

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

Continuing Project; Year 2 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

Biocontrol of Phytophthora root rot with mulches

In a mulch trial in soil heavily infested with *P. cinnamomi* in Somis Ca., yardwaste mulch increased health ratings, trunk diameter and canopy volume of Thomas rootstock by 76%, 4%, and 0% respectively. Mulch increased the health ratings, trunk diameter and

canopy volume of UC2011 rootstock by 61%, 17% and 22% respectively. Mulch increased the health ratings, trunk diameter, and canopy volume of Duke 7 rootstock by 65%, 28%, and 55% respectively (see Table).

**Sprinklings Mulch Trial Avocado Tree Ratings
January 1977**

Rootstock	Rating*		Trunk Diam (mm)		Canopy Volume (cu ft)	
	Mulch	No mulch	Mulch	No mulch	Mulch	No mulch
Thomas	0.07 A	0.29 A	60.14 A	57.93 A	92.07 A	94.43 A
Duke 7	0.73 A	2.08 C	54.55 A	42.50 B	81.54 A	52.75 B
UC 2011	0.42 A	1.08 B	55.25 A	47.25 B	95.11 A	78.16 AB

*Rating 0=no damage; 5=dead

Means followed by same letters are not significantly different according to Waller's k-ratio t test.

It is clear that mulching increases growth of the least *Phytophthora-resistant* rootstocks more than the most resistant rootstocks. In a second mulching trial on *Phytophthora* infested soil in Somis, CA, mulch is beginning to enhance growth of avocados compared to the unmulched controls. A new mulch trial has been established in a grove infested with root rot in Carpinteria, CA. A fourth mulch trial will be established on a root rot site in San Diego Co. These last two sites will explore the difference between raw yardwaste mulch and composted yardwaste mulch as they affect growth and root rot of avocado.

Leaf analysis of mulched and unmulched avocados indicates that the mulches significantly reduce Mg and Mn concentrations in the leaves but enhance the K levels in the leaves. P, Ca, Na, Zn, and Cu levels were unaffected by mulch treatments.

Mulches in young avocado groves can reduce water use by as much as 50%. Mulches enhance the total microorganism population more than 7-fold. The total fungal population is increased 541% by mulch at the soil surface and by 213% at a depth of 7.5 cm in the soil. Microbial activity is increased 867% by mulch at the soil surface and by 1500% at a depth of 7.5 cm in the soil. Cellulase and gluconase are two of the enzymes that degrade the hyphae of *Phytophthora cinnamomi*. Cellulase activity is increased 586% by mulch at the soil surface but is not increased at a depth of 7.5cm in the soil. Gluconase is increased 11,000% by mulch at the soil surface and by 30% at a depth of 7.5cm in the soil Actual hyphae of *Phytophthora cinnamomi* is degraded 6400% more readily on the mulched soil surface than on the unmulched soil surface. Hyphae of *P. cinnamomi* are not degraded more readily on mulched soil than on unmulched soil at a depth of 7.5cm. Inoculum potentials of *P. cinnamomi* are reduced by 40% by mulches at the soil surface and by 30% at a depth of 7.5cm. As a result avocado roots are increased 6763% by mulch at the soil surface but are actually reduced by 53% by mulches at a depth of 7.5cm in the soil. Through all soil depths examined avocado roots were increased 35% by mulching. It appears that microbial activity, cellulase activity and gluconase activity at the soil surface under mulches reduces *Phytophthora* populations and enhances avocado root growth. This effect is far less noticeable at a depth of 7.5cm in the soil. Cellulase produce By *Penicillium funiculosum* at IOunits per ml completely eliminated sporangial production and zoospore

encystment by *Phytophthora cinnamomi*. Cellulase produced by *Trichoderma harzianum* increased production of sporangia by *P. cinnamomi*. It is evident that all cellulases are not alike and some organisms produce cellulase which is far more toxic to *Phytophthora* than others.

Infection of avocado seedlings by *P. cinnamomi* was significantly decreased by gypsum amendments. Soil nitrogen was not altered by gypsum treatments. The pH of the soil was raised only slightly by the application of gypsum. Populations of soil microorganisms and soil microbial activity was unaffected by the addition of gypsum. Root and total seedling weight in soil not infested by *P. cinnamomi* was not increased by gypsum amendments. The amounts of avocado root exudates and the resistance of avocado to *Phytophthora* root rot were also not affected by gypsum treatments. However, sporangial production by *Phytophthora* and the length and width of sporangia were significantly reduced by gypsum amendments. Soil extracts made from gypsum amended soils also reduced *Phytophthora* sporangial numbers and sporangial size. Irrigation with gypsum solutions also reduced production of *Phytophthora* sporangial numbers and sporangial size. *Phytophthora* zoospore production was also reduced in soil amended with gypsum. However, gypsum did not affect zoospore movement or encystment in the soil. Additions of drywall gypsum caused the soil to drain more rapidly, while fine-grade gypsum caused the soil to drain more slowly. Since both gypsum products reduced *Phytophthora* root rot, it appears that the effect of gypsum on soil permeability is not the major factor responsible for the beneficial affects of gypsum on reducing root rot of avocado.

