Morphological, Physiological, Ecological, and Pathological Comparisons of *Phytophthora* Species Isolated from *Theobroma cacao*

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ABSTRACT

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Phytophthora citrophthora was isolated from black pods and trunk cankers on cocoa trees. Isolates of *P. citrophthora* from cocoa were compared with *P. palmivora* and *P. capsici* from cocoa and *P. citrophthora* from citrus with respect to colony morphology, sporangium production, sporangial stalk lengths, compatibility types, and pathogenicity to cocoa and citrus. After 8 wk of growth in soil artificially infested with chlamydospores of *P. palmivora* and *P. citrophthora* or oospores of *P. capsici*, 100, 93, and 40% of cocoa seedlings were infected with *P. palmivora*, *P. citrophthora*, and *P. capsici*, respectively. Mortality at 8 wk was 67, 53, and 0% for seedlings infected with *P. palmivora*, *P. citrophthora*, and *P. capsici*, respectively. Single isolates of all three species produced homothallic oospores in inoculated cocoa pod tissue after a minimum of 5 wk.

Black pod disease of cocoa (*Theobroma cacao* L.) is an economically serious problem in all areas of the world where the crop is grown (4). Until recently, all isolates of *Phytophthora* from cocoa were classified as *P. palmivora* (Butl.) Butler (3). In 1976, isolates of *Phytophthora* from cocoa from all over the world were compared for morphological and chromosomal differences and were placed into four groups (MF1, MF2, MF3, and MF4) (12). Later, Brasier and Griffin equated MF1 with *P. palmivora* and described MF3 as a new species, *P. megakarya* Brasier and Griffin (3). Based on morphological and physiological data, MF4 isolates are now generally considered to be *P. capsici* Leonian (1).

The occurrence and distribution of *P. palmivora*, *P. capsici*, and *P. citrophthora* (Smith & Smith) Leonian in Bahia, the principal cocoa-growing region of Brazil, have been reported (6,16). In this paper the morphological and physiological features of *P. citrophthora* from cocoa are described. In addition, the relative survival of *P. palmivora*, *P. capsici*, and *P. citrophthora* in cocoa pod tissue and in soil were compared, and the potential of chlamydospores and oospores to infect roots of cocoa seedlings was evaluated in the greenhouse.

MATERIALS AND METHODS

Isolates and media. Specific information on the isolates of *Phytophthora* used in all experiments is given in Table 1. Some cocoa isolates of *Phytophthora* were identified as *P. citrophthora* as a result of the comparisons described and will be referred to as this species throughout the text. Isolates of *P. capsici* and *P. palmivora* used were morphologically typical of previously identified cultures from Brazil. Culture media used in various experiments included carrot agar (CA), clear V-8 agar (CV8), potato-dextrose agar (PDA), a synthetic medium (SM) (28), and cornmeal agar (CMA).

Colony, sporangium, and chlamydospore morphology. Colony morphologies were compared after 3 days of growth at 24 C under continuous light from two cool daylight lamps (1,200 W/cm; Sylvania Lighting, Los Angeles, CA) on plates containing 15 ml of CMA, PDA, or CA. When the pattern of sporangium or chlamydospore production on CA appeared to differ from that of *P. palmivora* or *P. capsici* from cocoa, structures were observed and measured microscopically. The caducity of sporangia was determined by flooding plates with water as described by Kaosiri et al (14). Noncaducous sporangia were collected by scraping the flooded agar surface. Measurements of sporangia and chlamydospores included length and breadth of sporangia, dimensions of papillae, sporangial stalk lengths, and chlamydospore diameters.

Compatibility types. The compatibility types of isolates of *P. citrophthora* from cocoa were evaluated by pairing them with A1 and A2 isolates of both *P. palmivora* and *P. capsici* from cocoa. To facilitate evaluation of a large number of isolates, the following technique was developed for the production of oospores. Isolates of known and unknown compatibility types were grown in plates with 15 ml of CA for 5 days. Eight-millimeter-diameter mycelial disks of isolates to be paired were cut from the colony margins. A stack of three agar disks was made by sandwiching an 8-mm-diameter disk of plain CA between two 8-mm-diameter disks of CA that contained different isolates to be tested. Ten stacks of three disks each could be placed in a 90-mm-diameter petri plate. The lid of each plate was sealed with Parafilm. Plates were placed at 24 C for 24 hr in the dark, then incubated at 21 C in the dark. After 6 days, the three disks were separated and observed microscopically (×10) for oospores. Crosses were made between A1 and A2 isolates of *P. palmivora* and *P. capsici* from cocoa and isolates of *P. citrophthora* from cocoa, citrus, and *Euonymous* sp. as well as an isolate of unknown origin from New Zealand. Each cross was performed twice.

