

## COMPARISON OF VARIOUS METHODS FOR THE ISOLATION OF PHYTOPHTHORA CINNAMONI FROM AVOCADO SOILS

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For any disease survey to be effective it is essential that both the recovery and detection methods be efficient and practical. In surveys involving *Phytophthora* and *Pythium* spp., which because of their slow growth are rapidly overgrown by other fungi in culture, conventional isolation methods are normally ineffective. Various alternative isolation methods have therefore been proposed for this group of fungi. The object of this investigation was to determine which of these isolation techniques is most effective for the recovery of *Phytophthora cinnamomi* from infested avocado soils.

According to Hendrix and Kuhlman (1965), the poor recovery of *Phytophthora* spp. from the soil may be because these fungi normally give rise to fewer infective propagules per gram of soil than most other soil fungi. Manning and Crossan (1966), also mention that these fungi, because of their slow growth cannot compete with certain other fungi in non-sterile soil. There is evidence too, that various micro-organisms have a direct inhibitory effect on the growth of these fungi. These factors would account for the failure of *Phytophthora* spp. to develop on conventional soil dilution plates (Tsao, 1960).

Several methods have been proposed for the isolation of *P. cinnamomi* from the soil.

McCain, Holtzmann & Trujillo (1967) used a set of soil sieves. As the diameter of chlamydospores of *P. cinnamomi* varies from 40 to 135  $\mu$ , these spores would be trapped on a 400 mesh sieve (having a 38  $\mu$  aperture). With this technique the material remaining on this sieve after washing, is plated on selective medium and the number of *P. cinnamomi* colonies which develop per gram of soil tested, are counted and recorded.

A well-known means of isolation from the soil is qualitative and is based on the use of pieces of host tissue which are introduced into the soil. Uninjured mature Fuerte avocado fruits are commonly used for this purpose (Zentmyer, Gilpatrick & Thorn, 1960).

Another method described by the above authors is the direct isolation of *P. cinnamomi* from small dead feeder roots of infected avocado trees.

Several selective media have been proposed by various workers for the isolation of *Phytophthora* and *Pythium* spp. in culture. These media include corn meal, lima bean, and potato dextrose agars to which various antibiotics have been added. These antibiotics and other fungi-static compounds include pimarcin, polymyxin, vancomycin,

mycostatin, streptomycin, rose bengal and pentachloronitrobenzene (PCNB) (Hendrix & Kuhlman (1965), Singh & Mitchell (1961) and Tsao & Menyonga (1966)). These chemicals have been used in different combinations and are said to provide media which are specific for *Phytophthora* and *Pythium* spp.

In this investigation, several methods were tested and compared for the isolation of *P. cinnamomi* from root rot infested avocado soils.

### **1. Soil sieving technique.**

The method used is similar to that described by McCain *et al* (1967). This involves suspending 50 g of soil in 500 ml distilled water and agitating in a blender at a slow speed for one minute. Before the suspension settles, it is passed through a nest of sieves with aperture sizes of 149, 61, 44 and 38  $\mu$ . All material remaining in the blender is washed three times and the suspension decanted through the nest of sieves. Immediately after this, several 1 g samples of soil from the 38  $\mu$  aperture sieve are plated on to selective medium and incubated at 25 °C for 24 to 48 hours before the colonies are counted.

### **2. Isolations from infected roots.**

The small roots are first washed under running tap water to remove the soil, surface sterilized with 70% ethanol for 10 to 20 seconds, rinsed in distilled water and dried between absorbent paper. Sections of the infected root are plated on selective medium. After incubation at 25 °C for 3 days, the plates are examined for the presence of *P. cinnamomi*.

### **3. Fruit traps.**

A mature avocado is placed in a test soil/distilled water (1:1) for four to ten days at 20° to 25°C. The development of lesions on the fruit at the water-line is indicative of a positive infestation of the soil by the pathogen. This result is confirmed by plating pieces of tissue from the infected fruit skin, flesh, or pip onto a selective medium. However, instead of avocado fruits, other fruit and plant traps were found to be very effective. For example, improperly fertilized avocado fruits or "cukes," and Jacaranda and lupin seedlings.

