

# Differential Virulence of *Phytophthora parasitica* Recovered from Citrus and Other Plants to Rough Lemon and Tomato

M. E. MATHERON, Extension Plant Pathologist, and J. C. MATEJKA, Research Assistant, University of Arizona, Yuma Agricultural Center, Yuma 85364

## ABSTRACT

Matheron, M. E., and Matejka, J. C. 1990. Differential virulence of *Phytophthora parasitica* recovered from citrus and other plants to rough lemon and tomato. Plant Dis. 74:138-140.

The relative virulence of *Phytophthora parasitica* recovered from citrus and other plants to rough lemon (*Citrus jambhiri*, a citrus rootstock) and tomato (*Lycopersicon esculentum*) was examined. Isolates of *P. parasitica* from citrus were highly virulent to rough lemon seedlings, causing crown rot and significant reduction of root weight. Isolates of the pathogen from noncitrus hosts demonstrated low virulence to rough lemon, with no crown rot and only minor reduction of root weight. All tested isolates of *P. parasitica* were highly virulent to tomato seedlings, causing stem lesions and (usually) plant death. Apparently, isolates of *P. parasitica* from several noncitrus hosts do not pose a serious threat to citrus orchards. On the other hand, the high susceptibility of tomato plants to *P. parasitica* from several hosts, including citrus, suggests caution when planting tomatoes in areas adjacent to citrus orchards, other commercial crops, or landscape plantings that may be infested with this pathogen.

*Phytophthora parasitica* Dastur occurs widely and has a very broad host range (15). Important hosts include tomato (*Lycopersicon esculentum* Mill.) (11), citrus (*Citrus* spp.) (5), petunia (*Petunia hybrida* Vilm.) (10), watermelon (*Citrullus vulgaris* Schrad.) (9), carnation (*Dianthus caryophyllis* L.) (2), pineapple (*Ananas comosus* (L.) Merr.) (2), and hibiscus (*Hibiscus rosa-sinensis* L.) (2).

The host range and relative virulence levels vary greatly among isolates of *P. parasitica* (1,2,12). In one study, all isolates from several different hosts were pathogenic to peperomia (*Peperomia obtusifolia* (L.) Diétr.) (12). Conversely, an isolate of *P. parasitica* from parsley (*Petroselinum crispum* (Mill.) Nym.) was pathogenic to carnation but not to carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), or tomato (1). An earlier report in Arizona revealed that isolates of *P. parasitica* from rosemary (*Rosmarinus officinalis* L.), Texas ranger (*Leucophyllum frutescens* Johnston), palm (*Washingtonia* spp.), and petunia were all pathogenic to sweet orange (*Citrus sinensis* L. Osbeck) but differed markedly in virulence (16).

Our study determined the pathogenicity and relative virulence levels of isolates of *P. parasitica* from citrus and other hosts to rough lemon (*C. jambhiri* Lush., a common rootstock) and tomato.

This information is needed to determine the potential threat to commercial citrus plantings from *P. parasitica* infecting plants other than citrus. In addition, tomato cultivation is increasing in Arizona in areas previously devoted to citrus production. The potential threat to tomato production by *P. parasitica* associated with declining citrus trees also needs to be evaluated, so that appropriate disease management strategies can be implemented. A partial account of this work has been published (6).

## MATERIALS AND METHODS

**Isolates and mating type determination.** Fifteen isolates of *P. parasitica* were examined in this study (Table 1). The identities of all isolates were confirmed on the basis of sporangium morphology, cardinal temperatures for vegetative growth, colony morphology, production of chlamyospores, and pairing with an opposite mating type for production of oospores (13-15), using previously described procedures (8).

**Pathogenicity and relative virulence of isolates of *P. parasitica*.** Preparation of test seedlings and inoculum. Rough lemon and tomato (cultivar Peto 343) seedlings were grown in sterile potting mix (45% peat, 45% vermiculite, 10% sand). Four-month-old rough lemon and 2-mo-old tomato seedlings were removed carefully from the potting mix, washed thoroughly in tap water to remove potting mix adhering to the roots, and then placed in plastic cups (8-cm diameter, 13-cm depth) each containing 400 ml of distilled water. Each cup held 10 rough lemon or tomato seedlings with roots totally immersed in water.

