Identity of *Phytophthora* Isolated from Milkweed Vine

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ABSTRACT

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Comparative morphological, physiological, and pathological tests indicated that isolates of *Phytophthora* from dying milkweed vine (MWV) (*Morrenia odorata*) in Florida are *P. palmivora*, and not *P. citrophthora* as previously stated. Sporangia of six isolates were caducous with short stalks (2.7 μm). They were ellipsoid to ovoid, and averaged 46 × 27 μm, with a length/breadth (L/B) ratio of 1.7 and papilla width of 5 μm. Sporangia of isolates of *P. citrophthora* from citrus were persistent. They were highly variable in shape and size, and averaged 55 × 35 μm, with an L/B ratio of 1.6 and papilla width of 7 μm. MWV isolates formed oospores in pairings with A[1] isolates of *P. palmivora* and *P. parasitica*, whereas isolates of *P. citrophthora* were sterile. Sweet orange plants were resistant to isolates from MWV, but susceptible to isolates of *P. citrophthora* from citrus. Disc gel electrophoresis of hyphal proteins revealed high percentages of similarity between isolates from MWV and *P. palmivora* (Morphological Form 1), but not between isolates from MWV and *P. citrophthora*.

The milkweed vine (MWV), *Morrenia odorata* Lindl., is a weed pest in many Florida citrus groves (28,31,34). It competes for light, water, and nutrients; girdles tree limbs; and interferes with spraying, harvesting, and irrigation (28,31,34). Mechanical removal of established vines and the use of selective herbicides are currently the primary means of control (28,29,31,37). However, such methods are costly to the growers and only partial control of the vines may result (31,32).

Isolations from dying MWV with girdled stem and root rot consistently yielded a fungus identified as *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leon. (5,6). Isolates of *Phytophthora* from MWV did not attack citrus and related species, and race differences within this species were suggested (5,6). Investigations have been conducted in Florida to obtain data on the distribution, efficacy, host range, and stability of this pathotype of *P. citrophthora* in order to evaluate its potential as a biological control agent (5,6,24,32).

This paper reports the results of comparative morphological, physiological, and pathological studies of isolates from MWV and isolates of *P. citrophthora* from citrus that were performed to clarify the identity of the isolates from MWV.

MATERIALS AND METHODS

Isolates. The isolates of *Phytophthora* examined are listed in Table 1. One mutant isolate (P1185), from MWV, obtained by exposing motile zoospores to UV irradiation (32), was provided by C. L. Schoulties (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville), who was studying the possibility of genetic changes in the fungus then known as *P. citrophthora*. Cultures are maintained in the Phytophthora Collection, Department of Plant Pathology, University of California at Riverside. Single-zoospore isolates were used in all experiments. Twelve single-zoospore cultures were prepared from each mass culture and their colony morphology and growth rates on potato-dextrose agar were compared. The culture most representative of the mass culture was selected for further study.

Media used. The media used included: cleared V-8 juice agar (CV-8A) and broth (CV-8B) (45), fresh potato-dextrose agar (PDA) (45), Ribeiro's synthetic medium (SM) (30), yeast-peptone broth (GYP) (15), carrot agar (CA) (18), and cornmeal agar (CM) (Difco 17 g/L).

Colony morphology. Isolates were transferred to plates containing 15 ml of PDA medium, and colony morphology was observed after incubation at 24 ± 1 C in the dark for 5 or 6 days.

