Examination of Mitochondrial DNA Restriction Fragment Length Polymorphisms, DNA Fingerprints, and Randomly Amplified Polymorphic DNA of *Colletotrichum orbiculare*

J. C. Correll, D. D. Rhoads, and J. C. Guerber

First and third authors, associate professor and research specialist, respectively, Department of Plant Pathology, and second author, assistant professor, Department of Biological Sciences, University of Arkansas, Fayetteville 72701.
Corresponding author: J. C. Correll.
This work was supported in part by grants from the Arkansas Science and Technology Authority, USDA CSRS Grant 92-37303-8006, and DOE/NSF/USDA Program in Plant Biology (92-04428).
Accepted for publication 10 June 1993.

ABSTRACT

Correll, J. C., Rhoads, D. D., and Guerber, J. C. 1993. Examination of mitochondrial DNA restriction fragment length polymorphisms, DNA fingerprints, and randomly amplified polymorphic DNA of *Colletotrichum orbiculare*. Phytopathology 83:1199-1204.

Twenty-four isolates of *Colletotrichum orbiculare,* three isolates of *C. magna,* and two putative isolates of *Glomerella cingulata* var. *orbiculare,* representing diverse geographical and cucurbit host origins, vegetative compatibility groups, and races were examined for mitochondrial and nuclear DNA restriction fragment length polymorphisms (RFLPs) and randomly amplified polymorphic DNA (RAPD). Six isolates of *C. orbiculare* from cocklebur, a noncucurbit host, also were examined. Four mitochondrial DNA (mtDNA) haplotypes, designated A, A1, B, and C, were observed among the isolates examined. Twenty-two isolates, representing three vegetative compatibility groups and two races pathogenic to cucurbits, had an identical mtDNA RFLP haplotype (haplotype A) with each of seven restriction enzymes. Six cocklebur isolates (nonpathogenic on cucurbits) had an mtDNA RFLP haplotype identical to the pathogenic isolates with six of the seven restriction enzymes examined; however, restriction enzyme *Pvu*II detected a single additional restriction site in the mtDNA of the cocklebur isolates (haplotype A1). Three nonpathogenic isolates of *C. magna* had a third mtDNA RFLP haplotype (haplotype B). Two nonpathogenic isolates of *C. orbiculare* from honeydew melon and two nonpathogenic isolates of *Glomerella cingulata* var. *orbiculare* from cucuzzi gourd had a fourth mtDNA RFLP haplotype (haplotype C). DNA fingerprinting, which was conducted with the synthetic oligonucleotide probe $(CAT)_5$ or a human minisatellite DNA probe, identified these same four haplotypes. Five RAPD primers also resolved the four RFLP groups. One RAPD primer resolved two groups among isolates within mtDNA RFLP haplotype A that correlated with host origin, vegetative compatibility group, and race. Thus, one RAPD primer differentiated the two races of *C. orbiculare.* Overall, there was a strict correspondence between mtDNA RFLP haplotype, DNA fingerprint group, and RAPD group among the isolates examined.

Colletotrichum orbiculare (Berk. & Mont.) Arx (= *C. lagenarium* (Pass.) Ellis & Halst.), generally recognized as one of several *Colletotrichum* species with a relatively narrow host range, is found primarily on hosts in the Cucurbitaceae (1,3,28,29,33). Anthracnose of cucurbits, caused by *C. orbiculare,* is a destructive disease, particularly on cucumber, watermelon, and cantaloupe. The pathogen is capable of infecting foliage, stems, and fruit and can cause both severe yield losses and reductions in fruit quality (31). Although disease resistance, fungicides, and crop rotation are somewhat effective as management strategies to control anthracnose, favorable weather and virulent races contribute to disease outbreaks, and anthracnose remains an important cucurbit disease.

In addition to attacks on hosts in the Cucurbitaceae, *C. orbiculare* has been reported on safflower (*Carthamus tinctorius* L., Asteraceae) and celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers., Apiaceae) from Australia (27,33). More recently, an extensive taxonomic study by Walker et al (33) reported that *C. orbiculare* is a common pathogen of spiny cocklebur (*Xanthium spinosum*). In Australia, *C. orbiculare* has been observed to naturally control spiny cocklebur, and work has been initiated to determine if cocklebur isolates of *C. orbiculare* can be used as mycoherbicides to control spiny cocklebur. Cocklebur isolates of *C. orbiculare* also have been examined for their potential to control common cocklebur (*Xanthium strumarium* L.) in the United States and Australia (26). Walker et al (33) reported that some of the cocklebur isolates they examined are capable of causing leaf spotting on certain watermelon and honeydew cultivars but do not cause any symptoms on several cucumber cultivars.

