

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

Continuing Project; Year 3 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**

We currently have a collection of the following 7 biocontrol agents which were collected by William Casale and have shown activity against *Phytophthora cinnamomi*. Fp1(*Trichoderma harzianum*, Santa Barbara CA), Fp2(*Gliocladium virens*, Santa Barbara CA), Fp16(*Verticillium sp.*, Santa Barbara, CA), Fp 20(*Pseudomonas*

*rubrisubalbicans*, Santa Barbara CA), Fp21(*Paenibacillus macerans*, Santa Barbara, CA), Fp22(*Micrococcus varians*, Santa Barbara, CA), Fp23(*Pseudomonas fluorescens*, Santa Barbara, CA). We also have the following biocontrol agents against *Phytophthora cinnamomi* collected from various sources: Fp4(*Gliocladium roseum*, wood mulch Riverside), Fp5(*Cochlonema sp.*, wood mulch Riverside), Fp6(*Volutella ciliata*, avocado soil Ventura Co.), Fp7(*Mucor sp.*, avocado soil Ventura, CA), FpB(*Gilmaniella humicola*, avocado soil Riverside, CA), Fp9(*Penicillium funiculosum*, Dr. Tsao UCR), Fp11(*Myrothecium sp.*, avocado soil UCR), Fp12(*Chaetomium sp.*, Thailand), Fp13(*Agrocybepediades*, wood mulch, UCR), Fp14(*Chaetomium globosum*, UCR), Fp15(*Trichurus sp.*, mulch Ventura Co.), Fp16(*Verticillium sp.*, Ventura Co.), Fp17(*Verticillium sp.*, Ventura Co.), Fp18(*Fusarium sp.*, Ventura CA), Fp19(*Pythium sp.*, Ventura CA).

During the past year we have added Fp25(*Phanaerochete chrysorhiza*, wood mulch, Somis CA), Fp26(*Agrocybe dura*, wood mulch Somis, CA), Fp27(*Cyathus olla*, wood mulch Somis CA), Fp28(*Ceraciomyces tessulatus*, wood mulch Somis CA), Fp29(*Chaetomium globosum*, wood mulch Somis CA). All of these have been selected for their ability to produce cellulase and glucanase which dissolves the hyphae of *P. cinnamomi*. We also have isolated Pi1(*Dothierella, sp.* dead avocado roots Somis CA), Pi2(*Cylindrocarpon radicola*, dead avocado roots Somis CA), Pi3(*Saprolegnia sp.* dead avocado roots Somis CA), Pi4(*Mucor sp.* dead avocado roots Somis). These fungi have the ability to colonize dying avocado roots.

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

In a mulch trial in soil heavily infested with *P. cinnamomi* in Somis Ca., mulch improved the growth of avocado trees on Duke 7 by 117%, trees on UC2011 by 27% and trees on Thomas rootstock by 27%. Mulch plus phosphorus acid improved growth of trees on Duke 7 rootstock by 114%, trees on UC2011 rootstock by 40% and trees on Thomas rootstock by 42% (Table 1). However, yields were not influenced as much as growth, since stressed trees often yield more than unstressed trees. Mulch improved the yield of trees on Duke 7 rootstock by 82%, trees on UC2011 by 0% and trees on Thomas rootstock by 12%. However, mulch plus phosphorus acid increased yield of trees on Duke 7 rootstock by 200%, trees on UC2011 rootstock by 116% and trees on Thomas rootstock by 0%. It appears that mulching and fungicide applications help the less resistant rootstocks such as Duke 7 and UC2011 more than the more resistant Thomas rootstock. A new MPN (most probable number) method was devised which indicates that mulching reduces the population of *Phytophthora* by about 50%.

**Fig. 1. Effect of mulch on canopy volume of avocado trees in a *Phytophthora cinnamomi*-infested field plot at Somis, CA 1997<sup>1)</sup>**

Treatment	Rootstock					
	Duke 7		UC 2011		Thomas	
	Canopy volume (cu ft)					
No mulch	58	E	89	DE	147	BC
Mulch	126	CD	113	CD	182	AB
Phosphorous acid	114	CD	108	CD	147	BC
Mulch + Phosphorous acid	124	CD	125	CD	209	A

<sup>1)</sup> Values followed by the same letters are not significantly different at P=0.05 according to Waller's k-ratio t test.