Sporangial morphology. The method of Ribeiro and Baumer (*personal communication*) was used to study the sporangial morphology of six isolates from MWV and 10 isolates of *P. citrophthora* from citrus; 100 sporangia were measured for each isolate. A 5-mm-diameter mycelial disk was taken from actively growing cultures on CV-8A and transferred to 9-cm-diameter petri

TABLE 1. Origin of isolates of *Phytophthora*[a] used to study the identity of an isolate of *Phytophthora* from the milkweed vine (*Morrenia odorata*)

Culture no.[b]	Source host	Geographic origin
P992	*Morrenia odorata* Lindl.	Florida
P1182	*Morrenia odorata* Lindl.	Florida
P1183	*Morrenia odorata* Lindl.	Florida
P1184	*Morrenia odorata* Lindl.	Florida
P1185[c]	*Morrenia odorata* Lindl.	Florida
P1186	*Morrenia odorata* Lindl.	Florida
P1187	*Morrenia odorata* Lindl.	Florida
P479	*Citrus sinensis* (L.) Osbeck	California
P1152	*Citrus aurantium* L.	Argentina
P1153	*Citrus sinenis* (L.) Osbeck	Brazil
P1156	*Citrus* sp.	California
P1157	*Citrus paradisi* Macf.	California
P1158	*Citrus* sp.	California
P1159	*Citrus* sp.	California
P1160	*Citrus sinensis* (L.) Osbeck	California
P1161	*Citrus* sp.	California
P1163	Citrus sinensis (L.) Osbeck	California
P491	*Carica papaya* L.	Brazil
P492	*Citrus* sp.	Brazil
P255	*Theobroma cacao* L.	Costa Rica
P550	*Theobroma cacao* L.	Jamaica

[a] Isolates from: F. Albuquerque, A. A. Bitancourt, T. A. DeWolfe, E. Feichtenberger, J. A. Menge, W. H. Ridings, V. Rossetti, and G. A. Zentmyer.
[b] Stock culture numbers in the Phytophthora Collection at the Department of Plant Pathology, University of California, Riverside.
[c] Mutant milkweed vine isolate obtained by exposing motile zoospores of the isolate from MWV to UV irradiation (32). From C. L. Schoulties, Fla. Div. of Plant Industry, Gainesville.

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plates containing diluted CV-8B (2 ml of CV-8B in 10 ml of sterile demineralized water). The plates were incubated under two 40-W day-light-type fluorescent lamps suspended 40 cm above the colonies, at room temperature (24 ± 2 C). After 24 hr, the diluted CV-8B was removed aseptically, and 10 ml of sterile demineralized water was added to each plate. Plates were returned to the light system and were observed with a Leitz Ortholux microscope after 24–48 hr.

Sporangial stalk length. The method of Kaosiri et al (18) was used to determine the sporangial stalk or pedicel length of six isolates from MWV. Two isolates of *P. palmivora* (Morphological Form 1) from cacao and 10 isolates of *P. citrophthora* from citrus were included in this study for comparison.

Oospore production. Isolates of *Phytophthora* from MWV and isolates of *P. citrophthora* from citrus were initially tested for homothallism by placing a single 5-mm-diameter mycelial plug in the center of both CV-8A and SM plates. After 13 days at 24 ± 1 C in the dark, inoculated plates were observed for oospore production.

Isolates were also tested for production of sexual structures in crosses with *P. parasitica*, P492 (A^1) and P491 (A^2), and *P. palmivora*, P550 (A^1) and P255 (A^2). Five-millimeter-diameter mycelial plugs were placed 60 mm apart on CV-8A or SM and plates were incubated at 24 ± 1 C in the dark. The paired cultures were examined microscopically for oospore production 13 and 23 days later.

Disc gel electrophoresis of hyphal proteins. A modification of the gel electrophoretic method described by Gill and Zentmyer (15) was used to compare the soluble protein pattern bands of four isolates from MWV, an isolate of *P. citrophthora* from citrus, and an isolate of *P. palmivora* from cacao.

