Reexamination of Races of the Cucurbit Anthracnose Pathogen *Colletotrichum orbiculare*

L. A. Wasilwa, J. C. Correll, T. E. Morelock, and R. E. McNew

First and second authors, graduate research assistant and associate professor, respectively, Department of Plant Pathology; third author, professor, Department of Horticulture and Forestry; and fourth author, professor and department head, Department of Agricultural Statistics, University of Arkansas, Fayetteville 72701.

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Corresponding author: J. C. Correll.

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ABSTRACT

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Seven physiological races of the cucurbit anthracnose pathogen *Colletotrichum orbiculare* have been previously described on the basis of disease reactions on 12 differential cucurbit hosts. In this study, 89 isolates of *C. orbiculare* (35 from cucumber, 33 from watermelon, two from cantaloupe, four from cucuzzi gourd, two from honeydew, 10 from cocklebur, and three from unknown cucurbit hosts) and three isolates of *C. magna* (two from watermelon and one from acorn squash) were examined for vegetative compatibility with the use of nitrate nonutilizing mutants. The collection included a representative culture of each of the seven previously described races obtained from the American Type Culture Collection. Twenty-eight isolates, selected to represent all vegetative compatibility groups (VCGs) and several previously identified races, were examined in greenhouse cotyledon inoculation assays on 13 cucurbit hosts. Ten VCGs were identified among all isolates examined; however, only isolates in three VCGs (VCGs 1, 2, and 3) were pathogenic on the cucurbit differentials in greenhouse virulence tests. VCG 1 was composed of 28 cucumber and two cantaloupe isolates, and VCG 2 was composed of 33 watermelon, two cucuzzi gourd isolates, and a single isolate with an unknown host origin. Seven older cucumber isolates, collected prior to 1986, belonged to a third VCG (VCG 3). Of the three VCGs pathogenic on the cucurbit differentials, two virulence phenotypes could be identified. Isolates in VCG 1 gave disease reactions similar to isolates previously described as race 1, whereas isolates in VCG 2 gave disease reactions similar to isolates previously described as race 2. The cucumber isolates in VCG 3 also gave disease reactions typical of race 1-type disease reactions. The *C. orbiculare* population pathogenic on cucurbits in the United States appears to have a limited VCG diversity. Within this population, there was a distinct correspondence between host origin, VCG, and virulence (race) phenotype.

Colletotrichum orbiculare (Berk. & Mont.) Arx (=*C. lagenarium* (Pass.) Ellis & Halst.) is a widespread pathogen of cucurbits that causes anthracnose of cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*), muskmelon (*Cucumis melo* L.), squash (*Cucurbita* spp.), gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita pepo* L.), cantaloupe, honeydew (*Cucumis melo* L.), and *Luffa* spp. (5,7,22,34). *C. orbiculare* has also been reported on several noncucurbit hosts (36,49). In commercial production, *C. orbiculare* is most destructive on cucumber (3,24,48), watermelon (6,24), and cantaloupe (2,34). Squash and pumpkin cultivars apparently are highly resistant to anthracnose (3,22,44). Anthracnose is relatively common in warm, humid regions worldwide, and in the United States it is particularly destructive in the south, southeast, northeast, and midwest regions where field losses of up to 60% have been reported (48).

The race classification system of *C. orbiculare* includes differential host cultivars from the genera *Cucumis* (cucumber and cantaloupe), *Citrullus* (watermelon), and *Cucurbita* (squash). Seven races of *C. orbiculare* have been described on the basis of disease reactions on these differential hosts. Goode (23,24) initially described races 1, 2, and 3. Race 1 isolates were considered virulent on all cucumber cultivars tested (Model, Palmetto, and PI 163213), weakly virulent on the watermelon cultivars Charleston Grey, Congo, and Fairfax, and moderately virulent on the squash cultivar Butternut. In contrast, race 2 isolates were virulent on all watermelon and cucumber cultivars tested and moderately virulent on the squash cultivar Butternut. Race 3 was identical to race 1 except that Butternut squash was immune. Using additional differentials, Dutta et al (17) and Jenkins et al (30) described races 4, 5, 6, and 7. A summary of the disease reactions on the differential hosts for the described races is included (Table 1).

The virulence of *C. orbiculare* and disease reactions on differential hosts is influenced by host age, inoculum concentration and viability (23,35), nutritional status of the host (18,50,51), temperature and relative humidity (18), and time and method of inoculation (35). Consequently, many inconsistencies have been observed in both greenhouse and field virulence tests with *C. orbiculare*. For example, Barnes (6) recovered six isolates of *C. orbiculare* and reported that all six had different disease reactions on the set of differential hosts than did the seven previously reported races. We have observed that the time of year can also greatly influence the overall disease ratings (50,51). The use of fertilizer on curcurbit seedlings in winter months (even with greenhouse temperatures similar to summer months) dramatically increases disease severity and obscures differences in disease reactions on susceptible and resistant cultivars (50,51).

In addition to virulence, vegetative (heterokaryon) compatibility has been used to characterize genetic diversity in many plant-pathogenic fungi including species of *Fusarium* (10,19,33, 42,43), *Verticillium* (11,31,39,41), and others (1,4,8,38,54). Recently, vegetative compatibility has been studied in several species of *Colletotrichum* (9,13,16).

The objective of the present study was to reevaluate race diversity within the cucurbit anthracnose pathogen population. Isolates of *C. orbiculare* from throughout the United States were characterized for vegetative compatibility and virulence on 13 cucurbit hosts in greenhouse pathogenicity tests and were compared to a representative isolate of each of the seven previously described races.

MATERIALS AND METHODS

Isolates. The 92 isolates used in this study were recovered from symptomatic tissue by the authors, obtained from other researchers, or obtained from the American Type Culture Collec-

tion (ATCC, Rockville, MD) (Table 2). The majority of the isolates were collected between 1984 and 1992.

All isolates (except those from ATCC) were single-spored and grown on green bean agar (GBA). To prepare GBA, 20 g of agar and two jars (226 g) of green bean baby food (Gerber Production Co., Fort Smith, AR) were added to 1 L of double deionized water. Isolates were transferred to GBA, which was acidified by the addition of 3 ml of 80% lactic acid to reduce bacterial contamination. All cultures were stored on desiccated filter paper at 4 C (14).

