1999 California Avocado Research Symposium pages 65-68
California Avocado Society and University of California, Riverside

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

We have discarded a number of biocontrol agents this year which did not perform well in greenhouse tests. We currently have a collection of the following which have shown activity against *Phytophthora cinnamomi. Fp1(Trichoderma harzianum,* Santa Barbara CA), *Fp2(Gliocladium virens,* Santa Barbara CA), Fp1*6(Verticillium sp.,* Santa Barbara, CA), *Fp21(Paenibacillus macerans,* Santa Barbara, CA), *Fp9(Penicillium funiculosum,* Dr. Tsao *UCK),(Verticillium sp.,* Ventura Co.), *Fp17(Verticillium sp.,* Ventura *Co.),.Fp25(Phanaerochete chrysorhiza,* wood mulch, Somis CA), *Fp28(Ceraciomyces tessulatus,* wood mulch Somis CA), We also have isolated *Pil(Dothierella, sp.* dead avocado roots Somis CA), *Pi2(Cy/indrocarpon radicola,* dead avocado roots Somis CA), *Pi3(Saprolegnia sp.* dead avocado roots Somis CA), *Pi4(Mucor sp.* dead avocado roots Somis), *Pi5(Circinella spinosa* dead avocado roots Escondido CA), Pi6 *(Trichoderma sp,* dead avocado roots, Escondido CA), and Pi7 *(Trichoderma aureoviride,* dead avocado roots, Somis CA). These fungi have the ability to colonize dying avocado roots.

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

Mulching increased tree canopy volume in only one of the four avocado trials. In the Sprinkling trial mulch increased the canopy volume of trees on Duke 7 rootstock by 89%. In this same trial, mulch did not significantly increase the canopy size of avocados on the more Phytophthora resistant rootstocks UC2011 and Thomas. In the Vetter trial mulch reduced canopy volume by 27%, however, in this same trial composted mulch did not reduce canopy size. In the other two trials mulch had no significant affect on canopy volume, although in earlier years mulch appeared to reduce canopy volume in the Vanoni trial. Mulching increased trunk diameters in only one of the four avocado trials. In the Sprinkling trial mulch increased the trunk diameters of trees on Duke 7 rootstock by 38% and the diameters of trees on UC 2011 by 15% . The diameters of trees on the more Phytophthora resistant Thomas rootstock were not affected by mulch applications. In the other three trials mulch had no effect on trunk diameters. Mulching improved visual tree ratings in two of the four avocado trials. In the Vetter trial in 1998, compost but not mulch significantly improved visual appearance of the trees. In the Sprinkling trial, mulch significantly improved the visual appearance of trees on Duke 7 and UC 2011 rootstocks but not on the more resistant Thomas rootstock. In the two avocado plots in which yield was taken, mulch increased yield in one and decreased yield in the other. In the Vanoni trial, mulch reduced yield by 25%. In the Sprinkling trial, mulch significantly increased yield of trees by 61% when all rootstocks were combined. Yield was increased more on Duke 7 rootstock than the other more resistant rootstocks, but was not significantly increased by mulches for any of the rootstocks alone. Mulches did not affect overall fruit size in the Vanoni trial, however, in 1996 the mulched trees had smaller fruit while in 1998 the mulched trees had significantly larger fruit. In the Sprinkling trial mulches did not affect fruit size. Where mulches increased the growth, appearance or yield of avocado it is believed that mulches reduce avocado root rot and improve the ability of avocado to grow in the presence of the disease. The rootstocks less tolerant to root rot responded more favorably to mulching than did the more resistant rootstocks. In the Sprinkling trial, the mulches compared favorably to applications of the fungicide Aliette, which is used to control avocado root rot, and the best treatments were mulches combined with Aliette. Where mulches reduced growth and yields of avocado it is believed that the mulches prevented the soil from drying out. Avocado root rot is favored by wet soils. At the Sprinkling ranch, irrigation was monitored by tensiometer so that the trees were only watered when the soil became dry. In the Vanoni trial, trees were watered every two weeks by the calendar and this apparently kept the soil too wet and overcame the inhibitory effects of the mulch on avocado root rot. As the trees got older and used more water, the trees were no longer too wet and the mulches no longer inhibited growth or yield.