Pathogenicity to stems of citrus and cocoa seedlings. Pathogenicity tests were conducted in the greenhouse with 2-mo-old cocoa seedlings grown from seed of the cocoa tree of unknown parentage growing at the University of California, Riverside (UCR) campus and with 10-mo-old cultivar Pineapple sweet orange seedlings (*Citrus sinensis* Osbeck). Seedling stems were inoculated by cutting a small slice in the stem with a razor blade and placing a mycelial disk from the margin of a 5-day-old culture on CV8 on the wound. The wound site was wrapped with transparent tape to avoid desiccation. Cocoa seedlings were inoculated with *P. citrophthora* from cocoa (P1200, P1202, P1203, or P1211), *P. capsici* from cocoa (P623), *P. palmivora* from cocoa (P986), or a

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CV8 agar disk alone. The bark around the inoculation site was removed and discolored lesion areas were measured after 1 wk. Plants were rated for disease development by using the following scale: 0 = no lesion, 1 = lesion up to 2 cm in length, 2 = lesion 2–3 cm in length, 3 = lesion more than 3 cm in length, 4 = entire top wilted, and 5 = dead plant. In another experiment, stems of sweet orange seedlings were inoculated with *P. citrophthora* from cocoa (P1200, P1202, P1211), *P. capsici* from cocoa (P623), *P. citrophthora* from citrus (P1163), or CV8 agar disks alone. Inoculation sites were observed daily for gummosis. There were four replications of each treatment.

Pathogenicity to roots of cocoa seedlings. Chlamydospores of *P. palmivora* (P1240) and *P. citrophthora* (P1202) were produced as described by Tsao (22) except that the bottles were incubated on their sides to increase numbers of chlamydospores of *P. palmivora*. After 4 wk at 18 C, the mycelial mats were rinsed on a 44-μm nylon screen with distilled water, comminuted twice for 30 sec in a Waring blender at high speed with 50 ml of distilled water, and filtered through two layers of cheesecloth. Oospores of *P. capsici* (P623 × P795) were produced as previously described (15). After 4 wk at 21 C, three cultures containing oospores were comminuted twice for 30 sec with 100 ml of distilled water, washed by three low-speed centrifugations, frozen for 4 hr at −12 C to kill hyphal fragments, and thawed. Oospore or chlamydospore suspensions were subjected to 60% of the maximum sonication of the 100-W cycle of a Braunsonic 1510 ultrasonic system (Braun Company, Burlington, CA) for 30 sec. The number of spores present in ten 10-μl drops were counted. The viability of propagules in the inoculum suspensions was determined by streaking 1-ml samples onto each of three plates of 1.5% water agar, incubating the plates for 12–24 hr under continuous light at room temperature, and then counting the germinated spores. In preliminary experiments, spore viability counts were made before and after freezing or sonication. Subsequently, samples were taken from spore suspensions just prior to soil infestation.

A sandy-loam avocado field soil from the UCR campus with a pH of 7.7 was infested with chlamydospores or oospores in 1,500-g lots by adding aliquots of an inoculum suspension until soil moisture was at 20% and the final calculated propagule density was 200 propagules per gram of dry soil. The propagules were mixed thoroughly into the soil with a Hobart mixer (Hobart Corporation, World Headquarters, Troy, OH).

The initial detectable inoculum density in each lot of infested soil was assayed after 24 hr by dilution plating of subsamples from each lot. Fifty grams of infested soil was diluted with 100 ml of 0.25% water agar and 1-ml samples were spread onto each of 10 plates of a selective antibiotic medium (PARPH) (13). The amount of dry soil per plate was determined by drying and weighing a 1-ml sample of each dilution. After 2 days of incubation at room temperature in darkness, soil was washed from the surface of the agar plate and colonies of *Phytophthora* were counted. Inoculum densities were expressed as propagules per gram of dry soil.

