Biological Control of Milkweed Vine in Florida Citrus Groves with a Pathotype of Phytophthora Citrophthora[1]

by
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INTRODUCTION

Milkweed vine (*Morrenia odorata* Lindl.) has been well recognized as causing serious problems in Florida citrus groves. Some of these problems have been presented in other papers at this symposium (Habeck, Charudattan).

Studies on the control of milkweed vine with a pathotype of *Phytophthora citrophthora* (R. E. Sm. and E. H. Sm.) Leonian were initiated in the spring of 1973, following the isolation of this fungus in the fall of 1972 from dying milkweed vines in a citrus grove in Orange County, Florida. Burnett *et al.* (1, 2) reported in 1973 and 1974 that this fungus was highly pathogenic to milkweed vines in field trials and that it had a high degree of specificity for this noxious weed.

Since 1974, investigations have been directed to obtain data on the distribution, efficacy, host range, and stability of this pathotype so as to evaluate its potential use as a biological control agent. This paper reports the results obtained to date in this study.

MATERIALS & METHODS

The *Phytophthora* spp. and isolates used in the study are listed in Table 1. Cultures were maintained in test tubes on hemp agar slants (40 ml of oil extracted from hemp seed and 15 g agar in 1 liter of medium) under mineral oil at 10C in the Florida Type Culture Collection (FTCC) and in test tubes on hemp agar slants without mineral oil at room temperature with periodic transfers as needed.

Other media employed in this study were potato dextrose agar (PDA) (broth from 200 g fresh potatoes, 20 g dextrose, and 15 g of Difco agar per liter of medium), cornmeal agar (CMA) (17 g of Difco CMA and 2 g of dextrose per liter of medium), clear V-8 agar (200 ml of Campbell V-8 juice, 4.5 g calcium carbonate, and 15 g of Difco agar per liter of medium). The V-8 juice was cleared by centrifugation at 1275 g for 7 minutes prior to adding the agar and sterilizing. The liquid V-8 medium was prepared similiarly to the clear V-8 agar except that the agar was omitted. In certain mating studies, the clear V-8 agar was supplemented with 30 mg of β-sitosterol, 20 mg of tryptophan, 100 mg of $CaCl_2 \cdot H_20$, and 1 mg of thiamine per liter of medium (SCV-8 agar) as described by Chee *et al.* (1976). Lima bean broth was prepared by steaming 10 oz of baby lima beans (Birds Eye) in 500 ml of distilled water and adjusting the final volume to 2 liters of medium with distilled water following filtering of the beans. Chlamydospores were produced in liquid V-8 medium using a modification of the method described by Tsao (1967). This modification consisted of vigorously shaking 1-day-old cultures and placing the bottles upright immediately at 18 C in the dark for the remainder of the incubation time (4 to 5 weeks). The procedure for the sonication of Chlamydospores was the same as that described by Raimirez and Mitchell (1975) except that 80% maximum sonication was used. The number of chlamydospores in a given suspension was determined after sonication by averaging 8 sample counts in a standard hemacytometer.

Cultures for zoospore production were obtained by inoculating either 25 ml of lima bean broth in 16 oz prescription bottles or 15 ml of lima bean broth in petri plates and incubating at 25 C for 2

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Table 1. *Phytophthora* spp. and isolates used in milkweed vine studies

Species and isolate designation*	Obtained from	Host and origin	Compatibility type[e]
P. citrophthora			
484	WVU[a] - N170	Citrus roots, FL	A^1
485	WVU - N222	Unknown	A^2
514	G. R. Grimm, USDA Orlando, FL	*Citrus sinensis* 'pineapple', FL	A^2-DPI
524	J. O. Whiteside, PR-122; AERC, Lake Alfred, FL[b]	Citrus fruit 'Hamlin', FL	A^1-CPI
525	J. O. Whiteside, PR-136	Citrus fruit, FL	A^2-DPI
541	J. O. Whiteside, 209	Citrus fruit, FL	A^2-DPI
666	H. C. Burnett, FDACS[c]	*Morrenia odorata* roots, Winter Garden, FL	Unknown
666-1	Reisolate of FTCC 666 Jan, 1974	Roots of *M. odorata* infected with isolate 666, Greenhouse, Gainesville, FL	Unknown
666-2	Reisolate of 666-1 Jan, 1975	Roots of *M. odorata* infected with isolate 666-1, Greenhouse, Gainesville, FL	Unknown
666-3	Reisolate of 666-2 Jan, 1976	Roots of *M. odorata* infected with isolate 666-2, Greenhouse, Gainesville, FL	Unknown
693	UCR[d] P-717	CMI 32034	A^1
694	UCR P-667	*Citrus reticulata* 'Cleopatra' France	A^2
745	UCR P-479	*Citrus limon*, CA	A^2-DPI
747	UCR P-1007	*Euonymus japonicus*	A^2-DPI
P. palmivora (Butl.) Butl.			
664	UCR P-253	Unknown	A^1
665	UCR 255	*Theobroma cacao*	A^2
491	WVU, N130	*Theobroma cacao*, Ivory Coast CMI-74805	A^2
492	WVU, N137	Hevea fruit, Costa Rica	A^1
739	UCR, P-626	*Theobroma cacao*, Brazil	A^2
740	UCR, P-624	*Theobroma cacao*, Brazil	A^1
741	UCR, P-257	*Theobroma cacao*, Guatemala	A^1
742	UCR, P-370	*Hevea brasiliensis*, Malaga	A^1
743	UCR, P-488	*Theobroma cacao*, Brazil	sterile
744	UCR, P-1006	*Hedra* sp., CA	A^2

Table 1. *Phytophthora* spp. and isolates used in milkweed vine studies (cont.)

Species and isolate designation*	Obtained from	Host and origin	Compatibility type[e]
766	J. F. Knauss, AREC, Apopka, FL R9	*Dieffenbachia*, FL	A^2
767	J. F. Knauss, G17	*Dieffenbachia*, FL Reisolate of R9	A^2
768	J. F. Knauss, G21	*Dieffenbachia*, FL Reisolate of R9	A^2
769	J. F. Knauss, G24	*Dieffenbachia*, FL Reisolate of R9	A^2
P. parasitica Dastur			
493	WVU, N15	*Nicotiana tabacum*, N.C.	A^1
494	WVU, N34	Lemon fruit, CA	A^2
691	UCR, P-991	Pomelo soil, CA	A^1
692	UCR, P-731	Citrus soil, CA	A^2

*Number refers to Florida Type Culture Collection designation.
[a] Phytophthora culture collection in Division of Plant Sciences at West Virginia University, Morgantown.
[b] AREC=Agricultural Research and Education Center, University of Florida, Lake Alfred.
[c] FDACS=Florida Department of Agriculture and Consumer Services, Winter Haven.
[d] UCR=Phytophthora collection at University of California, Riverside.
[e] Compatibility types determined experimentally by interspecific pairings with A^1 and A^2 compatibility of *P parasitica* and *P. palmivora* by the authors are designated as DPI. Pairings were done on SCV-8 agar. Remaining compatibility types were already determined prior to receiving the isolates by the authors.

to 5 days. The lima bean broth cultures were rinsed 2 times and resuspended in a small quantity (ca 10 ml) of sterile tap water. The cultures were than incubated under fluorescent lights for 48 hours. Zoospores were released by treating the sporangia in the culture with chilled distilled water (10 C). Zoospores were filtered through 16 layers of cheesecloth to remove sporangia. A suspension of encysted zoospores was prepared for counting by placing a 5 ml sample in a test tube and agitating the suspension for 30 sec on a vortex mixer (Tokunaga & Bartnicki-Garcia, 1971). The number of zoospores was then determined by averaging 6 to 8 sample counts in a standard hemacytometer.

RESULTS

Distribution

Information on the occurrence in Florida of the milkweed vine pathotype of *P. citrophthora,* and citrus brown rot caused by *P. parasitica* and/or *P. citrophthora* was obtained from literature reports, field surveys, and personal communications. The field survey was conducted in the summer of 1975 for naturally dying milkweed vines caused by *P. citrophthora* in the central Florida counties of Lake, Orange, Polk, and Osceola. Personnel of the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, submitted specimens of dying vines to the Plant Pathology laboratory at Gainesville and recorded the degree of milkweed vine infestation in the groves inspected. Soil samples were assayed for this fungus from groves where the vine infestation was regarded as little or none.

