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. australis x Fortunella sp.) fruits were equally effective as traps for P. parasitica.

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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.), trifoliata orange [Poncirus trifoliata (L.) Raf.], ‘Cleopatra’ mandarin (C. reticulata Blanco), macrophylla (C. macrophylla Wester), kumquat [Fortunella margarita (Lour.) Swing.], calamondin

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.

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

<table>
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<tr>
<th>Dilutions</th>
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**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*.

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.

### LITERATURE CITED