Generation of mutants. Nitrate nonutilizing (*nit*) mutants were produced and characterized as previously described by Puhalla (40) and Correll et al (12). Briefly, isolates were transferred to minimal medium containing 1.5% potassium chlorate ($KCLO_3$)

TABLE 1. Results of previously reported pathogenicity tests that characterized races of *Colletotrichum orbiculare* using differential cucurbit cultivars and accessions

Differential host	Disease reaction[z]								
Cultivar	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	*C. magna*	Reference
Cucumber									
Fletcher	S	R	M						18
Long Marketer	S	M	S						18
Marketer	S	M	M	R					18
	S	S	S	S	R	R	S		30
	S	S	S					R	29
Model	S	M	S	M					18
	S	S	S						24
	S	S	S	S	R	R	S		30
	S	S	S					R	29
Palmetto	S	S	S						24
	S	S	S					R	29
Palomar	S	M	S						18
Pixie	S	R	S	S	R	R	R		30
Niagara	S	R	S						18
PI 163213	S	R	R	R					18
	S	S	R						24
	S	S	R	S	R	M	R		30
	S	S	S					R	29
PI 175111	R	R	R						18
PI 197087	M	R	R						18
Watermelon									
Black Diamond	S	S	S	S					18
	S	S	S						53
Charleston Grey	R	S	R	R					18
	R	S	R						24
	R	S	R	S	S	S	S		30
	R	S	R						53
	R	S	R					S	29
Chris Cross	S	S	M	M					18
	S	S	S	S	S	S	S		30
Congo	R		R	R					18
	R	S	R						24
	R	S	R					M	29
Fairfax	R	S	R						18
	R	S	R						24
	R	S	R						53
	R	S	R					S	29
Garrison	S	S	S						24
	S	S	S	S	S	S	S		30
	S	S	S						53
	S	S	S					M	29
Hope Diamond	R	S	R						18
New Hampshire Midget	S	S	S						18
	S	S	S						24
	S	S	S	S	S	S	S		30
	S	S	S						53
	S	S	S					S	29
Sugar Baby	S	S	S						53
	S	S							23
Squash									
Butternut	R	R	R						18
	M/R	S	R						24
	M	M	R	S	R	R	R		30
	M	S	R					S	29
Seneca Prolific Hybrid	R	S	M	S	R	S	M		30
Cantaloupe									
Edisto	S	S	S	S	S	R	S		30
Rio Gold	S	S	S	S	S	R	S		30
Muskmelon									
H.B. 36	S	S	S						18

[z]Disease severity was measured with a disease rating scale of 1–5 where 1 = no disease; 2 = slight flecking or small lesions on leaves; 3 = distinct leaf lesions or restricted stem lesions; 4 = many large leaf lesions and deep stem lesions; 5 = dead plant. R = Resistant (≤2.5); M = moderately resistant (2.6–3.5); S = susceptible (≥3.5) (24).

(MMC), and chlorate-resistant sectors were recovered after 10–21 days. Sectors were put on basal agar medium supplemented with sodium nitrate as the nitrogen source (MM). *Nit* mutant colonies grew as thin expansive colonies on MM.

Nit mutants were assigned to two phenotypic classes, *nit*1 and NitM, according to their growth on a basal medium supplemented with either nitrate, ammonium, or hypoxanthine as the nitrogen source (12). Complementing *nit* mutants were identified from selected isolates and used as testers for the vegetative compatibility tests. At least two complementing *nit* mutant testers were used per vegetative compatibility group (VCG) in subsequent tests.

Vegetative compatibility tests. Protocols described by Puhalla (40) and Correll et al (12) for *Fusarium oxysporum* were followed to determine the vegetative compatibility of isolates. Phenotypically distinct *nit* mutants (*nit*1 and NitM mutants) from all appropriate isolates were paired in all possible combinations. Mycelial plugs of two *nit* mutants were placed 2 cm apart on the surface of MM and incubated at room temperature. The formation of dense aerial wild-type mycelium where the *nit* mutants came in contact indicated that the isolates were vegetatively compatible. However, heterokaryons formed in complementation tests were quite variable. Some isolates formed strong, robust heterokaryons (>1.5 cm wide) in 7–10 days, whereas others formed weak, narrow (<1.0 cm wide) or discontinuous (tufts) heterokaryons after 10 days. Pairings were scored for complementation for up to 28 days after pairing.

Pathogenicity tests. Twenty-eight isolates were examined for virulence in greenhouse pathogenicity tests (Table 2). The isolates used in the pathogenicity tests were selected on the basis of VCG classification, host and geographic origin, and previously reported race classification. The 13 cucurbit hosts used included the cucumber cultivars Arkansas Little Leaf (H19) (Petoseed), Wisconsin SMR-58 (Asgrow), Pixie and Marketer (Hollar), Poinsett 76 (Harris Moran), GY14 (USDA, University of Wisconsin), and an accession PI 197087 from India; the watermelon cultivars included Charleston Grey, Crimson Sweet, Sugar Baby (Petoseed), and Black Diamond (synonymous with Florida Giant) (Asgrow). The cantaloupe cultivar Edisto (Asgrow) and the squash cultivar Butternut Waltham (Northrup King) were also included. The differential hosts Pixie, Marketer, PI 197087, Charleston

TABLE 2. Vegetative compatibility group (VCG), previously reported race identification, and host and geographic origin of isolates of *Colletotrichum orbiculare*

VCG	Previous race[v]	Isolate[w]	Origin		Year collected[x]
			Host	Location	
1		BB11	Cucumber (leaf)	Lane, Oklahoma	1991
		BB12	Cucumber (leaf)	Lane, Oklahoma	1991
		BB13	Cucumber (fruit)	Lane, Oklahoma	1991
		CP10	Cucumber	Oklahoma	1991
		CP11	Cucumber	Oklahoma	1991
		CP12	Cucumber	Oklahoma	1991
	*	CR7A	Cucumber (leaf)	Sumter Co., Florida	...[y]
		CR7B	Cucumber (leaf)	Sumter Co., Florida	...
		CT1	Cucumber	...	(1990)
		GW-409	Cucumber	...	(1985)
		GW-683	Cucumber	...	(1986)
	*	JC1	Cucumber (leaf)	Kibler, Arkansas	1990
		JC2	Cucumber (leaf)	Kibler, Arkansas	1990
		JC3	Cucumber (leaf)	Kibler, Arkansas	1990
		JC4	Cucumber (leaf)	Kibler, Arkansas	1990
		JC5	Cucumber (leaf)	Kibler, Arkansas	1990
		JD1	...	Wisconsin	(1991)
		JH1	Cucumber	...	(1991)
		LB1	Cucumber	Independence, Louisiana	1991
	*	LB4	Cataloupe	Angola, Louisiana	1991
		LB5	Cantaloupe	Angola, Louisiana	1991
		LB6	Cucumber	Crowley, Louisiana	1991
		MH1	Cucumber	Madison, Wisconsin	1979
	*	MH3	Cucumber	Hancock, Wisconsin	1982
		MH6	Cucumber	Madison, Wisconsin	1987
		NC2	Cucumber (leaf)	North Carolina	1991
	*	NC3	Cucumber (leaf)	North Carolina	1991
		NC4	Cucumber (leaf)	North Carolina	1991
		YB1	Cucumber	Tupelo, Arkansas	1991
		YB2	Cucumber	Tupelo, Arkansas	1991
		YB3	Cucumber	Tupelo, Arkansas	1991
2		AK1	Watermelon (fruit)	South Carolina	1991
		BB14	Watermelon (fruit)	Lane, Oklahoma	1991
		BB15	Watermelon (fruit)	Lane, Oklahoma	1991
		BB16	Watermelon (fruit)	Lane, Oklahoma	1991
		BH1	Watermelon	Oklahoma	1990
		BH2	Watermelon	Oklahoma	1990
		BH3	Watermelon	Oklahoma	1990
		CP1	Watermelon	Oklahoma	1990
		CP2	Watermelon	Oklahoma	1990
	*	CP3	Watermelon	Oklahoma	1990
		CP4	Watermelon	Oklahoma	1990
		CP5	Watermelon	Oklahoma	1990

(continued on next page)

[v] Previously identified races (18,24,30,53). Isolates with an asterisk were used in the greenhouse pathogenicity tests.
[w] ATCC = American Type Culture Collection.
[x] Year isolate was recovered. Parentheses indicate the year the culture was received, and the year of isolation is not known.
[y] Information not available.
[z] Isolate of *C. magna*.