Cocoa seedlings were produced from seed of the UCR cocoa tree. After removal of seed coats, seeds were incubated at room temperature in moist paper towels for 4 days. Forty cubic centimeters of sterile sand, 100 g (wet weight) of infested soil, and 50 g (wet weight) of uninfested soil, respectively, were layered sequentially into 6-cm-diameter pots and germinated cocoa seeds were transplanted into the layer of uninfested soil. Pots were incubated in the greenhouse and watered as needed to maintain normal plant growth. Eight weeks after planting, the number of dead plants was recorded. The root systems of the living plants were removed from soil, rinsed in sterile distilled water, briefly immersed in 80% ethyl alcohol, and blotted dry. The entire root system of a single plant was placed on a plate of PARPH. After 2–3 days of incubation at room temperature in the dark, colonies of *Phytophthora* growing from each root system were counted as infection sites. The number of recoverable propagules per gram of dry soil in each treatment was determined by dilution plating as described above. The experiment was performed twice with seven or eight replications.

Survival in cocoa pods. The survival of *P. palmivora* (P1240), *P. citrophthora* (P1202), and *P. capsici* (P623) in green cocoa pods was compared. The pod pericarp was removed and cut into 2-cm squares. Each square was placed, epidermal side up, on a filter paper disk in a petri plate. A small cut was made in the pod surface

TABLE 1. Origins of *Phytophthora* isolates used in morphological and physiological comparisons

Species Isolate[y]	Host	Origin	Compatibility type[z]
P. palmivora			
P255	Cocoa	Costa Rica	A2
P1240	Cocoa canker	Bahia, Brazil	A2
P986	Cocoa soil	Nigeria	A2
P. capsici			
P1091	*Capsicum* sp.	New Mexico	A2,H
P623	Cocoa pod	Bahia, Brazil	A2
P1043	Cocoa pod	Bahia, Brazil	A2
P622	Cocoa pod	Bahia, Brazil	A1
P1242	Cocoa husk pile	Bahia, Brazil	A1
P795	Cocoa pod	Cameroun	A1
P. citrophthora			
P1200	Cocoa canker	Bahia, Brazil	–
P1201	Cocoa seedling	Bahia, Brazil	–
P1202	Cocoa pod	Bahia, Brazil	–
P1203	Cocoa canker	Bahia, Brazil	–
P1210	Cocoa pod	Saõ Paulo, Brazil	–
P1211	Cocoa pod	Saõ Paulo, Brazil	A1
P1212	Cocoa pod	Saõ Paulo, Brazil	–
P1213	Cocoa pod	Saõ Paulo, Brazil	–
P449	Cocoa pod	Bahia, Brazil	A1
P620	Cocoa pod	Bahia, Brazil	–
P453	Cocoa pod	Bahia, Brazil	A1
P776	Cocoa leaves	Bahia, Brazil	A2
019†	Cocoa soil	Bahia, Brazil	A1
P318	Citrus root	Australia	A2
P479	Lemon bark	California	–
P895	*Euonymous* sp.	Oregon	–
P1153	*Citrus sinensis*	Rio Grande do Sul, Brazil	–
P1163	*Citrus sinensis*	California	–
P999	*Heuchera* sp.	California	–
P717	Unknown	New Zealand	A1

[y] University of California, Riverside, culture collection number (†, not in collection).

[z] Compatibility type was determined as described in this paper. Some isolates did not form oospores in any of the combinations tested (–) and one isolate formed oospores in single culture (H).

TABLE 2. Colony growth rates of *Phytophthora citrophthora* from cocoa and citrus and *P. palmivora* and *P. capsici* from cocoa at five temperatures

Species	Isolate[y]	Host[z]	Growth rate (mm/day)[x] at: 9 C	12 C	24 C	33 C	36 C
P. citrophthora	P1200	Cocoa	0.4	4.3	14.1	9.1	0.1
	P1201	Cocoa	0.5	4.2	13.9	9.3	0.2
	P1202	Cocoa	0.5	5.0	14.4	9.6	0.1
	P1203	Cocoa	0.2	4.6	12.5	7.8	0.4
	P1210	Cocoa	1.9	4.0	12.2	6.5	0.2
	P1211	Cocoa	1.4	4.1	13.8	7.5	0.1
	P1212	Cocoa	2.6	4.0	12.9	9.9	0.4
	P1213	Cocoa	1.5	4.9	14.0	8.4	0.1
	P1163	Citrus	1.4	3.2	9.6	4.0	0.3
	P895	Citrus	1.4	4.5	8.7	2.8	0
P. capsici	P1043	Cocoa	1.8	5.3	14.3	7.0	0.1
P. palmivora	P255	Cocoa	0.5	1.3	7.5	5.5	0

[x] Diameters of three replicate colonies of each isolate on a synthetic medium (28) were measured after 2 and 5 days of growth at each temperature. The average daily increase in colony diameters over the 3-day period were expressed in millimeters per day.

