Isolation of *Phytophthora palmivora* Pathogenic to Citrus in Florida

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ABSTRACT

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A *Phytophthora* sp. recovered from soil in a citrus orchard near Fort Pierce, Florida, was characterized and designated as *P. palmivora*. It produced papillate, caducous sporangia (50 × 27 μm) with a pedicel 3 μm long. All isolates were A^1 and mated readily with A^2 mating types of *P. palmivora* from cacao and *P. parasitica* from citrus. Oogonia, oospores, and chlamydospores averaged 30, 25, and 34 μm, respectively, in diameter. The optimum temperature for growth was 30 C, with little or no growth at 15 or 33 C. The citrus isolate of *P. palmivora* was as pathogenic as or more so than *P. parasitica* to fibrous roots of sweet orange, sour orange, and Swingle citrumelo. A *P. arecae* isolate from palm that was morphologically similar to the citrus isolate was not pathogenic to citrus roots. The *P. palmivora*-citrus, *P. arecae*-palm, and *P. parasitica* isolates produced stem lesions on cuttings of citron cv. Etrog and on sweet orange seedlings. The citrus isolate of *P. palmivora* was as pathogenic as *P. citrophthora* to citrus fruit, and *P. arecae* from palm was also pathogenic to fruit. This is the first report of *P. palmivora* pathogenic to citrus in the United States.

The two species of *Phytophthora* most commonly associated with disease problems in citrus are *P. parasitica* Dastur (syn. *P. nicotianae* Breda de Haan) and *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (8,13). Both species are widely distributed in citrus-growing areas and cause root rot, foot and crown rot, brown rot of fruit, and damping-off and blight of seedlings. *P. parasitica* is widespread in Florida citrus orchards (13,21), but *P. citrophthora* is localized and usually associated with outbreaks of brown rot (20). One other unidentified species of *Phytophthora* has been reported from citrus in Florida (2).

In California, *P. parasitica* and *P. citrophthora* are common, and *P. syringae* (Kleb.) Kleb. and *P. hibernalis* Carne are often associated with outbreaks of brown rot during the winter rainy season (8). *P. megasperma* Drechs. and *P. cinnamomi* Rands reportedly have been isolated from citrus in California (3,18) but are not commonly recognized citrus pathogens. *P. palmivora* (E.J. Butler) E.J. Butler has been isolated from citrus in Puerto Rico and caused necrosis of inoculated tender grapefruit shoots (17). This species also has been associated with seedling blight in nurseries in Puerto Rico (18). Neither *P. palmivora* nor *P. arecae* (Coleman) Pethybridge has been reported as a pathogen of citrus in the continental United States (3,18). Klotz (8), writing on brown rot and Phytophthora blight, mentioned that *P. palmivora* has been reported on citrus from India, Sri Lanka, Java, Malaysia, the Philippines, the West Indies, South America, Trinidad, and Tanzania but cited no references. The pathogenicity of *P. palmivora* and *P. arecae* to citrus roots, stems, and fruit has not been determined.

Recently, we isolated an unusual *Phytophthora* sp. from soil in a citrus orchard near Fort Pierce, Florida. This study reports the characteristics of this isolate and its pathogenicity to citrus.

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MATERIALS AND METHODS

Fungal characteristics and inoculum production. The isolate from citrus used throughout this study, and henceforth designated as *P. palmivora*-citrus, was recovered on soil dilution plates of selective media (7) from an orchard near Fort Pierce, Florida. It was recognized originally because it produced small compact colonies clearly distinguishable from the *P. parasitica* colonies normally recovered from Florida citrus soils.

A *P. arecae* isolate from palm described previously (12) and a *P. palmivora* isolate from cacao in Costa Rica were kindly provided by D. J. Mitchell (University of Florida, Gainesville). *P. parasitica* isolates used were recovered from citrus soils in the vicinity of Lake Alfred, Florida, and an isolate of *P. citrophthora* from Florida was provided by G. E. Brown (Florida Department of Citrus, Lake Alfred).

