

Biocontrol of Phytophthora Root Rot of Avocado With Mulch and Biocontrol Agents

Continuing Project; Year 5 of 5

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Benefit to the Industry

Biocontrol may provide an effective, environmentally acceptable method of controlling Phytophthora root rot of avocado through a combination of cultural methods and application of native or genetically engineered microorganisms. This approach will probably be most effective as part of an integrated system of resistant rootstocks, sanitation and cultural methods.

Objectives

1. Locate and collect biocontrol agents.
2. Manage Phytophthora root rot using continuous applications of biocontrol agents through the irrigation system.
3. Understand how effective populations of biocontrol agents can be maintained in the field.
4. Characterize mechanisms of antagonism against *P. cinnamomi* provided by mulch and biocontrol agents.

Summary

Locate and Collect Biocontrol Agents

A survey of California avocado groves is continuing to identify local groves, which have soils which are suppressive to *Phytophthora cinnamomi*. Two criteria are used to identify a suppressive soil. It is a soil which degrades *P. cinnamomi* hyphae or chlamydospores, or one which has high populations of *Phytophthora* but the trees continue to thrive. Twenty-nine groves have been surveyed with four showing suppressiveness to *Phytophthora*. Individual trees in other groves also show suppressive characteristics. We have identified two microorganisms which are directly pathogenic to *P. cinnamomi*. *Rozella* sp. and *Lyobacter mycophilus* are known parasites of fungi and will attack and kill *P. cinnamomi* under laboratory conditions. Work is ongoing in the laboratory to test the biocontrol ability of these microorganisms.

Epidemics of *Phytophthora* in individual groves have been studied. Populations of *P. cinnamomi* appear to decline precipitously immediately behind the leading edge of the epidemic in some groves. Biocontrol fungi are being isolated from this region. We postulate that microorganisms which fill a niche similar to that of the pathogen or that compete for chemical substrates important to the pathogen, such as root exudates, will be effective biological control agents. To identify such organisms, we have developed an in situ, culture-independent strategy to identify bacteria and fungi that rapidly grow in response to specified chemical substrates in environmental samples. To test this approach, we examined soils from a southern Californian avocado grove where a *Phytophthora cinnamomi* epidemic has produced three distinct zones. In Zone 1 where the pathogen epidemic has occurred, the trees are dying or dead but there is very little recoverable *Phytophthora*. Zone 2 is where the advancing margin of the *Phytophthora* epidemic is located and where the trees are relatively healthy but the roots are becoming infected and *Phytophthora* populations are high. In the region ahead of the advancing margin (Zone 3), the trees are healthy and the soil is conducive to avocado root rot. In Zone 1, microorganisms are reducing the populations of *P. cinnamomi* and the soil has become suppressive. Soil in Zone 1 is 467% more suppressive to *Phytophthora cinnamomi* than was Zone 3. Soil from Zone 1 is 10 fold more suppressive than fumigated soil that has removed all microorganisms. Using a substrate utilization approach, we molecularly identified six bacteria which responded to root exudates of avocado and were present only in Zone 1, where the *Phytophthora* epidemic had passed through. Four of these bacteria had

varying similarities to *Bacillus mycoides*. The other two species were *Renibacterium salmoninarum* and *Streptococcus pneumoniae*. We have recovered four of these *Bacillus sp.* and have tested them in greenhouse trials (see below). *Trichoderma aureovirde*, *Trichoderma harzianum*, *Gliocladium virens* and *Hyphodontia alutacea*, which were recovered behind the leading edges of *Phytophthora* epidemics, greatly damage *Phytophthora* chlamydospores. Using the new species specific DNA probes, many potential biocontrol agents are continuing to be identified from decomposing mats of *P. cinnamomi* hyphae. Attempts are continuously being made to isolate these microorganisms.