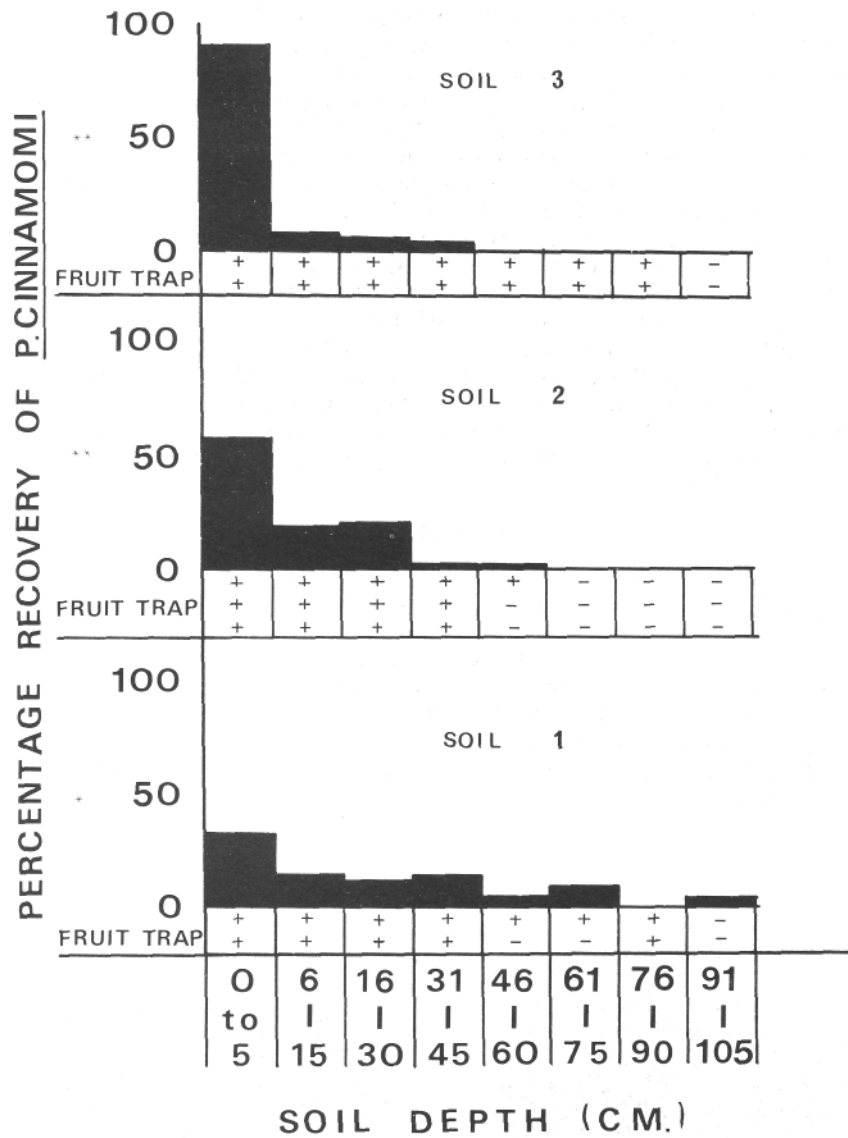
## **RESULTS**

In these experiments a modified selective medium (P<sub>10</sub>VP) was successfully used. The number of bacterial contaminants on the isolation plates was effectively reduced with Vancomycin while pimaricin and PCNB virtually eliminated fungi other than *Phytophthora*, *Pythium* and *Mortierella* ssp. from developing on the isolation plates.

In the one series of experiments the three isolation methods were compared for the recovery of *P. cinnamomi* from samples taken from various soils and different soil

depths.\* Results in Figure 1 show that fruit traps, direct plating of root pieces and soil sieving are all effective for the isolation of the pathogen from the soil. All three methods clearly demonstrated that the recovery of *P. cinnamomi* decreased significantly with increased soil depth. The pathogen could however, still be recovered to a soil depth of nearly one metre using these techniques.

