Citrus Leaf Pieces as Traps for Phytophthora parasitica from Soil Slurries

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ABSTRACT

Phytophthora parasitica readily infected citrus leaf pieces floating on a soil slurry. Numerous sporangia were produced along the cut edges of floating leaf pieces in 3 to 4 days at room temperature, but were absent from whole leaves or cut pieces that sank. Abundant sporangia were produced on leaf pieces of all citrus varieties, but only a few were produced on leaf pieces from other plants. Pure cultures of *P. parasitica* isolated from citrus and noncitrus leaf pieces were pathogenic to citrus seedlings. Citrus leaf pieces and calamondin (*Citrus reticulata* var. *austera* ? × *Fortunella* sp. ?) fruits were equally effective as traps for *P. parasitica*.

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Isolation of *Phytophthora* spp. from soil by the use of plant hosts and fruit traps is a well-known practice (1, 2, 5, 6, 9). The use of citrus fruits as traps in soil slurries for the isolation of *Phytophthora* spp. pathogenic to citrus is an established technique (5, 7). However, suitable citrus fruit is not always available, and lemon or calamondin fruit are inconvenient to use when large numbers (500-700) of soil samples must be indexed. For convenience and availability of traps, we investigated the possibility of using citrus leaves as a substitute for fruit.

Initial tests for the development of the leaf piece technique were made with prepared potting soil (3 parts peat:1 part vermiculite:1 part sand) that had been artificially infested with *P. parasitica* Dastur and used for inoculations of citrus seedlings.

Soil samples of 100-cc volume were placed in 473-ml waxed cardboard containers, and sufficient rainwater was added to flood the soil to a depth of 1 to 2 cm. Pieces 3- to 5-mm square, cut from mature citrus leaves, were floated on the surface of the water (Fig. 1) and allowed to remain 3 to 4 days at room temperature (22 to 28 C). During this time, Phytophthora zoospores gathered along the cut edges of floating leaf pieces and germinated, forming mycelium and sporangia (Fig. 2). Sporangia were not formed on leaf pieces that sank, nor along the natural margin of whole leaves. Identification of the fungus was made by observing the sporangia at X40 and X100 magnification.

Subculture of *P. parasitica* from infected leaf pieces was easily accomplished by submerging infected leaf pieces in molten cornmeal agar containing 10 μg/ml pimaricin, 200 μg/ml vancomycin, and 100 μg/ml pentachloronitrobenzene (PCNB) (8) and transferring hyphal tips as they grew in the medium.

The pathogenicity of pure cultures of *P. parasitica* subcultured from citrus and noncitrus leaf pieces was verified by root inoculations of citrus seedlings.

Abundant sporangia developed along the cut edges of leaf pieces from sweet orange [*Citrus sinensis* (L.) Osb.], 'rough' lemon [*C. limon* (L.) Burm. f.], sour orange (*C. aurantium* L.), grapefruit (*C. paradisi* Macf.), trifoliate orange [*Poncirus trifoliata* (L.) Raf.], 'Cleopatra' mandarin (*C. reticulata* Blanco), macrophylla (*C. macrophylla* Wester), kumquat [*Fortunella margarita* (Lour.) Swing.], calamondin