[y] University of California, Riverside, culture collection number.

[z] Host from which originally isolated.

with a razor blade and a 5-mm-diameter mycelial disk taken from 3-day-old CV8 cultures of each isolate was placed on each wound. The inoculum was covered with moist cotton and 10 ml of sterile distilled water was added to each plate. Plates were incubated at room temperature in the dark. The cotton and the remains of the mycelial disk were removed after 5 days when most of the inoculated pod pieces were at least 50% blackened. Plates were incubated further under dry or moist conditions. For dry treatments, the moist filter paper was replaced with dry paper. For moist treatments, an additional 5–10 ml of sterile distilled water was added to each plate. Plates were incubated at 24 or 18 C in the dark.

After 3, 5, 7, 10, 12, and 14 wk, one pod piece from each treatment was examined. Each piece was immersed in 95% ethyl alcohol, flamed briefly, and blended with 50 ml of sterile distilled water at high speed in a Sorvall Omnimixer (Sorvall Co., Norwalk, CT). The resulting pod slurry was diluted with water (1:10 or 1:100), and 1 ml of each dilution was spread over each of three replicate plates containing 15 ml of PARPH. Plates were incubated in the dark at room temperature and colonies were counted after 2 days. Two-milliliter samples of each pod slurry were air-dried and weighed so that fungal propagule counts could be computed on the basis of propagules per gram of dry pod tissue. On each sample date, pod slurries were prepared from each treatment and observed microscopically for structures of *Phytophthora*.

RESULTS

Colony morphology. The colony morphologies of *P. citrophthora* from cocoa were similar to previous descriptions of *P. citrophthora* from other hosts. On CMA all isolates of *P. citrophthora*, *P. palmivora*, and *P. capsici* examined produced colonies with finely radiate growth patterns. Slight variations in growth rates and patterns were noted among isolates when they were grown on CV8, PDA, and CA. All isolates grew most profusely on CA. On CA, all isolates of *P. citrophthora* from cocoa produced identical dense rosettes of hyphae that were very similar to those produced by isolates of *P. citrophthora* from citrus. In contrast, *P. palmivora* from cocoa (P255) formed dense compact colonies and *P. capsici* from cocoa (P623) grew in a diffuse rosette pattern.

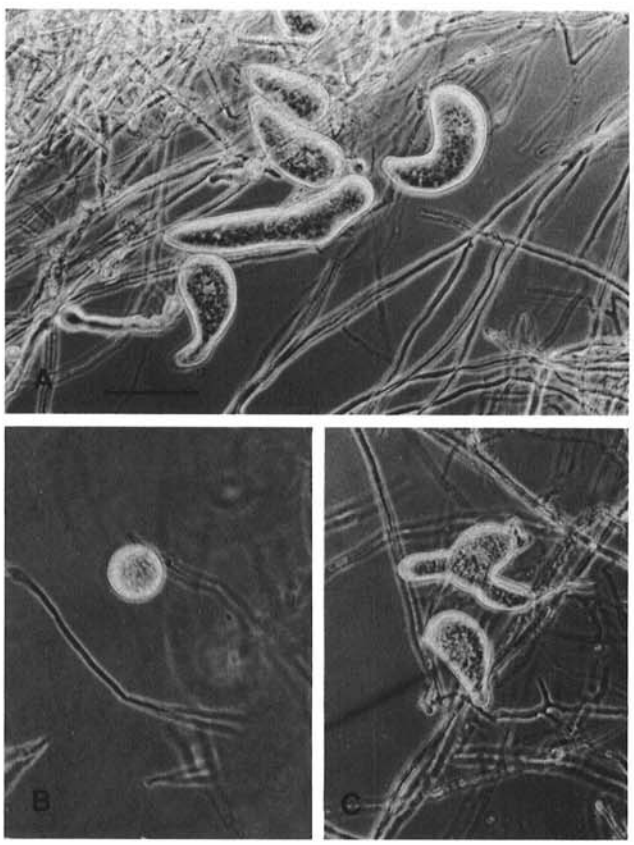

Fig. 1. A and C, Typical sporangia and **B,** chlamydospore of *Phytophthora citrophthora* isolate P1211 from cocoa. Structures were produced after 5 days of growth in carrot agar. Bar = 50 μm.