Various molecular protocols have been widely used to characterize strains of many plant-pathogenic fungi. These protocols have included mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis (5), DNA fingerprinting (4,5,21–23), and, more recently, randomly amplified polymorphic DNA (RAPD) analysis (2,9,12–14,24,36,37). However, there is a paucity of data on the integrated use of genetic and multiple molecular criteria to characterize plant-pathogenic fungi (15).

We previously examined vegetative compatibility group (VCG) diversity and re-evaluated races of *C. orbiculare* from a diverse collection of isolates (34). The objective of the current study was to evaluate the molecular diversity of isolates in this collection. Isolates of *C. orbiculare, Glomerella magna* S.F. Jenkins & Winstead (anamorph: *C. magna*) (18,20), and the putative teleomorph of *C. orbiculare, Glomerella cingulata* (Stoneman) Spauld. & Schenk var. *orbiculare* S.F. Jenkins & Winstead (18,19), were selected to represent diverse geographical and host origins and different VCGs and races. Isolates were examined for mtDNA RFLPs; for DNA fingerprints with two probes, HVR33.6, a human minisatellite probe (15,17), and an oligonucleotide probe, $(CAT)_5$; and for RAPD patterns. Preliminary reports of this work have been published (7,8).

MATERIALS AND METHODS

Isolates. Twenty-four isolates of *C. orbiculare,* three isolates of *C. magna,* and two isolates of the putative teleomorph of *C. orbiculare, G. c. orbiculare,* were examined for mtDNA RFLPs. The isolates were recovered from several cucurbit hosts from throughout the United States, Africa, and Australia (Table 1). Several cocklebur isolates from different locations in New South Wales, Australia, were also included. The isolates represented 10 VCGs and two distinct races (34). Isolates in VCGs 1, 2, and 3 were pathogenic on cucurbits. VCGs 1 and 3 represent race 1 isolates, and VCG 2 represents race 2 isolates (34). Isolates in VCGs 4–10 were nonpathogenic or very weakly pathogenic on cucurbits.

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mtDNA RFLP analysis. Purified mtDNA of reference strain JC1 was recovered as previously described (6,16). Plasmid clones were generated by partial digestion of mtDNA with *Eco*RI and ligation into the *Eco*RI site of pUC13 (32). Two specific clones, 2u18 and 4u40, were obtained that contained multiple *Eco*RI fragments. The insert in 2u18 included fragments of 2.1, 2.5, and 5.5 kb, while 4u40 included fragments of 6.4 and 7.3 kb. Detailed restriction mapping of the inserts and comparison to restriction maps deduced from the total mtDNA confirmed the contiguous nature of the fragments as cloned in 2u18 and 4u40. These clones encompass approximately 65% of the mtDNA genome of isolate JC1 of *C. orbiculare*.

A restriction endonuclease map of the mtDNA of JC1 was constructed on the basis of restriction digestions of purified mtDNA and Southern blots probed individually with the plasmid subclones 2u18 and 4u40. The final map was confirmed by paired enzyme digestions of purified mtDNA.

A "mini-prep" procedure was used to recover total DNA from all isolates as previously described (6,16). Total DNA was digested with one of seven restriction enzymes (*Bam*HI, *Pal*I, *Eco*RI, *Pvu*II, *Bgl*II, *Hin*dIII, and *Xba*I) according to the manufacturer's recommendations. Restricted DNA was separated electrophoretically on 0.7% agarose gels. Fragments of purified mtDNA were visualized directly by staining with ethidium bromide. Fragments of total DNA were transferred to nylon membranes. Both purified mtDNA or clones 2u18 and 4u40 in combination were used to determine the mtDNA RFLP phenotypes. An enhanced chemiluminescence DNA labeling kit (ECL, Amersham, Arlington Heights, IL) was used to label the purified mtDNA or the plasmid clones, which were then used to probe the Southern blots. For each enzyme, any isolates with the same mtDNA restriction fragment pattern were assigned a common mtDNA RFLP haplotype.