Isolates of *P. parasitica* used in this study were recovered from citrus, walnut

(*Juglans hindsii* (Jeps.) Jeps.), silk tree (*Albizia julibrissin* (Durazz.) Schneider), tomato, jojoba (*Simmondsia chinensis* (Link) Schneider), petunia, hibiscus, and peach (*Prunus persica* (L.) Batsch). These isolates (Table 1) were grown on V-8 agar (8) for 5 days at 24 C. Ten agar disks (6-mm diameter) were removed from the edge of an actively growing culture of each isolate and placed in individual plastic petri dishes (60-mm diameter), each containing 8 ml of 1.5% nonsterile soil extract (4). Numerous sporangia formed after incubating the agar disks for 5 days at 24 C in darkness; the sporangia were then induced to produce zoospores by chilling at 4 C for 15 min. Immediately after chilling, the contents of the petri dishes were used to inoculate the test plants.

**Inoculation and incubation.** The test plants were inoculated with agar disks bearing sporangia of an isolate of *P. parasitica*. One cup (10 plants) of each plant species was inoculated with each isolate. Control plants were incubated in distilled water without adding agar disks. After inoculation, plants were maintained in the laboratory for 4 hr at 22-24 C, during which time the agar disks remained on the bottom of the cup. Microscopic observation of sporangia immediately after chilling had revealed numerous ungerminated sporangia and no zoospores; after the 4-hr period of incubation, 90-100% of the sporangia had released zoospores.

The approximate number of zoospores produced by each isolate of *P. parasitica* was determined by letting one petri dish containing agar disks and soil extract rewarm to 24 C after the chilling treatment. A 0.5-ml aliquot of zoospore suspension was removed from the petri dish and placed in 100 ml of distilled water. After mixing thoroughly, a 0.5-ml aliquot of this dilute zoospore suspension was spread evenly over a surface of PARP selective medium (containing 17 g cornmeal agar, 5 mg pimaricin, 200 mg ampicillin, 10 mg rifampicin, and 100 mg PCNB per liter of water) (3). Duplicate plates were prepared for each isolate of *P. parasitica*. Excess moisture was removed from the surface of the selective medium by placing the plates in a laminar-flow transfer chamber for 30 min. The plates were then incubated in darkness for 48 hr at 24 C, after which colonies of *P. parasitica* were counted

to determine the concentration of zoospores in the original suspension. The approximate final concentrations of zoospores in the inoculation treatments ranged from 300 to 2,300 zoospores per milliliter, depending upon the isolate.

After the 4-hr incubation, test plants were removed from the cups. The water and agar disks in each cup were decanted and replaced with fresh distilled water. Roots of inoculated plants were rinsed in tap water, placed back into the cups, and incubated in the laboratory for an additional 18 hr at 24 C. Rough lemon and tomato seedlings then were planted individually in potting mix in plastic pots (10-cm diameter, 10-cm depth) in the greenhouse. Plants were arranged in a randomized complete-block design. Once every 2 wk, pots containing seedlings were immersed for 48 hr in water-filled plastic pans so that 1 cm of water covered the surface of the potting mix in each pot. Flooding treatments were continued for the duration of the experiment to stimulate disease development. Between floodings, seedlings were watered as needed and the potting mix was allowed to drain freely. Plants were fertilized weekly with water-soluble Miracle-Gro 15-30-15 fertilizer. The soil temperature ranged between 12 and 36 C during these tests.

**Analysis of results.** Our experiments lasted 3 mo. Final disease incidence and severity were evaluated by recording the occurrence of crown rot and the fresh weight of shoots and roots. Analyses of variance of the resultant data were performed and (when appropriate) Duncan's multiple range test was used to differentiate treatment means. The pathogenicity and relative virulence experi-

ments were performed three times with similar results; data are presented from one experiment only. Disease in rough lemon and tomato seedlings was confirmed as resulting from *P. parasitica* infection by placing 10 rootlets from each of 10 replicate plants per treatment onto PARP selective medium to reisolate the pathogen (3).

## RESULTS

**Isolate morphology.** The isolates of *P. parasitica* used in our experiments were similar in size and shape of sporangia as well as in colony morphology. Their minimum, optimum, and maximum temperatures for vegetative growth were similar, ranging from 9 to 12, 30 to 33, and 36 to 39 C, respectively. None of the *P. parasitica* isolates formed sexual reproductive structures in single culture. Isolates 33-1-5, 34-1-3, MI-10, MI-12, MI-22, MI-24, MI-29, C-5, and C-19 formed oospores when paired with A<sub>1</sub> mating types of *P. cryptogea* Pethybr. & Lafferty and *P. parasitica*. Isolates MI-23, C-2, C-7, C-40, C-63, and C-66 formed oospores when paired with A<sub>2</sub> mating types of *P. drechsleri* Tucker and *P. parasitica* (Table 1).