The rain forest of Papua New Guinea is thought to be the center of origin for *P. cinnamomi*. We have visited Papua New Guinea and verified that *P. cinnamomi* appears to be endemic to the Highlands region of New Guinea. This area has a rainfall of several hundred inches per year. Flowing water is plentiful and would be ideal to disseminate *P. cinnamomi*. Nearly all crops are grown on mounds to prevent the roots from standing in water. Many endemic plants susceptible but tolerant to *P. cinnamomi* were located in the highlands region including *Pandanus*, *Nothofagus*, *Acacia*, *Eucalyptus*, *Melanoleuca* and *Casuarina*. Many avocados were found growing and surviving in standing water in which *P. cinnamomi* was present. Either the avocado varieties in New Guinea are resistant to *P. cinnamomi* or there are natural microorganisms in New Guinea which suppress the activities of *P. cinnamomi*. We have enlisted the aid of Bob Tombe, University of Goroka, Papua New Guinea, to cooperate with us and send samples every two months for the next two years. Locations and plant associates were identified during the visit. These samples are being sent regularly. Five shipments have now been received totaling 39 locations. Of these 39 samples only 7 have provided suppressive microorganisms. It appears that, similar to California, the season strongly influences the suppressiveness of the soil. The warm, rainy season, when travel is difficult, appears to provide the most efficacious biocontrol organisms. Next year we will try to concentrate our collecting efforts during this period. Soil from around *Nothofagus* from the Mt Kubor region in New Guinea has proven to be extremely suppressive to *P. cinnamomi*. The microorganism involved appears to be a heat-stable bacterium, which would suggest a *Bacillus sp.* Efforts to isolate and identify this bacterium are now being made.

Samples of soil were also brought back to the US from Northern Australia during the Australian Avocado Symposium. Seventeen out of 40 samples were highly suppressive to *P. cinnamomi*. Nearly all of the suppressive soil samples were from rainforest areas. Soil samples from arid regions or avocado groves were not suppressive to *P. cinnamomi*. Efforts are now being made to isolate biocontrol microorganisms from these Australian soils.

Contacts have been made in Malaysia and we are expecting a shipment of suppressive soil from that country.

Biocontrol of *Phytophthora* root rot with microorganisms

A series of greenhouse experiments were conducted with the biocontrol bacterium *Pseudomonas putida* which has had some success controlling root rot of citrus when applied continuously through the Ecosoils Bioject machine. A summary of the results from several tests is shown in Table 2. Both bimonthly and monthly applications of *Pseudomonas putida* + its growth medium significantly improved shoot growth of avocado seedlings by 65-68% in *Phytophthora cinnamomi* infested soil. However, root health was significantly improved only by the bimonthly applications of *P. putida*. Root dry weight of avocado seedlings were improved 161% by bimonthly applications of *P. putida*. It appears that *Pseudomonas putida* may provide some biocontrol of avocado root rot when applied frequently. Further work is continuing with this bacterium.

Four *Bacillus sp.* isolates from regions behind an epidemic of *Phytophthora cinnamomi* were implicated as contributors of the suppressive soil by molecular techniques and were isolated and tested separately in a greenhouse trial. The results were extremely variable and none of the bacteria significantly reduced avocado root rot. However, *Bacillus 29* appeared to restore the original level of suppressiveness back to the soil when it was added to fumigated soil. Work is continuing with these bacteria.

Mechanisms of Biological Control

Five soils representing different levels of soil suppressiveness to *P. cinnamomi* have been selected from the Southern California survey. These five soils will be examined during the spring, summer, fall and winter for parameters which may cause the suppressiveness. The soils will be tested for: 1) soil mineral nutrients, pH, EC, soil type and soil permeability, 2) surfactant levels, 3) tree health, 4) populations of *Phytophthora cinnamomi* and *P. citricola*, 5) *P. cinnamomi* hyphal degradation, 6) *P. cinnamomi* chlamydospore degradation, 7) bacterial community structure, 8) fungal community structure, 9) rhizosphere community structure, 10) soil microbial activity, 11) microbial biomass, 12) soil respiration, 13) cellulase and laminarinase enzymes, 14) laminarin and cellulose utilization and 15) biocontrol abilities on avocado seedlings (autoclaved and natural soil). To date the suppressiveness in the soil seems to fluctuate with the season. The soils in general are more suppressive to hyphae and chlamydospores of *P. cinnamomi* in the summer than in the winter, yet populations of *P. cinnamomi* are usually greater in the summer than in the winter. The Vanoni soil loses its biocontrol ability when autoclaved indicating the cause of the suppressiveness is microbial. Two other soils are suppressive to hyphae and chlamydospores of *P. cinnamomi*, yet they do not reduce root rot in pots in the greenhouse. Other suppressive soils, as observed by healthy field trees growing in soils infested with *P. cinnamomi*, do not appear to be suppressive as a result of microorganisms.