DNA fingerprinting. Two DNA fingerprint probes were used

TABLE 1. Vegetative compatibility group (VCG), previously reported race identification, and host and geographic origin of isolates of *Colletotrichum orbiculare*

Isolate[a]	VCG	mtDNA haplotype[b]							Origin	
		A	B	C	D	E	F	G	Host	Location
BB11	1	–	–	–	–	–	–	–	Cucumber (leaf)	Lane, Oklahoma
BB12	1	–	–	–	–	–	–	–	Cucumber (leaf)	Lane, Oklahoma
BB13	1	–	–	–	–	–	–	–	Cucumber (fruit)	Lane, Oklahoma
CR7A	1	A	A	A	A	A	A	A	Cucumber (leaf)	Sumter County, Florida
JC1	1	A	A	A	A	A	A	A	Cucumber (leaf)	Kibler, Arkansas
JC2	1	–	–	–	–	–	–	–	Cucumber (leaf)	Kibler, Arkansas
JC4	1	–	–	–	–	–	–	–	Cucumber (leaf)	Kibler, Arkansas
LB1	1	–	–	–	–	–	–	–	Cucumber	Independence, Louisiana
LB4	1	A	–	–	–	–	–	–	Cantaloupe	Angola, Louisiana
LB5	1	A	A	A	–	–	–	A	Cantaloupe	Angola, Louisiana
LB6	1	A	–	–	–	–	–	–	Cucumber	Crowley, Louisiana
MH3	1	A	A	–	A	–	A	A	Cucumber	Hancock, Wisconsin
NC3	1	A	A	–	A	–	A	A	Cucumber (leaf)	North Carolina
AK1	1	–	–	–	–	–	–	–	Watermelon (fruit)	South Carolina
BB15	1	–	–	–	–	–	–	–	Watermelon (fruit)	Lane, Oklahoma
CP3	2	A	A	A	A	A	A	A	Watermelon	Oklahoma
CP6	2	A	A	A	A	A	A	A	Watermelon	Texas
JC7	2	A	A	A	A	A	A	A	Watermelon (fruit)	Kibler, Arkansas
JC9	2	A	–	A	–	A	A	A	Watermelon (fruit)	Kibler, Arkansas
LB2	2	A	A	A	–	–	–	A	Cucuzzi gourd	Ruston, Louisiana
LB3	2	–	–	–	–	–	–	–	Cucuzzi gourd	Ruston, Louisiana
ATCC 15094	2	A	A	A	A	–	–	A	Watermelon	North Carolina
ATCC 15470	2	A	A	A	A	–	–	A	Watermelon	Africa
ATCC 15471	2	A	A	A	A	A	–	A	Watermelon	Florida
ATCC 15472	2	A	A	A	A	–	–	A	Watermelon	Oklahoma
ATCC 15098	2	A	A	A	A	A	A	A	Unknown	Manhattan, Kansas
MH5	3	A	A	A	A	A	A	A	Cucumber (fruit)	Hancock, Wisconsin
ATCC 15093	3	A	A	A	A	A	A	A	Cucumber	North Carolina
ATCC 15095	3	A	A	A	A	A	A	A	Cucumber	North Carolina
DAR 55655	3	–	–	A	–	–	–	A	*Cucumis myriocarpus*	Narromine, Australia
DAR 61396	3	–	–	A	–	–	–	A	Cucumber	Parklea, Australia
AK2[c]	4	B	B	B	–	–	–	B	Acorn squash (fruit)	Charleston County, South Carolina
ATCC 15015[c]	5	B	B	B	B	B	B	B	Watermleon	North Carolina
ATCC 15016[c]	6	B	B	B	B	B	B	B	Watermelon	North Carolina
ATCC 15096[d]	7	C	C	C	–	–	–	C	Cucuzzi gourd	North Carolina
ATCC 15097[d]	7	C	C	C	C	–	–	C	Cucuzzi gourd	North Carolina
HD1	9	C	C	C	–	–	–	C	Honeydew (fruit)	Lane, Oklahoma
HD3	9	C	C	C	–	–	–	C	Honeydew (fruit)	Lane, Oklahoma
LW1	10	A	–	A	–	–	–	A1	Cocklebur (stem)	Coolah, Australia
LW2	10	A	A	–	–	–	–	–	Cocklebur (stem)	Merriwa, Australia
LW3	10	A	A	A	A	A	A	A1	Cocklebur (stem)	Scone, Australia
LW4	10	–	–	–	–	–	–	–	Cocklebur (stem)	Merriwa, Australia
LW5	10	A	A	A	–	–	–	A1	Cocklebur (stem)	Coolah, Australia
LW6	10	A	A	A	A	A	A	A1	Cocklebur (stem)	Pawnee Hills, Australia
LW7	10	–	–	–	–	–	–	–	Cocklebur (stem)	Coolah, Australia
DAR 55629	10	–	–	A	–	–	–	A1	Cocklebur	Orange, Australia

[a] ATCC = American Type Culture Collection; DAR = New South Wales Department of Agriculture culture collection (33).

[b] Mitochondrial DNA (mtDNA) haplotypes based on restriction fragment length polymorphisms (RFLP) with seven enzymes: A = *Bam*HI; B = *Pal*I; C = *Eco*RI; D = *Bgl*II; E = *Hin*dIII; F = *Xba*I; and G = *Pvu*II. Letters within a column indicate a mtDNA RFLP haplotype; – = information not available.

