Characteristics of *Colletotrichum* from Peach, Apple, Pecan, and Other Hosts

B. Bernstein, E. I. Zehr, and **R. A. Dean,** Department of Plant Pathology and Physiology, Clemson University, Clemson, S.C. 29634-0377, and **E. Shabi,** Department of Plant Pathology, ARO, Volcani Center, P.O.B. 6, Bet Dagan 50-250 Israel

ABSTRACT

Bernstein, B., Zehr, E. I., Dean, R. A., and Shabi, E. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan, and other hosts. Plant Dis. 79:478-482.

Seventy-two *Colletotrichum* isolates from peach, apple, pecan, and other hosts were examined morphologically and tested in vitro for benomyl sensitivity and for polymorphisms in the ribosomal 18S and 28S transcriptional unit. In general, the isolates separated into pink and gray colony types. Each type was isolated from peach, apple, and pecan. Regardless of host origin, pink isolates produced pink to pinkish-orange colonies on potato-dextrose agar (PDA), grew on benomyl-amended medium, produced mostly fusiform conidia, and had similar restriction fragment length polymorphism (RFLP) patterns. Gray isolates produced gray colonies on PDA, grew slightly or not at all on benomyl-amended medium, had conidia with rounded ends, and had similar RFLP patterns that were distinct from pink isolates. Symptoms on detached peach fruit following inoculation with gray and pink isolates were not visually distinguishable. The two types corresponded to *C. acutatum* (pink isolates) and *C. gloeosporioides* (gray isolates), respectively. We concluded that *C. acutatum* and *C. gloeosporioides* are separate species and are found on peach, apple, and pecan.

Glomerella cingulata (Stoneman) Spauld. & H. Schrenk, (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.) in Penz. causes diseases on many fruits including peach (*Prunus persica* (L.) Batsch) (11), pecan (*Carya illinoensis* (F. D. Wangenheim) K. Koch) (3), and apples (*Malus domestica* Borkh.) (7,19). Division of *C. gloeosporioides* into subspecies or forma speciales has not been reported on fruit hosts, although forma speciales are used in descriptions of *G. cingulata* (5). *Colletotrichum acutatum* J. H. Simmonds also has been reported on several fruit hosts including apple (4).

Colletotrichum taxonomy is in flux (6,8,21). In 1957 von Arx (21) defined *C. gloeosporioides* as the anamorph of all fungi with the *G. cingulata* teleomorphy, subsuming more than 500 species from the genera *Colletotrichum*, *Gloeosporium,* and *Vermicularia*. Currently, isolates of *Colletotrichum* lacking the sexual state are considered *C. gloeosporioides*, or may be considered related species such as *C. acutatum* or C. *fragariae* based on host, morphological features, and cultural characteristics. Two recent papers have described clear morphological and genetic differences between *C. gloeosporioides* and *C. acutatum* (8,16). Smith and Black (16) described cultural and morphological differences between *C. gloeosporioides, C. acutatum,* and *C. fragariae* isolated from strawberry. *Colletotrichum gloeosporioides* was described as fast-growing at 32°C, forming cylindrical conidia with rounded ends and dark gray mycelium, whereas *C. acutatum* was slower-growing at 32°C, formed fusiform conidia, and white to beige, orange, pink, or rose mycelium.

Bonde et al. (2) compared isozymes of isolates from many hosts including strawberry and nonhorticultural hosts such as velvetleaf (*Abutilon theophrastii* Medik.). Results indicated that although *C. gloeosporioides* is genetically diverse, *C. acutatum* forms a distinct group.

Sonoda and Pelosi (15) reported two types of *Colletotrichum* on citrus blossoms. One type was a fast-growing gray type that had conidia averaging 14.8 × 4.8 µm in size, and the other was a slow-growing orange-mycelium type that had conidia averaging 13.3 × 4.7 µm. The gray type was highly sensitive to benomyl, whereas the orange type was less sensitive. In other investigations of *Colletotrichum* on citrus (1), three types were found: fast-growing gray, a slow-growing orange, and a Key lime type. The fast-growing gray type grew two to three times faster than the other two and had larger conidia with mostly rounded ends, whereas the type with orange colonies grew slightly faster than Key lime isolates and conidia had one rounded end and the other fusiform. The Key lime type, besides growing slowly, had conidia with one rounded end and the other fusiform, similar to the orange-colony type. Genetic analyses (10) of these strains found restriction fragment length polymorphisms (RFLPs) between the fast-growing gray type and the slow-growing orange type. As reported by Sonoda and Pelosi (15), slow-growing orange isolates were less sensitive to benomyl than were gray strains. Fungicide sensitivity has been used by Shabi and Katan (13) and Shabi et al. (14) to distinguish isolates of *C. gloeosporioides* obtained from different hosts in Israel.