**Pathogenicity and relative virulence of isolates of *P. parasitica*.** Citrus isolates of *P. parasitica* were highly virulent to rough lemon seedlings, causing significant reductions in fresh weights of shoots and roots relative to control plants (Table 1). Isolates from noncitrus hosts caused less reduction of shoot and root weights. Only citrus isolates of *P. parasitica* caused lesions on crowns of rough lemon seedlings. Isolates of *P. parasitica* originally from citrus were recovered from 54% of the rootlets assayed from

rough lemon at the conclusion of the experiment. In contrast, isolates of *P. parasitica* originally obtained from noncitrus hosts were recovered from 0.5% of the rootlets of rough lemon seedlings.

Isolates of *P. parasitica* from citrus and other host plants were highly virulent to tomato seedlings and caused significant reduction of fresh weights of shoots and roots (Table 1). All isolates caused lesions on the stems of tomato plants, usually followed by plant death. Isolates of *P. parasitica* from citrus were recovered from 25% of rootlets of tomato samples at the conclusion of the experiment; isolates from tomato were recovered from 32% of rootlets; and isolates from six other host species were recovered from 40% of rootlets.

## DISCUSSION

Despite the morphological similarities of *P. parasitica* isolates tested in this study, the pathogenicity and relative virulence of individual isolates differed markedly. These differences in virulence varied according to the host from which the isolate was originally recovered and the test plant that was inoculated with the isolate. Similar results were reported by Wheeler and Boyle (16), who found that rosemary and sweet orange were infected by isolates of *P. parasitica* from Texas ranger, palm, petunia, rosemary, and citrus, while petunia was infected only by a petunia isolate.

Our results suggest that the tested isolates of *P. parasitica* exhibit differential virulence on citrus and tomato, as opposed to strict host specificity. Isolates of the pathogen from walnut, silktree, jojoba, petunia, peach, and tomato

**Table 1.** Pathogenicity and relative virulence of *Phytophthora parasitica* recovered from different hosts on rough lemon and tomato seedlings

Isolate	Host	Mating type	Rough lemon				Tomato			
			Fresh wt.(g) <sup>y</sup>		Plants with crown rot	Isolation from rootlets (%) <sup>z</sup>	Fresh wt.(g) <sup>y</sup>		Plants with stem lesions	Isolation from rootlets (%) <sup>z</sup>
			Shoots	Roots			Shoots	Roots		
Uninoculated control	...	...	24 a	9.5 a	0	0	16.0 a	3.3 a	0	0
33-1-5	Walnut	A <sub>2</sub>	20 bc	8.0 b	0	0	0.7 c	0.9 bc	10	42
34-1-3	Silktree	A <sub>2</sub>	20 bc	8.0 b	0	0	0.4 c	0.6 bc	10	36
MI-22	Jojoba	A <sub>2</sub>	21 abc	7.9 b	0	1	3.0 bc	1.2 bc	9	36
MI-23	Petunia	A <sub>1</sub>	20 bc	7.3 b	0	1	2.1 bc	0.9 bc	9	27
MI-24	Hibiscus	A <sub>2</sub>	22 ab	8.2 ab	0	0	0.4 c	0.5 c	10	41
MI-29	Peach	A <sub>2</sub>	20 bc	7.7 b	0	2	2.2 bc	0.9 bc	9	55
MI-10	Tomato	A <sub>2</sub>	21 abc	7.7 b	0	0	2.0 bc	1.0 bc	9	20
MI-12	Tomato	A <sub>2</sub>	18 c	6.9 b	0	0	0.3 c	0.5 c	10	43
C-2	Citrus	A <sub>1</sub>	5 d	2.1 c	10	13	0.5 c	0.6 bc	10	37
C-5	Citrus	A <sub>2</sub>	5 d	2.3 c	6	71	2.4 bc	1.3 bc	10	34
C-7	Citrus	A <sub>1</sub>	4 d	1.7 c	6	91	6.2 b	1.5 b	10	22
C-19	Citrus	A <sub>2</sub>	2 d	0.9 c	9	31	1.1 c	0.5 c	10	21
C-40	Citrus	A <sub>1</sub>	3 d	1.0 c	10	89	0.4 c	0.4 c	10	39
C-63	Citrus	A <sub>1</sub>	4 d	2.1 c	10	56	3.1 bc	0.8 bc	10	8
C-66	Citrus	A <sub>1</sub>	3 d	1.0 c	10	31	1.0 c	0.8 bc	10	17

<sup>y</sup>Each value is the mean of 10 replicate plants per treatment. Numbers within a column followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>Percentage of rootlets from which *P. parasitica* was isolated at the termination of the experiment. Values represent the mean of 10 rootlets from each of the replicate plants.

caused a small but significant reduction of root fresh weight on infected rough lemon seedlings. The occasional recovery of some of these isolates from rootlets of rough lemon seedlings suggests that noncitrus isolates of *P. parasitica* can infect roots of rough lemon. The low virulence of these isolates to rough lemon could be interpreted, however, as strict host specificity instead of differential virulence.