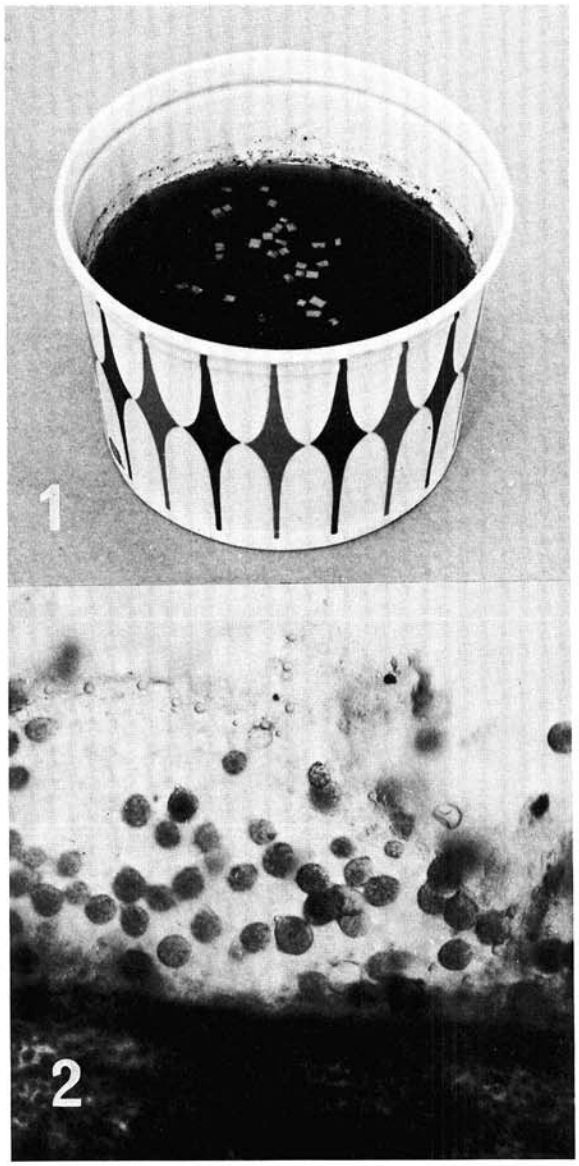

Fig. 1-2. 1) Citrus leaf pieces floating on a slurry of infested citrus soil for recovery of *Phytophthora parasitica*. 2) *P. parasitica* sporangia produced along the cut edge of a citrus leaf piece. (× 160).

TABLE 1. Comparison of citrus leaf pieces (L) and calamondin fruits (C) as traps for recovering *Phytophthora parasitica* in naturally and artificially infested soil slurries

	Soil A[a]			Soil B			Soil C		
	Replication			Replication			Replication		
Dilutions[b]	I	II	III	I	II	III	I	II	III
	LC	LC	LC	LC	LC	LC	LC	LC	LC
1/8	++[c]	++	++	++	++	++	++	++	++
1/16	++	−+	++	++	++	++	++	++	++
1/32	++	−+	+−	++	++	++	++	++	++
1/64	−+	−−	++	−−	++	+−	++	++	++
1/128	++	−−	−−	−−	−−	−−	+−	−+	+−

[a] Soil A and B was naturally infested. Soil C was artificially infested.
[b] Infested soil diluted by weight with addition of sterilized soil.
[c] Recovery (+) and no recovery (−) of *P. parasitica*.

(*C. reticulata* var. *austera* ? × *Fortunella* sp. ?), 'Carrizo' and 'Yuma' citrange (*P. trifoliata* × *C. sinensis*), 'Sampson' tangelo (*C. paradisi* × *C. reticulata*), 'Etrog' citron (*C. medica* var. *ethrog*), and *Severinia buxifolia* (Poir.) Ten. There was no apparent difference in the reaction of *P. parasitica* to the various varieties of citrus leaves tested, although these varieties vary greatly in their susceptibility to root and stem infections.

A few sporangia were formed along the cut edges of pieces of leaves from avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), litchi (*Litchi chinensis* Sonn.), peach (*Prunus persica* Sieb. & Zucc.), nightshade (*Solanum nigrum* L.), tomato (*Lycopersicon esculentum* Mill.), beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita pepo* L.), ligustrum (*Ligustrum japonicum* Thunb.), pineapple (*Ananas pativus* Schult), gloxinia (*Sinnongia speciosa* Benth. & Hook), and gynura (*Gynura sarmentosa* DC.). However, none of these was as satisfactory for trapping *P. parasitica* as citrus.

A comparison of the trapping efficiency of citrus leaf pieces and calamondin fruit for detection of *P. parasitica* from naturally and artificially infested soils was made using a modification of Tsao's serial dilution technique (7). The soil dilution series was prepared by combining weighed amounts of infested and sterile soil. A total of 240 g of soil thoroughly mixed in the proportion desired was divided into six 40-g samples for each replication. Three samples were trapped with leaf piece traps and three samples with calamondin fruit. A dilution level for each fungus trap was considered positive (+) if the sporangia of *P. parasitica* were observed on any leaf piece or calamondin. The results (Table 1) show that the traps are essentially equal in their efficiency to detect *P. parasitica* in soil slurries.

Citrus leaf pieces have been a good substitute for lemons and calamondins in detection of viable *P. parasitica* from soil samples from fumigation experiments (3, 4) and to culture *Phytophthora* spp. from naturally infested soil.

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