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Biocontrol of Phytophthora Root Rot of Avocado 2004

Continuing Project: Year 2 of 3

Project leader: John A. Menge (951-787-4130), e-mail John. Menge@UCR.edu
Department of Plant Pathology. UC Riverside
Cooperating personnel: D. Crowley, M. Crowley, E. Pond, J. Borneman, B. Mckee, B. Faber, G. Bender, P. Mauk, J. Downer, P. Clark, V. McDonald, Bob Tombe

Benefit to the Industry

Biocontrol may provide an effective, long-term, non-chemical, environmentally acceptable method of controlling Phytophthora root rot of avocado through a combination of cultural methods and application of selected microorganisms. This approach will probably be most effective as part of an integrated system of resistant rootstocks, sanitation and cultural methods.

Objectives

1. Identify new biocontrol agents using conventional and new molecular biology methods.
2. Evaluate new biocontrol agents in the greenhouse and field.

Summary

Identification and Isolation of new biocontrol agents for suppression of Phytophthora root rot

1. Survey for suppressive soils in California

Two criteria are used to identify a suppressive soil: 1) It is a soil which degrades *P. cinnamomi* hyphae or chlamydospores, or 2) one which has high populations of *Phytophthora* but the trees continue to thrive. Thirty 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. These cultures have not survived in laboratory cultures. Efforts are still being made to recover them.

We are utilizing several experimental approaches to extract biological control agents from local suppressive soils. One of them is to identify bacteria and fungi from soil mixtures with varying degrees of suppressiveness. We postulate that the microorganisms involved in the suppressiveness will be discovered by identifying species whose population levels correlate positively with the suppressiveness.

An avocado soil that is microbially suppressive to *Phytophthora cinnamomi* was identified in 2001 in Somis, CA. The suppression was proven to be microbial by a greenhouse test in which part of the soil was autoclaved which caused it to lose its suppression. This soil was then subjected to a fumigation gradient in which decreasing amounts of natural soil from the Somis grove were mixed with fumigated Somis soil. The results showed that the suppressive effect was able to be transferred to conducive soil in amounts as small as 1% natural to 99% fumigated soil, with seedlings planted in this soil as healthy as the nontreated control.

The Somis soil was also subjected to a temperature gradient in which soil samples were treated at 21, 45, 60, 75 and 90 degrees C, then planted with avocado seedlings and inoculated with *P. cinnamomi*. This showed a gradual elimination of the suppressive effect in the soils with increasingly higher temperatures. In order to try to bait the suppressive organisms from this soil, *P. cinnamomi* hyphal mats were buried for eight days in the soil samples taken from each pot after takedown. After eight days, these mats were plated on various selective media to isolate for fungi and bacteria that may be antagonistic to *P. cinnamomi*. The fungi identified by cultural methods include *Filobasidium, Verticillium, Trichoderma* and *Fusarium.*

Filobasidium has no known biological control role. *Verticillium, Trichoderma* and *Fusarium* have been shown to be antagonistic to *P. cinnamomi* in other studies but their presence did not correlate with the suppressiveness of the Somis soil.

The Somis soil has been evaluated over time to see how persistent the suppressive effect is. Soil samples were taken four times per year over a two year period (2001-2002) and evaluated for their effect on hyphal and chlamydospore degradation. The first year seemed to show a seasonal variation in the suppressive effect, with the highest activity in the summer and the lowest in the winter. However, the second year evaluation of the suppressive effect did not show the same seasonal variation. These results indicated that the suppressive effect was transient and not predictable based on the seasons.

Two hypotheses put forth to explain the above variability of suppression in 2002 look at the effect of moisture on the antagonistic microorganisms in the suppressive soil. First, the variable suppression in 2002 could be due to sampling at different times after irrigation (i.e. the suppressive organisms need a certain duration of moisture to build up their numbers in the soil). This hypothesis was tested with Somis soil at field capacity for different lengths of time before burying hyphal mats. The results from this study showed no difference in chlamydospore or hyphal degradation due to the duration of moisture in the soil.

The second hypothesis states that the variable suppression in 2002 is due to sampling at different moisture levels (i.e. the suppressive organisms need a certain amount of moisture to build up their numbers.) A preliminary experiment comparing the effects of soil matric potential at 0 mbar and -10 mbar showed a dramatic antagonist effect at -10 mbar but none at 0 mbar. This experiment was then expanded to evaluate the effects of matric potential at 0, 5, 10 and 15 mbar (Table 1). This showed a gradual increase in the antagonistic effect from 0 to 15 mbar, with decreasing numbers of chlamydospores and increasing hyphal mat degradation, with almost total degradation at 15 mbar.

