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 criterions 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. Fifteen groves have been surveyed with two showing suppressiveness to *Phytophthora*. However, the suppressiveness appears to fluctuate with season. Individual trees in other groves also show suppressive characteristics. 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. *Trichoderma aureovirde, Trichoderma harzianum, Gliocladium virens* and *Hyphodontia*

alutacea, which were recovered behind the leading edges of *Phytophthora* epidemics, greatly damage *Phytophthora* chlamydospores (Table 1). Other new potential biocontrol organisms, which have been isolated, include *Pseudomonas alcaligenes* and *Erwinia cypripedii*. 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. New Guinea is thought to be the center of origin for *P. cinnamomi*. We have obtained and examined soil samples from cabbage, *Casuarina*, papaya, avocado, tomato and pineapple in New Guinea. Four soil samples from *Casuarina*, avocado and tomato show outstanding biocontrol activity towards *P. cinnamomi*. We are attempting to identify the active microorganisms in these samples.

Preliminary results indicate we have devised a new molecular strategy to identify predators of *Phytophthora*. After verification, we will initiate experiments to identify *Phytophthora* predators, which cannot be isolated by normal techniques.

Biocontrol of *Phytophthora* root rot with mulches and earthworms

Earthworms reduced damage to avocado caused by *Phytophthora*. Earthworms also favored biocontrol agents such as *Trichoderma harzianum* and increased their dispersal in soil. It does not appear that earthworms destroy survival inoculum of *P. cinnamomi*, but they can reduce root infection. Mulch experimentation has been delayed by the sabbatical of Ben Faber.

Biocontrol of *Phytophthora* root rot with microorganisms

Eight biocontrol agents, including all the known commercial biocontrol agents, were tested to see if they would control avocado root rot in the greenhouse. These biocontrol agents were Promote (active ingredient *Tricoderma spp.*), Mycostop (active ingredient *Streptomyces griseoviride*), SoilGuard (active ingredient *Gliocladium virens*), Kodiak (active ingredient *Bacillus subtilis*), Agri50 (active ingredient organic colloid), Rootshield (active ingredient *Trichoderma harzianum*), Trichoderma aureoviride, and *Mucor sp.* Only Kodiak (active ingredient *Bacillus subtilis*) provided significant control of avocado root rot and none of the biocontrol agents significantly reduced spore production or damaged hyphae of *P. cinnamomi*.

New methods were designed to test biocontrol agents under field conditions. Six biocontrol agents including *Paenibacillus macerans, Bacillus subtilis, Trichoderma harzianum, Trichoderma auroviride*, *Gliocladium virens*, and *Verticillium sp.* were tested to see if they would control *P. cinnamomi* under field conditions but they were not successful. However, the method, which involves placing rooting cylinders into the ground around living avocado and treating the cylinders, has great potential for testing biocontrol agents under field conditions.

Mucor sp., Trichoderma aureoviride, Trichoderma sp., Trichoderma polysporum, Pestolotia sp., and *Hyphodontia alutaria* were tested to see what effect they had on *Phytophthora cinnamomi* chlamydospores formed inside avocado roots. *Trichoderma aureoviride* significantly reduced *P.*

cinnamomi chlamydospores in avocado roots while *Hyphodontia alutaria* eliminated them totally. The other biocontrol agents had no effect on the chlamydospores.

In a second experiment testing the effects of *Trichoderma aureoviride*, *Hyphodontia alutaria* and *Trichoderma harzianum* on *Phytophthora cinnamomi* in hyphal mats and in avocado roots. Only *Hyphodontia alutaria* reduced *Phytophthora cinnamomi* in both hyphal mats and avocado roots. *Hyphodontia alutaria* is a wood decay organism found commonly in mulch. It will not actively parasitize *Phytophthora cinnamomi*.

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. Work is underway to establish an avocado trial with the EcoSoils Bioject.

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 are now trying several novel techniques to identify and isolate the organisms responsible for suppression of *Phytophthora cinnamomi*.

Using mulch is a highly effective way to reduce populations of *Phytophthora cinnamomi*. Microorganisms associated with mulch such as *Hyphodontia alutaria* have proven to be the most effective biocontrol agents. These organisms do not attack and parasitize *Phytophthora cinnamomi* and mulch must be present for them to be effective.

It appears many biocontrol agents may be effective at reducing survival inoculum of *Phytophthora cinnamomi*. Although these organisms may not prevent *Phytophthora cinnamomi* from killing trees, they may be able to destroy the survival structures in the soil to facilitate replanting and aid control by other means.

Table 1. Effect of biocontrol agents on mycelial colonies and chlamydospores recovered from dilution of macerated avocado roots infested with *Phytophthora cinnamomi* in vitro.

Biocontrol agent	**Mycelial colonies[2] (Log10)**	**Chlamydospores[2] (Log10)**
Control	4.23 a	3.57 a
Mucor	3.75 ab	1.86 abc
Trichoderma aureoviride	1.96 c	0.80 bc
Trichoderma sp.	4.20 a	3.16 a
Trichoderma polysporum	3.15 b	1.92 ab
Pestalotia sp.	4.37 a	3.71 a
Hyphodontia alutaria	0.93 d	0.00 c

[1]Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test.
[2]Five 1-cm root pieces were macerated in water for each sample. One ml samples were counted under microscope for chlamydospore counts and plated onto PARPH plates to recover mycelial colonies.