Cultures were maintained and fungal structures were produced on clarified V8 juice agar (CV8A) or broth (CV8B) prepared as described by Mitchell et al (9). For sporangium production, 2-day-old cultures in CV8B were washed with sterile distilled water and incubated for two additional days at room temperature. Where needed, cultures were chilled for 15 min at 5 C and then returned to room temperature to induce zoospore production. Chlamydospores were produced by the method of Tsao (16). When used as inoculum, chlamydospores were separated from the mycelium by repeated blending and low-speed centrifugation (16). Oospores were produced by pairing isolates with the opposite mating type on CV8A and incubating them in the dark. Since only the A^1 mating type of *P. palmivora*-citrus was available, matings were done with A^2 isolates of *P. palmivora*-cacao and *P. parasitica*. The size of spore structures was measured with a Leitz Dialux microscope with an eyepiece micrometer. At least 30 spores were measured for each isolate and the mean and standard deviation of the measurements reported.

For determination of the optimum temperature for growth of these fungi, mycelial plugs were transferred to 15 × 100 mm plastic petri dishes of CV8A. Three plates of each species were used at each temperature. Colony diameter was measured and the area calculated when the fastest growing isolates had nearly covered the plate.

The PARPH (pimaricin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol) selective medium described previously by Kannwischer and Mitchell (7) and Mitchell et al (9) was used throughout this study. In this study, pimaricin was used at 5.0 mg/L and hymexazol at 30 mg/L of media and all other ingredients were as described previously (7,9). This medium was used in the original recovery of these isolates, for all assays for propagule densities in pathogenicity tests, and for reisolations from infected tissue. For determinations of propagule densities, the methodology of Timmer et al (14) was followed.

Pathogenicity tests. For root inoculations, the method of Graham (6) was used. Chlamydospores produced as described were used to infest several liters

of steam-sterilized Candler fine sand. This inoculum was incubated moist at room temperature for 2 days, then the propagule density was determined as described by Timmer et al (14). This infested soil was mixed with steam-sterilized Candler fine sand to give an inoculum level of one and four propagules per cubic centimeter of soil in the first and second tests, respectively.

Seedlings were grown in Pro-Mix BX (Premier Brands, Inc., New Rochelle, NY) in plastic tubes about 21 cm long and 4 cm in diameter. Seedlings about 15 cm tall were selected, rinsed free of potting mix, transplanted to the infested or noninfested soil in 15-cm-diameter clay pots, and maintained in the greenhouse at about 22–30 C. Plants inoculated with each species were grouped together on the greenhouse benches to avoid cross-contamination. Groups were rotated on the benches biweekly to reduce any possible location effects. All pots, including the noninoculated controls, were flooded for 48 hr each week. For the first experiment, seven single-plant replications of sour orange (*Citrus aurantium* L.) seedlings were used. For the second, six single-plant replications each of sweet orange (*C. sinensis* (L.) Osbeck), sour orange, and Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. × *C. paradisi* Macfady), which are highly susceptible, tolerant, and highly resistant, respectively, to *P. parasitica* (6), were evaluated.

Root systems were evaluated after 6 wk on the following scale: 0 = all roots healthy; 1 = rotted roots apparent, root system reduced; 2 = obvious root rot, root system small; 3 = severe fibrous root rot, taproot necrotic, few new roots; 4 = no healthy roots, taproot necrotic, stem girdled. Evaluations were made independently by two observers and the average rating presented. In addition, up to 100 root tips or all of those present on each seedling were rated as healthy or rotted and the data expressed as percent root rot. Roots and shoots were then dried to a constant level of moisture at 65 C and weighed.