Biocontrol of *Phytophthora* root rot of avocado with microorganisms

We currently have a collection of the following 21 biocontrol organism which have shown activity against *Phytophthora cinnamomi*: Fp1(*Trichoderma harzianum*, Santa Barbara CA), Fp2(*Gliocladium virens*, Santa Barbara CA), Fp4(*Gliocladium roseum*, wood mulch Riverside, CA), Fp5(*Cochlonema sp.*, wood mulch Riverside, CA), Fp6(*Volutella ciliata*, avocado soil Ventura CA), Fp7(*Mucor sp.*, avocado soil Ventura CA), Fp8(*Gilmaniella humicola*, avocado soil Riverside CA), Fp9(*Penicillium funiculosum*, Dr. Tsao UCR), Fp11(*Myrothecium sp.*, avocado soil UCR), Fp12(*Chaetomium sp.* Thialand), Fp13(*Agrocybe pediades*, wood mulch UCR), Fp14(*Chaetomium globosum*, UCR), Fp15(*Trichurus sp.*, mulch Ventura CA), Fp16(*Verticillium sp.*, Santa Barbara CA), Fp17(*Verticillium sp.*, Ventura CA.), Fp18(*Verticillium sp.*, Ventura CA), Fp18(*Fusarium sp.*, Ventura CA), Fp19(*Pythium sp.*, Ventura CA), Fp20(*Pseudomonas rubrisubalbicans*, Santa Barbara CA), Fp21(*Paenibacillus macerans*, Santa Barbara CA), Fp22(*Microccus varians*, Santa Barbara CA), Fp23(*Pseudomonas fluorescens*. Santa Barbara CA). These organisms are undergoing intensive screening in the greenhouse and laboratory in order to select the best isolates to try under field conditions. We are continuing to collect new biocontrol microorganisms by using *P. cinnamomi* as a bait in avocado soils. We are also collecting and testing a variety of commercial biocontrol microorganisms.

Bioenhanced yardwaste (a mulch colonized with biocontrol agents) has shown an ability to significantly reduce or suppress populations of *Phytophthora cinnamomi*, resulting in decreased root rot of avocado trees. Organic matter from the bioenhanced mulches trapped zoospores of *P. cinnamomi* and significantly reduced root infection of avocado.

Bioenhanced mulches actually increased sporangial production, but 29% of the sporangia aborted before zoospore release. *Phytophthora cinnamomi* hyphae were lysed by the bioenhanced mulches, but a high degree of lysis occurred in control treatments as well. Yardwaste infested with *Gliocladium virens* was found to contain significantly higher levels of carbon dioxide than control treatments. High levels of carbon dioxide are known to induce dormancy in *Phytophthora*. All mulch treatments increase microbial activity in the soil and reduced the survivability of *P. cinnamomi* zoospores.

In a 3-year-old field plot at Somis CA in soil heavily infested with *P. cinnamomi*, no significant differences were found in the growth of avocado trees treated with *Pseudomonas rubrisubablicans*, *Microccus variens*, *Pseudomonas fluorescens* or *Paenibascillus* and control trees without biocontrol agents. However, all bacterial treatments increased the size of the trees over untreated trees and trees with mulches and when taken together as a group the trees treated with biocontrol treatments were significantly larger than non-treated trees. The greatest growth of avocado trees occurred when mulch, *Gliocladium virens* and *Paenibascillus macerans* were used together. This treatment resulted in avocado trees that were 27% larger than untreated controls.

Two field plots in mature avocado groves with root rot were established in the Escondido area in 1995. In these field plots the bacterial biocontrol agent *Bacillus subtilis* was applied continuously in the irrigation water to these groves. The bacteria were grown in field fermentation tanks (Ecosoils Systems Inc.) in the field and automatically injected in the water at every irrigation. Newer, more sophisticated fermenters will be installed for 1996 and the biocontrol organism which will be injected will be *Pseudomonas fluorescens*. The injection of the biocontrol agents did not significantly enhance yield during the first year. However, the biocontrol bacteria did significantly increase P, S, K, Mn, Fe, Cu and Zn and reduce B in the leaves of one grove. In the other grove the biocontrol applications increased Fe concentrations in the leaves but decreased Mn concentrations. This technology will also be studied in detail in the greenhouse. It has tremendous potential for delivering biocontrol agents in the field. A third plot is planned for the Somis area in Ventura Co.