The occurrence of the milkweed vine pathotype of *P. citrophthora,* other isloates of *P. citrophthora,* and citrus brown rot are presented in Fig. 1. The reports of brown rot in Florida list the causal fungus as *P. parasitica* and/or *P. citrophthora.* However, recent work by Whiteside (1970) indicates brown rot is primarily the result of infection by *P. ctirophthora.* Occurrences of brown rot have been sporadic in the counties where *P. citrophthora* has been found. In 2 counties, Manatee and Sumter, brown rot has not been reported although the fungus has been detected (Fawcett, 1933).

The field survey in 1975 for the milkweed vine pathotype revealed its natural occurrence only in Lake and Orange counties. (Fig. 1).

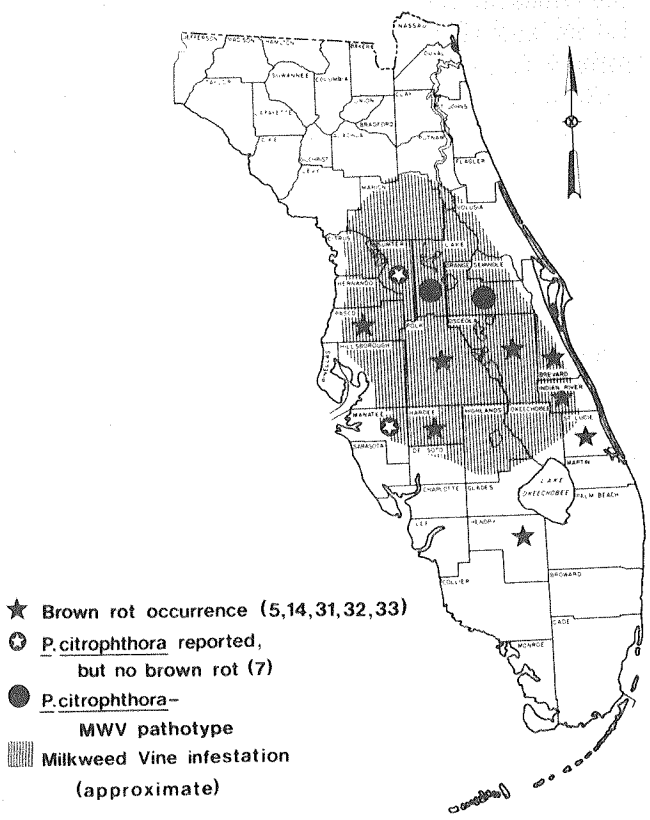

Figure 1. Distribution of brown rot, Phytophthora citrophthora, MWV pathotype of P. citrophthora, and milkweed vine in Florida (abbreviated map).

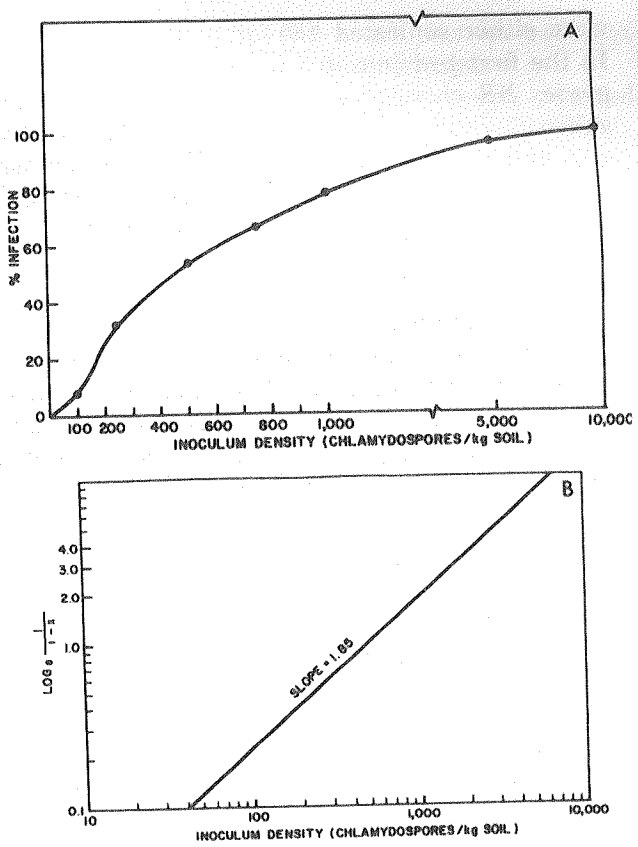

Figure 2. Arithmetic (A) and logarithmic (B) plots of infection of milkweed vine seedlings at different inoculum levels of the MWV pathotype (666-2) of Phytophthora citrophthora in the laboratory after 8 days at 25 C.

The distribution of the milkweed vine ranges from Marion County in the North to Hardee County in the South (Tucker *et al.*, 1971). There are reports that the vine is continuing to spread in a south-southeast direction and appears to be a problem confined primarily to citrus groves (Tucker, personal communication) (Fig. 1).

Efficacy

In the laboratory, sonicated chlamydospores (cs) of 666-2 were incorporated into steam-treated Astatula sand (typic quartzipsamment hyperthermic, uncoated) at concentrations ranging from 100 to 10,000 cs per kilogram of soil. Milkweed vine seeds were surface treated in 0.3% sodium hypochlorite for 4 minutes prior to placement on moist paper towels at room temperature (22-25 C) for germination. Germinated seeds (radicles approx. 5 mm long) were placed on steam-treated soil layered over infested soil containing different inoculum levels using the technique described by Mitchell (1975). Three germinated seeds were placed in each of 10 cups for each inoculum level tested. After incubation at 25C for 8 days with watering as needed to maintain moisture levels just short of field capacity, plants were removed, and the roots were washed, dipped in 70% ethyl alcohol, and rinsed in sterile distilled water prior to plating on CMA supplemented with 10 ppm of pimaricin, 200 ppm of vancomycin, and 100 ppm of PCNB (CMA-PVP). Root infection was obtained at all levels of incolum tested, and the percent root infection increased with corresponding increases in the levels of inoculum (Fig. 2A). Approximately 50% of the seedlings were infected at 500 cs per kilogram of soil. The slope determined by linear regression analysis in log-log transformations ($\log_e \frac{1}{1-x}$) to log of inoculum density was 1.85 (Fig. 2B).

Field studies were conducted in the summer of 1975 at 2 locations, the Grandview grove in Lake County and the Bay Lake grove in Polk County. Inoculum consisted of suspensions of chlamydospores from comminuted cultures of 666-2 which

were either sonicated (S) or nonsonicated (NS). In the first test initiated in June at the Bay Lake grove, NS chlamydospores were suspended in 1 gallon (3.78 liters) of water and were applied from a 2 gallon (7.56 liters) stainless steel pressure sprayer uniformly to the soil under the canopy of the citrus trees at rates of approximately 1.5 and 8.0 cs/cm^2. Six trees were treated at the level of 1.5 cs/cm^2 and 9 trees at the level of 8.0 cs/m^2. The mean number of vines was 9 and 25 per tree for the low and high inoculum levels, respectively. In the second test initiated in August at a grove in Lake County, both S and NS chlamydospores were applied at 1.5 and 8.0 cs/cm^2 with the same procedure used in the first test. Five trees were treated at each inoculum level. The number of vines varied from 49 to 86 for each tree. The height of the vines at both grove sites varied from 0.2 to 1.0 m, and the mean diameter of the tree canopy ranged from 5.2 to 5.8 m.