[c] Isolates of *Colletotrichum magna* (18,20).

[d] Isolate reported as *Glomerella cingulata* var. *orbiculare* (18,19).

on selected isolates representing each mtDNA RFLP haplotype. Southern blots of total DNA digested with either *Eco*RI or *Pvu*II were probed with a human minisatellite DNA oligonucleotide probe (HVR 33.6) according to the manufacturer's recommendations (SNAP DNA Fingerprinting Kit, Molecular Biosystems Inc., San Diego, CA) (15). The second probe was the oligonucleotide 5′-$(CAT)_5$-3′, provided by Dr. T. C. Harrington (Iowa State University) (10), which was end labeled with [^{32}P]dATP by terminal deoxynucleotidyl transferase to a specific activity of $>10^7$ cpm/μg. Hybridizations were performed in 7% sodium dodecyl sulfate, 250 mM sodium phosphate, pH 7.2, at 42 C for 18 h followed by three 5-min washes at 42 C with 6× SSC (1× SSC = 150 mM sodium chloride, 15 mM sodium citrate, pH 7.2) plus 3 mM sodium phosphate, pH 7.2. Wet membranes were wrapped in plastic wrap and exposed to X-ray film with an intensifying screen for 1–2 days at −85 C.

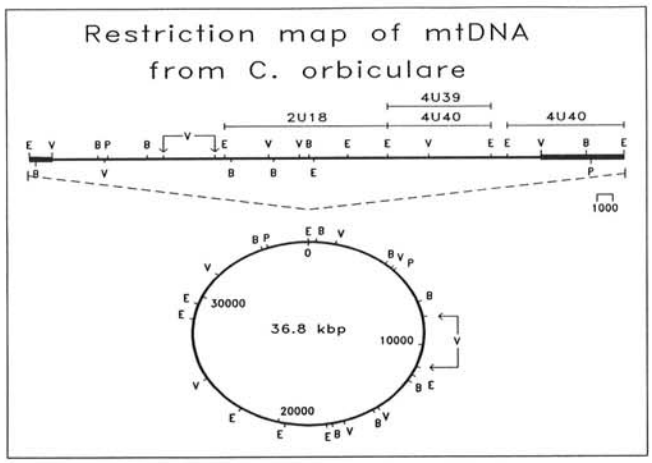

Fig 1. Map of selected restriction endonuclease cleavage sites in the mitochondrial DNA (mtDNA) of isolate JC1 of *Colletotrichum orbiculare*. This mtDNA is diagrammed as its native circular form (below) and as a linear form opened at one of the *Eco*RI cleavage sites (above). The positions of the mtDNA clones are indicated by the brackets above the linearized mtDNA. The heavier line indicates the region that contains an additional *Pvu*II cleavage site in cocklebur isolates. The *Pvu*II site could not be accurately mapped, and two of the possible positions are indicated by arrows. E = *Eco*RI (E); B = *Bam*HI; V = *Pvu*II; and P = *Pst*I.

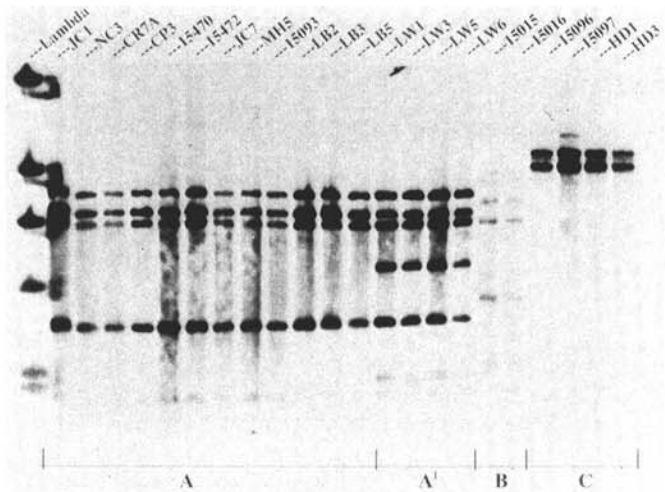

Fig 2. Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) patterns of *Colletotrichum orbiculare*. A Southern blot of total DNA was digested with *Pvu*II and probed with purified mtDNA from isolate JC1. The four different mtDNA RFLP phenotypes are indicated at the bottom. The first lane contains lambda DNA digested with *Hin*dIII as a size marker.

