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Analysis of Suppressive Soils and Development of Biological Control Methods for Phytophthora Root Rot of Avocado

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Introduction

Phytophthora root rot (PRR) of avocado *(Persea americana),* caused by *Phytophthora cinnamomi,* is a serious problem in California despite current approaches to disease control that include chemical fungicides, resistant rootstocks, and cultural methods. The current trend toward increasing restrictions on the use of chemical pesticides threatens to eliminate any benefits these materials may provide. This research project was initiated in response to the need for effective alternative approaches to control PRR that will shift the emphasis away from chemical fungicides. Our approach was to identify sites in which replants or older avocado trees were growing well despite the presence of *P. cinnamomi* in these or neighboring groves. Soils from these suspected "PRR-suppressive" sites were investigated as possible sources for biological control agents. Some of these soils have suppressed PRR in greenhouse experiments. Microorganisms isolated from these soils have reduced disease of test plants caused by *P. cinnamomi* in our greenhouse tests.

Identification of Phytophthora root rot (PRR)-suppressive soils

Dr. John Menge collaborated with us in surveying California avocado groves to identify sites which fit our criterion for disease suppressiveness; *i.e.,* soils in which *P. cinnamomi* is known or believed to be present, but on which trees are not dying and are growing well. We purposely used a liberal criterion for tentatively identifying soils as "PRR-suppressive" to reduce the chance of ignoring a soil that may be useful at yielding biological control agents. Soils have been collected from seven of these sites in Santa Barbara County (C1-7) and six sites in San Diego County (soils S1-4, S6, & S7). One additional soil from San Diego County (soil S5) with a history of being very conducive to PRR was included in some experiments for comparison.

Initial screenings of avocado grove soils were done in greenhouse flats with *Persea indica* as a test plant. *P. indica* is a relative of avocado which is extremely susceptible to *P. cinnamomi* and is also more convenient to grow than avocado. Soils were infested with ground millet seed cultures of *P. cinnamomi.* We were able to isolate *P. cinnamomi* in only one soil (S6) prior to experimentally introducing the pathogen. The greatest number of dead plants occurred in soils S4, S6, and S7; and survivors in these soils, in general, grew poorly. Two other soils, S1 and S2, had few dead plants, but the survivors were stunted or chlorotic. All these soils were considered to be conducive to PRR under greenhouse conditions. Of the San Diego County soils tested, only soil S3 indicated suppression of disease by having few dead plants and vigorous survivors. None of the Santa Barbara County soils had the high numbers of dead plants seen in the most conducive San Diego soils. However, under our greenhouse conditions many plants which were obviously not growing well (or at all) still remained alive. Soils C2, C4, and C5 were the most suppressive based on shoot growth and the percentage of roots visibly rotted.

We have begun testing the most promising soils using avocado variety Topa-Topa. Soils S5, C2, C4, and C5 were included in the first of these experiments (which has been repeated). We also included *sterilized* soil C4 to indicate the importance of biological factors *(i.e.,* soil microorganisms) in the suppression of disease. Six week-old Topa-Topa plants were transplanted into test soils infested with ground millet seed cultures of *P. cinnamomi* at one of four inoculum levels: 1) no *P. cinnamomi,* 2) 0.02 g inoculum/L soil, 3) 0.2 g inoculum/L soil, or 4) 1.0 g inoculum/L soil.

As expected, plant growth decreased with the severity of root rot. Plants in soil S5 ("conducive") grew the least and had the most severe root rot. Even at the 0 inoculum level root rot occurred, indicating the presence of *P. cinnamomi* in the field soil prior to experimental infestation. Soils C4 and C5 showed the greatest suppression of root rot, even at fairly high levels of *P. cinnamomi.* Soil C2 showed moderate suppression of disease. A comparison of plant growth in soil C4 and autoclaved C4 suggests that there is suppression of disease that is eliminated by autoclaving, presumably due to biological factors. This result made us optimistic about isolating potential biological control agents.

Determination of the physical and chemical characteristics of the test soils was delayed due to problems obtaining the necessary soil analysis services. Preliminary information, however, indicates a higher than usual amount of organic matter for the suspected-suppressive soils (this is consistent with observations of *P. cinnamomi*-suppressive soils in Australia). However, all soils with high organic matter were not necessarily suppressive.

Isolation of microorganisms associated with PRR-suppressiveness

Approximately 150 fungi and bacteria have been isolated from seven test soils so far. Since antibiotic production has been implicated in biocontrol of other plant pathogens, isolated microorganisms were screened for antibiosis against *P. cinnamomi.* The preliminary screening for antibiosis was as follows: *P. cinnamomi* and one of the test organisms were placed on opposite sides of agar growth medium in a petri dish. As the two organisms grew together, antibiosis was indicated by a zone of inhibited growth of *P. cinnamomi* in the vicinity of the test organism. To date, 26 microorganisms have demonstrated the ability to inhibit the growth of *P. cinnamomi* in culture. In some cases, inhibition of growth was accompanied by disruption of the hyphae of *P. cinnamomi.*

Effective biological control may require the application of two or more microorganisms together, and obviously antagonism among biological control agents would be detrimental. Fortunately, the microorganisms isolated so far that show antibiosis against *P. cinnamomi* do not, in general, show antibiosis against each other. This apparent specificity of antibiotics for *P. cinnamomi* is not completely unexpected due to the rather distant evolutionary relatedness of *Phytophthora* with other fungi and bacteria.

One of the bacteria we have isolated grows very well along the hyphae of *P. cinnamomi.* Inoculation at one point of a colony of *P. cinnamomi* results in complete colonization of the *P. cinnamomi* mycelium and inhibition of further growth of the fungus.

A genus of fungi isolated in six out of seven soils is *Trichoderma. Trichoderma* spp. have had some experimental success as biological control agents against other plant pathogens. The *Trichoderma* isolates from avocado rhizosphere soils have shown coiling around hyphae *of P. cinnamomi,* which suggests parasitism of *P. cinnamomi* by *Trichoderma.* Some of the-*Trichoderma* isolates also demonstrate antibiosis. We have isolated *P. cinnamomi* in only one of the seven avocado rhizosphere soils, although surrounding soil is believed to be infested with the fungus. Of possible importance is that *Trichoderma* was not detected in the soil from which *P. cinnamomi* was isolated.

The isolated microorganisms were tested in greenhouse experiments for biological control of PRR. Young *Persea indica* plants were transplanted into soil infested with the test fungi or had their roots dipped in a suspension of test bacteria prior to transplanting. A suspension of *P. cinnamomi* was then added to the soil and the plants observed for symptoms of disease caused by the pathogen. *P. indica* is extremely susceptible to *P. cinnamomi* and as such provides a stringent test for the potential biological control agents. To date, treatments with 15 different fungi and bacteria have reduced disease of *P. indica* caused by *P. cinnamomi* in these experiments. Isolation and testing of microorganisms from avocado grove soils are continuing.

Summary

Several California avocado grove soils that we tentatively identified as PRR-suppressive (based on field observations) have provided evidence of suppressing disease by *P. cinnamomi* in greenhouse experiments. The ability to conduct experiments in the greenhouse will facilitate our analysis of these soils to identify factors contributing to disease suppression. For at least one of these soils, a biological component appears to play a major role in the disease suppression. We have not, however, ruled out other possible contributing factors. Some microorganisms isolated from the avocado grove soils have reduced disease of test plants under greenhouse conditions. We are testing the most promising of these potential biological control agents more extensively, under a variety of environmental conditions and in various combinations.