Colony growth rates. *P. citrophthora* and *P. capsici* from cocoa exhibited better growth over a wider range of temperatures than did *P. citrophthora* from citrus or *P. palmivora* from cocoa on SM (Table 2). No isolates grew well at 36 C. At 33 C, *P. citrophthora* and *P. capsici* from cocoa grew faster than did the *P. citrophthora* from citrus and *P. palmivora* from cocoa. All isolates grew most rapidly at 24 C. At 12 C, growth was slow but comparable for all isolates except *P. palmivora* from cocoa which grew much slower than the others. At 9 C, *P. citrophthora* from cocoa from Bahia and *P. palmivora* from cocoa grew very little whereas the *P. citrophthora* from cocoa from Saõ Paulo, *P. citrophthora* from citrus, and *P. capsici* from cocoa grew more rapidly.

Sporangium and chlamydospore morphology. On CA, caducous sporangia with short or long stalks were produced by all cocoa isolates that previously had been identified as *P. palmivora* or *P. capsici*, respectively. *P. citrophthora* from citrus produced noncaducous sporangia that fit previous descriptions for these specific isolates (7,8). All *P. citrophthora* isolates from cocoa produced sporangia with morphologies similar to *P. citrophthora* (P1153 and P1163) from citrus (7). Noncaducous, irregularly shaped, papillate sporangia averaged 63.7 ± 7 μm in length and 35.2 ± 2 μm in breadth, with an average length:breadth ratio of 1.87 ± 0.2 μm (Fig. 1). Occasionally sporangia with two papillae were observed. Papilla width and depth averaged 7.2 ± 0.7 μm and 4.8 ± 0.4 μm, respectively. Abundant chlamydospores that averaged 26.5 ± 1.2 μm in diameter formed on the agar surface of all cultures of *P. citrophthora* from cocoa (Fig. 1).

Oospore production. The sandwich technique used to produce oospores reduced the amount of materials and space required to evaluate a large number of isolates for compatibility type. In positive reactions, oospores were present in the center disk after 6 days. The abundance of oospores produced in particular pairings varied. With some pairings, oospores that were not detected in large plates were observed with the disk technique. The isolates of *P. citrophthora* from cocoa did not form oospores when crossed among themselves in all possible combinations. However, some of these cocoa isolates (P1210, P449, P453, 019) formed oospores with P318 (A2), and one isolate of *P. citrophthora* from cocoa (P776) formed oospores with P717 (A1). Ferguson described P318 and P717 as A2 and A1, respectively, and classified both of these isolates as *P. citrophthora* (8).

Stem inoculations. *P. citrophthora* from cocoa induced severe stem lesions on cocoa seedlings but did not produce symptoms on citrus seedlings (Table 3). Severe lesions also were induced on cocoa by isolates of *P. palmivora* and *P. capsici* from cocoa. *P. citrophthora* from citrus induced gummosis and lesions on the citrus seedlings but not on cocoa seedlings.

Pathogenicity of isolates to roots of cocoa seedlings. The germination of chlamydospores from inoculum suspensions after 24 hr of incubation on water agar averaged 86 and 79% for *P. palmivora* (P1240) and *P. citrophthora* (P1202), respectively. Oospores of *P. capsici* (P623 × P795) did not germinate on water agar after freezing, although germination percentages similar to those previously reported (15) were obtained in unfrozen preparations. Oospores that did not germinate on water agar in the laboratory after freezing, germinated and produced colonies on PARPH after at least 24 hr incubation in soil.

Initial recovery of *P. palmivora* and *P. citrophthora* from infested soil was close to the calculated infestation density of 200 propagules per gram (dry soil basis), but only 27 propagules per gram (dry soil basis) were recovered for *P. capsici* (Table 4). More than half of the colonies of *P. capsici* observed on the dilution plates could be seen to originate definitely from oospores. After 8 wk, the propagule density declined for *P. citrophthora* and *P. capsici* while the inoculum density of *P. palmivora* remained at the

initial level. By 8 wk, 53% of the plants in the *P. palmivora* treatment had died and all remaining plants were infected. Of the plants inoculated with *P. citrophthora*, 67% were dead and 93% of the remainder were infected. In those inoculated with *P. capsici*, none of the plants died and only 40% were infected. Roots infected with *P. citrophthora* and *P. palmivora* were blackened and rotting, but no disease symptoms were observed on roots infected with *P. capsici*. The number of infection sites per root system averaged 6.1, 1.8, and 0.8 for *P. palmivora*, *P. citrophthora*, and *P. capsici*, respectively. There were no significant differences in root weights of plants remaining alive at 8 wk. In those treatments inoculated with *P. capsici*, 18 colonies from soil dilution plates and five colonies from roots were checked for compatibility type and all were A2.