A series of experiments with the Vanoni soil is summarized in Table 1. Natural Vanoni soil is approximately 6 times more suppressive to *Phytophthora cinnamomi* than the same soil after it has been fumigated to remove all microorganisms. As little as 1% of the natural soil added back to the fumigated soil will restore the original level of suppressiveness. In fact sterilized soil with 1-10% natural soil seems to be more suppressive than natural soil. This suggests that the microorganism(s) responsible for suppressiveness to *Phytophthora cinnamomi* are abundant in natural Vanoni soil and that they readily colonize the fumigated soil.

Field testing of biocontrol agents

The EcoSoils Bioject machine has been thoroughly tested and has been found to produce high quality biocontrol agent inoculum in the field and deliver it reliably in the irrigation water. Large acreages can be treated by this machine since numerous treatments with low density inoculum of biocontrol agents eventually results in the same soil populations as numerous high density treatments. Continuous application of biocontrol agents in the irrigation water results in far better soil colonization than does single applications. A field project to test the efficacy of continuous applications of a biocontrol agent on avocado root rot has been established at South Coast Field Station. The biocontrol agent is the BpO2 *Bacillus sp.* (P. Douillet Co.), which showed promise as a biocontrol agent of *Phytophthora cinnamomi* in greenhouse tests in previous years. The trial is progressing well but as of yet the avocado root rot disease is not causing enough damage to give significant growth differences among treatments. Soil population measurements of *Phytophthora cinnamomi* are now being conducted.

Conclusions

We have not yet identified an effective biocontrol agent of *Phytophthora cinnamomi*, but we feel that we have made great progress. We have identified three sources of soil suppressiveness: 1) Suppressiveness from Southern California, 2) Soils that have experienced an avocado root rot epidemic, and 3) Soils from the region where *P. cinnamomi* originated, namely Papua New Guinea. Soils are now regularly being examined from all of these sources. Some of these sources are 10-fold more suppressive than the same soils which are fumigated to remove the biocontrol microbes. In some of the soils the microbes and the suppressiveness are transferable to other soils. We have identified strong biocontrol candidates such as *Rosella sp.* and *Lytobacter mycophilus* which are vicious predators of *P. cinnamomi* in the laboratory. Using new and novel microbial techniques we have identified, isolated and tested our first microbes from suppressive soil. We are now poised to initiate a massive assembly-line procedure to isolate and test new biocontrol organisms for use against *Phytophthora cinnamomi*. There probably are several types of soil suppressiveness toward *P. cinnamomi* and not all of them appear to be of microbial origin.

Known biocontrol agents such as *Trichoderma*, *Gliocladium* and *Bacillus subtilis* have been shown to reduce survival inoculum of *P. cinnamomi* and they may be partially responsible for reducing damage caused by avocado root rot in the long term. However, they do not appear capable of preventing avocado infection or death of

individual trees. These organisms may be able to destroy the survival structures in the soil to facilitate replanting and aid control by other means. We are now concentrating on more predatory fungi which attack and kill *P. cinnamomi* directly.

Table 1. Effect of Somis suppressive soil diluted with fumigated soil and infested with *Phytophthora cinnamomi* on root health and root weight¹

Soil Treatment (%)	Root health (% healthy)	Root dry weight (gm)
Natural 100	60.45 ab	6.51 bc
Fumigated 100	10.58 d	3.85 d
Natural 50/Fumigated 50	49.13 bc	5.68 cd
Natural 10/Fumigated 90	77.08 a	8.24 ab
Natural 1/Fumigated 99	74.85 a	9.88 a

¹ Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test.

Table 2. Effect of the biocontrol bacterium *Pseudomonas putida* applied monthly or bimonthly on avocado root rot¹

Treatment	Healthy roots (%)	Root weight (gm dry wt)	Shoot weight (gm dry wt)
No <i>Phytophthora</i>	84 a	0.84 a	1.83 a
<i>Phytophthora</i> + <i>P. putida</i> Bimonthly	44 b	0.47 b	1.38 b
<i>Phytophthora</i> + <i>P. putida</i> Monthly	13 c	0.25 c	1.35 b
<i>Phytophthora</i> No biocontrol	9 c	0.18 c	0.82 c

¹ Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test.