Three 5-mm-diameter mycelial disks were added to 50 ml of GYP in a 250-ml Erlenmyer flask. Cultures were incubated in the dark at 24 ± 1 C for 7 days, then the contents of the flasks were blended in a sterile blender cup at high speed for 5 sec. Five milliliters of each homogenized mycelial suspension was added to 100 ml of GYP in an 800-ml Roux bottle. Cultures were incubated in the dark at 24 ± 1 C for 7 days. The mycelium was vacuum filtered onto a Whatman No. 1 filter paper and washed three times with distilled water. The method of Gill and Zentmyer (15) was modified by using a cell homogenizer MSK apparatus (B. Braun Instruments, South San Francisco, CA 94080) to extract buffer soluble proteins. Five grams of mycelium, which was blotted dry, and 5 ml of cold phosphate buffer (pH 7.0, 0.1 M potassium monobasic phosphate and 0.1 M sodium dibasic phosphate) were placed into a precooled 75-ml homogenizer glass bottle which had been filled with approximately 50 g of 0.45-mm-diameter glass beads prerinsed with phosphate buffer. The bottle was placed into the MSK homogenizer and shaken four times for 30 sec each at 3,450 rpm. The contents of the bottles were removed and centrifuged at 27,000 *g* (SORVAL superspeed RC-2) for 1 hr. The resultant clear supernatant from the fungal extract was decanted immediately and used for electrophoresis. A disc electrophoretic apparatus model 12 (CANALCO Corporation, Bethesda, MD 20014) was used (10,23,27). Each sample contained approximately 550 μg of fungal protein, as determined by the Lowry method (22). Electrophoresis was carried out at room temperature (24 ± 2 C) in precooled tris-glycine buffer at pH 8.3. The gels were then removed from the tubes and stained with amido black or Coomassie blue. Spectrophotometric scans of the destained gels were made at 560 nm. Each isolate was replicated four times and several runs were made from each protein preparation. The experiments were repeated three times with fresh extract prepared as outlined above.

Protein patterns were evaluated on the basis of number, position, and width of the bands. The R_f values were calculated by expressing the mobility of each band in relation to the tracking dye. The percent similarity values of the protein bands obtained were determined by using the formula:

$$\% \text{ similarity} = \frac{\text{No. pairs of similar bands}}{(\text{No. different bands}) + (\text{No. pairs of similar bands})} \times 100$$

Protein bands located at comparable positions in two gels were considered similar and given the same R_f value when they overlapped each other for more than 50% of their widths.

Pathogenicity. Rossetti's stem inoculation method (33), similar to that of Klotz and Fawcett (19), was used to evaluate the pathogenicity of one MWV isolate (P992) and five isolates of *P. citrophthora* from citrus to four citrus rootstocks: Pineapple sweet orange (*Citrus sinensis* (L.) Osbeck), Brazilian sour orange (*C. aurantium* L.), alemow (*C. macrophylla* Wester), and Troyer citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck). Ten seedlings for each rootstock were inoculated at a height of approximately 5 cm above soil level, by removing a 5-mm-diameter bark disk to expose the cambium layer and placing a mycelial disk from a PDA culture onto the exposed tissue. The bark disk was replaced and the stem was wrapped with a waterproof tape. After 40 days, the bark was stripped from the stem and lesions on the surface of the cambium and wood were measured (length × breadth).

TABLE 2. Sporangial characteristics[a] of isolates of *Phytophthora* from milkweed vine (MWV) (*Morrenia odorata*) and isolates of *Phytophthora citrophthora* from citrus