For stem inoculations, rooted cuttings of citron (*C. medica* L. 'Etrog') and seedlings of sweet orange cv. Pineapple were grown in 15-cm-diameter plastic pots until 30–50 cm in height and 5–8 mm in stem diameter at the base. For the first experiment with Etrog citron, inoculum was prepared as for sporangium production. Mats bearing sporangia were placed beneath a 2 × 35 mm flap cut from the bark on each side of the stem. Control plants were wounded but not inoculated. Stems were then wrapped in moist cotton and packed with moist peat moss held in place by a bottomless, inverted Styrofoam cup placed around the stem area. Each treatment was replicated five times on single plants. In the second experiment, inoculum of each isolate was prepared as for the root inoculation. Sweet orange seedlings were wounded by slicing away a small piece of bark on each side of the stem. Soil infested with chlamydospores and adjusted to 50 propagules per cubic centimeter then was packed around the stem and held in place by an inverted bottomless Styrofoam cup. Ten single-plant replications were used for each isolate. All plants were maintained in the greenhouse at 22–30 C.

After 2 mo, disease severity was evaluated as before on the following scale: 0 = wound completely callused; 1 = necrosis apparent, wound callus around entire wound margin; 2 = necrosis around most of margin, little callus present; 3 = necrosis around entire margin, no callus; 4 = severe necrosis, stem girdled.

For fruit inoculations, Navel or Valencia oranges were collected from the field, rinsed with water, and surface-disinfested by wiping with 70% ethanol. Fruit were placed in basins of water about 1–2 cm deep. Five droplets (about 0.05 ml each) of a suspension containing 10^5 zoospores per milliliter were placed on each fruit without wounding. Six fruit were inoculated with each isolate in each test. Basins were covered with plastic wrap and incubated in the laboratory at 23–25 C for 1 wk. The number of fruit with brown rot symptoms was counted, and reisolations were attempted from all of the fruit.

RESULTS

Fungal characteristics. Characteristics of the *Phytophthora* sp. recovered from the citrus orchard, which we have designated *P. palmivora*, and the palm isolate of *P. arecae* are compared in Table 1. The sporangia of *P. palmivora*-citrus were small, papillate, mostly ellipsoid, and caducous with a short pedicel and had wide sporangiophores (Fig. 1, Table 1). Abundant chlamydospores were produced using the method of Tsao (16). The *P. palmivora*-citrus and the palm isolate of *P. arecae* were both A^1 mating types and produced oospores readily when mated with A^2 isolates of *P. palmivora* from cacao or *P. parasitica*, but many oospores were abnormal in matings with *P. parasitica*. The *P. arecae* from palm and the *P. palmivora* from citrus were nearly identical morphologically.

The optimum temperature for growth of *P. palmivora*-citrus was about 30 C, with very little growth at 33 C (Fig. 2). Growth rate and optimum temperature were similar to those of the Florida isolate of *P. citrophthora*. *P. parasitica* grew much faster, had an optimum temperature of 27 C, and produced considerable growth at 33 C and some at 36 C.

Pathogenicity. In the initial experiment using sour orange seedlings and inoculum levels of one propagule per cubic centimeter of soil, *P. parasitica* and *P. palmivora*-citrus produced root rot ratings of 1.3 and 1.4 and percentages of rotted tips of 11 and 15%, respectively. Plants inoculated with *P. arecae*-palm and the noninoculated controls had root rot ratings of zero and less than 1% rotted root tips.

In the second experiment, in which three rootstock species were inoculated, *P. parasitica* and *P. palmivora*-citrus caused extensive root rot of all three species and reduced root and shoot weights in most cases (Table 2). Inoculation of seedlings with *P. arecae*-palm did not produce root rot but did reduce the root weights of sweet orange and sour orange. Disease ratings and root rot percentages

Table 1. Characteristics of isolates of *Phytophthora palmivora* from citrus and *P. arecae* from palm

Structure	*P. palmivora*-citrus	*P. arecae*-palm
Sporangia		
Length (μm)	49.6 ± 4.6[x]	51.6 ± 4.8
Breadth (μm)	27.3 ± 3.2	28.2 ± 3.6
L/B ratio	1.83 ± 0.19	1.85 ± 0.21
Pedicel length (μm)	3.1 ± 0.7	3.5 ± 1.1
Sporangiophore width (μm)	2.76 ± 0.54	2.30 ± 0.58
Chlamydospore diameter (μm)	33.8 ± 2.4	33.9 ± 3.7
Oogonium diameter (μm)	29.7 ± 1.1[y]	...[z]
Oospore diameter (μm)	25.2 ± 0.7[y]	...

