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Beneficial Microorganisms for Biocontrol of Phytophthora Root Rot

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Restrictions on the use of chemical pesticides have been increasing and will undoubtedly continue to do so in the foreseeable future. Growers are being challenged to maintain plant health with reduced input from agricultural chemicals. Disease management through biocontrol may be part of an effective response to this challenge. By biocontrol, I mean the suppression of a pathogen's (disease-causing organism's) population or its ability to cause disease through the activity of another organism. At UCR, in a collaborative effort between my laboratory and Dr. John Menge's research group, we are investigating the use of beneficial soil microorganisms in biocontrol of Phytophthora root rot (PRR) of avocado caused by *Phytophthora cinnamomi.* The discussion that follows will deal with microbial suppression in soil, although many points may also apply to non-soil environments.

Why do we believe biocontrol with beneficial microorganisms is possible? The suppression of soil-borne plant pathogenic microorganisms by other, so-called antagonistic, microorganisms under natural conditions has long been observed (1,2). A pathogen introduced into otherwise sterile soil, after steam sterilization or fumigation for example, will generally cause more severe disease than in soil containing a natural, diverse population of microorganisms. However, when conditions are favorable to the pathogen, natural microbial antagonism may not be sufficient to prevent an economically significant level of disease. The goal of biocontrol, therefore, is to tip the balance in favor of microbial antagonism at the expense of the pathogen.

It may be satisfying to think that some microorganisms suppress disease especially for our benefit. However, any advantage we gain from their activity is only a fortuitous consequence of their survival behavior. The existence of any organism testifies that it has been able to obtain the raw materials (nutrients) necessary for its survival, growth, and proliferation. In the process of acquiring these raw materials, each organism competes with others for available nutrients and avoids being itself consumed as a nutrient source. The designation "beneficial" simply indicates that, in the process, a microorganism's usefulness to us outweighs any harm it may cause us.

Organic and inorganic nutrients in the soil are present in many forms. Organisms have evolved to utilize nutrients in essentially all forms that the basic features of biochemistry allow. The efficiency with which nutrients can be extracted from various sources, and the energy required to extract them, vary greatly with the nutrient source and the organism. Some organisms rely on speed. They can disperse and grow very rapidly and utilize readily available nutrients. Other organisms are more plodding in their approach. These forfeit the most easily obtainable resources to the swifter organisms, but are persistent and able to extract nutrients from sources that are much more difficult to break down. Still others wait for other organisms to collect nutrients in their bodies, then they attack these organisms and consume the nutrients. Pathogens can avoid direct competition with other microbes by infecting otherwise healthy plants that resist invasion by non-pathogens. In becoming effective at utilizing a particular nutrient source, organisms have either lost or never acquired the ability to exploit other sources. The differences in the abilities of various microorganisms to exploit nutrient sources and avoid themselves being consumed is the basis of microbial antagonism of plant pathogens and its exploitation for biocontrol of disease.

Our approach to developing biocontrol of PRR has been to identify naturally occurring antagonists of *P. cinnamomi* and incorporate them into a system of biocontrol. Soils in Australia suppressive to PRR have been reported since the 1970s (for reviews see: (1, 3, 4)). these were natural forest soils (5-7) or avocado grove soils with a high input and buildup of organic matter (8). The suppression of disease in these soils was attributed to the activity of soil microorganisms (5-13). Borst (14) gave a brief description of PRR suppressive soils in California avocado groves. At the start of our project on biocontrol of PRR, we had two questions regarding PRR-suppressive soils. Were any California avocado grove soils particularly suppressive to PRR? And, if so, were any of these soils suppressive due to microbial activity? Avocado grove soils that suppress the disease in the field due to beneficial microorganisms might be a good source of biocontrol agents for PRR, especially if these were found in association with avocado roots. We looked for groves in which *P. cinnamomi* was present or in which disease was severe in neighboring groves, but in which replants or older trees had little or no root rot. We purposely used rather liberal criteria in our initial search to avoid overlooking potentially useful locations. Thirteen sites fitting our criteria for PRR-suppressiveness were identified in a survey of southern California avocado groves. Trees growing at these sites were often on highly susceptible root stocks such as Topa Topa.

We could not be sure any soil was suppressive based only on observation of disease incidence in the field. We also knew that low incidence or severity of disease could depend on physical soil characteristics such as superior drainage. To identify soils containing potential biocontrol agents, we tested soil collected from the root zones of avocado trees at suspected suppressive sites (15,16). Four of the thirteen suspected suppressive soils reduced disease in greenhouse experiments, apparently due to microbial activity. Figure 1 is an example from one such experiment in which avocado seedlings grown in two test soils, CARP 4 and CARP 5 (third and fifth rows, respectively, from the left), had less root rot and were larger than plants in soil that was very conducive to disease (SAGO 5, first row) or only moderately suppressive (CARP 2, second row). All of these test soils had been experimentally infested with *P. cinnamomi* prior to transplanting seedlings. So in answer to our first question, some California avocado grove soils were apparently more suppressive to PRR than were others.