RAPD analysis. Thirty-two individual 10-nucleotide random primers (20 from Kit X, Operon Technologies Inc., Alameda, CA, and 12 from BioSynthesis, Inc., Lewisville, TX) were initially screened for their ability to amplify DNA of *C. orbiculare*. Five primers (OPX3, 5′-TGGGGCAGTG-3′; OPX11, 5′-GGAGCTCAG-3′; 923GT, 5′-GGTGGTTGGG-3′; 947GT, 5′-GGTTGGTGGG-3′; and 935GT, 5′-GGGTTGTGGG-3′) yielded reproducible fragment patterns of multiple distinct bands and were therefore used to examine the remaining isolates.

Reaction mixtures (40 μl) consisted of 1× *Taq* polymerase buffer (Promega Corp., Madison, WI); 1.5 mM MgCl; 200 mM each dATP, dCTP, dGTP, and dTTP; 30 pmoles primer; 25 ng of total genomic DNA (obtained from the "mini-prep" procedure); and 1 U *Taq* Polymerase (Promega Corp.). RAPD protocols were 85 C for 2 min, 5 cycles of 94 C for 25 s, 35 C for 25 s, 72 C for 2 min followed by 25 cycles of 94 C for 25 s, 45 C for 25 s, 72 C for 2 min, and final extension of 72 C for 3 min in a thermal cycler (Biotherm Corp., Fairfax, VA).

The RAPD products were resolved in 1.7% agarose gels in 1× TAE buffer (0.04 M Tris acetate, 1 mM EDTA, pH 7.5), stained with ethidium bromide, and photographed. Each RAPD comparison was repeated at least twice. RAPD haplotypes were identified visually as those isolates yielding identical or highly similar fragment patterns.

RESULTS

mtDNA haplotypes. Restriction digests of purified mtDNA of strain JC1 of *C. orbiculare* indicate that the mitochondrial genome size is approximately 36.8 kb (data not shown). Various enzyme-probe combinations were used to construct a mtDNA restriction map of strain JC1 (Fig. 1).

Four distinct mtDNA restriction fragment patterns (mtDNA RFLP haplotypes), designated A, A1, B, and C, were observed among all isolates with the restriction enzyme *Pvu*II (Fig. 2, Table 1). With six other enzymes (*Bam*HI, *Pal*I, *Eco*RI, *Bgl*II, *Hin*dIII, and *Xba*I), haplotypes A and A1 could not be distinguished. Thus, only three mtDNA RFLP haplotypes (A, B, and C) were distinguished.

All isolates of *C. orbiculare* that were pathogenic on a set of cucurbit differentials (34) had an identical mtDNA RFLP haplotype (haplotype A) with each of the seven enzymes examined (Table 1). These isolates were from diverse cucurbit hosts (cucumber, watermelon, cantaloupe, and cucuzzi gourd) from throughout the United States, Africa, and Australia. In addition, the isolates belonged to one of three different VCGs (VCGs 1, 2, and 3) and represented two distinct races (races 1 and 2) (34).

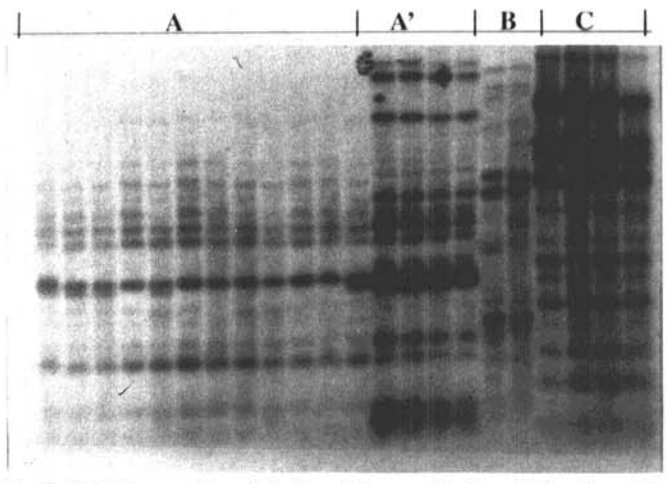

Fig 3. DNA fingerprints of *Colletotrichum orbiculare*. A Southern blot of total DNA was digested with *Pvu*II and probed with oligonucleotide $(CAT)_5$. The four different mitochondrial DNA restriction fragment length polymorphism haplotypes are indicated at the top (A′ = A1). The isolates (starting in lane 1) are JC1, NC3, CR7A, CP3, 15470, 15472, JC7, MH5, 15093, LB2, LB3, LB5, LW1, LW3, LW5, LW6, 15015, 15016, 15096, 15097, HD1, and HD3.