	Length (L)		Breadth (B)		L/B ratio		Papilla width	
Culture no.[b]	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Isolates from MWV								
P992	44 ± 6	29–60	25 ± 3	19–37	1.7 ± 0.2	1.2–2.3	5 ± 1	3–8
P1182	46 ± 8	29–73	28 ± 4	19–41	1.7 ± 0.3	1.1–3.0	5 ± 1	4–7
P1183	47 ± 6	33–60	29 ± 5	22–44	1.7 ± 0.2	1.2–2.4	5 ± 1	4–8
P1184	45 ± 8	27–83	29 ± 4	21–37	1.6 ± 0.3	1.2–2.5	5 ± 1	3–8
P1185	47 ± 9	27–73	27 ± 4	21–37	1.7 ± 0.3	1.1–2.7	5 ± 1	3–8
P1187	44 ± 6	28–67	27 ± 3	20–35	1.6 ± 0.2	1.2–2.4	5 ± 1	4–6
Isolates from citrus								
P479	63 ± 15	32–110	35 ± 6	21–54	1.8 ± 0.5	1.1–3.6	7 ± 1	4–10
P1152	60 ± 17	33–108	35 ± 6	23–64	1.7 ± 0.5	0.8–3.9	8 ± 2	4–12
P1153	52 ± 8	38–94	36 ± 5	25–53	1.5 ± 0.2	0.8–2.4	7 ± 1	4–10
P1156	53 ± 7	38–71	35 ± 5	24–58	1.6 ± 0.3	0.7–2.4	7 ± 1	5–11
P1157	47 ± 11	30–98	34 ± 6	23–56	1.4 ± 0.3	0.6–3.1	7 ± 1	4–11
P1158	55 ± 13	39–125	35 ± 4	25–48	1.6 ± 0.4	1.0–2.9	7 ± 1	4–10
P1159	60 ± 10	37–81	37 ± 7	27–60	1.7 ± 0.4	0.8–2.9	9 ± 1	6–11
P1160	50 ± 8	35–89	35 ± 4	25–50	1.5 ± 0.3	0.7–2.6	7 ± 1	4–12
P1161	51 ± 10	32–83	34 ± 4	22–46	1.4 ± 0.3	0.8–2.2	7 ± 1	4–10
P1163	63 ± 12	29–91	37 ± 7	23–79	1.7 ± 0.3	0.5–2.6	7 ± 1	4–9

[a] Average size (μm) of 100 sporangia produced in liquid medium (1 part CV-8B:5 parts sterile deionized water) ± standard deviation.
[b] Selected single-zoospore cultures.

