Proc. of Second World Avocado Congress 1992 pp. 123-128

Evaluation of *Phytophthora* Root Rot-Suppressive Soils from California Avocado Groves

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Abstract. **California avocado grove soils that appeared to suppress *Phytophthora* root rot (PRR) in the field were evaluated as part of a project to develop biological control methods of PRR. Sites were identified as possibly suppressive if replants or older trees, especially those on highly susceptible rootstocks, exhibited little or no PRR despite the presence of the pathogen *Phytophthora cinnamomi.* Soils from suspected suppressive sites were tested for suppression of PRR in the greenhouse. Soils collected from the root zones of avocado trees were infested with ground millet cultures of *P. cinnamomi* (0.1% w/v). Several, but not all, soils that indicated suppression of PRR in the field also suppressed root rot of susceptible avocado *(Persea americana* var. Topa Topa) and *Persea indica* in greenhouse experiments. Al soils that suppressed PRR in the greenhouse had high organic matter, but not all soils with high organic matter were necessarily suppressive. PRR-suppressiveness was eliminated by sterilization of the soil, suggesting a biological component was important for suppressiveness. Microorganisms isolated from suspected suppressive soils were screened for antagonism of *P. cinnamomi* in culture and for biocontrol of PRR in greenhouse experiments.**

Phytophthora root rot (PRR) of avocado (*Persea americana* Mill.), caused by *Phytophthora cinnamomi,* continues to be a serious problem in California despite current approaches to disease control including chemical fungicides, resistant rootstocks and cultural methods. In addition, the current trend is towards increasing restrictions on the use of chemical pesticides. This project was initiated in response to the need for effective alternative root rot control methods that will shift the emphasis away from chemical fungicides.

In the early 1970's, Broadbent and Baker (1974) reported on soils from avocado groves in Queensland, Australia that were suppressive to PRR. *P. cinnamomi* was present in these soils and avocados were growing on susceptible rootstocks, but there was little root rot, whereas other avocado groves in the area had significant root rot. In greenhouse tests in which soils were experimentally infested with *P. cinnamomi,* plants grown in the suppressive soils had significantly decreased root rot compared with plants in other, more conducive soils. Sporangium production was reduced in the suppressive soils.

Since sporangia are the fungal structures that release zoospores, the primary infectious stage of *P. cinnamomi,* decreased sporangium production means a lower potential for disease. Lysis of *P. cinnamomi* mycelium in the suppressive soils was also observed. When soil that was initially suppressive to root rot was steamed at 100C for 30 min, the soil no longer suppressed disease; soil steamed at 60C, however, retained its suppressiveness. This was interpreted as indicating soil microorganisms that survived steaming at 60C, but not 100C, were responsible for disease suppression.

Although conditions in California avocado groves are different from those in Queensland, our approach was to identify PRR-suppressive soils in California, then use these as a basis for developing biocontrol methods and a source for potential biocontrol agents. The objectives of this project were: (i) to identify sites in California avocado groves that appeared to suppress PRR, (ii) to select soils, collected from avocado roots at these sites, that suppress PRR in the greenhouse due to a biological component, and (iii) to identify potential biocontrol agents from among soil microorganisms isolated from PRR-suppressive soils.

Materials and Methods

Survey of avocado groves for PRR-suppressiveness. Southern California avocado groves were surveyed (in collaboration with Dr. John Menge, Dept. of Plant Pathology, University of California, Riverside) to identify sites on which trees had little or no root rot despite the presence of *P. cinnamomi* in the soil or the occurrence of PRR and *P. cinnamomi* in neighboring groves. These liberal criteria were used to tentatively identify soils as PRR-suppressive to reduce the likelihood of ignoring a soil that might be useful in yielding biocontrol agents or other valuable information.

Greenhouse tests for suppressiveness. Soil was collected from the root zones of avocado trees at the suspected suppressive sites identified during the survey described above. These soils were tested for suppression of root rot in the greenhouse. Greenhouse tests seemed more apt to select soils that were suppressive due to a biological component, eliminating soils that, for example, have reduced root rot because of superior drainage in the field. Also, the ability to observe disease suppression in the greenhouse would facilitate subsequent experiments and allow for greater control of environmental variables.

