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RECOVERY OF *PHYTOPHTHORA CINNAMOMI* FROM AVOCADO SOILS OF SOUTH FLORIDA

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ABSTRACT

Six selective agar media were compared for efficiency of recovery of *Phytophthora cinnamomi* (causal agent of Phytophthora root rot of avocado) from Immokalee fine sand (Collier County) and Rockdale soil (Dade County) in south Florida. A medium containing cornmeal agar (17 g/liter), pimaricin (10 mg/liter), ampicillin (250 mg/liter), rifampicin (10 mg/liter), PCNB (100 mg/liter), and hymexazol (50 mg/liter) provided detection of *P. cinnamomi* equal to or more sensitive than that provided by any of the five remaining media. This medium should prove useful in studies on this pathogen and the important disease of avocado it causes in south Florida.

Avocado (*Persea Americana* Mill.) production is influenced throughout subtropical and tropical regions of the world by many diseases (5). Of these, Phytophthora root rot is the most damaging. Root rot (caused by *Phytophthora cinnamomi* Rands) results in the loss of avocado feeder roots with corresponding reductions in canopy growth and fruit size; chlorosis and partial defoliation of the canopy may also occur (5, 17, 25). In severe cases, as during episodes of flooding (15, 16, 21), root rot can kill trees. Symptoms preceeding mortality may include chlorosis, necrosis, and wilt of foliage or defoliation.

Phytophthora root rot is an important, though often unappreciated disease in south Florida (17). Although they were not aware of the etiology of root rot at the time, Stevens and Piper (19) in 1941 were apparently the first to report this disease from Florida. Because symptoms of root rot are nondescript, it is difficult to determine its prevalence and the distribution of *P. cinnamomi* in Florida in the absence of confirmed diagnoses. Using fruit baits or isolations on cornmeal agar, Burns et al. (2) reported recovering the pathogen from soil and roots, respectively, from 8 of 16 avocado groves sampled in Dade County in 1965. They indicated that detection of *P. cinnamomi* is especially difficult when low levels of the pathogen are involved and that laboratory tests may not detect low levels of the fungus. Based on previous losses of avocado trees associated with flooding (C. W. Campbell and R. T. McMillan, Jr., personal communication) it is probable that *P. cinnamomi* is distributed in avocado production areas in the state not included in the survey of Dade County conducted by Burns, et al. (2). However, no reliable study has been reported to date on the distribution of this important pathogen in avocado groves in Florida.

Phytophthora cinnamomi is a good microbial competitor in soil (9, 22) possessing saprophytic (26) as well as parasitic capabilities. When high soil moistures were maintained in the absence of a host, the fungus survived for 6 years in one study (26) and 27 months in another (8). The survival of *P. cinnamomi* in relatively dry soil, however, is poor (26). In south Florida avocado groves, soil moisture fluctuates greatly in surface layers of soil (ca 0-10 cm) in which activity of the fungus would be expected to be the greatest. Populations of the fungus also fluctuate in these soils, decreasing at times to nondetectable levels (Ploetz, unpublished). Studies are needed on the population dynamics of *P. cinnamomi* in south Florida avocado groves to determine when its activity is greatest and to what factors periods of increased activity are related.

Studies on the activity of this pathogen and its distribution in south Florida require an accurate method for its detection. The present study was conducted to identify such a technique.

MATERIALS AND METHODS

Five experiments were conducted to determine which of several previously reported selective agar media (6, 7, 10, 12, 18, 20) would provide reliable detection of *P. cinnamomi* under conditions found in south Florida. The following media were dispensed in 9-cm-diameter plastic, disposable Petri plates and stored without light at room temperature the day before they were used: Flowers' (6) medium; McCain's (10) medium; modified Kerr's medium (7); PARPH medium (12); PCH medium (18); PVPH medium (20).

In four experiments, Rockdale soil from Dade County was used. In two of these, soil naturally free of *P. cinnamomi* was artificially infested with a single, virulent isolate of the pathogen recovered from avocado in Dade County. In the remaining two experiments, soil naturally infested with the fungus was recovered from beneath canopies of avocado trees in two different groves.

In each of the four Rockdale experiments, soil was sieved through nested 2-mm (no. 9 mesh) and 0.045-mm (no. 325 mesh) sieves under running tap water. In preliminary work, relatively few propagules of *P. cinnamomi* were detected in soil fractions >2 mm or <0.045 mm in diameter (Ploetz, unpublished). In the present study, soil collected on the 0.045 mm sieve (2 mm to 0.045 mm fraction) was assayed for the pathogen. In a fifth experiment, nonfractionated naturally infested Immokalee fine sand from an avocado grove in Collier County was assayed.

In all five experiments, partial or whole fractions of soil to be assayed for the fungus were suspended in sterile 0.25% Difco^{R} water agar and mixed on a stirring plate with a magnetic bar. One ml of these suspensions was dispensed per plate of a given medium; suspensions were spread evenly across the surface of each medium with the blunt end of a test tube surface disinfested with 95% ethanol. Two concentrations of soil suspensions (ca 0.3 and 0.03 g soil [oven dry wt] / ml) were assayed in each experiment.