Latham and Williams (9) described perithecial and chromogenic strains isolated from apple. The perithecial isolates grew more rapidly on potato-dextrose agar (PDA) and were olive gray to greenish black in culture. Chromogenic isolates grew relatively slowly on PDA and were purple-red to dark green with orange rings of conidia. Perithecial isolates produced larger lesions when used to inoculate detached apples. Sutton and Shane (20) reported similar results when they inoculated apples in the field with perithecial and chromogenic strains, respectively.

Molecular techniques, including RFLP and random amplified polymorphic DNA (RAPD) analyses, have been used to differentiate species, e.g., isolates of *C. acutatum, C. fragariae,* and *C. gloeosporioides* from strawberry (17). Isolates of *C. acutatum* were heterogeneous in molecular composition but were distinct from *C. gloeosporioides* and *C. fragariae*. Differences found between *C. gloeosporioides* and *C. fragariae* were insufficient to consider them separate species. In contrast, Freeman et al. (6) were able to distinguish among *C. acutatum, C. coccodes, C. fragariae, C. lindemuthianum, C. magna, C. orbiculare*, two groups of *C. graminicola* and three groups of *C. gloeosporioides*, and *C. musae* when DNA was compared.

Mating-type segregation has been used to classify isolates of *G. cingulata* (18) as plus or minus, where plus-type colonies were characterized by light gray mycelium and had perithecia with ascospores that developed into plus- or minus-type colonies, and minus-type colonies were characterized by dark gray mycelium and had perithecia with ascospores that formed only minus-type colonies. Chromogenic colonies also were noted, having pink to red mycelium and no perithecia.

We observed that when apple trees severely affected by bitter rot were growing adjacent to peach orchards, anthracnose of peach fruit was not always present. Isolates of *Colletotrichum* from such apple

Technical contribution No. 4051 of the South Carolina Agricultural Experiment Station, Clemson University.

Accepted for publication 22 February 1995.

orchards, and also from peach, have morphological characteristics corresponding to pink and gray colony forms on agar media. Our primary objective was to determine whether underlying genetic differences contributed to host specificity that might explain the absence of anthracnose in peaches growing near affected apple orchards. Because pecan groves also are planted near peach orchards, isolates from pecan also were studied.

MATERIALS AND METHODS

Source and maintenance of isolates. A total of 72 isolates of *Colletotrichum* were supplied by cooperating researchers or collected from apple, peach, pecan, and other hosts in the southeastern United States and Israel (Table 1). Isolations from fruit collected in South Carolina were made on PDA. Cultures were single-spored after pure cultures were obtained, and maintained on PDA slants at 4°C. Seven isolates collected from strawberry and previously identified as *C. acutatum* were obtained from Barbara Smith (USDA-ARS Small Fruit Research Station, Poplarville, Miss.) for purposes of comparison.

The seven *C. acutatum* isolates and 21 isolates of *Colletotrichum* collected from eight different hosts were tested for benomyl sensitivity, spore morphology, RFLP patterns and virulence on detached ripe peach fruit (Table 2). The remaining isolates were tested only for benomyl sensitivity and by RFLP analysis.

Conidial morphology. Cultures were grown in the dark on PDA at 26°C for 10 to 14 days. One hundred conidia per isolate, selected arbitrarily, were rated as oblong, obclavate, or fusiform, and 25 selected conidia per isolate were measured.

Growth on amended medium. Sensitivity of isolates to benomyl was tested on PDA amended with benomyl to obtain a final concentration of 2 µg per ml of benomyl. Culture dishes containing approximately 18 ml of amended medium were inoculated with 5-mm mycelial plugs removed from the margins of 3- to 4-day-old cultures grown in the dark at 26°C. Inoculated dishes were incubated in the dark at 26°C. Growth rates were determined by measuring colony diameters at 24-h intervals for 7 days. Each isolate was replicated three times per experiment and was tested at least twice. All isolates were tested, including those identified previously as *C. acutatum*.

Inoculation. Isolates were tested for virulence in firm, ripe peach fruit. Conidia scraped from 2-week-old cultures grown in the dark at 26°C were diluted to approximately 1×10^5 conidia per ml in sterile, distilled water. One drop of Tween 80 (Fisher Scientific, Fair Lawn, N.J.) was added to the spore suspension to reduce surface tension and prevent the spore suspension from rolling off the peach.