Rainfall and temperature records were summarized from the Florida Weather and Crop News pertinent to the weather station located in proximity to the test site. Milkweed vines were counted at the time of application of the fungus and at intervals of time following the inoculum application. The cumulative percent kill of milkweed vine at the Bay Lake grove increased rapidly 10 days after inoculum application and reached over 90% for both inoculum levels after 84 days (Fig. 3A). Using linear regression analysis and plotting the percent infection as the log e $\frac{x}{1-x}$ (x=percent infection versus time), the apparent infection rate

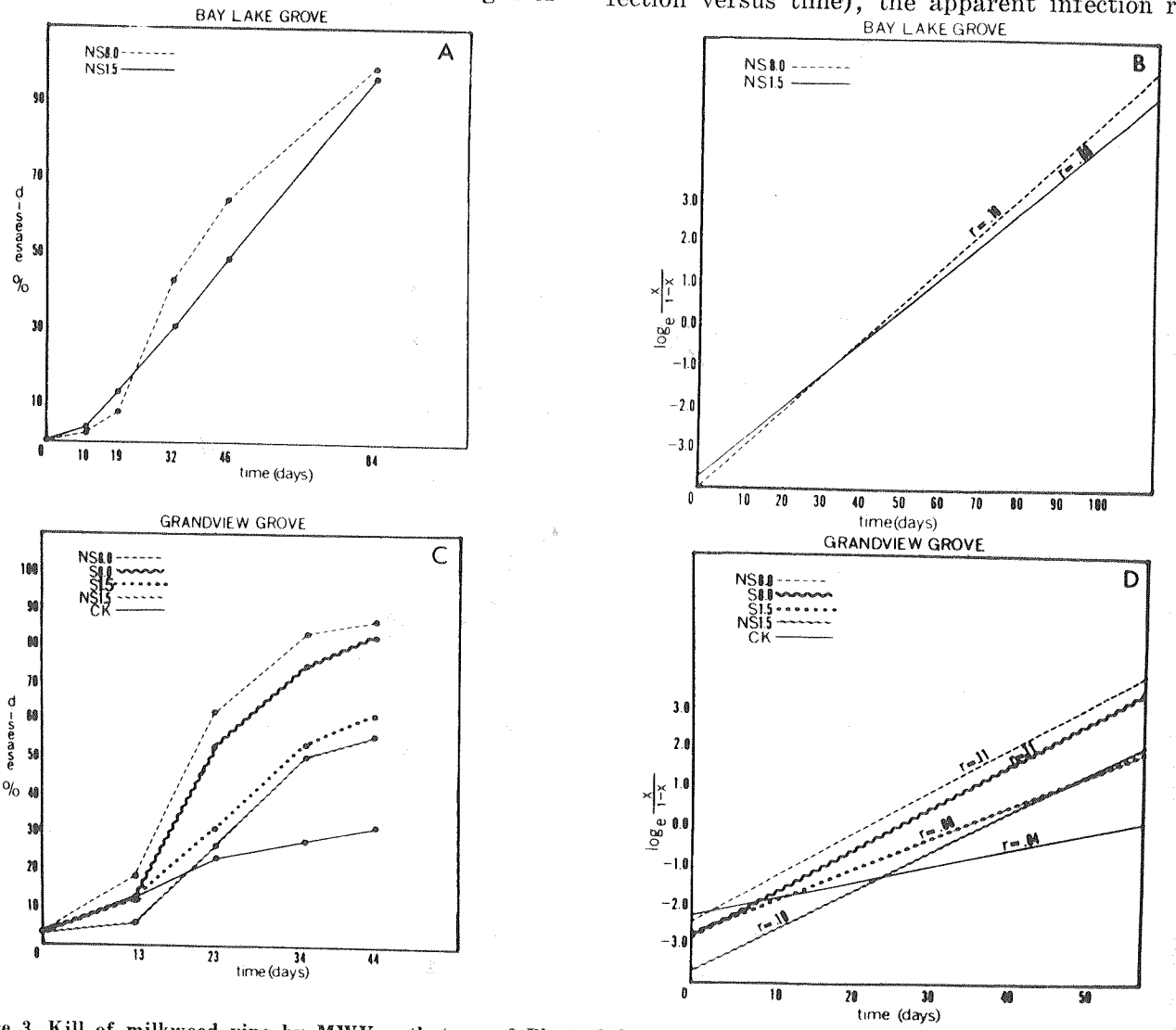

Figure 3. Kill of milkweed vine by MWV pathotype of Phytophthora citrophthora at Bay Lake and Grandview Groves. (A, C) Arithmetic plot of percent kill vs time. (B, D) Logarithmic plot for determination of apparent infection rate "r".

"r" (=slope) was 0.08 and 0.10 for the 1.5 and 8.0 cs/cm^2 levels, respectively (Fig. 3B). The weekly precipitation records from the field during the experiment for the time period of 27 June to 19 September were as follows: 0.79, 0.18, 3.68, 4.52, 0.69, 6.07, 6.43, 6.65, 0.43, 7.04, 5.74, and 3.45 cm.

Death of the milkweed vines at the Grandview grove began 13 days after application of the inoculum and thereafter increased rapidly (Fig. 3C). After 44 days, greater kill was obtained with the higher inoculum levels (8.0 cs/cm^2) regardless of whether or not the inoculum was sonicated. The kill of milkweed in the non-inoculated checks was attributed to spread of the MWV pathotype of *P. citrophthora* from plots in the area previously treated with the fungus. The irregular distribution of the fungus in the check treatments did not lead to a rapid kill of the vines as was found in the treated plots. An analysis of variance showed significant differences ($P<.05$) in the percent kill by S50 and NS50 versus the other treatments at 23 days. After 44 days, significant differences ($P<.05$) occurred wtih S10, S50, and NS50 versus the check. The apparent infection rate "r" was 0.08, 0.11, 0.10, 0.11, and 0.04 for the treatments S10, S50, NS10, NS50, and noninoculated check, respectively (Fig. 3D). The weekly precipitation records during the experiment for the time period of 6 August to 19 September were as follows: 4.98, 2.21, 6.88, 1.91, 7.85, 2.95, and 6.81 cm.

The air temperature fluctuated from 21 to 35 C at both grove sites. Isolations from dying milkweed vines during the experiments at both groves yielded the MWV pathotype (666-2) of *P. citrophthora* in all cases.

Host Range

Twelve plant families containing 58 representatives were subjected to pre-emergence, post emergence and foliage inoculation tests.

Inoculum in the pre-emergence tests consisted of infested soil prepared by inoculation of 7.5 m tall milkweed seedlings growing in steam-treated Astatula sand in flats. A zoospore inoculum was applied to the MWV seedlings as a drench, and the soil was mixed 2 to 3 days after the seedlings were dead. The soil was assayed for its inoculum density 1 to 2 weeks prior to use in the pre-emergence test. To conserve soil inoculum, 200 cc of steam-treated soil of the same type as infested soil was placed in the bottom of a 10 cm diameter clay pot followed by a 150 cc layer of infested soil. Five germinating seeds of the test plants were placed on the infested soil which was covered with 100 cc of steam soil of the same type.

Seeds, unless indicated otherwise, were obtained from the Seed Laboratory of the Division of Chemistry of the Florida Department of Agriculture and Consumer Services in Tallahassee and from commercial sources. The seeds were washed to remove any fungicide residue and treated in 0.52% sodium hypochlorite for 5 min. prior to incubating on moist sterile filter paper until germination was evident. Five pot replicates were used for each host tested. Percent emergence was determined after 10 days at 21 to 27 C (70-80F).

In the post-emergence tests, plants were allowed to develop in the steam-treated soil to the 1st or 2nd true leaf stage before zoospore inoculum was applied as a drench at a rate of 5 X 10^5 zoospores in a volume of 25mlper 10 cm diameter pot. Incubation at 21 to 27 C was for 3 to 4 weeks before plants were removed from the pots, and the roots examined for symptoms of infection relative to the non-inoculated plants. Root samples were rinsed and surface-sterilized in 0.3% sodium hypochlorite for 4 min before plating on CMA-PVP to detect infection. In the foliage inoculation tests, zoospore inoculum (1 X 10^5 zoospores/ml) was atomized onto the foliage of 2nd and 3rd true leaf stage plants until runoff occurred. Tween 20 was added to the inoculum at a rate of 0.4 ml per liter. Plastic bags were placed over the plants for 2 to 3 days, and the temperature monitored inside the bags. Disease readings were recorded the same or 1 day after the bags were removed. Plants were incubated for at least 7 to 14 days longer to observe any disease progression. The post-emergence tests for citrus were conducted by placing the roots of citrus seedlings in a zoospore suspension (5 X 10^4 zoospore/ml) for 15 min prior to potting in steam-treated soil. Fruit inoculations were conducted with 150 ml of zoospore suspension in 16 oz wax or styrofoam cups. A single fruit was placed in the zoospore suspension inoculum which covered ½ of the fruit from the calyx end. Distilled water was added as needed to maintain the original volume of zoospore inoculum in the uncovered cups. Zoospore inoculum was used at levels ranging from 1.5 X 10^3 to 1.5 X 10^6 zoospores per cup. Eight to 10 fruits were inoculated at each zoospore level tested.