The cocklebur isolates were identical to mtDNA haplotype A isolates with six of the seven restriction enzymes (Table 1). However, a single additional restriction site was detected in the cocklebur isolates with *Pvu*II, where one of two 7-kb fragments was cut into a 5- and a 2-kb fragment (Figs. 1 and 2), thus distinguishing haplotypes A and A1.

mtDNA RFLP haplotype B was composed of three isolates. The two isolates from the American Type Culture Collection (ATCC), ATCC 15015 and ATCC 15016, were collected before 1958 and described as *C. magna* (=*G. magna*) (18,20). Isolate AK2 was collected from a squash fruit in South Carolina in 1991. In general, the two mtDNA clones, 2u18 and 4u40, did not hybridize to the group B isolates as well as they did to the other isolates.

mtDNA RFLP haplotype C was composed of two older ATCC isolates, ATCC 15096 and ATCC 15097, and two isolates (HD1 and HD3) collected from honeydew fruit in Oklahoma in 1991. The two ATCC cultures were reported to be teleomorphic isolates of the anthracnose pathogen, *G. c. orbiculare* (19).

DNA fingerprints. The two DNA fingerprint probes readily resolved the four mtDNA RFLP haplotypes (Figs. 3 and 4). The oligonucleotide $(CAT)_5$ probe and the HVR 33.6 probe detected minor polymorphisms among isolates within mtDNA RFLP haplotype B (Figs. 3 and 4).

DNA fingerprints for the $(CAT)_5$ probe were compared for percentage of similarity (25) on the basis of the presence or absence of 13–20 bands (Figs. 3 and 4). Isolates within mitochondrial haplotypes A, A1, or C were identical (i.e., 100% similarity); several polymorphisms were detected among isolates in haplotype B (82–100% similarity) (Table 2). Haplotypes A and A1 had similar $(CAT)_5$ fingerprints (68–72%), whereas all other haplotypes had very dissimilar fingerprints.

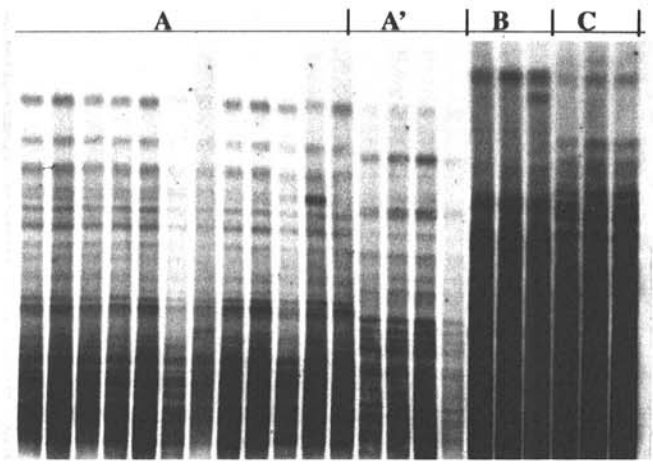

Fig 4. DNA fingerprints of *Colletotrichum orbiculare*. A Southern blot of total DNA was digested with *Eco*RI and probed with oligonucleotide HVR 33.6. The four different mitochondrial DNA restriction fragment length polymorphism haplotypes are indicated at the top (A′ = A1). The isolates (starting in lane 1) are JC1, NC3, CR7A, LB5, CP3, 15470, 15472, JC7, LB2, MHJ, 15093, 15098, LW1, LW3, LW5, LW6, 15015, 15016, AK2, 15096, HD1, and HD3.

TABLE 2. Similarity (%) of $(CAT)_5$ fingerprints among mitochondrial DNA haplotypes of *Colletotrichum orbiculare*[a]

Haplotype	A	A1	B	C
A	100	68–72	8	36–44
A1		100	6–18	42
B			82–100	14–22
C				100

[a] Calculated by Nei and Li's formula (25): $S_{xy} = 2n_{xy}/(n_x + n_y)$, where n_{xy} is the number of shared fragments, and n_x and n_y are the numbers of fragments in isolates x and y, respectively. Isolates of each mitochondrial DNA haplotype examined are listed in Figure 3.

RAPD haplotypes. All five RAPD primers revealed distinct patterns for each of the four mtDNA RFLP haplotypes. With three of the primers, OPX3, OPX11, and 923GT, the RAPD pattern was very similar among the isolates within a mtDNA RFLP haplotype (Fig. 5, only 923GT shown). However, with primer 947GT, minor polymorphisms were observed among the isolates in mtDNA RFLP haplotype A (Fig. 6). With primer 935GT, distinct polymorphisms were observed in groups A and B. Within mtDNA RFLP haplotype A, two distinct RAPD patterns were evident (Fig. 7). One pattern was associated with VCG1 isolates and the second pattern with VCG2 isolates. VCG1 isolates had a distinct fragment of approximately 1.0 kb that was absent from the VCG2 isolates, and VCG2 isolates had a fragment of approximately 1.8 kb that was absent from the VCG1 isolates. The RAPD pattern of VCG3 isolates was similar to that of the VCG1 isolates (Fig. 7).