[x]Mean ± standard deviation.
[y]When mated with A^2 isolate of *P. palmivora* from cacao.
[z]Not done.

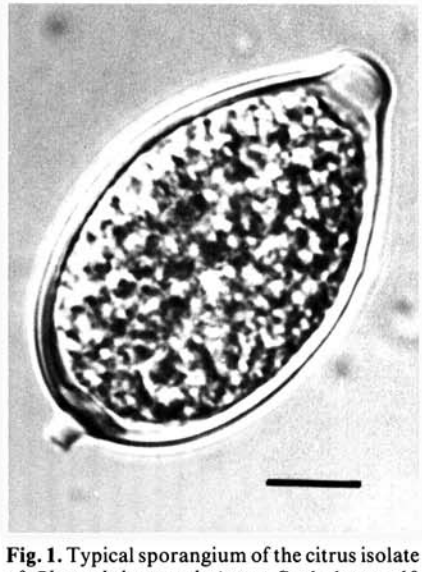

Fig. 1. Typical sporangium of the citrus isolate of *Phytophthora palmivora*. Scale bar = 10 μm.

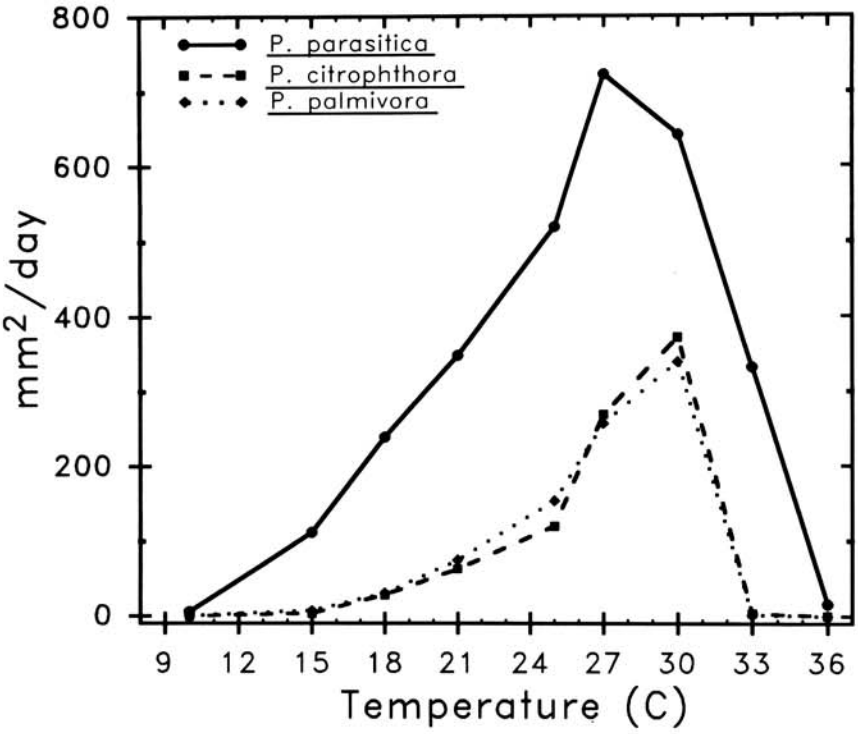

Fig. 2. Colony areas of *Phytophthora parasitica, P. citrophthora*, and the citrus isolate of *P. palmivora* grown at different temperatures.