Grey, Sugar Baby, Black Diamond, Edisto, and Butternut had previously been used to characterize races of *C. orbiculare* (Table 1).

Seeds were germinated on moist filter paper in petri plates in the dark at 28 C. Germinated seeds were planted in square pots (10 × 10 cm) in a 1:1 peat-perlite soil-less mix (Sunshine Mix #1). The plants were inoculated approximately 4 days after emergence or when the cotyledons on all cultivars were fully expanded.

Inoculation procedure. To produce conidial inoculum of *C. orbiculare,* cultures were grown on GBA for 7–10 days at room temperature (approximately 23 C) under 12 h of light per day. Conidia were washed off the agar surface, suspended in cold deionized water, and adjusted to a spore concentration of 8×10^4 spores per milliliter with a hemacytometer. Cotyledons were sprayed until run-off with a spray gun (Beseler-Dust Gun 22). After inoculation, plants were incubated in a dew chamber that could hold approximately 500 pots for 24 h at 21–25 C and were maintained at 100% relative humidity. A hygrothermograph was placed in the dew chamber to monitor temperature and relative humidity. Because of the number of isolates being examined, inoculations were done on two consecutive days. After inoculation, the plants were returned to the greenhouse, where temperatures ranged between 22 and 37 C, and observed daily for disease development.

Disease ratings. Cotyledons were scored for disease severity on a scale of 0–7. Disease severity ratings were given by visual assessment of the area of the cotyledon that showed symptoms (chlorosis and necrosis) of infection where 0 = no infection; 1 = 1–10% of cotyledon area with visible symptoms; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–90%; 6 = >90%; and 7 = complete collapse of the cotyledon (Fig. 1). Disease severity was rated daily from the onset of visible symptoms until 8 days after inoculation. The disease severity ratings collected 6 days after inoculation were used in the statistical analysis since there was no change in symptom development after this time.

Experimental design. All inoculation experiments were arranged in a completely randomized design with three replicates (pots) and three plants per pot. Disease ratings per replicate were averaged from six cotyledons. Each inoculation experiment was

TABLE 2. (*continued from preceding page*)

VCG	Previous race[v]	Isolate[w]	Origin		Year collected[x]
			Host	Location	
	*	CP6	Watermelon	Texas	1990
		CP7	Watermelon	Texas	1990
		CP8	Watermelon	Texas	1990
		CP9	Watermelon	Texas	1990
		CR1	Watermelon (fruit)	Leesburg, Florida	1978
		CR2	Watermelon (fruit)	Leesburg, Florida	1989
		CR3	Watermelon (fruit)	Leesburg, Florida	1989
		CR4	Watermelon (stem)	Sumter Co., Florida	1964
		CR5	Watermelon (fruit)	South Lake Co., Florida	1985
		JC6	Watermelon (fruit)	Kibler, Arkansas	1990
	*	JC7	Watermelon (fruit)	Kibler, Arkansas	1990
		JC8	Watermelon (fruit)	Kibler, Arkansas	1990
		JC9	Watermelon (fruit)	Kibler, Arkansas	1990
	*	JC10	Watermelon (fruit)	Kibler, Arkansas	1990
	1	JD2	Watermelon	Florida	(1991)
	2	JD3	Watermelon	Florida	(1991)
	*	LB2	Cucuzzi gourd	Ruston, Louisiana	(1991)
		LB3	Cucuzzi gourd	Ruston, Louisiana	(1991)
		PP2	Watermelon (leaf)	Kibler, Arkansas	1991
	2/*	ATCC15094	Watermelon	North Carolina	1958
	4/*	ATCC15098	Unknown	Manhattan, Kansas	1960
	5	ATCC15470	Watermelon	Africa	1964
	6/*	ATCC15471	Watermelon	Florida	1964
	7	ATCC15472	Watermelon	Oklahoma	1964
3	*	MH2	Cucumber	North Carolina	1958
		MH4	Cucumber (leaf)	Madison, Wisconsin	1985
	*	MH5	Cucumber (fruit)	Hancock, Wisconsin	1985
	1/*	RH1	Cucumber	...	(1990)
	1/*	ATCC15093	Cucumber	North Carolina	1953
	3/*	ATCC15095	Cucumber	North Carolina	1958
	1/*	ATCC16974	Cucumber	North Carolina	...
4	*	AK2[z]	Acorn squash (fruit)	Charleston Co., South Carolina	1991
5	*	ATCC15015[z]	Watermelon	North Carolina	1962
6	*	ATCC15016[z]	Watermelon	North Carolina	1962
7	1/*	ATCC15096	Cucuzzi gourd	...	1961
	2/*	ATCC15097	Cucuzzi gourd	...	1961
8		KD1	Unknown	Beltsville, Maryland	(1961)
9	*	HD1	Honeydew (fruit)	Lane, Oklahoma	1990
	*	HD3	Honeydew (fruit)	Lane, Oklahoma	1990
10	*	LW1	Cocklebur (stem)	Coolah, Australia	1984
		LW2	Cocklebur (stem)	Merriwa, Australia	1984
		LW3	Cocklebur (stem)	Sconc, Australia	1984
		LW4	Cocklebur (stem)	Merriwa, Australia	1984
		LW5	Cocklebur (stem)	Coolah, Australia	1984
	*	LW6	Cocklebur (stem)	Pawnee Hills, Australia	1984
		LW7	Cocklebur (stem)	Coolah, Australia	1984
		LW8	Cocklebur (stem)	Inverell, Australia	1984
		LW10	Cocklebur (stem)	Manilla, Australia	1984
		LW11	Cocklebur (stem)	Manilla, Australia	1984

repeated five times. Three tests were grouped as summer inoculation tests in May, June, or July of 1991, and two tests were grouped as winter inoculations tests in December 1991. The summer and winter tests differed in that the plants were watered daily with a fertilizer solution (200 ppm Peters N-P-K, 20-20-20) in the tests conducted in the summer, and tap water was used for tests conducted in the winter.

Statistical analysis. Because of the use of fertilizer in the summer inoculation tests, the summer and winter tests were treated as separate experiments. For the summer tests, the disease severity ratings from three independent inoculation experiments were averaged. Each independent inoculation experiment was treated as a replicate (block), and a *t* test (Statistical Analysis System, SAS Institute Inc., Cary, NC) was used for mean separation. Thus, the data were analyzed as a randomized complete block by analysis of variance with each experiment used as a block and the replicate × treatment used as the error term.

RESULTS

***Nit* mutant recovery.** *Nit* mutants were readily recovered from all isolates of *C. orbiculare* after 7–28 days. Approximately 80% of the transfers made to MMC yielded a *nit* mutant sector for most isolates. Approximately 5% of the *nit* mutants recovered from a given isolate were NitMs.

Vegetative compatibility tests. Ten VCGs were identified among the 92 isolates examined (Table 2). VCG 1 contained 29 isolates from cucumber, two from cantaloupe, and one from an unknown cucurbit host. VCG 2 contained 33 isolates from watermelon, two from cucuzzi gourd, and one with an unknown host origin. Six of the watermelon isolates had previously been characterized as race 1, 2, 4, 5, 6, or 7. VCG 3 contained seven isolates from cucumber. Three of the isolates in VCG 3 were previously characterized as race 1 and one isolate as race 3.