Mulch significantly increased nitrogen concentrations in avocado leaves in one of the three avocado trials tested. In the remaining two trials, concentrations of nitrogen in avocado leaves were unaffected by mulch. Phosphorous concentrations in avocado leaves were unaffected by mulches in the three avocado trials tested. Potassium concentrations in avocado leaves were increased by mulch applications in two of the three avocado trials tested. In the remaining trial, potassium concentrations were unaffected by mulch. Calcium concentrations in avocado leaves were unaffected by mulches in the three avocado trials tested. Magnesium concentrations in avocado leaves were reduced by mulch applications in one of the three avocado trials tested. In the other two avocado trials, magnesium concentrations in the avocado leaves were unaffected by mulch. Zinc concentrations in avocado leaves were increased by mulch applications in one of the three avocado trials tested. In the other two avocado trials, zinc concentrations in the avocado leaves were unaffected by mulch. Manganese concentrations in avocado leaves were unaffected by mulch applications in all three of the avocado trials tested. Copper concentrations in avocado leaves were unaffected by mulch applications in all three of the avocado trials tested. Iron concentrations in avocado leaves were unaffected by mulch applications in all three of the avocado trials tested. It appears that many soil nutrients are available to avocado from the mulches. Whether they are increased or decreased in plant tissue depends upon whether the nutrients are abundant or deficient in any given soil and also upon the normal soil chemistry and competition among nutrients that is present in all soils. The nutrients which are most deficient in California avocado soils and therefore need to be supplied, are nitrogen, zinc, manganese and copper. Nitrogen and zinc were shown to be supplied by mulch to avocados. No toxic nutrients like sodium or boron were increased in avocado tissues by the application of mulch.

Root length of avocado in the top 15cm of soil was significantly increased by mulch applications in both of the trials where it was measured. Root length was increased by 184% in the Sprinkling avocado trial and by 43% in the Vanoni avocado trial. Root length was measured at several depths under mulched and unmulched trees at the Vanoni trial. Mulches were found to change the depth of rooting in avocado trees. Roots were found to grow into the mulch and significantly fewer avocado roots were found at 7.5 and 15cm in the soil below the mulch than at the interface with the mulch. In unmulched trees the greatest number of roots formed at 7.5cm in the soil. When soil was taken from beneath mulched and unmulched trees in the Sprinkling trial and used as potting soil for Topa Topa avocado seedlings in the greenhouse, soil from mulched trees resulted in nearly a 7-fold increase in healthy roots and 98% increase in root length compared to soil from unmulched trees. These large growth increases of avocado roots in mulched soil are attributed to the fact that avocado roots will grow prolifically in the mulched layers of the soil and that these layers are inhibitory to avocado root rot.

Phytophthora populations were unaffected by mulch applications in the two avocado trials tested. When soil from underneath mulched and unmulched trees in the Sprinkling trial were used as potting soil for Topa Topa avocado seedlings in the greenhouse, the soil from mulched trees had no significant effect on the amount of root infection despite the fact that there were many more roots produced in the mulched soil. However, when root infections were measured at different depths under mulched and unmulched trees in the Vanoni trial, it was found that root infections caused by *Phytophthora* were very low in the mulch and at the mulch-soil interface, but significantly higher in the soil under the mulch. Similarly *Phytophthora* populations were found to be low in the mulch and at the mulch-soil interface but significantly higher in the soil whether it was mulched or unmulched soil. Finally when mycelium of *Phytophthora cinnamomi* was buried at different levels in the mulch, it could be shown that zoospore production at the mulch-soil interface was lower than at some depths lower in the soil. Hyphal lysis or death *of Phytophthora* hyphae was at a maximum at the mulch-soil interface and it was significantly higher than lysis in the unmulched soil. Parasitism *of Phytophthora* hyphae also reached its maximum at the mulch-soil interface.

Biocontrol of Phytophthora root rot with microorganisms

A greenhouse experiment was conducted which examined the effects of *Verticillium sp, Gliocladium virens, Gliocladium roseum, Penicillium funiculosum* and *Trichoderma harzanianum* on avocado root rot. In this experiment both the microorganisms and the spent media they were growing in were added to the avocado trees infected with *Phytophthora cinnamomi.* None of the biocontrol agents affected shoot weight, root weight or the % healthy roots of the avocados. However, the *Verticillium sp.* significantly reduced root infection by *Phytophthora cinnamomi.* This verified earlier greenhouse experiments that *Verticillium sp.* has activity against *Phytophthora.*

A second series of greenhouse experiments has been conducted using *Pseudomonas putida, Bacillus subtilis, and Paenibacillus macerans* as biocontrol agents which were added with their spent media. Again there were no effects of any biocontrol agent on shoot or root weight of avocado. However, *Bacillus subtilis, and Paenibacillus macerans* reduced root infection by *Phytophthora cinnamomi.* These bacteria will be examined carefully in future tests.