The results of the host range tests with vegetable and field crops are given in Table 2. In the pre-emergence tests, the following hosts showed one or more varieties with less than 50% emergence:

Table 2. Host range testing with MWV pathotype of *Phytophthora citrophthora*

Family, plant and varieties	Pre-emergence test % emergence relative to non-inoculated control	Post-emergence root infection (+ or 0)[b]	Foliage infection (+ or 0)
Amaryllidaceae			
Onion			
Texas Grand	6(2)[a]	0(2)	0(2)
Asclepiadaceae			
Milkweed Vine (*Morrenia odorata*)	0(6)	+(2)	+(3)
Chenopodiaceae			
Sugarbeet			
US 420[d]	100(1)	0(1)	0(1)
Compositae			
Endive			
Batavian Full Heart	56(1)	0(1)	0(2)
Deep Heart	76(1)	0(1)	0(2)
Ruffer	78(2)	0(2)	0(3)
Lettuce			
Black Seeded Simpson	100(1)	*	0(1)
Boston	100(1)	0(1)	0(1)
Valousine	97(2)	0	0(2)
Cruiferae			
Cabbage			
Charleston Wakefield	100(1)	0(1)	0(1)
Hybrid Precision	100(1)	0(1)	0(1)
Rio Verde Hybrid	96(2)	0(2)	0(2)
Collard			
Georgia	95(1)	0(1)	0(1)
Mustard			
Florida Broadleaf	90(2)	*	0(2)
Radish			
Red Prince	100(1)	0(1)	*
White Icicle	96(2)	0(2)	0(1)
Turnip			
Purple Top White Globe	97(3)	0(3)	0(2)
Cucurbitaceae			
Cantaloupe			
Morgan[e]	21(3)	0(2)	0(1)
Planters Jumbo	53(2)	*	0(1)
Cucumber			
Ashley	100(1)[a]	0(1)	0(1)
Crockula	62(1)	0(1)	*
Poinsett	80(2)	*	0(1)
Squash			
Bush Scallop	100(1)	0(1)	0(1)
Early Summer Crookneck	99(3)	+(2)	0(1)
Table Queen	100(1)	0(1)	*
Zucchini	100(2)	*	0(1)
Watermelon			
Charleston Gray	30(3)	0(2)	0(1)
Crimson Sweet	11(4)	+(3)	0(1)
Jubilee	9(3)	+(2)	*
Gramineae			
Corn			
Florida Sweet[f]	85(2)	0(2)	0(1)
Iobelle Sweet	100(2)	0(2)	0(1)
Silver Queen	100(1)	0(1)	*

Table 2. Host range testing with MWV pathotype of *Phytophthora citrophthora*

Family, plant and varieties	Pre-emergence test % emergence relative to non-inoculated control	Post-emergence root infection (+ or 0)[b]	Foliage infection (+ or 0)
Leguminosae			
Bush Bean			
Astro	98(2)	*	0(1)
Blue Lake 274	100(1)	0(1)	0(1)
Cherokee Wax	98(2)	0(2)	0(1)
Sprite	87(2)	*	0(1)
Pole Bean			
Dade	88(1)	0(1)	0(1)
McCaslan	98(2)	0(2)	0(1)
Lima Bean			
Henderson	100(2)	*	0(1)
Cowpea			
Purple Hull Pinkeye	98(2)	0(2)	0(1)
Purple Hull 49	100(1)	0(1)	0(1)
Zipper Cream	82(2)	*	0(1)
English Pea			
Little Marvel	63(6)	+(3)	+(2)
Malvaceae			
Okra			
Clemson Spineless	47(5)	0(3)	0(2)
Solanaceae			
Eggplant			
Florida Market	100(1)	0(1)	0(1)
Pompano	77(2)	*	0(1)
Pepper			
Cuban	85(2)	0(2)	0(1)
Early Calwonder	100(2)	0(2)	0(1)
Yolo Wonder	100(2)	0(1)	*
Potato (Irish)			
Hudson	*	+[c]	0(1)
Red LaSoda	*	+	0(1)
Sebago	*	+	+(1)
Tomato			
Florida MH-1	40(2)	0(2)	+(1)
Walter	39(4)	0(2)	+(1)
Umbelliferae			
Carrot			
Danvers	100(2)	0(2)	0(1)
HiColor	54(2)	0(2)	0(1)
Imperator 58	52(1)	0(1)	0(1)
Celery			
Earlibelle[g]	90(1)	0(1)	0(1)

*=Tests either inconclusive or not conducted.
[a] Number in parenthesis equals number of tests on which value is based.
[b] +=infection; 0=no infection.
[c] Tuber infection by wound inoculation.
[d] Leonard J. Herr, Dept. Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, OH.
[e] James Montelaro, IFAS Vegetable Crops, University of Florida, Gainesville.
[f] Stephen R. Kostewicz, IFAS Vegetable Crops, University of Florida, Gainesville.
[g] Thomas A. Zitter, Plant Pathologist, AREC Belle Glade, University of Florida, Belle Glade.

onion, milkweed vine, cantaloupe, watermelon, okra, and tomato. Several other hosts (ie., endive, cucumber, English pea, and carrot) gave emergence percentages close to 50%. Of these, reduced vigor relative to the non-inoculated controls was observed in English pea and okra. Isolations from the roots of these hosts using surface sterilization with 0.3% sodium hypochlorite for 4 min resulted in the recovery of the MWV pathotype as well as *Fusarium* sp.

In the post-emergence tests, root infection was detected in milkweed vine, squash, watermelon, and English pea. Wound inoculation of Irish potato tubers resulted in infection and recovery of the fungus from the margin of the brown necrotic areas. Infection of MWV and English pea resulted in death of these hosts.

In the foliage inoculation tests, milkweed vine, English pea, Irish potato, and tomato showed infection resulting in death of the milkweed vine and English pea. Limited stem infection occurred in the potato and restricted small leaf lesions resulted on the tomato foliage.

In the citrus pre-emergence tests, Carrizo and sour orange seedlings gave from 89 to 100% emergence. However, root rot was observed on the tap root of the Carrizo seedlings, and the MWV pathotype was isolated from the necrotic tissue. Although the fungus was isolated from the roots of sour orange, visual observations showed no root discoloration. Additional testing with other rootstocks and varying inoculum levels are planned. Gordon Grimm, USDA Plant Pathologist, Orlando, FL, is conducting pathogenicity tests on a large number of citrus rootstock seedlings.

In the post-emergence tests, the roots of Carrizo seedlings were examined 3 weeks after immersing in a zoospore suspension. Root rot was present, and the fungus was isolated. Additional tests are planned with other rootstocks.

Infection of Hamlin, Temple, Navel, and Pineapple oranges was obtained 7 to 10 days after inoculation of single fruit with 1 to 1.5 X 10^6 zoospores of isolates 666-1 and 666-2 (Table 3). Isolates of *P. citrophthora* from citrus caused infection at the much lower inoculum levels of 1.5 to 7.5 X 10^3 zoospores per fruit.

Stability

Colony growth characteristics

Three media (PDA, clear V-8 agar, and CMA) were utilized in distinguishing differences of single zoospore colonies of the MWV pathotype from those of other isolates of *P. citrophthora* and species of *Phytophthora*. Inocula of the test isolates consisted of 5 mm diam. discs cut from the margin of actively growing cultures on water agar (WA) (15 g Difco agar in 1 liter of medium). The inocula plugs were inverted on the test media in petri plates, and the cultures were incubated at 27 ± 1 C for 2 days prior to describing and characterizing the resultant colonies.

Table 3. Comparison of infection of citrus fruit by milkweed vine pathotype and other isolates of *Phytophthora citrophthora*

P. citrophthora Isolate	Number of zoospores needed to infect 50% of fruit[a]
666	1-1.5 x 10^6
524	7.5 x 10^3
514	1.5 x 10^3

[a]Data summarized from 3 or more experiments.