Twenty-seven of 31 cucumber and cantaloupe isolates in VCG 1 represented isolates collected after 1985 (Table 2). Of the seven cucumber isolates in VCG 3, four were collected in the 1950s. Twenty-eight of 33 watermelon and cucuzzi gourd isolates in VCG 2 were collected after 1986. Six isolates in VCG 2, including the five ATCC isolates, were collected prior to 1965.

Two watermelon isolates (ATCC15015 and ATCC15016) and an acorn squash isolate (AK2) of *Glomerella magna* (anamorph *C. magna*) each belonged to a unique VCG (designated VCGs 4, 5, and 6) (Table 2). Two cucuzzi gourd isolates (ATCC15096 and ATCC15097) collected prior to 1961 belonged to a single VCG (designated VCG 7). These two cucuzzi gourd isolates were previously described as races 1 and 2, respectively. A single isolate (KD1) from a unknown cucurbit host belonged to VCG 8, and two honeydew isolates (HD1 and HD3) belonged to VCG 9. All of the cocklebur isolates from diverse locations throughout New

Fig. 1. Disease rating scale on cucumber cotyledons. 0 = Healthy, no infection; 1 = 1–10% of the cotyledon area with visible symptoms; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–90%; 6 = >90%; and 7 = complete collapse of cotyledon.

South Wales, Australia, belong to a single VCG (designated VCG 10) (Table 2).

Although 67 isolates in VCG 1 and VCG 2 were classified into discrete VCGs, 25 of these isolates apparently represent "bridge type" isolates that were generally weakly vegetatively compatible across the two VCGs. That is, pairings between *nit* mutants of bridge isolates in VCG 1 and VCG 2 formed tufts or very narrow heterokaryons when paired and only after an extended period of time (21 days). Bridge isolates in VCG 1 included BB11, CP12, JC1, JC5, MH3, NC2, NC3, NC4, and YB2. *Nit* mutants of these isolates formed strong, robust heterokaryons only 7–10 days after pairing commenced with all the *nit* mutant tester strains in VCG 1. Bridge isolates in VCG 2 included BB14, CP1, CP2, CP3, CP4, CP5, CP6, CP7, CP8, CP9, CR3, JC7, JC10, JD2, LB2, and ATCC15471. As was the case with VCG 1 bridge isolates, robust heterokaryons were formed between *nit* mutants of these isolates and the *nit* mutant tester strains in VCG 2 after only 7–10 days. *Nit* mutants of an exceptional cucumber isolate, CP12 (from Oklahoma), initiated weak discontinuous heterokaryons 10 days after pairing with *nit* mutants of the watermelon bridge isolates (CP1, CP2, CP3, CP4, CP5, CP6, CP7, CP8, CP9, and JD2) but formed strong robust heterokaryons with all watermelon bridge isolates after 21 days. CP12 formed strong robust heterokaryons with all other VCG 1 isolates after only 7–10 days.

Five ATCC isolates (ATCC15094, ATCC15096, ATCC15470, ATCC15471, and ATCC15472) were vegetatively self-incompatible. That is, a *nit*1 and NitM from a given isolate showed no signs of complementation even after 28 days. However, these isolates could be assigned to VCGs because the *nit* mutants from these four isolates did strongly complement *nit* mutant testers from other isolates. One unusual isolate, LW2 from cocklebur, was vegetatively self-incompatible, and *nit* mutants of LW2 formed only tufts with the other cocklebur *nit* mutant testers.

Virulence tests. Disease symptoms typically first appeared on cotyledons 3 days after inoculation. On all cucumber and cantaloupe cultivars, anthracnose symptoms on cotyledons initially appeared as small, pale yellow, water-soaked, restricted lesions that were either chlorotic or necrotic and that progressed into large lesions that often coalesced and killed the cotyledons. On all watermelon cultivars, symptoms initially appeared as small water-soaked areas near veins or as isolated distinct lesions on the entire cotyledon that progressed into more severe lesions that would coalesce and kill the cotyledons. The color of the lesions on watermelon was somewhat dependent on the cultivar. For example, lesions on the watermelon cultivars Charleston Grey and Black Diamond were dark brown, and lesions on Sugar Baby and Crimson Sweet were black.

On the basis of host disease reactions, isolates could generally be characterized as either avirulent (mean disease reactions ≤0.5), or weakly (0.6–2.5), moderately (2.6–5.0), or highly virulent (5.1–7.0). Correspondingly, cucurbit hosts could be characterized as immune (no disease symptoms evident), highly resistant (mean disease reactions 0.6–2.5), moderately resistant (or moderately susceptible) (2.6–5.0), or highly susceptible (5.1–7.0).

All of the isolates in VCGs 1, 2, and 3 that were tested were virulent (mean disease rating >0.5) on most of the cucurbit hosts used in this study (Tables 3 and 4). All of the isolates in VCGs 7, 9, and 10 were avirulent (mean disease rating ≤0.5) in each of the five inoculation tests on all of the cucurbit tested (data not shown). Three isolates of *C. magna* in VCGs 4, 5, and 6 were avirulent on all cultivars examined except watermelon cultivar Black Diamond (data not shown). These isolates were weakly virulent (disease ratings ranged from 0.6 to 1.8) on cultivar Black Diamond after 6 days in several of the inoculation tests (data not shown). Virulence of an individual isolate in VCG 8 (from an unknown cucurbit host) was not determined due to its inability to sporulate.

Although a considerable amount of virulence diversity was observed among isolates of *C. orbiculare* in greenhouse pathogenicity tests (Tables 3 and 4), isolates within a VCG gave similar disease reactions on the various cucurbit hosts. In addition, different levels of host resistance were detected among both the cucumber and the watermelon cultivars tested (Tables 3 and 4). Overall, the isolates could be characterized into one of two distinct virulence phenotypes. For virulence phenotype one, all cucumber cultivars, the cantaloupe cultivar Edisto, and the watermelon cultivars Black Diamond, Sugar Baby, and Crimson Sweet were moderately to highly susceptible, whereas the watermelon cultivar Charleston Grey and squash cultivar Butternut Waltham were highly resistant. In contrast, for virulence phenotype two, all cucumber cultivars tested were moderately to highly resistant (with the exception of Marketer, which was highly susceptible), and all watermelon cultivars, including Charleston Grey, were highly susceptible.