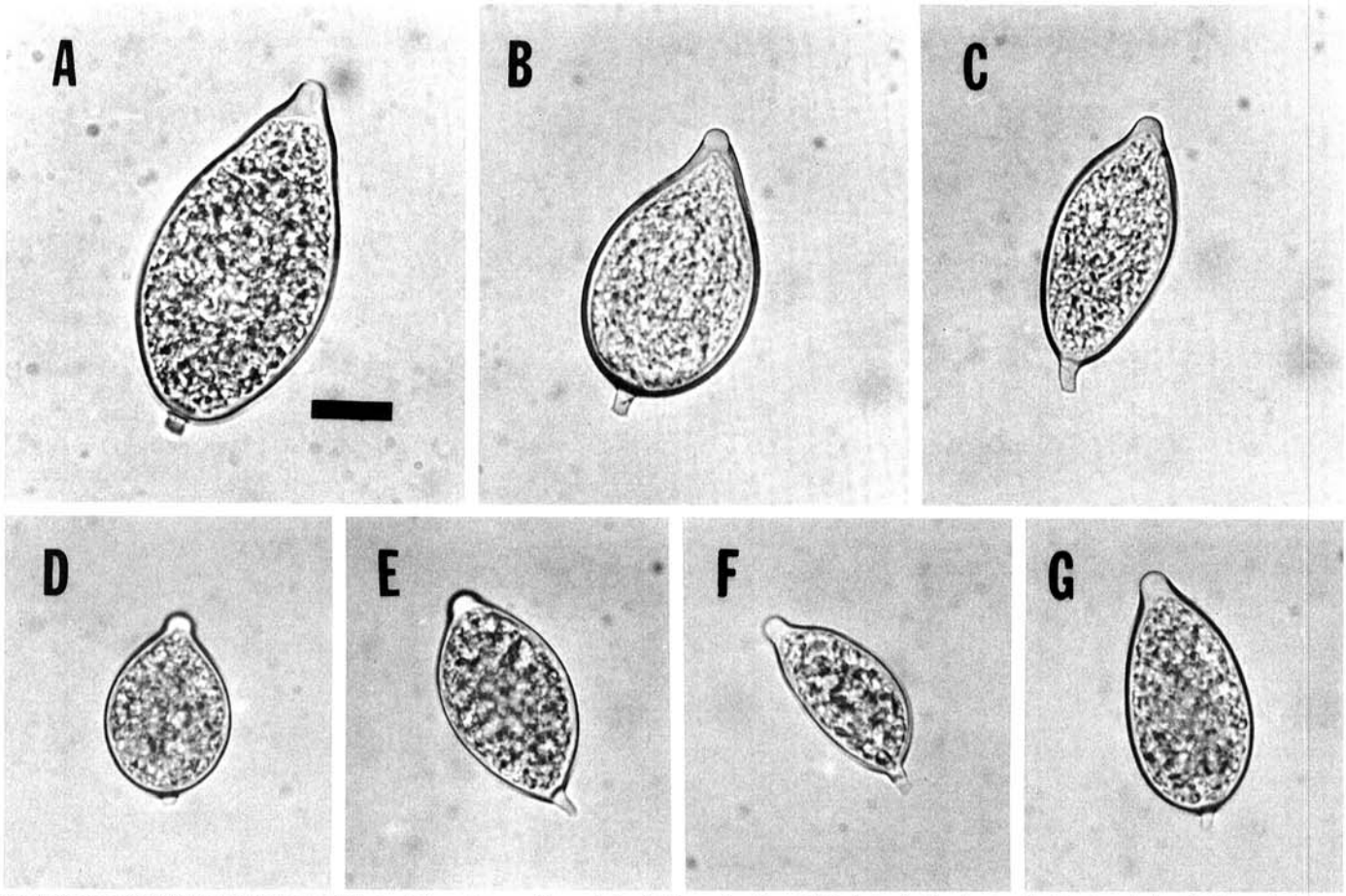

Fig. 1. Sporangia observed in isolates of *Phytophthora* from milkweed vine (*Morrenia odorata*). Note typically short pedicel and variation in size and shape. **A, B, C** = isolate 992; **D** = 1186; **E** = 1183; and **F, G** = 1182. Scale bar represents 20 μm.

RESULTS

Phenotypic stability of the cultures. Great variability among single-zoospore cultures was observed only with MWV isolate P992. Single-zoospore cultures of all other isolates of *Phytophthora* from MWV and ten isolates of *P. citrophthora* from citrus did not exhibit significant variation. The mutant MWV isolate (P1185) could not be studied for phenotypic stability of the cultures since normal cleavage of the protoplasm to form motile zoospores from sporangia did not occur.

Colony morphology on PDA. All isolates grew well on PDA, and colony morphology of all isolates from MWV was similar to that reported by Brasier and Griffin (4) for *P. palmivora* MF 1. Aerial mycelium was sparse, being more or less absent from the peripheral areas of the colony but more dense in the center.

Isolates of *P. citrophthora* from citrus produced cultures with a pronounced petalloid pattern and moderately aerial mycelium. This morphology has been described by Waterhouse and Waterson (44).

Sporangial morphology and dimensions. One hundred sporangia produced on dilute CV-8B were measured for each isolate. Six isolates of *Phytophthora* from MWV produced elongated, ellipsoid to ovoid sporangia, averaging 46×27 μm with length/breadth (L/B) ratio of 1.7 and papilla width of 5 μm (Table 2).

Ten isolates of *P. citrophthora* from citrus produced sporangia that were highly variable in shape and size, averaging 55×35 μm with an L/B ratio of 1.6 and papilla width of 7 μm (Table 2).