The colony growth features of the MWV pathotype (666-1) on the 3 media are presented in Fig. 4. Aerial mycelium was slight on PDA and CMA and slight to moderate on V-8. The edge of the colony was uneven and coarse on PDA while slightly uneven and smooth on CMA and V-8. A radiate growth pattern was found on CMA and V-8 while no distinct colony feature was found on PDA. The mean diameter of colony growth was 26, 34, and 32 mm on PDA, CMA, and V-8, respectively.

The colony features of the MWV pathotype particularly on PDA were distinct from those growth characteristics shown by other isolates of *P. citrophthora* and species of *Phytophthora* except isolate 744 of *P. palmivora*. Approximately 150 single zoospore cultures of the MWV pathotype (666, 666-1, and 666-2) did not vary in colony feature. This was observed also in cultures of the MWV pathotype subjected to inoculation and isolation from citrus fruit. The colony features of several other isolates of *P. citrophthora* (524, 485, and 514) showed variability in colony growth diameter and colony edge, but none resembled the colony features of the MWV pathotype (Fig. 4). Variability was found in isolates (494 and 665) of the other species tested (Table 4).

PATHOGENICITY OF ISOLATES OF P. CITROPHTHORA, P. PALMIVORA, AND P. PARASITICA TO MILKWEED VINE

Zoospore inocula of *P. citrophthora, P. parasitica,* and *P. palmivora,* produced from cultures grown in lima bean broth, were applied as soil

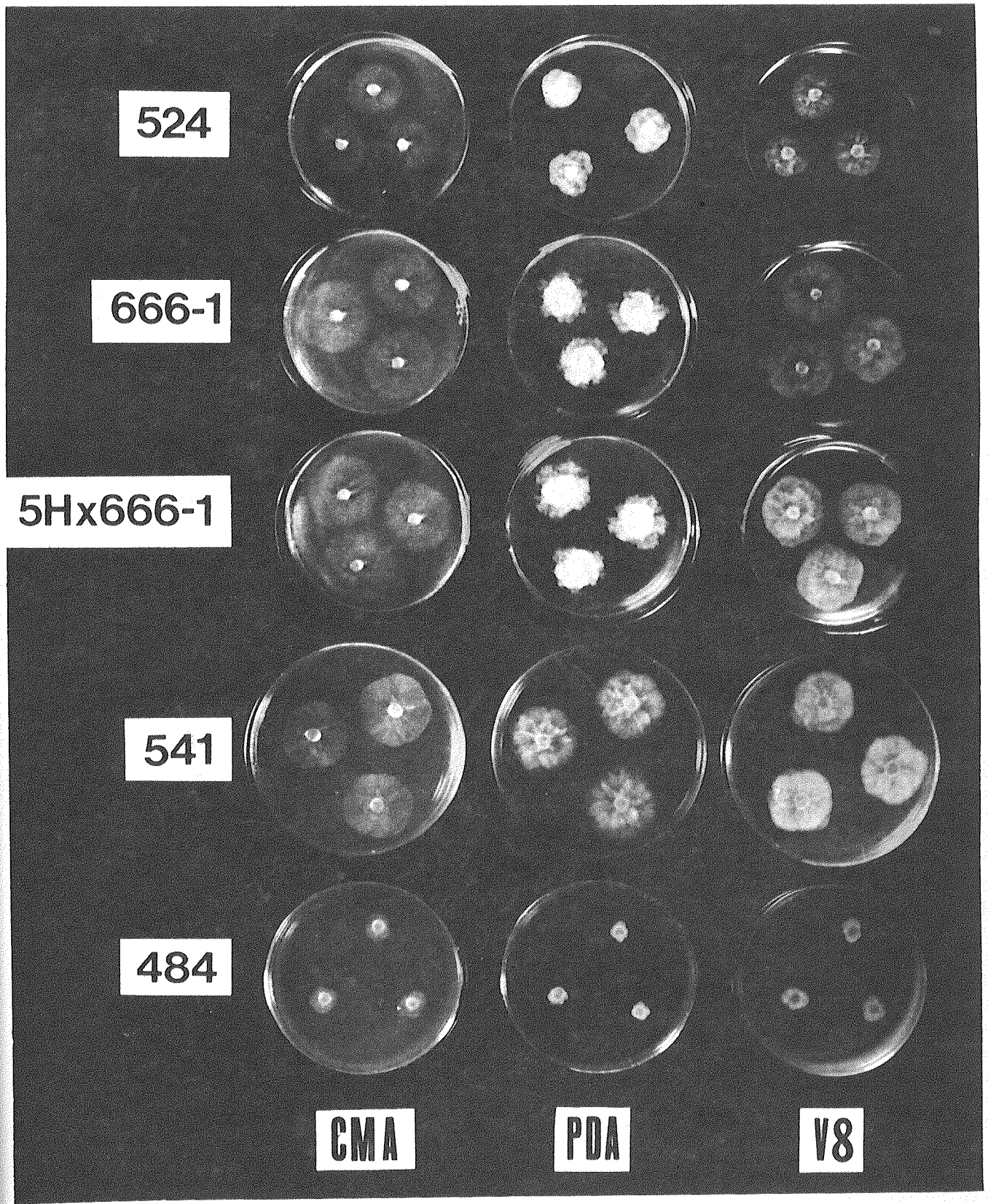

Figure 4. Single zoospore culture development by different isolates of Phytophthora citrophthora (524, 666-1, 5H X 666-1, 484, and 541) on 3 media after 2 days of incubation at 27 C.

Table 4. Variability in single zoospore colony types and similarity to MWV pathotype of isolates of *Phytophthora citrophthora*, *P. palmivora*, and *P. parasitica* on 3 media after 2 days at 27 C

Species	Isolates	No. of single zoospore cultures observed	No. of different single zoospore colony types[a]			Similarity of colonies to MWV pathotype[b]
			CMA	PDA	V-8	
P. citrophthora	666	9	0	0	0	s
	666-1	9	0	0	0	s
	666-2	132	0	0	0	s
	12H X 666[c]	18	0	0	0	s
	7H X 666	18	0	0	0	s
	5H X 666-1	18	0	0	0	s
	7P X 666	18	0	0	0	s
	7P X 524	18	1	0	0	ns
	484	9	0	0	0	ns
	485	9	3	4	3	ns
	514	18	1	1	1	ns
	524	18	0	0	0	ns
	541	9	0	0	0	ns
	694	9	0	0	0	ns
	745	9	0	0	0	ns
	747	9	0	0	0	ns
P. parasitica	493	9	0	0	0	ns
	494	9	0	1	0	ns
	691	9	0	0	0	ns
	692	9	0	0	0	ns
P. palmivora	664	9	0	0	0	ns
	665	9	1	2	1	ns
	739	9	0	0	0	ns
	740	9	0	0	0	ns
	741	9	0	0	0	ns
	742	9	0	0	0	ns
	743	9	0	0	0	ns
	744	9	0	0	0	s

[a] Number of colony types based on sum of differences in colony diameters and colony edges for each isolate and medium. Zero equals only one colony type among single zoospore cultures observed.
[b] s=similar; ns=not similar.
[c] Number of successive fruit inoculations with MWV pathotype (666) before isolation; H=Hamlin; P=Pineapple.

drenches to milkweed vines in 5.1 cm diam. pots (3 vines per pot). Five pot replicates were used for each isolate. The milkweed vines were grown to the 1st or 2nd set of true leaves. Several isolates did not produce sufficient zoospores; therefore, the mycelial mats were comminuted and applied as a drench. Isolates 666, 666-1, and 666-2 gave the expectant pathogenicity to milkweed vine but the most recent reisolate (666-2) apepared to be more virulent than the other isolates of the MWV pathotype (Table 5). The only other isolate of *P. citrophthora* to give infection and kill was 525, but only at high levels of inoculum. Isolate 524 of *P. citrophthora* was not included in this study but has been reported previously to attack milkweed vine (Burnett *et al.*, 1974). Two isolates of *P. palmivora*, 491 and 665, infected milkweed vine. Isolate 665 appeared to be equal or more virulent than 666-2 of *P. citrophthora* to milkweed vine. However, single zoospore cultures showed variability in virulence to milkweed vines. None of the *P. parasitica* isolates infected milkweed vine.