Table 2. Effect of inoculation with *Phytophthora* species on root rot severity and root and shoot weight of three citrus rootstocks

Rootstocks	Isolates			Noninoculated control
	P. parasitica-citrus	*P. palmivora*-citrus	*P. arecae*-palm	
Disease rating[y]				
Sweet orange	2.0	3.7	0.0	0.0
Sour orange	1.5	2.5	0.0	0.0
Swingle citrumelo	1.3	1.2	0.0	0.0
LSD = 0.61				
Root rot (%)[z]				
Sweet orange	49.3	76.3	1.1	0.0
Sour orange	41.2	66.5	0.2	0.0
Swingle citrumelo	43.2	28.9	1.1	0.3
LSD = 20.4				
Root dry weight (g)				
Sweet orange	0.36	0.11	0.46	0.71
Sour orange	0.32	0.24	0.43	0.75
Swingle citrumelo	0.53	0.38	0.64	0.66
LSD = 0.12				
Shoot dry weight (g)				
Sweet orange	0.95	0.46	1.32	1.76
Sour orange	1.05	0.65	1.26	1.84
Swingle citrumelo	1.20	0.71	1.72	1.45
LSD = 0.32				

[y] Rated on a scale of 0 = all roots healthy to 4 = no healthy roots, taproot necrotic, stem girdled.
[z] Percentage of 100 root tips per plant or all of the root tips present.

Table 3. Disease severity after stem inoculation of sweet orange cv. Pineapple with four isolates of *Phytophthora* species

Isolate	Visual rating[w]	No. reisolated/ no. tested[x]	Propagules/ cm³[y]
P. parasitica-citrus	0.6 bc[z]	6/10	1.8 bc
P. palmivora-citrus	1.3 ab	6/10	13.6 bc
P. arecae-palm	1.9 a	8/10	18.4 b
P. palmivora-cacao	0.1 c	1/10	47.4 a
Noninoculated control	0.0 c	0/10	0.0 c

[w] Rated on a scale of 0 = wound completely callused to 4 = severe necrosis, stem girdled.
[x] Number of the 10 plants inoculated from which the original isolate was recovered.
[y] Propagule density in the inoculum packed around the stem at the conclusion of the experiment.
[z] Mean separation by Duncan's multiple range test ($P \leq 0.05$).

were greater after inoculation with *P. palmivora*-citrus than with *P. parasitica* on sweet orange and sour orange but not on Swingle citrumelo. When an analysis of variance was conducted considering only these two species, rootstock and *Phytophthora* species had a significant effect on all parameters, except that rootstock had no significant effect on shoot weight. The interaction between rootstock species and *Phytophthora* species was significant in all cases, indicating a differential relationship of these *Phytophthora* species to resistance in these rootstocks. In general, the decline in disease severity observed from the highly susceptible sweet orange to the moderately resistant sour orange to the highly resistant Swingle citrumelo was more pronounced with *P. arecae* than with *P. parasitica*. When isolations from rotted roots were made from plants inoculated with *P. parasitica* and the *P. palmivora*-citrus, only the species inoculated was reisolated. The *P. arecae*-palm isolate was not recovered from inoculated citrus roots. Noninoculated control plants showed no root rot, and *Phytophthora* species were not isolated from these roots.

When stem inoculations were made on Etrog citron cuttings, *P. parasitica, P. palmivora*-citrus, and *P. arecae*-palm produced disease ratings of 2.3, 3.0, and 2.6, respectively, compared with a rating of 0.3 in the noninoculated control. The species inoculated was reisolated from the lesions in each treatment, and no *Phytophthora* sp. was recovered from the noninoculated control.

In the second stem inoculation experiment using sweet orange, *P. parasitica, P. palmivora*-citrus, and *P. arecae*-palm produced stem lesions but *P. palmivora* from cacao did not (Table 3). The inoculated species was reisolated from most of the affected plants, but only one of 10 plants inoculated with *P. palmivora*-cacao yielded the fungus and no *Phytophthora* sp. was recovered from controls. By the end of the experiment, propagule densities in the inoculum had diminished significantly from the original 50 propagules per cubic centimeter.

When six Valencia orange fruit were inoculated with the different isolates, the following number became infected: three fruit with *P. parasitica*, six with *P. palmivora*-citrus, four with *P. arecae*-palm, and six with *P. citrophthora*. All developed typical brown rot symptoms with extensive external mycelial development. *P. palmivora*-citrus, *P. arecae*-palm, and *P. citrophthora* produced abundant sporangia on the fruit surface, whereas *P. parasitica* produced none. In all cases, reisolations yielded only the same species inoculated. Similar results were observed with Navel oranges.