Figure 1. Avocado (Topa Topa) seedlings from an experiment testing four avocado grove soils for suppression of Phytophthora root rot. All soils were experimentally-infested with Phytophthora cinnamomi. *In one treatment (CARP 4 AUTOCL.), CARP 4 soil was sterilized prior to addition of* P. cinnamomi.

We also obtained results in greenhouse experiments that indicated that disease suppression was due to microbial activity. An example of this can be seen also in Figure 1. When CARP 4 soil, which suppressed PRR (Figure 1, third row), was sterilized by autoclaving (CARP 4 AUTOCL. fourth row) prior to infestation with *P. cinnamomi,* the suppressiveness was removed and disease was as severe as in soil highly conducive to PRR. This effect can be more clearly seen in Figure 2 showing the roots of plants from this experiment. Whereas the plant on the left grown in natural CARP 4 soil has a fairly healthy root system, the root systems of the plants on the right grown in sterilized CARP 4 soil (CARP 4 AUTOCL.) are completely rotted.

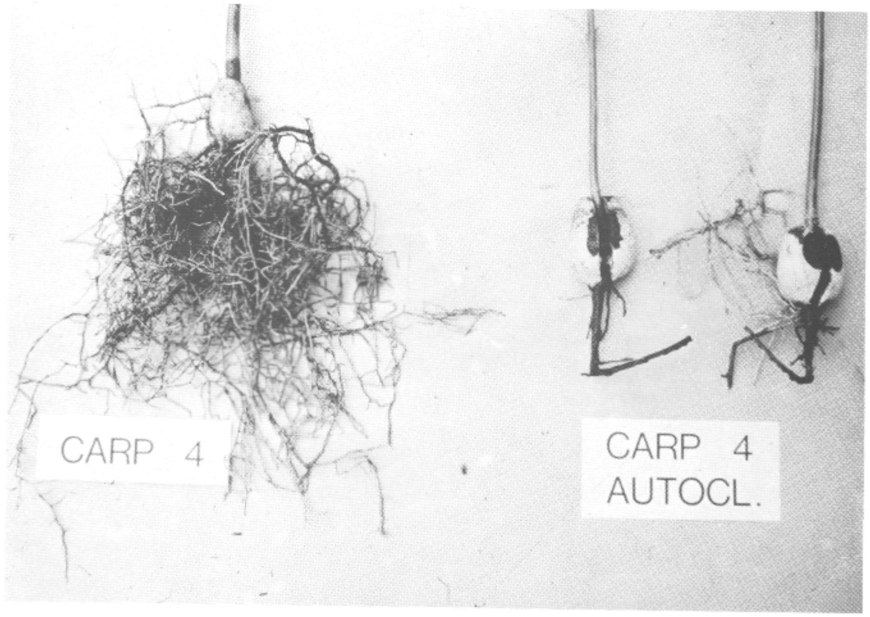

Figure 2. Root systems of avocado seedlings, shown in Fig. 1, grown in natural (CARP 4) and sterilized (CARP 4 AUTOCL.) soil experimentally-infested with Phytophthora cinnamomi.

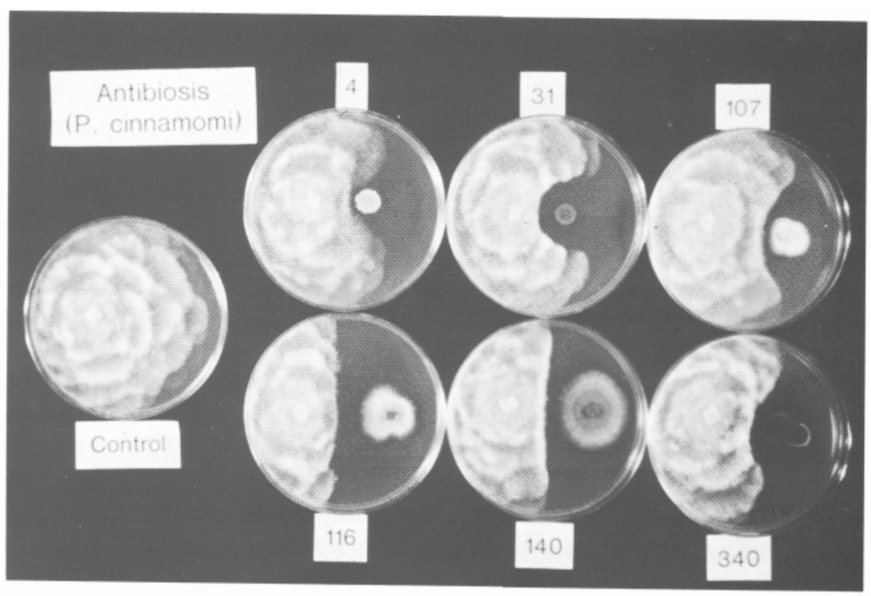

Figure 3. Inhibition of growth of Phytophthora cinnamomi *by antibiotics produced by several fungi isolated from suppressive avocado grove soil. Growth of* P. cinnamomi *in the absence of antibiotics is shown at the left (Control).*

