated as early as 1982 that this compound could be equal to metalaxyl in its efficacy. The keys to the improved strategy were using a preplant root drench of a quart of the product at a high concentration (1500-3000 parts per million) and follow-up applications using monthly foliar sprays at the same concentration. It should be stressed that this compound has yet to be registered for use with avocados. Aliette® is remarkable in that the active principle moves down so effectively to the roots. Thus it is possible to minimize one of the potential problems with fungicides: the breakdown of the product by soil microbes. The other potential problem remains, however: the possibility for development of Aliette®-resistant strains of Phytophthora.

Research with fungicides and their use with replants of clonal rootstocks must therefore focus on using more sophisticated, more reliable control strategies. He are studying the best ways to use Ridomil® and Aliette® in combination. This work has been in progress since 1985. The early results are very encouraging. Aliette® should be registered for nonbearing trees later this year (1987). Soon the reality of cost-effective and reliable chemical control may be achieved, at least with nonbearing avocados.

A major challenge remains and that is to develop cost-effective and efficacious chemical control methods for bearing avocados. This is now the major focus of our continuing control program. The idea of bringing large potentially-bearing avocado trees back to life using fungicides is a daunting one. The earliest work, begun in late 1970s with Ridomil®, showed that the stage of decline of the trees could be stabilized in some instances. In other words a stage 3 could be kept at a stage 3.

The wider possibilities of cost-effective control only became apparent in 1983, when the South African plant pathologist Dr. Joe Darvas reported that he could obtain efficacy against root rot by injecting the chemical Aliette® into trees. In 1983 we began our own evaluations of this procedure. Almost three years later we are beginning to get some feel for the methods. Unlike reports in both South Africa and Australia, we do not see a dramatic turn around where trees go from a stage of 2.5 or 3.0 to a stage 1.0 in less than a year. Rather over a 2-3 year period we are able to stabilize the trees and prevent their further decline. The climate conditions here in California are much more marginal for growth of avocados and our soils are generally poorer and much shallower than those in South Africa and parts of Australia. The potential for root recovery is therefore probably less under our conditions. This may explain the poorer responses. For the first time, however, we have the possibility of a cost-effective chemical control with large avocado trees. The amounts of material required for injection of trees appears to be 20-100 fold less than those needed with conventional treatments.

The need to evaluate such injection procedures further and also test new compounds as they appear is critical. We have seven different fungicides under test for their possible ability to control avocado root rot by trunk injection. My aim is to develop alternative fungicide strategies using chemicals with different modes of action. In addition, it is very important to optimize the performance of these chemicals. Do we need one, two or three injections per year? What is the dosage of chemical required? It is a long way from the days when all we had was methyl bromide fumigation and Topa Topa rootstocks. Research money being used for chemical control work is providing answers that were unthought-of of just a few years ago. The future looks bright, we are now beginning to test new compounds, some of which are more active even than Ridomil®. The ability to use even less fungicide than we currently do will make disease control more economical and more environmentally-acceptable in the years ahead.

The registration of new fungicides and new methods of using them is a very real need for effective root rot control. The costs of registration are so prohibitive that chemical companies have to feel that there is good efficacy and a major market for their use before they will proceed with the process. Supplying data for registration is only part of our job, we also have to convince chemical companies that avocados are a worthwhile investment. Our track record has been good. We obtained some of the earliest registrations for Ridomil® and we are on target with a nonbearing label for Aliette®, expected sometime during 1987.

The alternative scenario where there is no legal registration for these fungicides would be a disaster indeed. The recent strong adverse publicity regarding pesticides, and especially their wrongful use, make it essential to pursue legitimate registration goals if we are not ultimately to damage the good reputation of the avocado industry.

We need these new registrations to use with clonal rootstocks like G755. As groves become established on these rootstocks we can also begin to re-evaluate the rates of fungicides necessary for satisfactory root rot control in a downward direction.

4. Cultural and Biological practices. Good cultural practices are critical for proper establishment of clonal rootstocks. Research on this important aspect of the problem has been underway for several years. The success of mounding as a method for initiating good growth of young trees is well established in Santa Barbara County. This same practice is just beginning to be tried by growers in San Diego County. Sensible irrigation practices are perhaps the most critical factor of all. Frequent irrigations, 2 to 3 per week, combined with planting on mounds may provide a recipe for success in that early replant phase. Only too commonly young trees actually receive much less frequent irrigations, perhaps only every 7-10 days, and the growth results are generally

poor.