Initial tests for suppressive soils were performed using an indicator plant, *Persea indica,* that is related to avocado but is smaller and somewhat more convenient to use than avocado. *P. indica* is a very sensitive indicator of *P. cinnamomi* on which symptoms develop quickly: roots rot and the stem becomes blackened.

After field soils were screened to remove large stones and other debris, the soils were infested with ground millet seed cultures of *P. cinnamomi* at 1 g/L soil. Soils were mixed thoroughly to distribute the inoculum evenly. *P. indica* seedlings were transplanted into the infested field soils. Plant growth and root rot was monitored over several months.

Similar experiments were performed using the highly susceptible avocado cultivar, Topa Topa.

Importance of biological component to suppressiveness. The contribution of soil microorganisms to reducing disease caused by *P. cinnamomi* was tested by comparing disease in soil sterilized by autoclaving with disease in natural soil. Soil was autoclaved for 1 h, then infested with ground millet seed cultures of *P. cinnamomi* at 1 g/L soil. Natural (non-autoclaved) soil was similarly infested. Six week-old Topa Topa seedlings were transplanted into these soils. Plant growth (shoot length, shoot dry weight and root dry weight) and percent root rot were determined after 8 weeks.

To determine whether autoclaving itself was in some way detrimental to the plants, and also to determine the proportion of natural soil required for disease suppression, natural and autoclaved soils were mixed in various proportions. The soil mixtures were immediately infested with *P. cinnamomi* and planted with Topa Topa seedlings as before.

Isolation of soil microorganisms and antagonism of *P. cinnamomi* in culture. Microorganisms from soils tentatively identified as suppressive were isolated in an attempt to obtain potential biocontrol agents for PRR. Soil samples were diluted in sterile water and spread onto a variety of growth media. As expected, a mixture of many fungi and bacteria grew on the culture plates. Pure cultures of the microorganisms were obtained by transferring individual colonies to fresh growth medium.

Laboratory assays for the ability of the isolated soil microorganisms to inhibit growth of *P. cinnamomi* in culture provided a means of assigning priority to test organisms for subsequent greenhouse screening for bio-control of disease, and these tests also indicated possible mechanisms of antagonism of *P. cinnamomi* in soil and biocontrol of PRR. *P. cinnamomi* and individual 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. These interactions were microscopically observed for any other detrimental effects on *P. cinnamomi.*

Screening soil microorganisms for biocontrol in the greenhouse. Some mechanisms of antagonism observed in culture may not occur in soil; on the other hand, there may be mechanisms of antagonism in soil that are not expressed under the culture conditions we selected. Therefore, all isolated microorganisms are being tested for the ability to reduce disease caused by *P. cinnamomi* in the greenhouse.

Initial screening for potential biocontrol agents was performed using *P. indica.* Soil was infested as before with ground millet seed cultures of *P. cinnamomi* at 1 g/L soil. This inoculum level caused severe disease in controls and thus provided a fairly stringent screening procedure. Fungi to be tested for biocontrol ability were added to soil as wheat bran cultures (8 g/L soil); bacteria to be tested were applied by dipping roots in a

liquid suspension of the organisms, then transplanting the test plants into infested soil. Plant growth and root rot was monitored over several months.

Results

Survey of avocado groves for PRR-suppressiveness. Seven sites in Santa Barbara Co. and 6 sites in San Diego Co. fit our criteria for PRR-suppressiveness. At several of these sites trees approximately 35 years old or older were on highly susceptible rootstocks (e.g., cv. Topa Topa) but these trees were not suffering from severe root rot.

Greenhouse tests for suppres-siveness. Three (soils CARP2, CARP4, and CARP5) of the 7 Santa Barbara Co. soils, and 1 (SAGOS) of the 6 San Diego Co. soils were judged suppressive in greenhouse tests with *P. indica.* There was significantly less root rot and better growth of plants in these soils that were tentatively identified as suppressive in the greenhouse than in the other 9 soils that showed possible PRR-suppressiveness in the field. An example of *P. indica* seedlings growing in a conducive (CARP6) and a suppressive soil (CARP4) infested with *P. cinnamomi* is shown in Figures 1 and 2.