With a few exceptions, all cucumber isolates in VCG 1 were highly virulent on all of the cucumber hosts in both the summer and winter inoculation tests. Marketer was the most susceptible cucumber to all isolates representing the three VCGs in both the summer and winter inoculation tests (Tables 3 and 4). Cucumber isolate JC1 was significantly less virulent on H19 and Poinsett-76 than were most other VCG 1 isolates in the summer inoculation test (Table 3). In the winter inoculation test, cucumber isolate CR7A was significantly less virulent on GY14 than were most other VCG 1 isolates (Table 4). Disease reactions of the cantaloupe isolate (LB4) in VCG 1 were similar to those of the

TABLE 3. Summer disease severity ratings of 15 isolates of *Colletotrichum orbiculare* on a set of differential cucurbit hosts

		Cucumber							Watermelon				Canta-loupe	Squash
VCG[y]	Isolate	H19	SMR-58	GY14	Poinsett-76	Marketer	Pixie	PI 197087	Black Diamond	Charleston Grey	Sugar Baby	Crimson Sweet	Edisto	Butternut Waltham
1	NC3	7.0 Aa[z]	6.9 Aa	7.0 Aa	7.0 Aa	7.0 Aa	6.9 Aa	7.0 Aa	4.8 ABb	1.5 BCc	7.0 Aa	6.3 Aab	6.1 Aab	1.3 ABCc
	MH3	6.8 Aa	6.9 Aa	6.7 ABa	6.8 Aa	7.0 Aa	7.0 Aa	6.8 Aa	4.9 ABab	1.1 BCd	6.9 Aa	2.0 Ccd	4.3 ABbc	0.8 BCd
	CR7A	6.5 ABab	6.9 Aa	5.9 ABCab	7.0 Aa	7.0 Aa	6.8 ABa	7.0 Aa	4.3 Bbc	0.8 BCd	6.5 Aab	2.7 BCcd	4.5 ABbc	0.6 BCd
	JC1	4.7 BCbc	5.6 ABabc	5.4 ABCDabc	4.1 Bc	6.9 Aa	6.1 ABCab	6.9 Aa	7.0 Aa	7.0 Aa	7.0 Aa	7.0 Aa	4.5 ABbc	1.1 ABCd
2	CP3	2.0 Dcd	3.6 Bbc	3.8 CDEb	2.0 BCcd	6.4 Aa	2.7 Ebc	2.9 BCDbc	6.8 Aa	6.6 Aa	7.0 Aa	7.0 Aa	2.4 BCbc	0.6 BCd
	JC10	1.3 Dde	3.7 Bbc	3.0 Ebcd	1.9 Ccde	6.6 Aa	2.9 DEcd	3.0 CDbcd	5.2 ABab	7.0 Aa	7.0 Aa	7.0 Aa	4.0 ABbc	0.2 Ce
	15471	2.5 Dc	4.4 Bb	4.0 CDEb	2.5 BCc	6.4 Aa	4.4 BCDEb	5.4 ABab	6.3 ABa	6.5 Aa	7.0 Aa	7.0 Aa	4.1 ABb	0.9 BCd
	15094	2.2 Df	4.6 ABde	4.4 BCDEde	3.8 BCe	6.9 Aa	5.5 ABCDbcd	5.4 ABbcd	6.7 Aab	6.8 Aab	7.0 Aa	7.0 Aa	5.3 Acde	1.4 ABCf
	JC7	2.5 Def	5.1 ABbcd	3.7 DEde	2.4 BCef	7.0 Aa	5.0 ABCDEbcd	5.3 ABabcd	6.5 ABab	6.2 Aabc	7.0 Aa	7.0 Aa	4.8 Acd	0.9 BCf
	CP6	1.1 Dcd	3.3 Bbc	2.8 Ec	1.4 Ccd	6.6 Aa	2.4 Ecd	1.6 Dcd	6.1 ABab	5.8 Aab	6.9 Aa	7.0 Aa	2.4 BCcd	0.2 BCd
	15098	2.5 Def	4.3 Bcd	3.1 Ecdef	1.9 Cf	6.8 Aab	3.9 CDEcde	5.1 ABCabc	6.4 Abab	2.6 Bdef	7.0 Aa	5.2 Ababc	4.9 Abc	1.6 ABCf
3	MH5	6.6 ABa	6.7 Aa	6.6 ABa	6.6Aa	7.0 Aa	6.6 ABab	6.9 Aa	5.5 ABab	2.4 Bc	7.0 Aa	6.3 Aab	5.0 Ab	2.1 Ac
	15093	6.7 ABa	6.9 Aa	6.0 ABab	6.3 Aab	6.9 Aa	6.9 Aa	6.1 Aab	5.8 ABab	1.6 BCd	6.8 Aa	2.3 Ccd	4.4 ABbc	1.2 ABCd
	15095	3.3 CDb	4.2 Bab	3.2 Eb	2.6 BCbc	6.1 Aa	3.8 CDEb	3.8 ABCDab	1.5 Ccd	0.0 Cd	3.6 Bbc	0.0 Cd	0.6 Cd	0.2 Cd
	16974	3.1 CDabc	3.8 Bab	3.3 Eabc	2.8 BCabc	4.8 Ba	3.2 DEabc	2.9 BCDabc	4.5 Ba	1.9 BCbc	4.1 Bab	0.8 Cbc	2.3 BCabc	1.0 BCc

[y] Vegetative compatibility group.

[z] Means followed by the same letter within a column (uppercase, isolate comparison) or within a row (lowercase, cultivar comparison) are not significantly different from each other on the basis of a *t* test means comparison ($P = 0.05$). Numbers are the means of three inoculation experiments rated 6 days after inoculation (0 = no disease; 1 = 1–10% of the cotyledon area with visible symptoms; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–90%; 6 = >90%; and 7 = dead cotyledons).

cucumber VCG 1 isolates (Table 4).

All of the isolates in VCG 2 tested were weakly to only moderately virulent on all cucumber hosts tested with the exception of Marketer, which was moderately to highly susceptible (Tables 3 and 4). In general, the isolates in VCG 2 were significantly less virulent on most of the cucumber hosts than were most of the VCG 1 isolates (Tables 3 and 4). In particular, the cultivars H19, and to a lesser extent Poinsett-76 and GY14, gave good differential disease reactions to the VCG 1 and 2 isolates. The cucumber VCG 1 isolates were significantly more virulent than the watermelon VCG 2 on H19 in the summer test (Table 3) and on H19, Poinsett-76, and GY14 in the winter test (Table 4) isolates.

In VCG 3, four of the six cucumber isolates (RH1, MH2, MH5, and ATCC15093) gave statistically similar disease reactions to the cucumber VCG 1 isolates on all of the cucumber hosts (Tables 3 and 4). However, two VCG 3 isolates (ATCC15095 and ATCC16974) gave significantly lower disease reactions on most cucumber cultivars in the summer test (Table 3), and three isolates (ATCC15095, ATCC16974, and MH5) generally gave significantly lower disease reactions on most of the cucumber cultivars in the winter test (Table 4).

Most isolates from VCGs 1, 2, and 3 were moderately to highly virulent on the watermelon cultivars Black Diamond and Sugar Baby in the summer and on Black Diamond in the winter inoculation tests (Tables 3 and 4). However, differential disease reactions were observed on Charleston Grey. Most isolates in VCG 2 were highly virulent on Charleston Grey, whereas all isolates (except JC1 in the summer test) in VCGs 1 and 3 were weakly virulent or avirulent in both the summer and winter tests (Tables 3 and 4). These trends were also observed on Crimson Sweet with the exception of two VCG 1 isolates (JC1 and NC3) and one VCG 3 isolate (MH5), which were highly virulent in the summer inoculation test. Also, the cucuzzi gourd isolate in VCG 2 was weakly virulent on Charleston Grey (Table 4). Thus, Charleston Grey showed significant levels of resistance to most isolates in VCGs 1 and 3 and the cucuzzi gourd isolate in VCG 2 but not to any of the watermelon VCG 2 isolates.

The VCG 2 isolate ATCC15098, from an unknown cucurbit host, gave disease reactions similar to the cucuzzi gourd isolate (LB2) in VCG 2 (Table 4).

On Edisto, all isolates in VCGs 1, 2, and 3 were moderately to highly virulent except ATCC16974, which was weakly virulent; on butternut squash, all isolates were either weakly virulent or avirulent.