In an effort to find microorganisms which reduce inoculum *of Phytophthora cinnamomi* we have isolated microorganisms from roots which have been killed by *Phytophthora cinnamomi.* These organisms, *Circinela spines, Saprolegnia sp., Mucor sp. and Trichoderma aureoviride,* were inoculated onto avocado roots which contained chlamydospores (survival spores) *of Phytophthora cinnamomi.* Only *Trichoderma aureoviride* significantly damaged the chlamydospores. It reduced the number of viable chlamydospores by 90%.

Biocontrol of Phytophthora root rot using continuous application of biocontrol agents

We are currently setting up a new field trial to test the effect of the EcoSoils Bioject on young trees in a root rot infested area.

Understanding how effective biocontrol agents can be maintained in the field

Phytophthora root rot of avocado continues to be a serious problem affecting the

California avocado industry. Management techniques include the use of root rot resistant root stocks, chemical control, mulching, and application of biocontrol agents. Optimization of these strategies is presently limited by the poor understanding of the soil ecology *of Phytophthora* and the inability to monitor changes in the microflora that colonize plant roots and that help to maintain conditions that are suppressive to the disease. In particular mulching is thought to play a role in suppression of root rot by causing a general suppression due to production of cellulases and general antagonism of the pathogen. Changes in root exudate secretion are also thought to influence the pathogen, and may be influenced by plant nutrition, root stock type, and mulching.

In ongoing research, we are currently investigating repeated application of disease suppressive bacteria to control avocado root rot. A large, 3 year experiment was also recently completed in which we examined the interactions of plant root stocks, mulching and chemical control *of Phytophthora.* However, to better understand how all of the different factors that are involved in suppression of root rot it is necessary to begin examining microbial community effects in the rhizosphere and develop diagnostic methods for prediction of soils that are conducive or suppressive to the disease. Thus new research conducted initiated this past year examined the use of genetic fingerprinting techniques to characterize microbial community structure and species composition in the rhizosphere of healthy and infected avocado roots.

To generate the microbial community fingerprints, root tips were collected from healthy and infected trees that had been treated with repeated applications of the biocontrol bacterium, *Pseudomonas putida,* using the BioJect system developed by EcoSoils, Inc. Six 1 cm root tip samples were taken from 4 healthy and 4 infected trees, and were split longitudinally. One half of each root tip was placed on a selective agar medium for detection of *Phytophthora* root rot. The other halves were frozen until the plate assay was completed. Following determination of which roots were infected, 8 healthy and 8 infected root tips, matching those that were analyzed on the agar plates, were extracted for bacterial DNA associated with the root surface and adhering rhizosphere soil. The bacterial DNA was then subjected to PCR amplification using primers for 16S rDNA. This particular sequence of DNA enables taxonomic classification of all bacteria and can be used to ascertain the identity and relative abundance of different bacteria associated with plant roots.

Results shown in Figure 1 include an electrophoresis gel which reveals the microbial community structure associated with healthy and infected roots. The banding pattern in each lane of the gel resembles a bar code in which each bar or band represents a different species or group of bacteria. The intensity of the band corresponds to the relative abundance of each bacterial group or species. These image data were then subjected to image analysis to produce a line graph that can be more easily inspected (Figure 2). The band peaks from this line graph were then analyzed using a statistical procedure called principal components analysis that plots the communities in relation to their overall similarities.

As shown in the ordination diagram in Figure 3, the points representing the communities associated with healthy roots are clustered in a tight group, whereas the points for the microbial communities from infected roots are scattered and cluster in a different area than that of the healthy roots. The interpretation of this finding is that healthy avocado roots have a distinct bacterial microflora that is consistent among samples taken from different trees. When the roots become infected, however, the microbial communities break out into a chaotic, largely unpredictable structure that is comprised of diverse, opportunistic bacteria that are not normally part of the avocado rhizosphere.

In addition to providing insight into the microbial community structure associated with healthy and infected roots, this novel genetic fingerprinting technique has the potential to improve our understanding of the survival and activity of plant beneficial microorganisms in the rhizosphere and how these bacteria are influenced by various cultural practices such as mulching, root stocks, plant nutrition, and chemical control. A primary question at this point is the degree to which a healthy community confers stability and resistance to avocado root rot. It can be hypothesized that certain communities may confer resistance to Phytophthora by suppressing the pathogen through competition or antibiosis. The finding that healthy roots have a stable, consistent community also suggest that new statistical methods for pattern recognition may eventually permit diagnostic analysis of soil health.

During the next year, we will continue biocontrol treatments using repeated applications of disease suppressive bacteria to evaluate the usefulness of biocontrol as an approach for suppression of avocado root rot. As part of these experiments, we will also continue to explore the use of the genetic fingerprinting methods for evaluation of treatment effects on Phytophthora and the microbial communities that are associated with healthy and infected roots.