PATHOGENICITY OF MWV PATHOTYPE TO CITRUS FRUIT

Several subcultures of the milkweed pathotype of *P. citrophthora* were serially inoculated into wounded citrus fruits 7 to 12 times. After inoculation into the final fruit, the fungus was isolated and tested for any changes in virulence compared to the parent isolate. Pathogenicity tests with

Table 5. Pathogenicity of isolates of *Phytophthora citrophthora*, *P. palmivora*, and *P. parasitica* to milkweed vine seedlings

Phytophthora species	Isolate	Zoospore inoculum level(s) tested x 10^4	Percent milkweed vines diseased
P. citrophthora	666	2.0	100
		1.0	0
	666-1	2.0	100
		1.0	33
	666-2	2.0	100
		1.0	58
		0.2	33
	485	10.0	0
	525	10.0	17
	541	5.6	0
	693	cc[a]	0
	694	cc	0
P. palmivora	491	67.5	7
	492	77.5	0
	664	55.0	0
	665	2.0	100
		0.2	80
		0.02	39
	766	50.0	0
	767	42.5	0
	768	16.0	0
	769	56.0	0
P. parasitica	493	70.0	0
	494	46.0	0
	691	cc	0
	692	cc	0

[a] cc=comminuted cultures containing zoospores, sporangia, and mycelial fragments.

zoospore suspensions of the MWV pathotype (666 and 666-1) on nonwounded Hamlin and Pineapple oranges resulted in the same level of infection (50% @ 1-1.15 X 10^6 zoospores per fruit) as found with the parent isolate. Also, single zoospore subcultures did not differ from the parent culture in colony morphology (Table 4).

Assay for MWV pathotype from citrus trees

Citrus root samples were collected from trees treated with the MWV pathotype of *P. citrophthora* in July, 1973. Root samples were collected randomly from under each of the same 3 trees in 1974, 1975, and 1976. Roots were surface disinfested with 0.3% sodium hypochlorite for 4 min prior to rinsing and plating on APDA and CMA-PVP. Soil samples were assayed each time for the presence of the MWV pathotype of *P. citrophthora*.

The MWV pathotype of *P. citrophthora* was detected in the soil for each assay date but was not isolated from the roots of citrus (Table 6). However, cultures of *P. parasitica* were obtained and confirmed to be distinct from the MWV pathotype by colony morphology data and lack of pathogenicity to milkweed seedlings.

Table 6. Assay for MWV pathotype of *Phytophthora citrophthora* in citrus roots and soils from grove artificially infested with the fungus in July, 1973.

Assay date	Detection of MWV pathotype of *P. citrophthora* in Citrus soil	Citrus roots
12/74	+	0
4/75	+	0
9/75	+	0
6/76*	+	0

*Milkweed vines were detected under all trees. Some vines were dying; healthy vines were not established on the tree branches.

Oospore production

Inoculum plugs (5 mm diam.) from the periphery of actively growing cultures on clear V-8 agar were used to make 2 point pairings (50 mm apart) of the MWV pathotype (666-2) of *P. citrophthora* with A^1 and A^2 compatibility types of *P. citrophthora*, *P. parasitica*, and *P. palmivora* on SCV-8 agar at 18 C in dark. After 20 days, microscopic examinations were made at the interface of the cultures for oospore formation. These pairings were replicated 3 times and repeated twice. Oospores were observed only in pairings of the 666-2 isolate with A^1 compatibility types of *P. citrophthora*, *P. parasitica*, and *P. palmivora* (Table 7). Thus, the MWV-pathotype (666-2) of

Table 7. Determination of mating type of the MWV pathtype (666-2) of *Phytophthora citrophthora* when paired with isolates of 3 species of *Phytophthora* on a supplemented clear V-8 medium after 10 days at 18 C

Species	Isolates	Mating type of isolate	Oospore formation (+ or 0) in pairing with MWV pathotype[a]
P. citrophthora	524	A^1	+
	485	A^2	0
	514	A^2	0
	525	A^2	0
	541	A^2	0
	745	A^2	0
	747	A^2	0
P. palmivora	740	A^1	+
	739	A^2	0
P. parasitica	493	A^1	+
	691	A^1	+
	494	A^2	0

[a] Oospores observed = +; oospores not observed = 0.

P. citrophthora was determined to be an A^2 compatibility type. No data were recorded at this time on oogonia or oospore sizes. Presence of pigmentation of the oogonia was noted but was highly variable within the different isolate pairings.

UV irradiation

Zoospores of a single zoospore culture of 666-2 were prepared according to the procedure outlined in Materials and Methods for lima bean broth in petri plates.

Following zoospore release from sporangia, 10 ml aliquots of a zoospore suspension were placed in sterile glass petri plates. A one ml aliquot was taken from the control plate for determination of zoospore concentration by counts in the hemacytometer. Also, viability counts on CMA were conducted at the same time. The plates with 10 ml of the motile zoospores were used for irradiation at 30 or 40 sec interval exposure at 30 cm from UV light emitted from a GE germicidal lamp W8 of ca 254 nm wavelength. Following the irradiation treatment, 0.5 ml of the motile zoospore suspension as well as 0.5 ml of a 1/10 dilution was plated on WA and CMA. No dilution was made for the 40 sec exposure.

Single zoospores (approximately 30-50) showing germ tube development were picked at random and placed on CMA-PVP. Colonies developing on this medium were then subcultured on PDA. Only a small percentage of these single zoospore transfers established colonies.

In tests with colony morphology, the irradiated zoospore colonies were subcultured on PDA, CMA, and V-8 for inoculum production. This procedure was necessary for survival of the colonies and differed from the previous one where WA was used. Incubation was at 25-27 C for 2 days before colony morphology (colony edge and diameter) were recorded.

Ultraviolet light induced mutations that altered colony morphology (Table 8). Over one-half of the UV-exposed zoospores showed at least one

Table 8. Camparison of colony morphology of single zoospore cultures of UV-exposed zoospores to nonexposed zoospores of *Phytophthora citrophthora*, isolate 666-2 #1[a] on growth media.

Culture derived from UV-exposed zoospores of 666-2 #1	Culture's resemblance to 666-2 #1 on 3 growth media						Percent similarity of culture to 666-2 #1
	Colony edge[b]			Colony diameter[c]			
	CMA	PDA	V-8	CMA	PDA	V-8	
UV- 1	0	+	0	+	+	0	50
UV- 2	0	0	0	0	0	0	100
UV- 3	0	0	0	0	0	0	100
UV- 4	0	0	0	0	0	0	100
UV- 5	0	0	0	0	0	0	100
UV- 6	+	0	0	0	0	0	83
UV- 7	0	+	0	0	+	+	50
UV- 8	0	0	0	0	0	0	100
UV- 9	0	0	0	0	0	0	100
UV-10	0	0	+	0	0	0	83
UV-11	0	0	+	0	+	0	67
UV-12	0	0	0	+	+	0	67
UV-13	0	0	0	0	0	0	100
UV-14	0	0	+	+	+	+	33
UV-15	0	0	0	0	0	0	100
UV-16	+	0	+	0	+	0	50
UV-17	+	0	0	+	+	0	50
UV-18	0	0	0	0	0	0	100
UV-19	0	0	0	0	+	0	83
UV-20	0	0	0	0	0	0	100
UV-21	0	0	0	0	0	0	100
UV-22	0	0	0	0	0	0	100
UV-23	+	0	+	+	+	+	17
UV-24	0	0	0	0	0	0	100

[a] Isolate 666-2 #1 is a single zoospore isolate derived from *P. citrophthora*, isolate 666-2.

[b] Characteristics used to compare colony edge were smooth vs rough and even vs uneven. The symbol + designates a difference between the exposed culture and the parent; the symbol 0 indicates no apparent differences.

[c] If colony diameter of the UV-exposed culture was less or greater than 7.0 mm from the mean diameter of the parent, the symbol + designates a difference; the symbol 0 indicates no difference.

change in colony morphology. The similarity of these colonies to the MWV pathotype ranged from 17 to 100% based on colony diameter and colony edge characteristics on CMA, PDA, and V-8 agar. All 3 media proved useful in detecting differences in the cultures.