Each soil suspension was dispensed into five plates of each medium in experiments with naturally infested soil and into ten plates in experiments with artificially infested soil.

Propagule densities listed in Table 1 are those attained with the former concentration.

Except for the PCH medium, the scheme described above for utilizing these media was generally consistent with those described by the authors. Shew and Benson (18) dispensed soil on plates of PCH in slurries of approximately 2.5 ml of tap water; for the sake of consistency and convenience, 1.0 ml of 0.25% water agar was used in the present studies.

After soil suspensions were dispensed on the media, they were incubated without light for 72 hr at 25°C. At the end of the incubation period, soil suspensions were washed from the surface of all media with running tap water and colonies of *P. cinnamomi* were counted. The fungus was identified on the basis of its characteristic colony morphology and mycelial growth (25).

RESULTS AND DISCUSSION

The PARPH medium was consistently as or more sensitive at detecting *P. cinnamomi* in soil than any of the remaining media used in the present studies (Table 1). Although it did not detect the fungus in four soils in the present work which were believed to be infested with the pathogen, neither did any of the remaining media tested in these experiments. *Phytophthora cinnamomi* can be a difficult fungus to isolate from soil or plant tissue when agar media are used (1, 9). Development of assays for this pathogen more sensitive than those utilized in the present work may depend on the use of tools other than selective media. For example, *P. megasperma* Drechs. f. sp. *glycinea* Kuan & Erwin has been detected in infected tissue with immunological techniques (13).

An accurate method for detecting *P. cinnamomi* would obviously benefit studies on this pathogen and the important disease it causes on avocados grown in south Florida. Recent work (15) indicates that avocado is extremely sensitive to flooding when infected with this pathogen; even when levels of the fungus in soil and corresponding levels of root infection are very low, plant vigor is significantly reduced. Considering the high water tables and excessive rainfall which occasionally occur in south Florida, it is probable that avocado groves in this area with any level of *P. cinnamomi* are at risk. Identifying these groves will depend on the ability to detect low levels of the fungus.

In a similar vein, a reliable and accurate assay for this pathogen would aid studies on its population dynamics and the control of Phytophthora root rot. In general, positive correlations exist between populations of soilborne plant pathogenic fungi and the development of disease (11). Control of root rot with either fungicides (3, 4, 14, 23, 24) or cultural practices (3, 14, 23, 24) may depend on knowing when and where populations of the pathogen in soil are greatest.

Based on the present results, it is apparent that PARPH is a relatively sensitive medium for detecting *P. cinnamomi* in soil in avocado groves in south Florida. It should prove to be a valuable component of studies on Phytophthora root rot and the population dynamics of the pathogen in this area.

Table 1. Recovery of *Phytophthora cinnamomi* from soil with selective agar media.

Expt.	Site	Medium[t,u,v] Flowers'	McCain's	Modified Kerr's	PARPH	PCH	PVPH
1[w]	1	25.0 ab	6.3 b	0 b	37.5 a	0 b	0 b
2[x]	1	0 a	0 a	0 a	0 a	0 a	0 a
	2	0 b	0 b	0 b	2.5 a	0.5 b	0 b
	3	0 b	0 b	0 b	2.6 a	0.5 b	0 b
	4	0 a	0 a	0 a	0 a	0 a	0 a
3[w]	1	1.0 bc	3.3 b	0.5 bc	9.5 a	0 c	2.5 bc
4[y]	1	0 a	2.1 a	0 a	2.1 a	0 a	0 a
	2	0 a	0 a	a a	0 a	0 a	0 a
	3	0 a	0 a	0 a	0.9 a	a a	0 a
	4	0 a	0 a	0 a	0 a	0 a	0 a
5[y]	1	—[z]	7.8 a	—[z]	6.3 a	0 b	—[z]
	2	—	14.5 a	—	15.9 a	6.2 b	—
	3	—	0.6 b	—	2.8 a	0 b	—

[t]Mean propagule densities [per g soil (oven dry wt)].
[u]Within a row means are separated on the basis of Duncan's Multiple Range Test at $P<0.05$.
[v]Flowers' = Flowers' (6) medium; McCain's = McCain's (10) medium; Modified Kerr's = modified Kerr's medium (7); PARPH = PARPH medium (12); PCH = PCH medium (18); PVPH = PVPH medium (20).
[w]2-mm to 0.045-mm fraction of artificially infested Rockdale soil was assayed for *P. cinnamomi* in this experiment.
[x]Nonfractionated naturally infested Immokolee fine sand was assayed for *P. cinnamomi* in this experiment.
[y] 2-mm to 0.045mm fraction of naturally infested Rockdale soil was assayed for *P. cinnamomi* in this experiment.
[z]This medium was not used in this experiment.

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