Table 1. Daily radial growth rate of 54 pink and 18 gray isolates of *Colletotrichum* on potato-dextrose agar (PDA) or PDA amended with 2 µg per ml of benomyl (PDA + B)

Isolate	Source	Colony color	Growth (mm per day)[a]	
			PDA	PDA + B
SF-1[b]	Authors, Clemson, S.C., peach	pink	8.9	2.6
SF-2	Authors, Clemson, S.C., peach	pink	9.1	3.4
SF-3	Authors, Clemson, S.C., peach	pink	7.9	2.3
SF-4	Authors, Clemson, S.C., peach	pink	8.9	2.2
SF-5	Authors, Clemson, S.C., peach	pink	8.8	2.4
SF-6	Authors, Clemson, S.C., peach	pink	9.4	2.8
SF-7	Authors, Clemson, S.C., peach	pink	8.0	1.9
SF-8	Authors, Clemson, S.C., peach	pink	8.2	2.2
SF-9	Authors, Clemson, S.C., peach	pink	8.3	2.4
SF-10	Authors, Clemson, S.C., peach	pink	8.5	1.9
SF-11	Authors, Clemson, S.C., peach	pink	8.9	3.0
SF-12	Authors, Clemson, S.C., peach	pink	9.2	3.4
SF-13	Authors, Clemson, S.C., peach	pink	8.3	2.9
SF-14	Authors, Clemson, S.C., peach	pink	8.4	2.5
SF-15	Authors, Clemson, S.C., peach	pink	8.4	3.6
PCH-1	Authors, Clemson, S.C., peach	pink	8.4	2.9
PCH-8	Authors, Spartanburg, S.C., peach	pink	9.2	2.9
PCH-10	Authors, Sandy Springs, S.C., peach	pink	8.6	2.3
APL-1	R. Milholland, N.C., apple	pink	7.0	2.7
APL-2	Authors, Sandy Springs, S.C., apple	pink	8.6	2.6
APL-3	Authors, Sandy Springs, S.C., apple	pink	7.5	2.8
APL-5	F. Nichols, Ark., apple	pink	9.4	3.3
APL-6	M. Hotchkiss, Ga., apple	pink	9.5	3.1
APL-11	T. Sutton, N.C., apple	pink	6.7	2.6
APL-12	T. Sutton, N.C., apple	pink	8.5	2.6
APL-13	T. Sutton, N.C., apple	pink	9.4	1.9
APL-21	K. Hickey, Pa., apple	pink	8.0	2.7
APL-22	K. Hickey, Pa., apple	pink	7.9	2.7
APL-23	K Yoder, Winchester, Va., apple	pink	8.1	2.7
APL-24	A. Latham, Ala., apple	pink	7.8	2.7
APL-30	Authors, Spartanburg, S.C., apple	pink	7.2	2.3
APL-31	Authors, Spartanburg, S.C., apple	pink	10.4	1.8
APL-32	Authors, Spartanburg, S.C., apple	pink	7.4	2.1
APL-33	Authors, Spartanburg, S.C., apple	pink	8.5	2.6
APL-34	Authors, Spartanburg, S.C., apple	pink	8.4	2.6
APL-35	Authors, Spartanburg, S.C., apple	pink	8.5	2.6
APL-36	Authors, Spartanburg, S.C., apple	pink	8.2	3.1
APL-37	Authors, Spartanburg, S.C., apple	pink	7.5	1.9
APL-38	Authors, Spartanburg, S.C., apple	pink	8.9	3.1
APL-39	Authors, Spartanburg, S.C., apple	pink	8.5	2.3
APL-40	Authors, Spartanburg, S.C., apple	pink	8.7	2.1
APL-41	Authors, Spartanburg, S.C., apple	pink	8.8	3.4
APL-42	Authors, Spartanburg, S.C., apple	pink	9.2	3.1
PCN-5	A. Latham, Ala., pecan	pink	8.5	3.8
PCN-6	A. Latham, Ala., pecan	pink	8.4	3.0
PCN-8	A. Latham, Ala., pecan	pink	9.3	2.9
STR-2	R. Miller, S.C., strawberry	pink	8.3	2.8
STR-3	R. Miller, S.C., strawberry	pink	7.8	3.1
STR-4	R. Milholland, N.C., strawberry	pink	8.7	3.6
STR-5	R. Miller, S.C., strawberry	pink	7.2	2.9
STR-6	R. Miller, S.C., strawberry	pink	8.2	2.6
BLB-1	R. Miller, S.C., blueberry	pink	9.0	3.2
MYR-1	R. Milholland, N.C., crape myrtle	pink	8.3	2.7
GRP-1	R. Miller, S.C., grape	pink	8.9	2.8
AVO-1	Authors, Israel, avocado	gray	12.9	0
AVO-5	Authors, Israel, avocado	gray	15.0	0
PCH-13	Authors, Estil, S.C., peach	gray	13.4	0
APL-7	T. Sutton, N.C., apple	gray	14.3	0
APL-8	T. Sutton, N.C., apple	gray	14.0	1.6
APL-9	T. Sutton, N.C., apple	gray	14.7	0
APL-18	T. Sutton, N.C., apple	gray	13.8	0
APL-19	T. Sutton, N.C., apple	gray	12.4	0
APL-51	L. Pusey, Byron, Ga., apple	gray	14.0	0
APL-52	L. Pusey, Byron, Ga., apple	gray	13.3	0
APL-53	L. Pusey, Byron, Ga., apple	gray	13.0	0
APL-54	L. Pusey, Byron, Ga., apple	gray	12.9	0
APL-55	L. Pusey, Byron, Ga., apple	gray	12.7	0
PCN-1	M. Hotchkiss, Ga., pecan	gray	13.8	0
PCN-2	M. Hotchkiss, Ga., pecan	gray	13.7	0
PCN-4	M. Hotchkiss, LA, pecan	gray	14.1	0
PCN-7	A. Latham, Ala., pecan	gray	13.8	0
CAM-1	L. Barker, S.C., camellia	gray	12.0	0
Mil-1[c]		*C. acutatum*	5.3	2.4
Mil-2[c]		*C. acutatum*	8.3	2.5
Goff[c]		*C. acutatum*	8.4	2.8
Cal-A[c]		*C. acutatum*	6.4	2.9
Cal-B[c]		*C. acutatum*	6.6	2.5
Cal-C[c]		*C. acutatum*	8.7	4.3
al-D[c]		*C. acutatum*	8.7	3.3