Avocado cv. Topa Topa also had significantly greater growth and less root rot in soils CARP4 and CARP5 compared to a conducive soil (SAG05) (Fig. 3). Soil CARP2 was also suppressive in the experiments with Topa Topa', but to a lesser degree than were CARP4 and CARP5 (Fig. 3).

Importance of biological component to suppressiveness. Suppressiveness of soils CARP4 and CARP5 was eliminated after sterilizing these soils by autoclaving for 1 h (CARP4 example shown in Fig. 3 and 4). This indicated that suppressiveness in these soils was due to soil microorganisms.

When natural soil was mixed with the same soil sterilized by autoclaving, mixtures containing 25% or greater natural soil had less root rot and greater growth of Topa Topa plants (Table 1). Interestingly, 50% natural soil was actually more suppressive than 100% natural soil in the experiment shown in Table 1. Apparently, therefore, autoclaved soil was itself not detrimental to plants.

Isolation of soil microorganisms and antagonism of *P. cinnamomi* in culture. Approximately 200 fungi and bacteria have been isolated from suppressive soils so far. Of the organisms isolated, 26 demonstrated inhibition of growth of *P. cinnamomi* in culture due to antibiosis. In some cases, inhibition of growth was accompanied by an obvious disruption of the hyphae of *P. cinnamomi.* One of the fungi were observed tightly coiling around hyphae of *P. cinnamomi,* which suggested mycoparasitism.

Screening soil microorganisms for biocontrol in the greenhouse. Whereas most of the test microorganisms were not effective at controlling disease of *P. indica* in the greenhouse, 15 fungi and bacteria tested so far have shown significant reduction in the

level of disease caused by *P. cinnamomi.* The microorganisms that were most effective with *P. indica* are currently being tested using Topa Topa.

Discussion

A survey of southern California avocado groves suggested 13 sites that were suppressive to PRR in the field. Soil collected from avocado root zones at 4 of these sites suppressed disease in greenhouse tests and disease suppressiveness was apparently due to a biological component of the soil. Microorganisms were isolated from the suppressive soils in an attempt to obtain potential biocontrol agents for PRR. In culture, 26 different fungi and bacteria isolated from soils tentatively identified as suppres-sive inhibited the growth of *P. cinnamomi.* Fifteen microorganisms actually reduced disease caused by *P. cinnamomi* in greenhouse experiments. Further testing of these potential bio-control agents is underway and ways to enhance their effectiveness are being examined.

Literature Cited

Broadbent, P. and K.F. Baker. 1974. Behavior of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Austral. J. Agric. Res. 25:121-137.

