Techniques

A Modified Selective Medium for Detecting *Phytophthora cinnamomi* on Avocado Roots

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We thank Rachel Messinger and Arna Kariv for technical assistance.
Contribution 796-E, 1983 Series, from the Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.
Accepted for publication 6 January 1984.

ABSTRACT

Solel, Z., and Pinkas, Y. 1984. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. Phytopathology 84:506-508.

Isolation of *Phytophthora cinnamomi* from avocado roots on Phytophthora-selective media P_{10}VPH and PARPH was accompanied by the development of oomycetous contaminants which interfered with detection of the pathogen. Addition of iprodione to the media markedly inhibited the growth of the contaminants and *Pythium proliferum*. This weak pathogen of avocado was shown to be insensitive to hymexazol. More avocado seedlings were found to be infected with *P. cinnamomi* when iprodione-amended PARP medium was used.

Additional key words: Persea americana.

Selective media containing antibacterial and antifungal agents have been successfully used for the detection and isolation of *Phytophthora* spp. from plant tissues (2,5,6,9). After the first detection of *Phytophthora cinnamomi* Rands in Israel in avocado (*Persea americana* Mill.) plantations (3), an extensive survey was conducted to determine the extent to which the pathogen was established in avocado groves, and to detect any source of inoculum in nurseries. It was anticipated that contamination by *Pythium* spp. might interfere with the detection of *P. cinnamomi*, as reported by Tsao and Guy (7). Therefore, avocado roots were placed on Phytophthora-selective media P_{10}VP or PARP + hymexazol, which is selectively toxic to *Pythium* (2,7). Nevertheless, many of the culture plates contained rapidly developing oomycetes along with *P. cinnamomi*; the most frequently found were *Pythium proliferum* De Bary, a pathogen of avocado under excessive soil moisture (4), and other *Pythium* species which are common in soils.

The objective of this study was to modify a Phytophthora-selective medium to minimize the interference caused by oomycetes associated with avocado roots.

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MATERIALS AND METHODS

Selective media. Iprodione (Rovral 50% WP, Rhone-Poulenc Agrochemie, Lyon, France) at rates of 100 or 400 μg/ml a.i. was suspended in sterile water and added to molten sterilized P_{10}VP (8), or to cornmeal agar which contained 10 mg pimaricin, 125 mg ampicillin, 10 mg rifampicin, and 100 mg pentachloronitrobenzene per liter (1) (PARP), with or without the addition of 50 μg/ml hymexazol. Media supplemented with formulated iprodione appear somewhat turbid. This inconvenience can be minimized by using technical iprodione homogenized in water with a tissue grinder and added to the molten medium before it is poured into plates.

Selective toxicity of iprodione. Iprodione, 400 μg/ml, was incorporated into both P_{10}VP and PARP selective media without or with hymexazol (50 μg/ml). Six 3-mm-diameter agar disks removed from the margin of a young culture of each test organism were placed on agar, and the radial growth of the colony was measured after 2 days of incubation at 27 C. *P. cinnamomi* and four isolates of *Pythium* spp. were selected for this test: *P. proliferum*, a pathogen of avocado roots; *P. aphanidermatum* (Edson) Fitzp., which is common in soils in Israel; and *P. paroecandrum* Drechsler, and *P. rostratum* Butler, which were randomly selected because of their rapid growth in culture. Growth inhibition was calculated as:

TABLE 1. Inhibition of radial growth of fungi on selective media supplemented with hymexazol (H) and iprodione (I)[y]

Medium	*Phytophthora cinnamomi*	*Pythium proliferum*	*Pythium aphanidermatum*	*Pythium paroecandrum*	*Pythium rostratum*
P_{10}VP+H	3 a,m[z]	5 a,m	86 b,n	85 b,n	92 c,n
P_{10}VP+I	47 a,o	64 b,o	60 b,m	48 a,m	42 a,m
P_{10}VP+H+I	53 a,p	64 b,o	96 c,o	95 c,o	99 c,o
PARP+H	17 a,n	14 a,n	87 b,n	88 b,n	96 c,o
PARP+I	50 b,p	72 d,o	59 c,m	48 b,m	41 a,m
PARP+H+I	56 a,p	70 b,o	95 c,o	95 c,o	96 c,o

[y] H = hymexazol 50 μg/ml, and I = iprodione, 400 μg/ml.
[z] Growth inhibition = 100 − (radial growth on supplemented medium × 100/radial growth on unsupplemented corresponding medium). Means of six replicates, measured after incubation for 48 hr. Means in each row followed by the same letter a–d and means in each column followed by the same letter m–p do not differ significantly, $P = 0.01$, according to Duncan's multiple range test.

100−(radial growth on fungicide-supplemented medium × 100)/(radial growth on the unsupplemented corresponding medium).

Analysis of variance was used to determine the statistical significance of the differences observed.

Recovery of *P. cinnamomi* from roots. Feeder roots (~2 mm in diameter) were collected from two diseased avocado trees, one with mild and the other with severe symptoms. One hundred root pieces were taken from each tree, and each root piece was divided into three segments (~1 cm long). The root segments were rinsed in water, blotted on a paper towel and plated on PARP and PARP+ iprodione at both 100 and 400 μg/ml. After 48 hr of incubation at 27 C, each root segment was visually and microscopically examined for colonies of *P. cinnamomi* and other fungi. The χ^2 test was used to analyze the statistical significance of the differences observed.