DISCUSSION

VCG diversity in the cucurbit anthracnose pathogen (*C. orbiculare*) population appears to be limited. Although 10 VCGs were identified among the 92 isolates examined, only isolates in VCGs 1, 2, and 3 were pathogenic on the cucurbit hosts used in this study. Representative isolates in these three pathogenic VCGs could be characterized into two distinct virulence phenotypes. Most isolates representing virulence phenotype one were highly virulent (mean disease rating 5.1–7.0) on all cucumber, cantaloupe, and certain watermelon cultivars but weakly virulent on the watermelon cultivar Charleston Grey (Tables 3 and 4). In contrast, most isolates representing virulence phenotype two were only moderately virulent (mean disease rating 2.5–5.0) on all of the cucumber cultivars tested except Marketer, on which isolates were highly virulent (5.1–7.0). Isolates representing virulence phenotype two also were highly virulent on all of the watermelon cultivars tested, including Charleston Grey. Isolates in both virulence phenotypes were weakly virulent on the squash cultivar Butternut Waltham. Although a direct comparison is not possible, virulence phenotypes one and two are similar in disease reactions on the various differentials to the previously reported race 1 and race 2, respectively (24).

There was a correspondence between VCG, host origin, and virulence phenotype among the isolates examined in this study. All cucumber and cantaloupe isolates from throughout the United States belonged to VCG 1 or 3 and had a race 1 phenotype (Tables 3 and 4), whereas all watermelon isolates from throughout the United States belonged to VCG 2 and had a race 2 virulence phenotype. VCG 1 contained all the contemporary cucumber isolates (those collected after 1985) as well as two older cucumber isolates, MH1 and MH3, which were collected in 1979 and 1983, respectively. VCG 3 contained seven cucumber isolates, all of which were collected prior to 1986, including an isolate from Africa.

Although the sexual stage of *C. orbiculare* has been reported under laboratory conditions (27,28,46,52), the limited VCG diversity observed in the pathogen population would argue that it is reproducing asexually in nature. Only three VCGs were identified among the pathogenic isolates. Moreover, VCGs 1 and

TABLE 4. Winter disease severity ratings of 18 isolates of *Colletotrichum orbiculare* on a set of differential cucurbit hosts

		Cucumber							Watermelon		Canta-loupe	Squash
VCG[y]	Isolate	H19	SMR-58	GY14	Poinsett-76	Marketer	Pixie	PI 197087	Black Diamond	Charleston Grey	Edisto	Butternut Waltham
1	LB4	6.6 Aa[z]	7.0 Aa	6.5 Aa	6.8 Aa	7.0 Aa	7.0 Aa	7.0 Aa	7.0 Aa	0.4 Cc	7.0 Aa	1.0 Ab
	NC3	6.7 Aa	7.0 Aa	6.6 Aa	7.0 Aa	7.0 Aa	6.8 Aa	7.0 Aa	6.4 ABa	0.2 Cb	7.0 Aa	0.8 ABb
	MH3	5.2 ABa	6.8 Aa	6.0 Aa	6.6 Aa	7.0 Aa	6.4 Aa	7.0 Aa	5.8 ABa	0.5 Cb	7.0 Aa	0.8 ABb
	JC1	4.3 ABCa	6.1 ABa	5.0 ABa	5.8 ABa	6.0 ABa	5.5 ABa	5.3 ABCa	5.4 ABa	0.1 Cb	7.0 Aa	0.7 ABb
	CR7A	4.1 ABCa	4.7 ABCa	3.7 BCa	5.7 ABa	5.7 ABa	5.9 ABa	4.9 ABCa	4.3 ABCa	0.0 Cb	5.3 ABa	0.4 ABCDb
2	CP3	0.7 Dc	2.2 CDEFbc	0.7 DEc	0.6 Ec	4.3 ABCab	2.0 CDEbc	1.8 DEFbc	6.5 ABa	7.0 Aa	4.4 ABCab	0.0 Dc
	JC10	0.6 Dc	2.2 CDEFb	0.6 DEc	0.5 Ec	5.7 ABa	0.7 Ec	2.2 DEFb	7.0 Aa	7.0 Aa	6.0 ABa	0.1 CDc
	15471	0.6 Dcd	1.8 DEFbc	0.4 DEd	0.4 Ed	5.5 ABCa	1.2 DEbcd	2.2 DEFb	6.7 Aa	6.3 ABa	6.8 ABa	0.5 ABCDcd
	LB2	0.6 Dc	2.2 CDEFbc	0.6 DEc	0.3 Ec	4.1 ABCab	1.3 DEbc	2.2 DEFbc	6.7 Aa	0.4 Cc	5.3 ABCa	0.3 BCDc
	15094	0.0 Db	1.1 Fcd	0.0 Eb	0.4 Eb	4.5 ABCa	0.4 Eb	1.6 DEFb	6.7 Aa	6.7 Aa	4.8 ABCa	0.7 ABCb
	JC7	0.2 Dd	1.2 Fcd	0.4 DEd	0.2 Ed	3.2 BCbc	1.4 DEcd	1.2 Fcd	5.8 ABa	5.0 Ba	5.6 ABab	0.0 Dd
	CP6	0.7 Dc	2.3 CDEFbc	0.5 DEc	0.4 Ec	4.1 ABCab	0.8 DEc	2.0 DEFbc	5.0 ABCab	7.0 Aa	5.6 ABa	0.0 Dc
	15098	0.7 Da	1.6 EFa	0.3 DEa	0.7 Ea	3.3 BCa	1.1 DEa	1.5 EFa	3.4 BCDa	0.5 Cd	3.6 BCa	0.3 BCDa
3	RH1	4.7 ABCb	7.0 Aa	5.2 ABb	5.6 ABa	7.0 Aa	6.8 Aa	6.8 Aa	7.0 Aa	0.5 Cc	7.0 Aa	0.8 ABc
	MH2	4.1 ABCb	6.1 ABab	5.5 ABab	4.7 Bab	5.6 ABab	6.6 Aab	6.5 ABab	6.9 Aa	1.0 Cc	6.2 ABab	0.8 ABc
	MH5	2.4 BCDab	4.0 BCDE a	2.2 CDab	3.0 Cab	4.0 ABCa	4.1 BCa	3.7 CDEa	4.6 ABCa	0.1 Cb	5.2 ABCa	0.3 BCDb
	15095	2.1 Ccde	4.2 BCDbc	2.1 CDEcde	2.6 CDbcd	7.0 Aa	3.0 CDbc	4.0 BCDbc	2.2 DCcde	0.0 Ce	4.7 ABCab	0.2 BCDde
	16974	0.6 Dab	1.3 Fab	0.5 DEab	1.3 DEab	2.3 Ca	0.9 DEab	1.1 Fab	0.5 Db	0.2 Cb	2.3 Ca	0.4 ABCDb

[y] Vegetative compatibility group.

[z] Means followed by the same letter within a column (uppercase, isolate comparison) or within a row (lowercase, cultivar comparison) are not significantly different from each other on the basis of a *t* test means comparison ($P = 0.05$). Numbers are the means of two inoculation experiments rated 6 days after inoculation (0 = no disease; 1 = 1–10% of the cotyledon area with visible symptoms; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–90%; 6 = >90%; and 7 = dead cotyledons).