In a second trial in soil infested with *P. cinnamomi* in Somis CA, mulching did not significantly increase yields of avocados after two years. Mulch appears to slightly reduce the growth of trees. This occurs despite the fact that mulch increases root growth, increases parasitism of *Phytophthora* and reduces *Phytophthora* populations. Further evidence was obtained indicating cellulase and gluconase produced by wood decay organisms in the mulch are responsible for reducing populations of *Phytophthora*. Avocado roots grow up into the mulch where they are relatively safe from *Phytophthora*.

In the 1-year-old trial at Carpinteria CA., neither mulch nor compost had any significant effect on tree growth or health. A new trial was established at Escondido CA comparing compost and mulch in a *Phytophthora* infested avocado grove.

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

A greenhouse experiment was conducted which examined the effects of *Pseudomonas fluorescens*, *Paenibacillus macerans*, *Gliocladium roseum*, *Gliocladium viride*, *Verticillium sp.*, *Penicillium funiculosum*, *Pythium sp.* and *Bacillus subtilis* on *Phytophthora cinnamomi* and avocado root rot. None of the biocontrol agents had any effect upon avocado plant weight, root weight, % healthy roots, % infected roots, *Phytophthora* sporangia, zoospore production or hyphal survival. However, *Verticillium sp.* did increase the number of chlamydospores produced by *Phytophthora*, which indicates that it is antagonizing the *Phytophthora* hyphae. In a second set of experiments with the same biocontrol fungi, none of the organisms had any effect upon mycelial survival, zoospore production or chlamydospore production. However, both *Paenibacillus macerans* and *Verticillium sp.* reduced sporangia production compared to some of the other biocontrol agents. It appears that without some organic matter to keep the biocontrol organisms active they are not particularly effective. It also appears

that the fungal spores are not penetrating the soil effectively. However, we will run another series of experiments before we begin to discard the biocontrol agents. The most promising biocontrol agents to date are *Verticillium sp.* and *Paenibacillus macerans*.

A second series of experiments has been conducted using biocontrol microorganisms which were selected because they produced abundant cellulase and glucanase, which are known to dissolve the hyphae of *Phytophthora*. *Chaetomium globosum*, *Ceraceomyces tessulatus*, *Agrocybe dura* and *Phanaerochaete chrysorhizon*, were grown on mulch and the mulch was placed around the base of *Phytophthora* infested avocado seedlings. None of the biocontrol organisms had any significant effect upon avocado shoot or root weight, zoospore production or hyphal survival. However, *Chaetomium globosum* significantly increased the chlamydospore formation of *Phytophthora* indicating that the hyphae was being antagonized. Similarly, increased sporangia or zoospores were found with *Agrocybe dura*. It appears these biocontrol organisms are only effective when the roots grow up into the mulch.

### **Biocontrol of Phytophthora root rot using continuous application of biocontrol agents**

Using the bioreactor provided by EcoSoils Inc. we have applied the biocontrol bacterium *Pseudomonas fluorescens* to an avocado grove infested with *Phytophthora cinnamomi*. In 1996 *Bacillus subtilis* was applied. Populations of *Pseudomonas fluorescens* was consistently high in the bioreactor reaching populations of 10<sup>9</sup>/ml. Populations of *Pseudomonas fluorescens* measured at the emitters in the field were about 10<sup>4</sup>/ml. However, one day later the *P. fluorescens* populations in the soil were only 2.8 x 10<sup>2</sup>/gm. Six days later the bacterium was no longer detectable. Further studies indicated that the bacterium does not survive well in the soil and populations of more than 10<sup>7</sup>/ml must be applied to obtain a 10<sup>4</sup>/gm population after 7 days. It appears that the bioreactor will produce an abundant supply of bacteria and that it will function properly in the field. However, *Pseudomonas fluorescens* will not make a good biocontrol agent because of its short survival period in the soil. Despite the seeming ineffectiveness of the biocontrol agent, populations of *Phytophthora cinnamomi* were reduced 60% in the treated trees and the yields were increased 262% per tree. Care should be taken when interpreting this data since two different biocontrol agents had been applied, statistical tests were not done on the data, and the disease in the grove is not uniform but is moving in from one side.

### **Understanding how effective biocontrol agents can be maintained in the field**

Two diverse sequence areas were found through DNA sequencing of 16S-23S rDNA spacer regions in five closely related *Pseudomonas fluorescens* and *P. putida* biocontrol bacteria. The wild type *Pseudomonas fluorescens* biocontrol bacteria can be separated from all others by using a specific primer (5TCGCGATTAACACTCAC3') and the PCR method. We can now use this method to follow the biocontrol bacterium in the soil. Its population can be detected using the most probable number PCR method.