

# Biocontrol of Phytophthora Root Rot of Avocado with Mulch and Biocontrol Agents

## Continuing Project; Year 4 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. Effectively manage Phytophthora root rot using mulch alone and biocontrol agents in conjunction with mulches.
3. Manage Phytophthora root rot using continuous applications of biocontrol agents through the irrigation system.
4. Understand how effective populations of biocontrol agents can be maintained in the field.
5. 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 has been initiated 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 chlamydo spores, or one which has high populations of *Phytophthora* but the trees continue to thrive. Twenty-four 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 *Lytobacter 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. We postulate that in Zone 1, microorganisms are reducing the populations of *P. cinnamomi* and the soil has become suppressive. We used this new experimental approach to identify six bacteria whose population levels correlated with the differing levels of suppressiveness. These bacteria have varying similarities to several *Bacillus* species and *Arthrobacter globiformis*. We are currently in the process of testing these organisms in greenhouse trials. *Trichoderma aureovirde*, *Trichoderma harzianum*, *Gliocladium virens* and *Hyphodontia alutacea*, which were recovered behind the leading edges of *Phytophthora* epidemics, greatly damage *Phytophthora* chlamydospores. Other new potential biocontrol organisms, which have been isolated, include *Pseudomonas alcaligenes* and *Erwinia cyripedii*. Using the new species specific DNA probes, many potential biocontrol agents were identified from decomposing mats of *P. cinnamomi* hyphae. These organisms, which could not be cultured, included an unidentified protozoa-like organism, *Trichosporon* sp., which is a biocontrol agent of a corn disease, *Tritirachium* sp., which is closely related to parasites of insects, *Arthrobotrys dactyloides*, which is a parasite of nematodes, and *Hypomyces chrysospermus*, which is a parasite of many fungi. Attempts are now being made to isolate these fungi.

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. Preliminary data indicates that many of the samples from New Guinea are extremely suppressive to *P. cinnamomi*.

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.

### **Biocontrol of Phytophthora root rot with mulches and earthworms**

Mulch experimentation has been delayed by the sabbatical of Ben Faber.

### **Biocontrol of Phytophthora root rot with microorganisms**

A greenhouse test examined the ability of *Trichoderma aureovirde*, *Gliocladium virens*, *Hyphodontia* sp., and *Bacillus subtilis* to control *P. cinnamomi* root rot when inoculated onto a yardwaste mulch substrate. Neither the yardwaste nor any of the biocontrol agents controlled root rot of avocado under these greenhouse conditions. We have worked with these known biocontrol organisms for three years now without positive results. While these biocontrol organisms can damage survival structures of *P. cinnamomi* and may play a role in reducing soil inoculum in the long run, they are apparently unable to prevent avocado root infection and subsequent death of avocado trees. We are now abandoning these organisms in favor of some of the more direct parasites mentioned above.

Six biocontrol agents were tested in the greenhouse to determine if they could reduce avocado root rot. The biocontrol agents were: Spectrum (mix of biocontrol organisms-Soil Technology Co), BPO1 (*Pseudomonas*-P. Douillet Co.), BpO2 (*Bacillus* sp.- P. Douillet Co. ), extract from the Friedrich suppressive soil in California, polymer (Soil Technology Co.). The Spectrum and BpO2 products appeared to greatly reduce avocado root rot. The Friedrich suppressive soil appeared to reduce populations of *P. cinnamomi*. Experiments are now being conducted to verify these results. The polymer product, a commonly used soil supplement in Southern California, does not control avocado root rot and may increase the disease.

### **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 (Table 1). 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.

### Biocontrol of Phytophthora root rot using continuous application 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.).

### Conclusions

We still have not yet identified an effective biocontrol agent of *Phytophthora cinnamomi*. However, we have identified several soils which contain these organisms. We have identified strong biocontrol candidates such as *Rosella sp.* and *Lytobacter mycophilus* which are vicious predators of *P. cinnamomi* in the laboratory. We have now identified Papua New Guinea as the center of evolution for *P. cinnamomi* and have shown that the soils from Papua New Guinea and Northern Australia are highly suppressive toward *P. cinnamomi*. Soils from these locations are now being screened for biocontrol agents. We are now trying several novel techniques to identify and isolate the organisms responsible for suppression of *Phytophthora cinnamomi*. Suppressiveness in California soils may fluctuate with the season and the spatial location in the grove. 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.

We have identified two new commercial products, BPO2 (P. Douillet Co.) and Spectrum (Soil Technology Co.), which may prove to be useful biocontrol agents.

**Table 1.** Seasonal fluctuation in suppressive effects of Vanoni soil against *Phytophthora cinnamomi*

Month of sample	Damaged chlamydospores from mycelial mats buried in soil (%)	Damaged chlamydospores from infested roots buried in soil (%)
January	23	50
April	37	75
August	48	100