DISCUSSION

The study of the use of a pathotype of *P. citrophthora* to control milkweed vines in citrus groves in Florida has been directed toward obtaining information on the distribution, efficacy, host range, and stability of the fungus. These investigations should provide the information necessary to decide how effectively and safely this fungus can be utilized under various environmental conditions.

The distribution study showed that the MWV pathotype occurred naturally in Orange and Lake counties. However, it could become established in other counties where the milkweed vine is present (Fig. 1). Burnett *et al.* (1974) reported that a pathotype of *P. citrophthora,* capable of attacking citrus fruit as well as milkweed vine, occurred in Hardee County.

Citrus brown rot caused by *P. citrophthora* and/or *P. parasitica* has been reported from 8 counties but has not been detected in Orange and Lake counties (Cohen & Knorr, 1960; Fawcett, 1933; Knorr, 1956; West *et al.*, 1954; Whiteside, 1970; Whiteside & Oswalt, 1973). The origin of the MWV pathotype in these 2 counties is speculative, but *P. citrophthora* may have persisted here undetected. *Phytophthora citrophthora* has been detected in Manatee and Sumter counties where there has been no reported incidence of brown rot (Fawcett, 1933).

Whiteside (1970) has attributed the restricted occurrence of brown rot in Florida to the lack of favorable environmental conditions for fruit wetness as well as to the limited distribution of the fungus. Other factors which could affect the recovery of *P. citrophthora* from citrus grove soil and or roots are 1) the widespread distribution of *P. parasitica* which could mask *P. citrophthora* by its more aggressive nature on the citrus rootstocks, 2) the time of year and soil temperature under which *Phytophthera* isolation is attempted, and 3) the relative suppressive nature of soils to *P. citrophthora* versus *P. parasitica.* Future survey work hopefully will further define the parameters involved in the occurrence and distribution of *P. citrophthora.*

Distribution of the milkweed vine in Florida was reported by Tucker *et al.* (1971). Cultural conditions for citrus and evidently quite conducive for growth of the milkweed vine which has not become a recognized problem in any other Florida crop despite its geographically widespread occurrence. The first report of milkweed vine as a problem in citrus groves was in 1957 from Orange County (Swanson, 1975). This is the same place where the MWV pathotype of *P. citrophthora* was first isolated in 1972 (Burnett *et al.*, 1973). Thus, it appears after 15 years that the buffering and balancing effect of nature has produced an organism (MWV pathotype of *P. citrophthora*) capable of effectively controlling a highly populated plant species (milkweed vine).

The efficacy of the fungus appears very high at low inoculum levels in laboratory and field tests. In the laboratory, infection of milkweed vine seedlings compared closely with infection rates of other *Phytophthora* spp.; fifty percent of papaya and tobacco seedlings were infected at less than 500 cs per kilogram of soil with *P. palmivora* and *P. parasitica* Dast. var. nicotianae (B. deHaan) Tucker, respectively (Ramirez & Mitchell, 1975); M. E. Kannwischer and D. J. Mitchell, personal communication). In the field, the fungus interacted with the environment favorably as shown by the apparent infection rates which were close to those obtained in the laboratory using autoclaved soil. The percent kill of milkweed vine was comparable to the results obtained by Burnett *et al.* (1973) using variable inoculum levels in the field. The nearly continuous precipitation and highly favorable soil temperatures in the citrus groves during the field experiments were most likely the primary factors in contributing to the pathogenic activity of the fungus. Field studies will be repeated to further test the ability of the fungus to be effective under various environmental conditions at low inoculum levels. Also, the ability of this fungus to persist and control milkweed vine will be monitored. The low inoculum levels of 1.5 and 8.0 cs/cm^2 of soil surface provided effective control and should minimize any significant contamination of nontarget areas or plants in any future applications. Studies in the laboratory are underway to monitor the longevity of the fungus in citrus grove soil maintained at different moisture levels without the milkweed vine host. Also, a comparable study on the survival of the fungus in grove soil sustaining citrus trees but not milkweed vines would be useful in predicting pathogenicity changes in the

fungus. Previous work by Burnett *et al.* (1974) showed no infection of citrus roots in greenhouse studies after 6 months.

The host range testing in the laboratory showed some plant susceptibility in the families Amaryllidaceae, Asclepiadaceae, Cucurbitaceae, Malvaceae, Solanaceae, and Umbelliferae. However, most of these plants (onion, cantalope, okra, and carrot) were susceptible in the pre-emergence tests where no seed fungicide was used and where the inoculum levels were higher than was necessary for infection of milkweed vine. Additional tests using seed fungicide protectants, varying inoculum levels, and field plot applications may be necessary before the potentiality of the fungus can be critically assessed.

The host range of *P. citrophthora* is regarded as extensive (Gerlach *et al.*, 1976; Smith, 1937; Waterhouse & Waterson, 1964), and therefore it is not surprising to find hosts susceptible to the MWV pathotype. The amount of inoculum of the MWV pathotype needed to infect citrus fruit was found to be approximately 1/133 to 1/1000 that of citrus pathotypes previously isolated from citrus having brown rot. Burnett *et al.* (1974) reported infection of fruit by the MWV pathotype only after wound inoculation. In these early tests, an inoculum density high enough to obtain fruit infection was not employed. The infectivity of the MWV pathotype to several citrus rootstocks in pre-emergence tests showed that high inoculum densities along with nonfungicide-treated seed allowed a favorable situation for infection by the fungus. Additional tests will be conducted on fungicide protection, inoculum density versus infection of citrus seedlings, relative susceptibility of different age citrus plants, and relative susceptibility of various rootstocks.

No variability in colony morphology of single zoospore isolates from a mass culture of the MWV pathotype was detected on the 3 media tested. Pathogenicity and sporulation studies with single zoospore cultures should further indicate the degree of stability of the MWV pathotype. The stability of the colony morphology has proven useful in distinguishing the MWV pathotype from other Florida *Phytophthora* spp. in the field. However, stability of *Phytophthora* spp. in respect to colony morphology, sporulation, and pathogenicity has not been common to several of the species studied (Caten & Jinks, 1967; Erwin *et al.*, 1963; Gallegly, 1968; Satour & Butler, 1968; Zentmyer & Erwin, 1969). Only one isolate (744 of *P. palmivora*) showed a colony morphology similar to the MWV pathotype, and this isolate came from *Hedera* sp. in California (Table 1). The pathogenicity of isolate 744 to milkweed vine and its sporangial characteristics are being investigated to determine its relationship to the MWV pathotype.

Pathogenicity tests with the MWV pathotype and other isolates of *P. citrophthora, P. palmivora,* and *P. parasitica* revealed several isolates of *P. citrophthora* and *P. palmivora* to be pathogenic to milkweed vine. Thus, it appears that pathogenicity to milkweed vine is not unique to the MWV pathotype.

Changes in virulence, pathogenicity, or colony morphology of subcultures serially passed through citrus fruit by wound inoculation were not detected. The stability of pathogenicity characteristics of the MWV pathotype to milkweed vine and citrus was also supported by the recovery of the MWV pathotype from infested grove soil but not from citrus roots over the past 3 years.

Oospore production in pairings of the MWV pathotype with A^1 compatible types of *P. palmivora, P. citrophthora,* and *P. parasitica* indicated a potential source of variation in cultures produced from oospores which could originate from matings in the field. Variability in *Phytophthora* spp. has been found to be significant in areas where both compatibility types of heterothallic species exist (Gallegly, 1968; Laviola & Gallegly, 1967; Savage *et al.*, 1968; Watson, 1970; Zentmyer & Erwin, 1969; Zentmyer & Erwin, 1970). In Florida, *P. parasitica* is widespread in citrus groves (Whiteside, 1970), and both compatibility types do occur (Ridings & McRitchie, 1974); thus, a potential for genetic exchange exists because interspecific mating may occur.

Exposure of zoospores to UV light resulted in variations in colony morphology (Table 8). Additional tests are being conducted to determine UV-induced variability in pathogenicity and sporulation. Other sources of variability such as spontaneous mutation, parasexuality, hetercaryosis, physiological adaptation, and cytoplasmic control may also occur in *Phytophthora* spp. and give rise to new characteristics in the progeny (Erwin *et al.*, 1963). Mutation is thought to be the primary mechanism for variability in new pathogenic-race characters of *P. infestans* (Mont.) d By. (Gallegly, 1970).