DISCUSSION

We have designated the species recovered from citrus as *P. palmivora*. We

had originally reported this isolate as *P. arecae* (15), based primarily on its similarity to the isolate from palm (12; Table 1). The available keys and descriptions (10,17,19) do not clearly distinguish between the two species. Waterhouse (19) indicated that *P. palmivora* has larger sporangia (50–60 μm) with thin sporangiophores (1 μm), whereas *P. arecae* has smaller sporangia (40–50 μm) with wide sporangiophores (2.5 μm). The *P. palmivora*-citrus and *P. arecae*-palm isolates have intermediate-sized sporangia with wide sporangiophores (Table 1). The sporangia of *P. palmivora*-citrus are more ellipsoid, whereas those of *P. arecae* are typically more spherical (19). Thus, *P. arecae* may not be sufficiently different morphologically from *P. palmivora* to allow ready identification of isolates. In addition, analysis of isozymes and mitochondrial DNA restriction patterns indicate that some isolates of *P. palmivora* and *P. arecae* are identical and should be grouped in the same species (5,11).

P. arecae, to the best of our knowledge, has never been isolated from citrus or associated with any citrus disease. *P. palmivora* has been reported from citrus in many humid tropical areas (8) and has been associated with a blight of grapefruit nursery seedlings in Puerto Rico (18). Some isolates of *P. palmivora* produced necrosis of inoculated tender shoots of grapefruit, but several other species not normally associated with citrus diseases produced similar reactions (17). Thus, the pathogenicity of *P. palmivora* to citrus has not been demonstrated unequivocally.

The *P. palmivora*-citrus isolate from Florida now has been shown to be pathogenic to citrus roots, stems, and fruit. The *P. arecae* from palm was pathogenic to aboveground plant parts but failed to cause root rot of citrus in two tests. In the one test where *P. palmivora* from cacao was used, it did not affect stems. Thus, it appears that isolates from various sources may differ considerably in their effect on citrus.

P. parasitica is widespread in citrus orchards in Florida and *P. citrophthora* occurs only locally (2,20,21). The only other report on citrus from the state is an unidentified species associated with winter outbreaks of brown rot (2). This isolate was probably not *P. arecae* or *P. palmivora*, since it rarely produced sporangia and had a low temperature optimum in direct contrast to our isolate. The *Phytophthora* species used for biological control of milkweed vine (*Morrenia odorata* (Hook. & Arnott) Lindl.) in Florida citrus orchards was originally classified as *P. citrophthora* (1) but has been reclassified as *P. palmivora* (4). The milkweed vine isolates differ slightly morphologically from *P. palmivora*-citrus, are all A^2 mating types, and are not pathogenic to citrus (4) and thus are not the same as the *P. palmivora*-citrus isolate studied here.

The *P. palmivora*-citrus isolate has been recovered from only one other orchard, about 5 km from the site where it was originally isolated. The origin of the isolate is unknown. Both orchards have serious root rot problems, but *P. parasitica* is also present at both sites. Nevertheless, the presence of *P. palmivora* isolates that are highly pathogenic to roots, stems, and fruit of citrus trees in an orchard situation is cause for some concern. Serious damage could result should it become widespread. In recent tests where *P. palmivora* and *P. parasitica* were coinoculated on sweet orange seedlings, *P. palmivora* outcompeted *P. parasitica* and became the dominant species (Zitko and Timmer, *unpublished*). However, *P. palmivora* may not compete well with soil saprophytes under field conditions. For example, *P. citrophthora* has been present in Florida for many years and, for unknown reasons, remains localized (20). Perhaps the same factors that limit spread of *P. citrophthora* will restrict the spread of *P. palmivora*. Swingle citrumelo, which is resistant to *P. parasitica*, also appears to tolerate *P. palmivora*. Thus, a source of resistance to *P. palmivora* is available.

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