Sporangial stalk length. The sporangia of isolates of *Phytophthora* from MWV and isolates of *P. palmivora* (MF 1) from cacao were caducous in water. Sporangia of the isolates from MWV had short, thick pedicels (Fig. 1) averaging 2.7 ± 0.2 μm in length (Table 3). Sporangial stalks of two isolates of *P. palmivora* from cacao were also short and thick, averaging 2.2–2.3 μm in length.

TABLE 3. Sporangial stalk length[a] of isolates of *Phytophthora* from milkweed vine (MWV) (*Morrenia odorata*) and isolates of *P. palmivora* from cacao

Source host	Isolates[b]	Stalk length	
		Mean	Range
Morrenia odorata	P992	3.3 ± 1.0	2.1–6.2
	P1182	2.2 ± 0.6	1.0–4.2
	P1183	2.8 ± 1.0	1.0–4.6
	P1184	2.2 ± 0.7	0.6–4.2
	P1185	3.0 ± 1.3	0.8–6.2
	P1187	2.6 ± 1.0	0.8–4.6
Theobroma cacao	P255	2.2 ± 0.8	1.0–4.2
	P550	2.3 ± 0.8	1.0–4.6

[a] Average size (μm), with standard deviations, of 100 sporangia produced in carrot agar (18).

[b] Selected single-zoospore cultures, except isolate P1185 (mutant from MWV).

Most sporangia of ten isolates of *P. citrophthora* from citrus were persistent in water. Very few sporangia were detached from the sporangiophores when mature, and the few detached sporangia that were observed carried either no pedicel or a piece of hypha of irregular length.

Oospore production. Oospores were observed in crosses between wild-type isolates from MWV and A^1 mating types of *P. palmivora* or *P. parasitica* on both CV-8A and SM. None of the isolates from MWV produced sexual structures in single culture, and the mutant isolate from MWV did not form oospores in crosses.

Ten isolates of *P. citrophthora* from citrus did not produce sexual structures in single culture or when paired with A^1 or A^2 mating types of *P. palmivora* or *P. parasitica* on CV-8A or SM.

TABLE 4. Percentage similarity values of protein bands by disc gel electrophoresis, obtained when protein bands of four isolates of *Phytophthora* from milkweed vine (*Morrenia odorata*) (MWV) isolates were compared with each other and with protein bands of typical isolates of *Phytophthora palmivora* from cacao and *P. citrophthora* from citrus

Source plants and isolates	Milkweed vine				Cacao (*P. amylovora*)	Citrus (*P. citrophthora*)
	P1182	P1183	P1184	P1187	P255	P1157
MWV						
P1182	100	85	84	77	77	41
P1183		100	84	84	66	37
P1184			100	90	60	38
P1187				100	60	32
P. palmivora						
P255					100	38
P. citrophthora						
P1157						100

Fig. 2. Protein band patterns for four isolates of *Phytophthora palmivora* from milkweed vine (P1182, 1183, 1184, and 1187) compared with an isolate of *P. citrophthora* (P1157) from citrus.

Disc gel electrophoresis of hyphal proteins. The modified method proved to be reproducible. The protein band patterns for each isolate were identical in different electrophoretic runs, and also from cultures of the same isolate grown at three different times under the same conditions. The protein band patterns of the four isolates from MWV were similar (Fig. 2), with percent similarity values ranging from 77 to 90% (Table 4). When the protein band patterns of four isolates from MWV were compared to that of a typical isolate of *P. palmivora*, percent similarity values ranged from 60 to 77% (Table 4).

Protein band patterns of isolates from MWV were quite different from that of a typical isolate of *P. citrophthora* from citrus (Fig. 2). Percent similarity values obtained when four isolates from MWV were compared with the pattern of *P. citrophthora* ranged from 32 to 41% (Table 4).