DISCUSSION

RFLP analysis of mtDNA revealed four distinct haplotypes among the isolates examined (A, A1, B, and C). All isolates of *C. orbiculare* pathogenic on cucurbit hosts had an identical mtDNA RFLP haplotype (haplotype A). These isolates, from

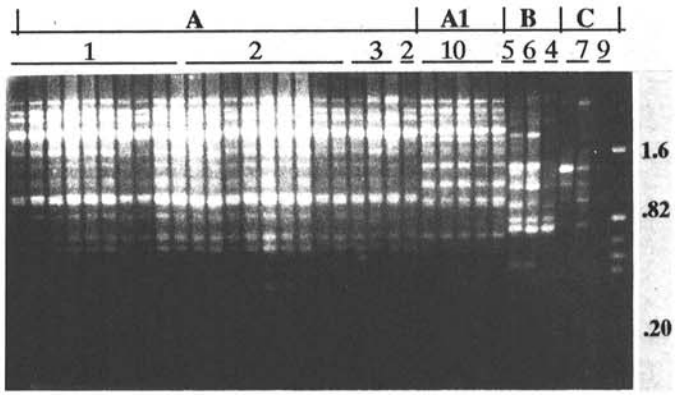

Fig 5. Randomly amplified polymorphic DNA patterns of *Colletotrichum orbiculare* with primer 923GT. The four different mitochondrial DNA restriction fragment length polymorphism haplotypes are indicated at the top. Vegetative compatibility groups are delineated numerically. Numbers on the right are size markers in kilobases. The isolates (starting from the left) are BB11, BB12, CR7A, JC1, JC4, LB1, LB4, LB6, MH3, NC3, AK1, BB15, CP3, CP6, JC7, JC9, LB2, 15094, 14371, 15472, MH5, 15093, 15095, 15098, LW3, LW4, LW5, LW6, LW7, 15015, 15016, AK2, 15096, 15097, and HD1.

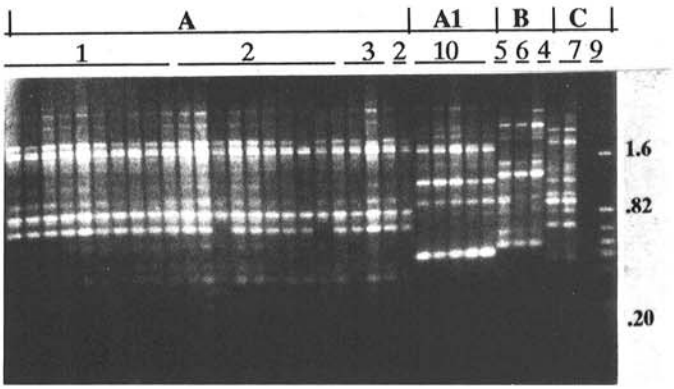

Fig 6. Randomly amplified polymorphic DNA patterns of *Colletotrichum orbiculare* with primer 947GT. The four different mitochondrial DNA restriction fragment length polymorphism haplotypes are indicated at the top. Vegetative compatibility groups are delineated numerically. Numbers on the right are size markers in kilobases. The isolates (starting from the left) are BB11, BB12, CR7A, JC1, LB1, LB5, LB6, MH3, NC3, AK1, BB15, CP3, CP6, JC9, LB2, 15094, 15470, 15471, 15472, MH5, 15093, 15095, 15098, LW3, LW4, LW5, LW6, LWY, 15015, 15016, AK2, 15096, 15097, and HD3.

three different VCGs, originated from numerous cucurbit hosts from throughout the United States, Africa, and Australia, indicating that the global cucurbit anthracnose pathogen population may be homogeneous with respect to mtDNA RFLPs.

Three mtDNA RFLP haplotypes were detected among the isolates that were nonpathogenic on cucurbits. Cocklebur isolates apparently are closely related to the cucurbit pathogen isolates on the basis of mtDNA RFLP and DNA fingerprint similarities. No differences were detected in the mtDNA between the cucurbit pathogen isolates and the cocklebur isolates with six of the restriction enzymes examined. A seventh enzyme, *Pvu*II, detected a single additional restriction site in the mtDNA of the cocklebur isolates. The similarity between these nonpathogenic isolates from a weed host and the cucurbit pathogens would indicate a close relationship between these two populations. However, one can only speculate whether the nonpathogenic cocklebur isolates gave rise to the cucurbit pathogens or whether the cucurbit pathogens gave rise to a host-specialized population on cocklebur.