The use of legumes as an interplant crop is a favored practice in other avocado-growing regions of the world e.g., Australia. Here in California we are just beginning to examine this practice. Similarly, the incorporation of organic matter in soils (e.g., chicken manure) prior to planting and the use of organic mulching with a mixture of straw and manure after planting is a proven method in the 'muck and magic' avocado culture found in parts of Australia, especially Queensland. Such organic farming practices, while they do exist here in California, have not been properly evaluated. A field program has begun to get some basic facts about these various cultural practices, and their usefulness or otherwise in the Californian environment.

Biological control using specific antagonistic microbes is a practice which has recently emerged as a viable approach to controlling soilborne pathogens like Phytophthora. I believe that avocados present us with an ideal situation for developing such biocontrol practices. Our certification program which dictates that avocado nursery trees must be raised in a sterile growing medium. Thus an ideal opportunity exists for introducing into the nursery mix highly antagonistic microbes capable of actively suppressing P. cinnamomi.

Soils exist in California that are suppressive to Phytophthora. These are extremely rare, however, perhaps largely due to the general lack of a 'muck and magic' philosophy among growers. However a few sites have been identified. At one site in particular we have been successful in isolating many different bacteria and fungi from an extremely rich microbe population associated with the feeder roots of avocado. Phytophthora has been in that grove for forty years with no adverse effect on the trees. Several fungi and bacteria, among the hundred or so tested, proved to have good antagonistic properties. In a highly sensitive test with highly susceptible Persea indica seedlings one fungus was as effective in controlling P. cinnamomi as either of two systemic fungicides also included as controls. Our aims are to develop suitable systems whereby we can stabilize biocontrol in a nursery context. When clonal rootstocks developed with biocontrol agents are planted in root rot situations, our hopes are that they will become established much more easily than is currently possible. In the next few years some of these researcher's dreams will hopefully become a reality. At least this is the sincere hope of this researcher in his endeavor to find that elusive, final solution to the root rot problem.

Crown rot of avocado

Crown rot of avocado is caused by Phytophthora citricola, a species which forms oospores. **Oospores** are resistant structures and can survive extremes of cold and heat, drying and much higher levels of toxicants such as methyl bromide than can chlamydospores. This type of disease was first recorded on avocados in 1916, though it was not until the 1970s that George Zentmyer correctly identified its cause as P. citricola. In a recent avocado survey we established that 16% of growers were aware of the problem in their groves. In other words at this moment in time about one-sixth of the industry may be affected by the disease.

The basic mechanisms of pathogenesis, the process whereby the fungus gains entry to the avocado tree and establishes this disease, are still unknown. The biology of the fungus in soil is also poorly understood.

Other hosts of P. citricola include apples, hops, citrus, maple, eucalyptus, tomato, Ceanothus, Hibiscus, Rhododendron, Rosa and Syringa. P. citricola, along with P. cinnamomi, causes major problems on walnuts in California.

Where does P. citricola come from? It was originally characterized in 1927 as a pathogen of citrus leaves in Formosa. In Western Australia it is the major cause of brown rot on citrus fruit. However here in California it has never been isolated from citrus, and yet four other Phytophthora species have been detected on that crop. Another source of P. citricola introduction may be on nursery avocado trees. The disease has the ability to remain latent for many years and consequently growers do not necessarily associate the problem with the original purchase of the trees. This possibility needs active research. We need to determine whether the procedures designed for P. cinnamomi are adequate for P. citricola. in view of the oospore-forming capabilities of P. citricola.

The design of effective control strategies may be helped by the patterns already set for P. cinnamomi. Of paramount importance is the need to establish if there are useful sources of resistance to P. citricola among our clonal rootstocks. An effective preventative strategy for chemical control needs to be researched. The effect of cultural and biological control practices requires investigation. Here we have a disease that in my estimation is slowly gaining a stranglehold on the industry. We need to strike back before it gains the hold that root rot (P. cinnamomi) has, to wait until it spreads through an entire industry would surely be unwise. With the control technology being developed for root rot at my disposal, I am confident of gaining the upper hand in time. The urgent need is for more research now, and the funds to support it.