The consideration for use of the MWV pathotype in Florida citrus groves to control milkweed will be studied critically to evaluate the benefit: risk ratio. Foot, root, and fruit rot caused by *P. citrophthora* appears to be of limited importance

in Florida (Whiteside, 1970). This disease is also of limited importance in Queensland, but can be severe in such places as California, Brazil, and Irael (Klotz, *et al.*, 1958; Klotz, *et al.*, 1969; Oxenham & Stone, 1969; Rossetti, 1969; Schiffman-Nadel, 1969). Thus, additional studies are required to elucidate the roles of pathogen variation, host resistance, and environmental conditions in disease development. Fawcett and Lee (1926) defined such fatcors as susceptible rootstocks, favorable soil and air temperatures, bark wounds, and heavy, wet soil as highly conducive for infection by *P. citrophthora* and *P. parasitica*. The danger of foot, root, and fruit rot caused by the MWV pathotype does not appear at this stage of the study to be any greater than that caused by the *P. parasitica* which is present in most Florida groves.

The greatest concern over the use of the MWV pathotype, however, is the possibility of it contributing to new pathotypes through genetic recombination or other mechanisms that could give rise to pathotypes virulent to citrus. The potential for this fungus to form new genotypes through sexual or asexual procedures should be evaluated as thoroughly as possible. The actual formation of oospores with the MWV pathotype and other isolates of *Phytophthora* spp. in the grove is not unexpected, but the ability of oospores to germinate and infect roots and fruit must be more fully determined to evaluate the significance of the oospores as actual sources of new pathotypes.

REFERENCES

Burnett, H. C., D. P. H. Tucker, M. E. Patterson, and W. H. Ridings. 1973. Biological control of milkweed vine with a race of Phytophthora citrophthora. Proc. Fla. State Hortic. Soc. 86: 111-115.

Burnett, H. C., D. P. H. Tucker, and W. H. Ridings. 1974. Phytophthora root and stem rot of milkweed vine Plant Dis. Rep. 58: 355-357.

Caten, C. E., and J. L. Jinks. 1967. Spontaneous variability of single isolates of Phytophthora infestans. I. Cultural variations. Can. J. Bot. 46: 329-348.

Chee, K. H., G. A. Zentmyer, K. M. Foong, and L. J. Klure. 1976. Mating types of Phytophthora palmivora in Malaysia. Plant Dis. Rep. 60: 866-867.

Cohen, Mortimer, and L. C. Knorr. 1960. Winter outbreaks of citrus brown rot in Florida. Plant Dis. Rep. 44: 578-579.

Erwin, D. C., G. A. Zentmyer, J. Galindo, and J. S. Niederhauser. 1963. Variation in the genus Phytophthora. Annu. Rev. Phytopathol. 1: 375-396.

Fawcett, Howard S. 1933. New locations for Phytophthora citrophthora and P. hibernalis on citrus. Phytopathology 23: 667-669.

Fawcett, Howard S., and H. Atherton Lee. 1926. Citrus diseases and their control. McGraw-Hill Book Co., Inc., New York. 582 p.

Gallegly, M. E. 1968. Genetics of pathogenicity of Phytophthora infestans. Annu. Rev. Phytopathol. 6: 375-396.

Gallegly, M. E. 1970. Genetics of Phytophthora. In the Tucker Memorial Symposium on Phytophthora. Phytopathology 60: 1135-1141.

Gerlach, W. W. P., H. A. J. Hoitink, and A. F. Schmitthenner. 1976. Survival and host range of Phytophthora citrophthora in Ohio nurseries. Phytopathology 66: 309-311.

Klotz, L. J., T. A. DeWolfe, and Po-Ping Wong. 1958. Decay of fibrous roots of citrus. Phytopathology 48: 616-622.

Klotz, L. J., T. A. DeWolfe, and M. P. Miller. 1969. Control of Phytophthora problems. California Citrograph 54(6): 228, 279.

Knorr, L. C. 1956. Progress of citrus brown rot in Florida, a disease of recent occurrence in the State. Plant Dis. Rep. 40: 772-774.

Laviola, C., and M. E. Gallegly. 1967. Heterothallism in Phytophthora citrophthora. Phytopathology 57: 646 (Abstr.).

Mitchell, D. J. 1975. Density of Pythium myriotylum oospores in soil in relation to infection of rye. Phytopathology 65: 570-575.

Oxenham, B. L., and Yolande N. Stone. 1969. Citrus brown rot in Queensland. Queensland Agric. and Animal Sciences 26: 173-180.

Ramirez, B. N., and D. J. Mitchell. 1975. Relationship of density of chlamydospores and zoospores of Phytophthora palmivora in soil to infection of papaya. Phytopathology 65: 780-785.

Ridings, W. H., and J. J. McRitchie. 1974. Phytophthora leaf spot of Philodendron oxycardium and related species. Proc. Fla. State Hortic. Soc. 87: 442-447.

Rossetti, Victoria. 1969. Studies on Phytophthora species on citrus in Brazil. Pages 1211-1216 in H. D. Chapman, ed., Vol. 3, Proc. 1st Intern. Citrus Symp., Univ. California, Riverside. 1838 p.

Satour, M. M., and E. E. Butler. 1968. Comparative morphological and physiological studies of the progenies from intraspecific matings of Phytophthora capsici. Phytopathology 58: 183-192.

Savage, E. J., C. W. Clayton, J. H. Hunter, J. A. Brenneman, C. Laviola, and M. E. Gallegly. 1968. Homothallism, heterothallism, and interspecific hybridization in the genius Phytophthora. Phytopathology 58: 1004-1021.

Schiffmann-Nadel, Mina. 1969. Research on Phytophthora diseases of citrus fruit in Israel. Pages 1201-1205 in H. D. Chapman, ed., Vol. 3, Proc. 1st Intern. Citrus Symp., Univ. California, Riverside. 1838 p.

Smith, Clayton O. 1937. Inoculation of some economic plants with Phytophthora cactorum and P. citrophthora. Phytopathology 27: 1106-1109.

Swanson, Henry F. 1975. Count down for agriculture. Designers Press of Orlando, Inc., Orlando, Fla. 338 p.

Tokunaga, J., and S. Bartnicki-Garcia. 1971. Cyst wall formation and endogenous carbohydrate utilization during synchronous encystment of Phytophthora palmivora zoospores. Arch. Mikrobiol. 79: 283-292.

Tsao, P. H. 1967. Production of chlamydospores of Phytophthora parasitica in liquid cultures. Ann. Phytopathol. Soc. Jap. 33: 73.

Tucker, D. P. H., R. L. Phillips, and J. L. Jackson. 1971. The milkweed vine-current recommendations and promising new approaches. Proc. Fla. State Hortic. Soc. 84: 41-44.

Waterhouse, G. M., and J. M. Waterson. 1964. Phytophthora citrophthora. C.M.I. Descriptions of Pathogenic Fungi and Bacteria No. 33. Commonwealth Mycological Institute, Kew, Surrey, England. 2 p.

Watson, I. A. 1970. Changes in virulence and population shifts in plant pathogens. Annu. Rev. Phytopathol. 8: 209-230.

West, Erdman, Mortimer Cohen, and L. C. Knorr. 1954. Brown rot of citrus fruit on the trees in Florida. Plant Dis. Rep. 38: 120-121.

Whiteside, J. O. 1970. Factors contributing to the restricted occurrence of citrus brown rot in Florida. Plant Dis. Rep. 54: 608-612.

Whiteside, J. O., and T. W. Oswalt. 1973. An unusual brown rot outbreak in a Florida citrus grove following sprinkler irrigation with Phytophthora-infested water. Plant Dis. Rep. 57: 391-393.

Zentmyer, G. A., and D. C. Erwin. 1969. Physiological and genetical studies in the genus Phytophthora. Pages 1217-1220 in H. D. Chapman, ed., Vol. 3, Proc. 1st Intern. Citrus Symp., Univ. California, Riverside. 1838 p.

Zentmyer, G. A., D. C. Erwin. 1970. Development and reproduction of Phytophthora. In the Tucker Memorial Symposium on Phytophthora. Phytopathology 60: 1120-1127.