TABLE 3. Pathogenicity of *Phytophthora citrophthora* and *P. palmivora* to cocoa and citrus seedlings as determined by lesion areas measured 8 wk after wound inoculation of seedling stems

Species	Host[w]	Isolate	Disease rating[v] Cocoa[x]	Citrus[y]
P. citrophthora	Cocoa	P1200	3.0	0
		P1202	3.7	0
		P1203	3.7	0
		P1211	4.7	...[z]
	Citrus	P1163	0	2.0
P. palmivora	Cocoa	P986	1.0	...
P. capsici	Cocoa	P623	4.7	...
Control			0	0

[v] Disease rating based on the following scale: 0 = no lesion, 1 = lesions up to 2 cm, 2 = lesions 2–3 cm, 3 = lesions more than 3 cm, 4 = top wilt, and 5 = dead plant. Each value is the mean of four replications.
[w] Original host from which fungus was isolated.
[x] One-month-old cocoa seedlings from cocoa tree of unknown parentage.
[y] Ten-month-old cultivar Pineapple sweet orange seedlings.
[z] Inoculation not made with this combination.

TABLE 4. Average number of propagules of *Phytophthora* recovered from soil artificially infested with chlamydospores of *P. palmivora* (P1240) and *P. citrophthora* (P1202) or oospores of *P. capsici* (P623 × P795) after 24 hr and 8 wk of incubation

Species	Incubation time[x] 24 hr	8 wk
P. palmivora	227 a[y,z]	237 a
P. citrophthora	188 b	59 b
P. capsici	27 c	11 c
Control	0 d	0 c

[x] Soil was infested with 200 propagules per gram of dry soil and incubated in polyethylene bags for 24 hr or planted with cocoa seedlings and maintained under greenhouse conditions for 8 wk prior to being assayed for fungal populations by soil dilution plating.
[y] Propagules per gram of dry soil.
[z] Each value is the mean of ten samples. Values in columns not followed by the same letter are significantly different from each other according to Duncan's multiple range test ($P = 0.05$).

TABLE 5. Average number of propagules recovered from cocoa pod tissue inoculated with *Phytophthora palmivora* (P1240), *P. citrophthora* (P1202), or *P. capsici* (P623) and incubated for 14 wk

Phytophthora species	Incubation treatment Moist[w] 18 C	Moist[w] 24 C	Dry[x] 18 C	Dry[x] 24 C
P. palmivora	3,175 a[y,z]	1,856 ab	3,017 ab	2,086 ab
P. citrophthora	989 b	364 b	736 b	720 b
P. capsici	406 b	69 b	317 b	240 b
Control	0	0	0	0

[w] Inoculated pod pieces were incubated on moist filter paper in covered petri dishes in the dark at 18 or 24 C.
[x] Inoculated pod pieces were incubated on dry filter paper in covered petri dishes in the dark at 18 or 24 C.
[y] Average number of fungal propagules per gram of dry pod tissue as determined by dilution plating of pod slurries on a selective antibiotic medium (PARPH) (13). Values represent the average number of propagules recovered on six sampling dates over a 14-wk period after pod inoculation.
[z] Data not followed by the same letter are significantly different according to Duncan's multiple range test ($P = 0.05$).

Survival of isolates in pods. The average number of propagules recovered from inoculated pod pieces are given in Table 5. More propagules were recovered from pods infected by *P. palmivora* than from pods infected with the other two species. At 18 C, survival of each species was similar in moist and dry pod pieces. At 24 C, survival was higher in the dry treatments of *P. citrophthora* and *P. capsici* than at 18 C. Propagules of *P. palmivora* were recovered in high numbers in moist and dry treatments at 18 and 24 C.

Chlamydospores, and less frequently sporangia, of *P. palmivora* and *P. citrophthora* were observed in the pods in all treatments. More chlamydospores were produced by *P. palmivora* than by *P. citrophthora*. Few sporangia and no chlamydospores of *P. capsici* were observed in any treatments. By 12 and 14 wk, many of the chlamydospores and sporangia of all three species were highly vacuolated and appeared to be nonviable.