*\*This work was conducted by the senior author in G. A. Zentmyer's laboratory while on a study tour to Riverside in 1971.*



+ = ROOT PIECES                      \*\* = SOIL SIEVING

Figure 1. Isolation techniques compared for the recovery of *P. cinnamomi* from soil taken at several depths and from different root rot infested avocado soils. In soils 2 and 3, figures are given as percentage of propagules recovered at each soil depth.

Of the three methods, only the soil sieving technique gives an actual quantitative measure of the infestation. With this method it appears that the majority of the infective propagules of *P. cinnamomi* occur in the upper few centimeters of the soil.

In another experiment, improperly fertilized avocados or "cukes" were used instead of the normal Fuerte fruit. The efficiency of the experiment was greatly increased since due to their small size (approx. 7cmx2cm) several "cukes" could be placed in each soil sample instead of a single normal fruit. Results could therefore be presented semi-quantitatively. In the same way as with normal Fuerte fruit, positive reactions on "cukes" showed up as brown lesions at the water-line (see Figure 2). The pathogen was easily recovered from infected fruit-trap pieces by plating these on culture medium.

Beside "cukes," lupin and Jacaranda seedlings were compared with avocado fruits for the recovery of *P. cinnamomi*. Within a few days after being transferred into the infested soil solutions, the seedlings wilted and died. Once again the fungus was readily recovered from brown lesions on the roots by plating out pieces of the tissue on a selective medium.

## SUMMARY

1. From these experiments it appears that the soil sieving method gives a quantitative measure of the number of chlamydospores (infective propagules) in the soil.
2. The majority of the infective propagules were recovered from the top 15cm of soil. However, small numbers of these spores were recovered at a depth of nearly one meter.
3. The conventional fruit trap method is sufficiently sensitive and reliable to be used on a practical scale for the recovery of *P. cinnamomi* from infested soils.

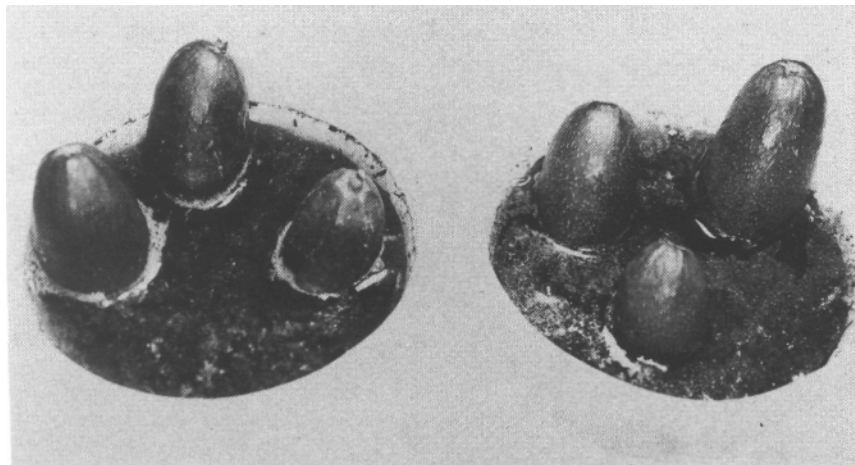


Figure 2. "Cukes" (improperly fertilized avocado) used as a fruit trap for the recovery of *P. cinnamomi* from the soil. Left: Lesions on "cukes" at the water-line in *Phytophthora* infested sample. Right: No lesions visible on "cukes" in the sterilized soil (Control).

4. The direct plating of root pieces onto a selective medium was shown to be an

effective and practical method. The selective medium P<sub>10</sub>VP was extensively tested in these trials and shown to be highly satisfactory of the recovery *P. cinnamomi*.

5. Because of their availability and small size, "cuke" avocados can be effectively used as baits. Several "cukes" can be placed in each soil sample instead of a single large fruit thus increasing the replication and hence efficiency of this type of test. Jacaranda and lupine seed lings are also effective as traps for the recovery of this pathogen.

6. The various isolation methods mentioned above are comparatively simple and relatively inexpensive to apply. The advantages obtained by the early recognition of root rot disease, especially for the large grower or nurseryman, warrant the adoption of one or several of these techniques on a routine basis.

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