The determination of relative or differential virulence could be affected by the criteria used to evaluate disease incidence and severity. If the presence of crown rot was the only factor used to determine disease incidence on rough lemon seedlings in this study, for example, one could conclude that isolates of *P. parasitica* from several noncitrus hosts were not pathogenic to rough lemon. When other factors are taken into consideration, such as fresh weight of shoot and root tissue and recovery of the pathogen from rootlets, the picture of nonpathogenicity is replaced by one of weak virulence.

The weak virulence of isolates of *P. parasitica* from several noncitrus hosts suggests that these pathogens are not a serious threat to citrus orchards should they spread from adjacent landscape or commercial plantings. On the other hand, tomato plants appear to be highly susceptible to *P. parasitica* recovered from several hosts other than tomato, including citrus. These findings suggest

caution when planting tomatoes in areas adjacent to citrus orchards, other commercial crops, or landscape plantings that may be infested with *P. parasitica*. Propagules of the pathogen could be spread easily from infested areas to tomato plantings by farm machinery or by runoff from excess rainfall and irrigation. In Arizona, planting tomatoes on sites formerly occupied by citrus could also lead to severe losses caused by *P. parasitica*, because this pathogen is found in a high percentage of the state's declining citrus groves (7).

#### ACKNOWLEDGMENTS

We thank S. M. Mircetich of USDA-ARS, University of California, Davis; E. Bernhardt of Petoseed Co., Inc.; J. B. Ristaino of North Carolina State University; and R. A. Haygood of Clemson University for supplying some of the isolates used in this study.

#### LITERATURE CITED

- Hine, R. B., and Aragaki, M. 1963. Influence of soil temperature on a crown rot disease of parsley caused by *Phytophthora parasitica*. *Phytopathology* 53:1113-1114.
- Hine, R. B., and Aragaki, M. 1963. Pathogenicity, vitamin nutrition, and cultural characteristics of isolates of *Phytophthora parasitica* from carnation and other hosts in Hawaii. *Phytopathology* 53:1194-1197.
- Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 70:1038-1043.
- Matheron, M. E., and Matejka, J. C. 1988. In vitro activity of sodium tetrathiocarbonate on sporulation and growth of six *Phytophthora* species. *Phytopathology* 78:1234-1237.
- Matheron, M. E., and Matejka, J. C. 1988.

Persistence of systemic activity for fungicides applied to citrus trunks to control *Phytophthora* gummosis. *Plant Dis.* 72:170-174.

- Matheron, M. E., and Matejka, J. C. 1989. Relative virulence of *Phytophthora parasitica* isolated from several hosts to rough lemon and tomato. (Abstr.) *Phytopathology* 79:910.
- Matheron, M., Matejka, J., and Bacon, D. 1988. Distribution of two species of *Phytophthora* within the citrus acreage in Arizona. Pages 19-20 in: *Citrus Rep. Univ. Ariz. Coll. Agric. Ser. P-76*.
- Matheron, M. E., Young, D. J., and Matejka, J. C. 1988. *Phytophthora* root and crown rot of apple trees in Arizona. *Plant Dis.* 72:481-484.
- Norton, D. C., and Rosberg, D. W. 1954. Watermelon fruit rot caused by *Phytophthora parasitica* in Texas. *Plant Dis. Rep.* 38:854.
- Phillips, D. J., and Baker, R. 1962. *Phytophthora* crown rot of petunia. *Plant Dis. Rep.* 46:506-508.
- Satour, M. M., and Butler, E. E. 1967. A root and crown rot of tomato caused by *Phytophthora capsici* and *P. parasitica*. *Phytopathology* 57:510-515.
- Siradhana, B. S., Ellett, C. W., and Schmitthenner, A. F. 1968. Pathogenic and cultural variation in *Phytophthora nicotianae* var. *parasitica* from greenhouse plants. *Phytopathology* 58:718-719.
- Waterhouse, G. M. 1963. Key to the species of *Phytophthora* de Bary. *CMI Mycological Papers* 92. 22 pp.
- Waterhouse, G. M. 1970. The genus *Phytophthora* de Bary. Diagnosis (or descriptions and figures) of the original papers. *CMI Mycological Papers* 122. 59 pp.
- Waterhouse, G. M., and Waterston, J. M. 1964. *Phytophthora nicotianae* var. *parasitica*. Descriptions of pathogenic fungi and bacteria. *CMI Mycological Papers* 35.
- Wheeler, J. E., and Boyle, A. M. 1971. Identification of four *Phytophthora* isolates previously unreported from Arizona. *Phytopathology* 61:1293-1296.