2 apparently are closely related in that bridge isolates (i.e., isolates that show some degree of vegetative compatibility with isolates from both VCGs) were identified. Isolates that bridge VCGs have been identified in *Fusarium oxysporum* (32,37) and *Verticillium dahliae* (31). All three virulent VCGs also have the same mitochondrial DNA haplotype, which is suggestive of a common genetic origin (15).

Although isolates of *C. orbiculare* had previously been characterized into seven physiological races, the data presented in this study indicate that seven races cannot be distinguished. Disease reactions of all isolates in VCGs 1 and 3 correspond to what was previously described as race 1, whereby all cucumber and watermelon cultivars tested were reported to be susceptible with the exception of the watermelon cultivar Charleston Grey, which was reported to be resistant (17,24,30). Similarly, disease reactions of all isolates in VCG 2 correspond to what was previously described as race 2, whereby all cucumber cultivars tested were moderately resistant and all watermelon cultivars were highly susceptible (24). These virulence phenotypes were significantly different from each other ($P = 0.05$) on watermelon differentials Black Diamond and Charleston Grey. Isolates in VCGs 1 and 3 (race 1) were highly virulent on Black Diamond and weakly virulent on Charleston Grey. These results agree with those of Dutta et al (17) and Jenkins et al (30) for what was previously called race 1. Isolates in VCG 2 (race 2) were highly virulent on all watermelon cultivars, and these results are in agreement with those of Goode (24), Dutta et al (17), and Jenkins et al (30), who reported that all watermelon cultivars tested were susceptible to race 2 isolates.

Virulence phenotypes of two cucuzzi gourd isolates in VCG 2 were different on watermelon cultivar Charleston Grey than other watermelon VCG 2 isolates and could possibly represent a third virulence phenotype (Table 4). Virulence diversity within a VCG has been reported in *F. oxysporum,* where multiple races have been found to belong to a single VCG (10,26,37). It also is possible that the cucurbit host of origin may influence pathogen virulence (47).

Eight of the 13 cucurbit cultivars used in this study had previously been used as differentials to characterize races of *C. orbiculare.* These included Marketer, Pixie, PI 197087 (cucumber), Black Diamond, Charleston Grey, Sugar Baby (watermelon), Butternut (squash), and Edisto (cantaloupe). In general, disease reactions observed on all watermelon cultivars in our tests are in agreement with those previously reported (17,24,30,53). However, on cucumber, cantaloupe, and squash cultivars, our results differ somewhat from those previously reported (17,24,30). For example, Dutta et al (17) reported that Marketer was resistant to race 4. Jenkins et al (30) disputed these findings and reported that Marketer was susceptible to race 4 but was resistant to isolates of races 5 and 6 of *C. orbiculare.* In this study, Marketer was highly susceptible to all of the isolates in VCGs 1, 2, and 3.

In our tests, the cucumber cultivar Pixie was highly susceptible to all five cucumber and cantaloupe isolates in VCG 1 and VCG 3, moderately resistant to all seven watermelon and cucuzzi gourd isolates in VCG 2 used in summer virulence tests, and highly resistant to watermelon and cucuzzi gourd isolates in the winter tests. These results are in agreement with those of Jenkins et al (30), who reported that Pixie was resistant to races 2, 5, 6, and 7 (all watermelon isolates) and susceptible to races 1, 3, and 4.

The cucumber accession PI 197087, which has been reported to be highly resistant to races 1, 2, and 3 (Table 2), was highly susceptible to cucumber and cantaloupe isolates in VCG 1 and VCG 3 and moderately resistant to watermelon isolates in VCG 2.

The butternut squash cultivar was highly resistant to all isolates in VCGs 1, 2, and 3 tested. These results are in agreement with those reported by Akai et al (3), Hadwinger and Hall (25), Gardner (22), and Sitterly (45), who reported that squash cultivars were resistant or immune to *C. orbiculare.* However, Goode (24) used butternut squash to differentiate between races 1 and 3, with isolates described as race 1 being moderately virulent to butternut and those described as race 3 being avirulent.

Cantaloupe cultivar Edisto was moderately to highly susceptible to all isolates in VCGs 1, 2, and 3, except two isolates in VCG 3 (ATCC15095 and ATCC16974). However, these isolates were generally less virulent on all the other cultivars used in this study and may represent attenuated isolates that have lost virulence due to prolonged storage.

Two ATCC isolates (15096 and 15097, both in VCG 7) were avirulent on all of the differential cucurbits in all five inoculation tests. These isolates had previously been reported to be virulent and have a race 1 (15096) or a race 2 (15097) phenotype (28). Two honeydew isolates (HD1 and HD3) in VCG9 and two cocklebur isolates (LW1 and LW3) in VCG 10 also were avirulent on all of the cucurbit hosts in all tests.

The role of *C. magna* in anthracnose of cucurbits is unclear. *C. magna* was originally reported in North Carolina on watermelon (27,29). The original cultures (ATCC15015 and 15016) were avirulent (to very weakly virulent on the watermelon cultivar Black Diamond in several tests) under our inoculation conditions. One recently recovered isolate of *C. magna* (AK2) also was avirulent under our inoculation conditions. However, two isolates of *C. magna* (DxD and L2.5) were recently reported to be virulent on cucumber and watermelon under a more severe inoculation procedure than that used in our study (i.e., the inoculum concentration was 30–40 times higher, and the dew period was 24 h longer) (20). These two isolates apparently are ascospore descendants of the two original ATCC cultures (R. J. Rodriguez, *personal communication*). Thus, it is apparent that isolates of *C. magna* are much less virulent than isolates of *C. orbiculare.* It is also apparent that more wild-type isolates of *C. magna* need to be examined to determine whether this is a pathogen of cucurbits under field conditions (20,21).