Table 1. Effect of matric potential of Somis suppressive soil on *P. cinnamomi* total chlamydospore numbers and mat degradation, Nov. 2003.[1]

Moisture Level (mbars)	Total Chlamydospores	Mat Rating 0-5 (0=healthy)
0	22750 a	0.33 d
-5	17450 bcd	1.33 c
-10	12000 ef	2.83 b
-15	10000 f	3.83 a

[1]Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test.

From subsequent laboratory experiments, this antagonistic effect at -15 mbar is present all the way down to -40 mbar. Further testing below -40 mbar is in progress.

In addition, an experiment evaluating disturbance of the Somis soil on the antagonistic effect was carried out. The antagonistic effect of sieved versus intact Somis soil cores on *P. cinnamomi* hyphal mats was compared both in the lab and in the field. The soil samples in the lab were kept at a constant matric potential of -40 mbar. The highest antagonistic activity was noted in the sieved soil cores kept in the lab.

Part of each *P. cinnamomi* hyphal mat is also being evaluated by novel molecular techniques through identification of fungi and bacteria by rDNA analysis. We postulate that the antagonistic microorganisms will most likely be found on or near the *P. cinnamomi* hyphal mat. The preliminary molecular data has identified *Fusarium* and *Tritirachium* as close matches to the rDNA from the mats. As mentioned above, *Fusarium* did not correlate with suppression in the temperature gradient soil. *Tritirachium* has no known biological control role.

To summarize, each of the above experiments consists of suppressive and non-suppressive treatments. The strategy to identify antagonistic microorganisms from these samples will utilize rDNA fingerprinting. Each experiment will have the suppressive microorganisms identified and contrasted with the non-suppressive microorganisms. Then, the microorganisms from each experiment will be correlated with the microorganisms identified from every other experiment. From this comparison and contrast of the microorganisms from all of the experiments, a pattern should be apparent between what is present in the suppressive treatments versus what is present in the non-suppressive treatments (Table 2).

Table 2. Strategy for identifying suppressive microorganisms via rDNA fingerprinting.

	Experiment	**Suppressive**	**Non-suppressive**
1.	**Fumigation Gradient 2001**	Natural 1% Fumi 99%	Fumi 100%
2.	**Temperature Gradient 2002**	21 C	90 C
3.	**Moisture Gradient 2004**	-10 to -15 mbar	0 to -5 mbar
4.	**Soil Cores 2004**	Lab Sieved (-40 mbar)	Lab Intact (-40 mbar)

2. Foreign exploration

We are continuing to import potential biocontrol agents from Papua New Guinea. Recent samples have not contained organisms which provide good soil suppressiveness. Political unrest in Papua New Guinea has limited our sampling procedure. However, there are still plenty of accessible sites from which to gather samples. From earlier samples, we have identified two bacteria – *Pseudomonas constantinii* and *Pseudomonas tolaasii* from New Guinea which are excellent biocontrol agents of *Phytophthora cinnamomi.* They are effective at low numbers and colonize the hyphae of *Phytophthora.* However, they do best at temperatures above 24C, which is warmer than most soil temperatures in our avocado groves. Greenhouse trials are pending USDA permits to test in our quarantine facility.

Evaluation of biocontrol agents

A greenhouse experiment was done with a commercial biocontrol agent and a *Bacillus sp*. that had been isolated from field soil where an epidemic of *P. cinnamomi* had passed through. In the zone of the epidemic where the soil was collected, the trees were dying or were dead, with very little *Phytophthora* recoverable. The soil from this zone was shown to be very suppressive to *P. cinnamomi*. The *Bacillus sp.* isolated from this soil was tested as a soil drench applied at regular intervals for three months. No significant suppressive effect was noted in the commercial biocontrol agent tested or in the *Bacillus* treatment compared to the nontreated control.

Conclusions

Further progress has been made in isolating and identifying biocontrol agents of *P. cinnamomi*. The molecular identification of the microorganisms in the suppressive Somis soil, which is the culmination of four years' worth of work, has begun and should be done in six months. *Pseudomonas constantinii* and *P. tolaasii* have been identified from New Guinea soil as excellent biocontrol agents of *P. cinnamomi*. Further evaluation of these organisms will begin pending receipt of USDA permits. Greenhouse evaluation of other potential biocontrol agents, including from commercial sources, has been unsuccessful but is ongoing.