Oospores with amphigynous antheridia were observed for all three species on at least two sample dates (Table 6). Because of their low frequency, exact quantification was not possible, but from 1 to 20 oospores were observed for each positive record. Oospores of *P. capsici* were found most frequently and oospores were observed more often at 18 than at 24 C. Attempts to germinate the oospores of *P. capsici* in water were unsuccessful. Oospores were similar to each other in size (22–24 μm) and thick-walled.

DISCUSSION

Although *P. citrophthora* has been reported as a pathogen of members of 28 plant families (20), it remains a relatively poorly

TABLE 6. Oospores observed in cocoa pod tissue inoculated with *Phytophthora palmivora* (P1240), *P. citrophthora* (P1202), or *P. capsici* (P623) after various incubation times and conditions

Treatment[y]	*Phytophthora* species	Incubation time (wk) and presence of oospores[x] 3	5	7	10	12	14
Moist pod 18 C	*P. palmivora*	–[z]	–	–	–	+	–
	P. citrophthora	–	–	–	–	–	–
	P. capsici	–	+	+	+	+	–
	Control	–	–	–	–	–	–
Moist pod 24 C	*P. palmivora*	–	–	+	–	–	–
	P. citrophthora	–	–	–	–	–	–
	P. capsici	–	+	–	+	–	–
	Control	–	–	–	–	–	–
Dry pod 18 C	*P. palmivora*	–	–	–	+	+	–
	P. citrophthora	–	–	–	–	+	+
	P. capsici	–	+	–	+	–	–
	Control	–	–	–	–	–	–
Dry pod 24 C	*P. palmivora*	–	–	–	–	–	–
	P. citrophthora	–	–	–	–	–	–
	P. capsici	–	–	+	+	+	–
	Control	–	–	–	–	–	–

[x] Two-centimeter pieces of inoculated pericarp in 50 ml of sterile distilled water were blended into slurries and samples were examined microscopically for the presence of oospores at indicated times after inoculation.
[y] Inoculated pods were incubated on wet or dry filter paper in covered petri dishes at 18 or 24 C in the dark.
[z] Oospores were observed (+) or not observed (–) in slurries of inoculated pod tissue.

characterized species. Some of the features of *P. citrophthora* described in Waterhouse's key (27) are not consistent with descriptions of this species given by other workers (8,9,24). The isolates of *P. citrophthora* from cocoa described here differ in some features from descriptions of *P. citrophthora* given by Waterhouse (27).

The length:breadth ratios of sporangia of the isolates of *P. citrophthora* from cocoa ranged from 1.6 to 2.2. To arrive at *P. citrophthora* in Waterhouse's key, a length:breadth ratio of 1.2 to 1.4 must be selected early in the keying process. However, other workers have reported length:breadth ratios of 1.6 or more for *P. citrophthora* (8,9), and size and shape of sporangia vary with culture media and environmental conditions (26).

P. citrophthora has been reported to be sexually sterile in single culture or in interspecific crosses with A1 or A2 isolates (8,9,20,24,26). Other isolates described as *P. citrophthora* are known to form oospores in intraspecific (8,18) and interspecific crosses (18,21). Five of the 10 isolates of *P. citrophthora* from cocoa examined formed oospores with a *P. citrophthora* from citrus (A2) or from an unknown New Zealand host (A1). The existing data on *P. citrophthora* suggest that a compatibility system more complex than the A1/A2 system operates within the species.

Caducity of sporangia is a useful taxonomic feature for some *Phytophthora* species (14). Sporangia of *P. citrophthora* have been described as caducous (19,23) or noncaducous (8,9,24). Inconsistencies can result if the tearing of sporangial stalks is misinterpreted for caducity. The *P. citrophthora* isolates studied here produced large, irregular, noncaducous sporangia in repeated experiments. The dimensions of the sporangia and papillae, and the production of sporangia with two papillae, conform to previous descriptions of *P. citrophthora* (9,27).

Colony growth patterns on CMA were not good diagnostic features for differentiating *P. citrophthora* from cocoa and *P. palmivora* or *P. capsici*. On CA, colony morphology was a slightly more distinctive characteristic, but due to the variations observed among isolates of the same species, colony morphology cannot be relied upon heavily for species determination.