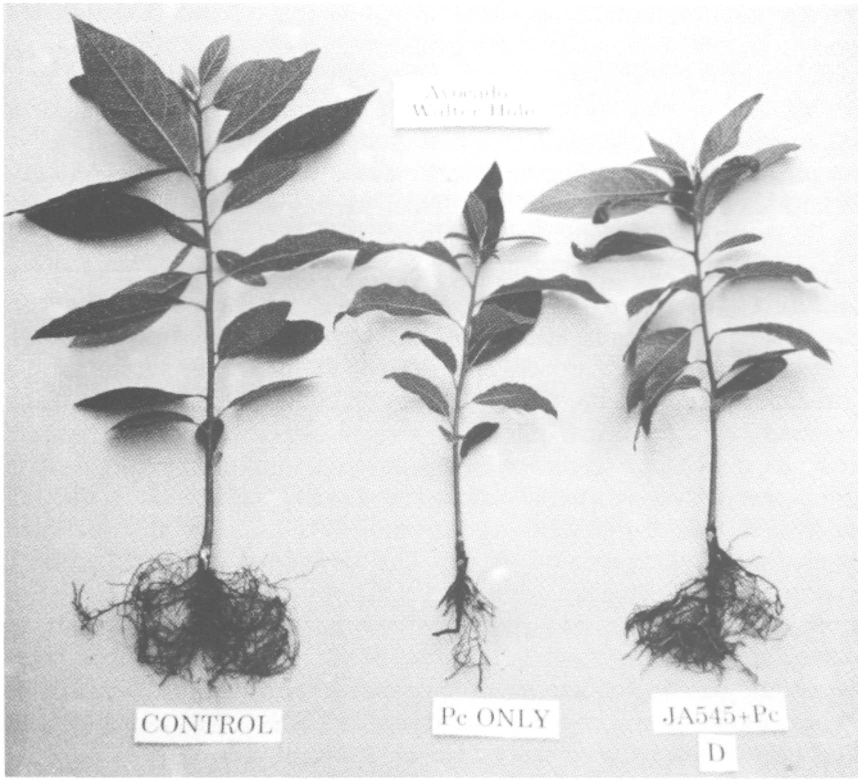

Figure 4. Biocontrol of root rot by bacterial isolate JA545. The plants in the center and on the right were grown in soil experimentally-infested with Phytophthora cinnamomi; *the control plant on the left was grown in soil without* P. cinnamomi. *The plant on the right was treated with biocontrol agent JA545.*

We isolated several hundred bacteria and fungi from the PRR suppressive avocado grove soils. Some of these produced antibiotics that significantly inhibited growth of *P. cinnamomi* in culture (Figure 3). More importantly, we identified several promising biocontrol agents among these microbial isolates. An example of biocontrol of root rot by one of the isolates is shown in Figure 4. Notice that when grown in *P.* cmnamomz-infested, soil, the plant on the right, treated with the biocontrol agent, has suffered less root rot and had better growth than the untreated plant in the center.

In collaboration with Dr. John Menge, we are addressing how best to apply these biocontrol agents in the field. Our current approach is to apply mulches that have been well colonized by the biocontrol agents. Mulches alone provide many benefits for plant growth and health, including improved soil porosity, increase of available nutrients, more efficient water use, and suppression of disease. The mulch system allows continued, periodic introduction of biocontrol agents by the application of colonized mulch at appropriate intervals. By introducing high populations of specific beneficial microorganisms determined to be effective in reducing Phytophthora root rot, we may be able to boost the disease suppressing characteristics of mulching. We hypothesize that a high population of a specific effective antagonist or combination of a few effective antagonists, of *Phytophthora* may offer a higher level of biocontrol than merely stimulation of a diverse population of resident microorganisms.

We selected 19 different mulches that are readily available in southern California. These were tested in the greenhouse for beneficial effects on growth and low toxicity to avocado. We then determined the mulches' abilities to support good growth and high populations of several fungal and bacterial biocontrol agents. Chemical analyses of the mulches are now being correlated with plant and microbial growth characteristics to derive a predictive model for selection of suitable mulches to be used in combination with biocontrol agents. We selected a wood, leaf, and straw combination mulch to set up our first field experiment in 1992 to insure an initial high population of our biocontrol agents. This developed into a rather large experiment that includes various combinations of mulching, biocontrol agents, pre-fumigation, and gypsum (there is evidence that gypsum, in conjunction with organic mulch, is suppressive to root rot). We are optimistic that a multiple component system, employing beneficial microorganisms as one component, will be effective at managing PRR and supporting general plant health with reduced input from agricultural chemical.

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