Pathogenicity to citrus rootstocks. Lesion sizes on four citrus rootstocks are given in Table 5. Differences in virulence among the isolates from citrus tested were found only with Pineapple sweet orange. Troyer citrange, Brazilian sour orange, and alemow were comparatively resistant to stem inoculations with all isolates. Among the five isolates of *P. citrophthora* from citrus, two (P1156 and P1158) caused large lesions on Pineapple sweet orange and three caused moderate lesions. All isolates of *P. citrophthora* from citrus caused lesions on this host larger than those produced by the MWV isolate. Lesions on Pineapple sweet orange inoculated with the isolate from MWV were comparable to those produced on resistant Troyer citrange, Brazilian sour orange, and alemow plants.

TABLE 5. Size[y] of lesions developed after 40 days in four citrus rootstocks inoculated with an isolate of *Phytophthora* from milkweed vine (*Morrenia odorata*) and isolates of *Phytophthora citrophthora* from citrus

Isolates	Source host	Pineapple sweet orange	Troyer citrange	Brazilian sour orange	Alemow
P1156	*Citrus* sp.	36.6 a[z]	2.8 c	1.8 c	1.7 c
P1158	*Citrus paradisi*	30.7 a	2.1 c	1.4 c	1.5 c
P1163	*Citrus sinensis*	12.5 b	2.5 c	1.4 c	1.7 c
P1153	*Citrus sinensis*	11.9 b	2.4 c	1.3 c	1.2 c
P1161	*Citrus* sp.	9.4 b	2.6 c	1.6 c	1.3 c
P992	*Morrenia odorata*	1.6 c	2.7 c	0.9 c	1.7 c

[y] Values (cm^2) are the means of 10 replicates.
[z] Figures followed by the same letter do not differ significantly according to Duncan's multiple range test, $P = 0.05$.

DISCUSSION

Our studies indicate that isolates from MWV are *P. palmivora* (MF1), not *P. citrophthora* as reported previously by Burnett et al (6).

Many authors have described morphological groups within isolates of *P. palmivora* (3,4,8,18,26,36,38–40,42,46). Brasier and Griffin (4) studied isolates from cacao and other hosts and found significant variation in both morphological and physiological characteristics among isolates that had been referred to as *P. palmivora*. They used such characters as chromosome type, sporangial pedicel length, and colony morphology to separate these isolates into three main groups (MF1, MF3, and MF4). On PDA, the isolates from MWV produced a distinct and uniform colony morphology, which was like that produced by the *P. palmivora* MF1 of Brasier and Griffin (4). This differed markedly from the typical petalloid pattern Waterhouse and Waterston described (44) for *P. citrophthora*, which was produced by all isolates of *P. citrophthora* that we studied from citrus. Our results agree with data reported by Ridings et al (32) who found that colony characteristics of single zoospore cultures of the isolate from MWV, particularly on PDA, were distinct from those of *P. citrophthora* and were like those of one isolate of *P. palmivora*.

Sporangium morphology has been emphasized by several workers as a taxonomic parameter in *P. palmivora* (3,4,12,20,38,39,42). The average size of sporangia for six isolates from MWV was 46 × 27 μm with a L/B ratio of 1.7 and papilla width of 5 μm. Ten isolates of *P. citrophthora* from citrus produced sporangia averaging 55 × 35 μm with a L/B ratio of 1.6 and papilla width of 7 μm. These dimensions differ from those reported for *P. palmivora* and *P. citrophthora* in Waterhouse's key (41); this is probably due to differences in media and environmental conditions (3,4,21).

Sporangium caducity and stalk length have proved to be highly reliable characters in identifying *Phytophthora* species (1,2,4,16,36,42,43,46). Comparative studies on CA reported here with isolates from MWV, isolates of *P. palmivora* from cacao, and isolates of *P. citrophthora* from citrus indicated that isolates from

MWV and cacao were similar to each other with regard to caducity and sporangial stalk length, while isolates of *P. citrophthora* from citrus produced persistent sporangia. Values of sporangial stalk length of isolates from MWV and isolates of *P. palmivora* from cacao were comparable to each other and to those reported by several authors (1–3,18) for *P. palmivora* MF1 (stalk length <5 μm). Sporangia of isolates of *P. citrophthora* were considered persistent, according to Al-Hedaithy and Tsao's (1) concept of caducity. Very few sporangia of our isolates of *P. citrophthora* dislodged from the sporangiophore, and these few detached sporangia carried either no pedicel or a piece of hypha of irregular length.