Several additional isolates, also nonpathogenic to cucurbits, had two additional unique mtDNA RFLP haplotypes. Isolates ATCC 15015 and ATCC 15016, described as *C. magna* (18,19), originally were reported as pathogens of cucurbits but were avirulent or only weakly virulent under our inoculation conditions (34). In contrast, Freeman and Rodriguez (11) reported that isolates of *C. magna* were virulent on susceptible cucurbits, although they did not compare these isolates to the apparently more common anthracnose pathogen *C. orbiculare*. The two older isolates, as well as a more recently collected isolate (AK2) from a squash fruit, had the same mtDNA RFLP haplotype (haplotype B). The two older isolates and the more recently collected isolate were avirulent or only very weakly virulent on cucurbits (34).

Isolates ATCC 15096 and ATCC 15097, reported as teleomorphic isolates of *C. orbiculare* (*G. c. orbiculare*), and two more recently collected isolates from honeydew fruit had the same mtDNA RFLP haplotype (haplotype C). One of these nonpathogenic isolates, HD3, was observed to be homothallic, producing perithecia and ascospores (J. C. Correll, *unpublished*). Thus, the association between pathogenic isolates of *C. orbiculare* and the purported teleomorph, *G. c. orbiculare*, should be re-examined (18,20,30,35).

Isolates with mtDNA RFLP haplotypes B and C were collected from cucurbit fruit and represent populations distinct from the more common anthracnose pathogen population (haplotype A). mtDNA RFLP haplotype A was recovered from numerous cucurbit hosts from throughout the United States, Africa, and Australia and was the only group virulent on cucurbits in greenhouse inoculation tests (34). It is possible that isolates in haplotypes B and C represent genetically distinct populations that are avirulent or only weakly virulent on cucurbit foliage and may be more commonly associated with fruit rots of cucurbits.

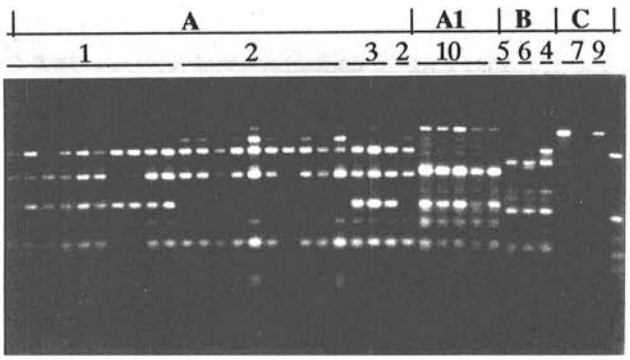

Fig 7. Randomly amplified polymorphic DNA patterns of *Colletotrichum orbiculare* with primer 935GT. The four different mitochondrial DNA restriction fragment length polymorphism haplotypes are indicated at the top. Vegetative compatibility groups are delineated numerically. Numbers on the right are size markers in kilobases. The isolates (starting from the left) are BB11, BB12, BB13, CR7A, JC1, LB1, LB4, LB5, MH3, NC3, AK1, BB15, CP3, JC7, JC9, LB2, LB3, 15094, 15471, 15472, MH5, 15093, 15095, 15098, LW3, LW4, LW5, LW6, LWY, 15015, 15016, AK2, 15096, 15097, and HD1.

The genomic markers, such as the two DNA fingerprint probes, indicate that there is limited nuclear genetic diversity among isolates within the mtDNA RFLP haplotypes found. These data, along with the very low VCG diversity observed among pathogenic isolates (34), would indicate that the cucurbit anthracnose pathogen is probably reproducing asexually in nature and that the population is somewhat fixed. In contrast, nuclear DNA polymorphisms were observed among the three isolates of *C. magna* (haplotype B); these isolates are capable of sexual reproduction (20).

RAPDs have been used with increasing frequency to examine fungal plant pathogens (2,9,12–14). They often are quick and inexpensive in comparison to techniques that require the processing of Southern hybridizations. In our study, the RAPD groups observed closely correspond to the mtDNA RFLP haplotypes as well as to the DNA fingerprint groups. Thus, RAPD analysis may provide a relatively quick and easy method for the analyses of *Colletotrichum* isolates from cucurbits. Furthermore, one RAPD primer, 935GT, differentiated two races of the pathogenic isolates within mtDNA haplotype A. This primer produces a 1.0-kb fragment that was present for all race 1 (VCGs 1 and 3) isolates but was absent from all race 2 (VCG 2) isolates examined. Thus, this race-specific primer could be useful for epidemiological studies of the *C. orbiculare* population on cucurbits.

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