Cardinal temperatures are used as aids in identification, but different isolates within a well-defined species may demonstrate differences in growth response to temperatures (28). Maximum growth temperatures reported for *P. citrophthora* range from 30 to 37 C (8,23,27) and minimum temperatures range from 5 to 10 C (24,27). Considering these variations in temperature responses, our isolates of *P. citrophthora* from cocoa fall within the range of temperatures considered characteristic of *P. citrophthora* even though some isolates exhibited poor growth at 9 C and a little growth at 36 C.

The pathogenicity of isolates of *Phytophthora* of the same species to different host plants may vary (23). Therefore, it was not unexpected that isolates of *P. citrophthora* from cocoa were not pathogenic to citrus and conversely that isolates of *P. citrophthora* from citrus examined were not pathogenic to cocoa.

Until sexuality in *Phytophthora* is better understood, and the more technically difficult classification methods such as chromosome counts and electrophoresis become more routine, morphological and physiological data must be relied upon to distinguish *Phytophthora* species. Based on previously published descriptions and the available keys, our data indicate that the group of isolates of *Phytophthora* pathogenic to cocoa and distinct from *P. capsici* and *P. palmivora*, can best be described as *P. citrophthora*. In the future *P. citrophthora* should be included in experiments to screen for cocoa material resistant to black pod disease and in fungicide evaluations. Also, quarantine measures should be enforced to restrict the spread of this pathogen to other cocoa-growing areas of the world.

Based on isolate surveys (6,16) *P. citrophthora* has been found more commonly than *P. palmivora* within the region of Bahia, Brazil. The ability of isolates of *P. citrophthora* to produce chlamydospores might increase its chance of survival in soil or plant parts. *P. capsici*, the most widely occurring species in Bahia, does not produce chlamydospores. Our results indicate that *P. citrophthora* has the potential to be a severe pathogen of cocoa roots, and more work is needed to determine the distribution of this species within Brazil.

Sonication did not reduce the viability of the propagules used in soil infestation experiments, confirming previous reports (2,13). Germinating chlamydospores and oospores have been recovered from artificially infested soil (2,10,13), and oospores of homothallic *Phytophthora* species typically germinate at high percentages in vitro. However, to our knowledge this is the first report of the recovery of germinating oospores of a heterothallic *Phytophthora* sp. from artificially infested soil. Freezing of the oospores may have induced dormancy, and incubation in soil for at least 24 hr stimulated the oospores to germinate. Further work with this system might provide information about the factors responsible for this stimulation.

Chlamydospores and oospores served as inoculum for infecting the roots of cocoa seedlings. The mortality rate of seedlings was higher for *P. palmivora* and *P. citrophthora* than for *P. capsici*. *P. palmivora* and *P. megakarya* have been isolated from naturally infected roots of mature cocoa trees in West Africa (25), but *Phytophthora* has not been isolated from cocoa roots in the field in Brazil. Since roots produced by cocoa seedlings are similar to feeder roots produced by mature trees (5), results of our experiments indicate that all three species found in Brazil have the potential for root infection in the field. *P. citrophthora* and *P. palmivora* have the potential to actively destroy feeder roots but no disease symptoms were observed on roots infected with *P. capsici*. Ward and Griffin (25) in Nigeria found that cocoa roots infected with *P. palmivora* and *P. megakarya* in the field did not always show symptoms of disease. Gregory (11) hypothesized that cocoa root infection by *Phytophthora* might serve as a site of fungal survival in the soil environment where it is otherwise not a good competitor. A pathogenic or nonpathogenic association with roots could perpetuate inoculum for above-ground infection.

In pod survival experiments, the most propagules of all three species were recovered from moist pod pieces at 18 C. This suggests that broken pods in husk piles that tend to hold water and are less exposed to direct sunlight are more suited to fungal survival than are pods hanging on trees which are more exposed to drying and warmer temperatures. Based on the differences in propagule numbers among species, pods may have different significance in disease epidemics depending on which species of the fungus predominates in a particular location.

There are a few well-documented reports in the literature of heterothallic *Phytophthora* species producing oospores in naturally infected host material (10,17). In two of these reports, both A1 and A2 compatibility types were recovered from the plant tissue in which the oospores were found. Isolates that produced oospores in inoculated cocoa pod tissue were never observed to produce oospores in single-isolate culture on agar. The variation in the natural population resulting from sexual recombination in homothallic oospores must be considered in control programs for black pod disease.

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