Oospores were observed in pairings of wild-type isolates from MWV with A^1 mating type cultures of *P. palmivora* and *P. parasitica*. Similar results were obtained by Ridings et al (32) with one isolate from MWV. It is important to consider that the production of sexual structures in both inter- and intraspecific crosses may provide the *Phytophthora* associated with MWV with a means of overwintering and with a means of undergoing genetic recombination. This might be important for this fungus in overcoming host resistance (32).

Under our conditions, sexual structures were not produced by citrus isolates of *P. citrophthora* in single culture nor in crosses with A^1 or A^2 mating-type isolates of *P. parasitica*. Our results are in agreement with the recent tabular key to the species of *Phytophthora* prepared by Newhook et al (25). However, some authors (11,30,35) have observed oospore production by some isolates identified as *P. citrophthora*. Future work is needed to clarify the nature of the sexual stage of this species.

Protein band patterns produced by disc gel electrophoresis are useful as an additional tool for identification of *Phytophthora* species (9,11,13–15). Our comparison of proteins of isolates from MWV, isolates of *P. palmivora* from cacao, and isolates of *P. citrophthora* from citrus gave additional proof that isolates from MWV are not *P. citrophthora*. The protein patterns of the isolates from MWV were similar to each other and to that of *P. palmivora*, but quite distinct from that of *P. citrophthora*. Percent similarity values obtained when protein bands of isolates from MWV and isolates of *P. palmivora* from cacao were compared with each other may suggest differences between them at the race level rather than at the species level. Some authors (14,15,17) reported little intraspecific variation in *Phytophthora* by electrophoretic protein patterns. Our modified method (E. Feichtenberger and G. A. Zentmyer, *unpublished*) is capable of detecting more bands than the original method of Gill and Zentmyer (15). This improved sensitivity made it possible to detect quantitative differences among the isolates from little MWV.

Pathogenicity tests by Ridings et al (32) with isolates from MWV and other isolates of *P. citrophthora, P. palmivora*, and *P. parasitica* revealed that several isolates of *P. citrophthora* and *P. palmivora* were pathogenic to MWV.

Ridings et al (32) also found that representatives of several plant families were susceptible to the isolate from MWV and that this isolate was capable of infecting several citrus rootstocks in preemergence tests. Burnett et al (6) observed the infection of citrus fruits by the isolate from MWV after wound inoculation. These reports are consistent with the known host range of *P. palmivora*, which parasitizes over 51 genera of plants, including *Citrus* spp. (7). With the recent revision in the taxonomy of *P. palmivora* (4,46), it is likely that several *Phytophthora* species are included in Chee's (7) host list.

In stem inoculations on several citrus rootstocks, the isolate from MWV was less virulent than were isolates of *P. citrophthora* from citrus on Pineapple sweet orange plants, which are moderately susceptible to *P. citrophthora* (33). As expected, Troyer citrange, Brazilian sour orange, and alemow exhibited sufficient resistance to all isolates of *Phytophthora* tested to prevent expression of any isolate differences in virulence. Considering our identification of the isolate from MWV as *P. palmivora* (MF 1), our inoculation results are in agreement with those of Rossetti (33), who reported that, of six *Phytophthora* species (including *P. palmivora*), *P. citrophthora* was by far the most virulent to sweet orange plants.

In conclusion, our comparative morphological, physiological, and pathological studies indicate that isolates from MWV are actually *P. palmivora* rather than *P. citrophthora*.

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