LITERATURE CITED

1. Adams, G., Hammer, S., and Proffer, T. 1990. Vegetative compatibility in *Leucostoma persoonii.* Phytopathology 80:287-291.
2. Agrios, G. N. 1988. Anthracnose disease caused by ascomycetes and imperfect fungi. Pages 379-390 in: Plant Pathology. 3rd ed. Academic Press, San Diego, CA.
3. Akai, S., Yasumori, H., and Terazawa, H. 1958. On the resistance of cucumber varieties to anthracnose and the behavior of the causal fungus in the invasion of the host tissues. Plant Dis. Rep. 42:1074-1079.
4. Anagnostakis, S. L. 1987. Chestnut blight: The classical problem of an introduced pathogen. Mycologia 79:23-27.
5. Arx, J. A., von. 1970. A revision of the fungi classified as *Gloeosporium.* Bibl. Mycol. 24:1-203.
6. Barnes, G. L. 1972. Differential pathogenicity of Oklahoma isolates of *Colletotrichum orbiculare.* Plant Dis. Rep. 56:35-38.
7. Baxter, A. P, Van der Westhuizen, G. C. A., and Eicker, A. 1983. Morphology and taxonomy of South African isolates of *Colletotrichum.* S. Afr. J. Bot. 2:259-289.
8. Brasier, C. M. 1988. Rapid changes in genetic structure of epidemic populations of *Ophiostoma ulmi.* Nature 332:538-541.
9. Brooker, N. L., Leslie, J. F., and Dickman M. B. 1991. Nitrate nonutilizing mutants of *Colletotrichum* and their use in studies of vegetative compatibility and genetic relatedness. Phytopathology 81:672-677.
10. Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum.* Phytopathology 81:1061-1064.
11. Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum.* Phytopathology 78:1017-1021.
12. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
13. Correll, J. C., Morelock, T. E., and Guerber, J. C. 1993. Vegetative compatibility and virulence of the spinach anthracnose pathogen, *Colletotrichum dematium.* Plant Dis. 77:688-691.
14. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396-400.
15. 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.
16. Correll, J. C., Weidemann, G. J., TeBeest, D. O., and Guerber, J.C. 1991. Genetic and molecular diversity in *Colletotrichum gloeosporioides.* (Abstr.) Phytopathology 81:1219.
17. Dutta, S. K., Hall, C. V., and Heyne, E. G. 1960. Observation on the physiological races of *Colletotrichum lagenarium.* Bot. Gaz. (Chicago) 121:163-166.
18. Dutta, S. K., Hall, C. V., Heyne, E. G., and Hansing, E. D. 1959. Nutritional control of pathogenicity of *Colletotrichum lagenarium* (Pass.) Ell. & Halst. Page 100 in: Proc. Int. Bot. Congr., 9th. (Abstr.) University of Toronto Press, Toronto, Canada.
19. Elmer, W. H. 1991. Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. Phytopathology 81:852-857.
20. Freeman, S., and Rodriguez, R. J. 1992. A rapid, reliable bioassay for pathogenicity of *Colletotrichum magna* on cucurbits and its use in screening for nonpathogenic mutants. Plant Dis. 76:901-905.
21. Freeman, S., and Rodriguez, R. J. 1993. Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. Science 260:75-78.
22. Gardner, M. W. 1918. Anthracnose of cucurbits. U.S. Dep. Agric., Agric. Inf. Bull. 727.
23. Goode, M. J. 1956. Physiological specialization in the cucurbit anthracnose fungus *Colletotrichum lagenarium.* Ph.D. thesis. North Carolina State College, Raleigh.
24. Goode, M. J. 1958. Physiological specialization in *Colletotrichum lagenarium.* Phytopathology. 48:79-83.
25. Hadwinger, L. A., and Hall, C. V. 1963. A biochemical study of the host-parasite relationship between *C. orbiculare* Ell. & Halst. and cucurbit hosts. Proc. Am. Soc. Hortic. Sci. 82:378-387.
26. Jacobson, D. J., and Gordon, T. R. 1991. *Fusarium oxysporum* f. sp. *melonis:* A case study of diversity within a forma speciales. Phytopathology. 81:1064-1066.
27. Jenkins, S. F. 1962. Genetic, taxonomic, and physiological studies of *Glomerella* species pathogenic on cucurbits. Ph.D. thesis. North Carolina State College, Raleigh.
28. Jenkins, S. F., and Winstead, N. N. 1961. Observations on the sexual stage of *Colletotrichum orbiculare.* Science 133:581-582.
29. Jenkins, S. F., Jr., and Winstead, N. N. 1964. *Glomerella magna,* cause of a new anthracnose of cucurbits. Phytopathology 54:452-454.
30. Jenkins, S. F., Jr., Winstead, N. N., and McCombs, C. L. 1964. Pathogenic comparisons of three new and four previously described races of *Glomerella angulata* var. *orbiculare.* Plant Dis. Rep. 48:619-622.
31. Joaquim, T. R., and Rowe, R. D. 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. Phytopathology 80:1160-1166.
32. Katan, T., Zamir, D., Sarfatti, M., and Katan, J. 1991. Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Phytopathology 81:255-262.
33. LaMondia, A., and Elmer, W. H. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues. Can. J. Bot. 67:2420-2424.
34. Layton, D. V. 1937. Parasitism of *Colletotrichum lagenarium* (Pass.) Ell. & Halst. Iowa Agric. Exp. Stn. Res. Bull. 223.
35. Littrell, R. H., and Epps, W. M. 1965. Standardization of a procedure for artificial inoculation of cucumbers with *Colletotrichum lagenarium.* Plant Dis. Rep. 49:649-653.
36. Nikandrow, A., Weidemann, G. J., and Auld, B. A. 1990. Incidence and pathogenicity of *Colletotrichum orbiculare* and a *Phomopsis* sp. on *Xanthium* spp. Plant Dis. 74:796-799.
37. Ploetz, R .C. 1990. Variability in *Fusarium oxysporum* f. sp. *cubense.* Can. J. Bot. 68:1357-1363.
38. Proffer, T. J., and Hart, J. H. 1988. Vegetative compatibility groups in *Leucocytospora kunzei.* Phytopathology 78:256-260.
39. Puhalla, J. E. 1979. Classification of isolates of *Verticillium dahliae* based on heterokaryon compatibility. Phytopathology 69:1186-1189.
40. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
41. Puhalla, J. E., and Hummel, M. 1983. Vegetative compatibility groups within *Verticillium dahliae.* Phytopathology 73:1305-1308.
42. Puhalla, J. E., and Spieth, P. T. 1983. Heterokaryosis in *Fusarium moniliforme.* Exp. Mycol. 7:328-335.
43. Puhalla, J. E., and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp. Mycol. 9:39-47.
44. Sitterly, W. R. 1972. Breeding for disease resistance in cucurbits. Annu. Rev. Phytopathol. 10:471-490.
45. Sitterly, W. R. 1973. Cucurbits. Pages 278-306 in: Breeding Plants for Disease Resistance, Concepts and Applications. R. R. Nelson, ed. Pennsylvania State University Press, University Park.
46. Stevens, F. L. 1931. The ascigerous stage of *Colletotrichum lagenarium* induced by ultra-violet irradiation. Mycologia 23:134-139.
47. Thomas, C. E., and Jourdain, E. L. 1992. Host effect on selection of virulence factors affecting sporulation by *Pseudoperonospora cubensis.* Plant Dis. 76:905-907.
48. Thompson, D. C., and Jenkins, S. F. 1985. Influence of cultivar resistance, initial disease, environment, and fungicide concentration and timing on anthracnose development and yield loss in pickling cucumbers. Phytopathology 75:1422-1427.
49. Walker, J., Nikandrow, A., and Millar, G. D. 1991. Species of *Colletotrichum* on *Xanthium* (Asteraceae) with comments on some taxonomic and nomenclatural problems in *Colletotrichum.* Mycol. Res. 95:1175-1193.
50. Wasilwa, L. A. 1992. Examination of vegetative (heterokaryon) compatibility and virulence in the cucurbit anthracnose pathogen *Colletotrichum orbiculare.* M.S. thesis. University of Arkansas, Fayetteville.
51. Wasilwa, L., Morelock, T. E., and Correll, J. C. 1991. Influence of fertilizer on pathogenicty of *Colletotrichum orbiculare* on *Cucumis sativa.* HortScience 26:613.
52. Watanabe, T., and Tamura, M. 1952. Studies on the perfect stage of the causal fungus of the anthracnose of cucumber. Ann. Phytopathol. Soc. Jpn. 16:137-140.
53. Winstead, M. N., Goode, M. J., and Barham, W. S. 1959. Resistance in watermelon to *Colletotrichum lagenarium* races 1, 2, and 3. Plant Dis. Rep. 43:570-577.
54. Zambino, P. J., and Harrington, T. C. 1990. Heterokaryosis and vegetative compatibility in *Leptographium wageneri.* Phytopathology 80:1460-1469.