TABLE 2. Recovery of *Phytophthora cinnamomi* colonies and contaminating oomycetes from avocado root segments plated on PARP medium supplemented with 0, 100, and 400 μg of iprodione per milliliter[y]

	Isolations from roots of trees:					
	Severely infected			Lightly infected		
Occurrence of contaminants	0	100	400	0	100	400
P. cinnamomi only	7 b[z]	17 a	28 a	0 a	1 a	2 a
P. cinnamomi accompanied by contaminants	16 a	7 b	5 b	2 a	0 a	1 a
Fast-growing contaminants	66 a	15 b	10 b	89 a	18 b	24 b
Slow-growing contaminants	10 b	44 a	35 a	9 c	74 a	51 b

[y] Colonies per 100 root segments.
[z] In each tree, figures in each row followed by the same letter do not differ significantly, $P = 0.05$, according to the χ^2 test.

RESULTS AND DISCUSSION

Selective toxicity of iprodione. In a preliminary study, two groups of fungicides were evaluated for differential toxicity towards representative contaminating *Pythium* species on one hand and towards *P. cinnamomi* on the other hand: toxicants to oomycetes—ethazol, fenaminosulf, metalaxyl, phosethyl Al, and propamocarb; broad-spectrum toxicants—captan, chlorothalonil, etaconazole, fentin acetate, iprodione, mancozeb, and phenylmercuric chloride (*unpublished*). The most selective toxicant was iprodione and it, therefore, was subjected to further evaluation.

The growth inhibition effects of either iprodione or hymexazol were similar with both P_{10}VP and PARP media (Table 1). While hymexazol was selectively toxic towards *P. aphanidermatum, P. paroecandrum,* and *P. rostratum,* it did not inhibit the growth of *P. proliferum*. Iprodione had an inhibitory effect on *P. proliferum* (64–72% growth inhibition) and *P. aphanidermatum* (59–60% growth inhibition) and to a lesser extent (47–50% growth inhibition) towards *P. cinnamomi*. Since hymexazol did not inhibit growth of *P. proliferum*, which is frequently associated with decayed avocado roots, iprodione was studied further in spite of its partial toxicity to *P. cinnamomi*.

Recovery of *P. cinnamomi* from avocado roots. Iprodione, at both concentrations, markedly reduced the number of rapidly growing oomycetes, some of which were completely inhibited, while others appeared to be suppressed and presumably were counted with the slow-growing contaminants (Table 2). As a result, inspection of the culture plates became more reliable and easier, especially with the 100 μg/ml concentration. In the case of the severely infected tree, the number of roots that yielded *P. cinnamomi* without accompanying contaminants increased significantly ($P = 0.05$) when the roots were plated on PARP + 100 or 400 μg/ml iprodione. The increase in the total number of colonies of *P. cinnamomi* was, however, not statistically significant. With the lightly infected tree, although there was no difference in the recovery rate, it was easier to inspect the plates supplemented with iprodione. The growth inhibition effect of iprodione on *P. cinnamomi*, which was observed in the toxicity test, was not discernible in the colonies which developed from assayed roots. This might be due to a difference in toxicity of iprodione against various stages of *P. cinnamomi*, ie, chlamydospores in roots versus mycelium in culture. Some change in the form of both the colonies and the mycelium (less coralloid) was observed.

In a nursery survey to detect root rot, feeder roots were randomly collected from 43 symptomless 18-mo-old seedlings. The root segments, 60 from each plant, were assayed for *P. cinnamomi* on PARP with or without the addition of iprodione (400 μg/ml). *P. cinnamomi* was found growing from roots of 13 of 43 seedlings on both media. Infection in one additional seedling was detected only on the unamended PARP, while on PARP plus iprodione five other seedlings were proven to be infected. In this test, contaminants were common on all plates. Iprodione-sensitive *P. proliferum* occurred infrequently, presumably because of the good aeration and the quality of the planting mixture used in the nursery. This organism interferes in isolation of *P. cinnamomi* from trees grown in poorly drained soils.

We may surmise that the combination of both selective fungicides, iprodione and hymexazol, can broaden the spectrum of selective activity and thus further enhance recovery rates of *P. cinnamomi* from infected roots as well as the convenience of inspecting uncrowded culture plates. If iprodione medium is to be used for the isolation of other *Phytophthora* spp., additional testing is necessary.

LITERATURE CITED

1. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
2. Masago, H., Yoshikawa, M., Fukada, M., and Nakanishi, N. 1977. Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soil and plants. Phytopathology 67:425-428.
3. Pinkas, Y. 1983. (Avocado root rot in Israel). Hassadeh 63:734-737 (in Hebrew).
4. Pinkas, Y., Nachmias, A., Tomer, E., and Kariv, A. 1981. (Avocado decline—a new disease caused by *Pythium proliferum*). Alon Hanotea 35:739-743 (in Hebrew).
5. Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J.

Cramer, Vaduz, Liechtenstein. 417 pp.
6. Tsao, P. H. 1970. Selective media for isolation of pathogenic fungi. Annu. Rev. Phytopathol. 8:157-186.
7. Tsao, P. H., and Guy, S. O. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. Phytopathology 67:796-801.
8. Tsao, P. H., and Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223:636-638.
9. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the disease it causes. Phytopathological Monograph 10, American Phytopathological Society, St. Paul, MN. 96 pp.