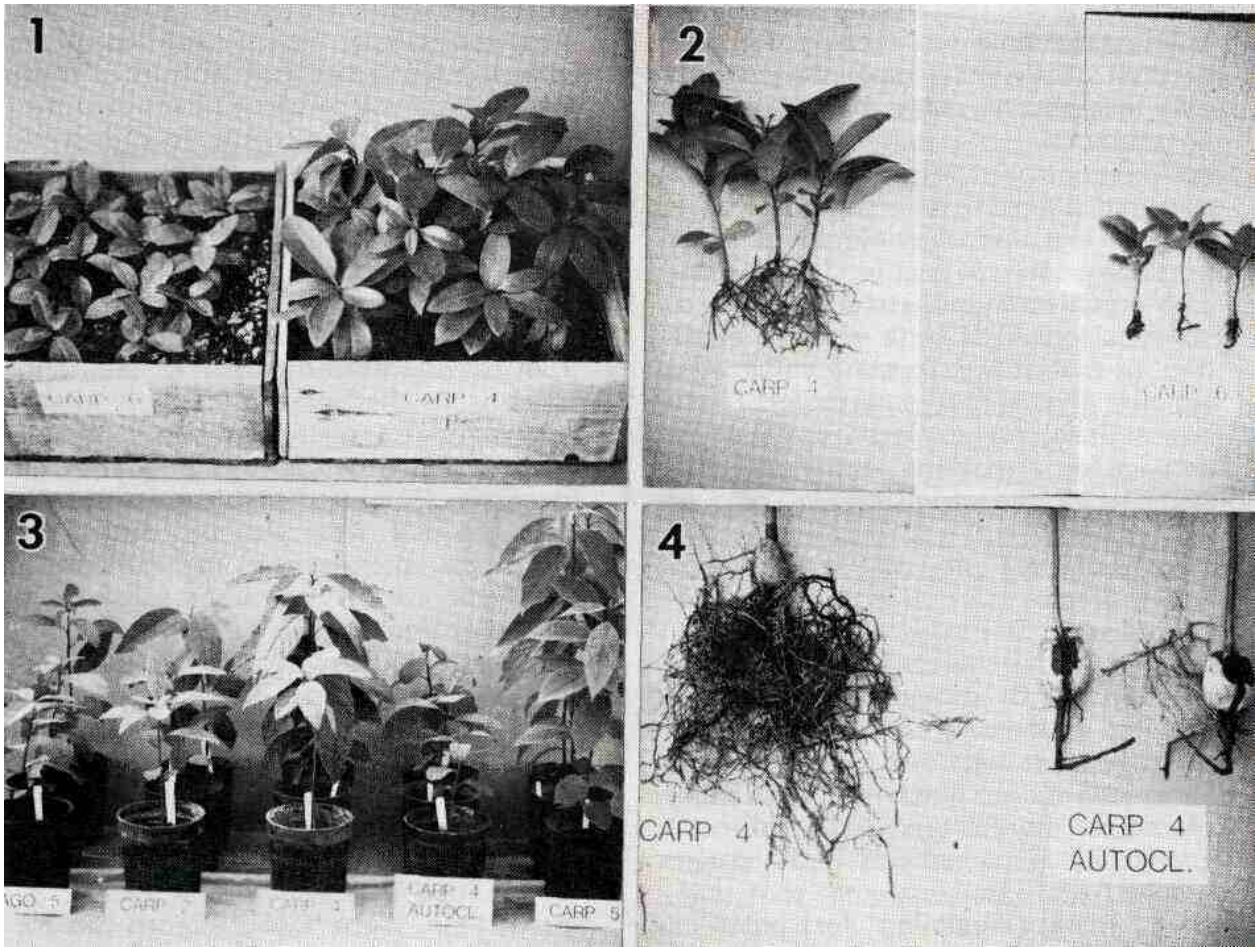

Fig. 1. Growth of *Persea indica* seedlings in conducive (CARP 6) and suppressive (CARP 4) soils infested with *Phytophthora cinnamomi.*

Fig. 2. *Persea indica* seedlings grown in suppressive (CARP 4) and conducive (CARP 6) soils infested with *Phytophthora cinnamomi.*

Fig. 3. Avocado (cv. Topa Topa) seedlings growing in natural conducive (SAGO 5) or suppressive (CARP 2, 4, 5) soils, or autoclaved CARP4 soil, infested with *Phytophthora cinnamomi*.

Fig. 4. Roots of avocado seedlings (shown in Fig. 3) grown in natural (left) or autoclaved CARP4 soil (right) infested with *Phytophthora cinnamomi.*

Table 1. Growth and root rot of avocado (cv. Topa Topa) plants in avocado grove soil (CARP4) infested with *Phytophthora cinnamomi.*

Soil mixture *P. cinnamomi*	Natural:Autoclaved[z]	Shoot length increase (cm)[Y]	New shoot dry weight (g)[x]	Root Rot (%)[w]
Non-infested	0:100	14.3 a[v]	3.58 a	6 d
lnfested[u]	0:100	1.0 d	0.12 d	80 a
Infested	10:90	3.6 c	0.80 cd	80 a
Infested	25:75	3.4 cd	1.01 c	69 b
Infested	50:50	10.5 b	2.55 b	27 cd
Infested	100:0	4.8 c	2.54 c	51 be

[z] Six week-old avocado seedling were transplanted into mixtures comprised of various proportions of natural:autoclaved (for 1 h) [v:v] CARP4 soil.

[y] Increase in shoot length since transplanting.

[x] Dry weight of the segment of shoot tip of a length equal to the increase in shoot length of individual plants since transplanting.

[w] Visual estimate of percentage of roots rotted.

[v] Means with a common letter within columns are not significantly different according to LSD ($P<0.05$).

[u] Soil mixtures were infested with ground millet seed cultures of *Phytophthora cinnamomi* at 1 g/L soil.