[a] Average of radial growth measurements of at least 6 colonies.

[b] SF-strains are isolates collected from peach at one location in South Carolina.

[c] Isolates of *C. acutatum* supplied by Barbara Smith, USDA-ARS, Poplarville, Miss.

Firm-ripe peaches purchased at local markets or obtained from local growers were washed with soap and water to remove fungicide residues, then surface sterilized for 2 min with 0.5% NaOCl and rinsed in sterile, distilled water. Six peaches were placed on metal screens over wet paper towels in each of 18 Rubbermaid dishpans. Three drops of spore suspension were placed on three peaches in each pan. Control treatments consisted of water with Tween 80 added. Three fruit were inoculated per isolate in each of three pans for a total of nine peaches per isolate and nine control fruit. Treatments were arranged in a completely randomized design. Containers were placed in clear plastic bags that were closed with wire ties and incubated at room temperature for 7 days. The bags were opened once a day for measurements of the diameters of the developing lesions. This experiment was performed three times.

RFLP analysis. Mycelium for DNA extraction was grown in 100 ml of half-strength clarified V8 juice containing 20 mg yeast extract, or in petri dishes containing approximately 20 ml of potato-dextrose broth, for 3 days at 28°C. Mycelial mats were taken from the culture medium, dried on paper towels, placed in Eppendorf tubes, lyophilized, and stored at –20°C in Parafilm-sealed Eppendorf tubes.

To extract DNA, lyophilized mycelium was ground in 1 ml of extraction buffer containing 50 µM EDTA and 0.2% sodium dodecyl sulfate in distilled water. The resulting slurry was incubated at 68°C for 20 min, then centrifuged at 16,000 × *g* for 2 min. The supernatant was recovered, 3 M potassium acetate amounting to one-tenth the volume of the supernatant added, and the mix kept on ice for 5 min before repetition of the centrifugation procedure. The supernatant was transferred to a new Eppendorf tube and one volume of 100% isopropanol was added, mixed briefly, and kept on ice for 20 min or overnight. The crude DNA precipitate was collected by centrifugation and resolubilized in 100 µl of sterile, distilled water. DNA was re-precipitated by adding 10 M LiCl amounting to one-tenth the volume of the solution and 2.5 volumes of 95% ethanol. The crude DNA was pelleted by centrifugation for 5 min. This pellet was resolubilized in 100 µl of Tris-EDTA, and treated with 4 µl of DNase-free RNAse (10 mg per ml) for 30 min at 37°C. A phenol-chloroform extraction was performed and the DNA was precipitated with a 0.1-volume 3 M sodium acetate and one volume 100% isopropanol followed by centrifugation. The purified DNA was resolubilized in 100 to 200 µl of Tris-EDTA and maintained at 4°C until use.

One microgram of extracted DNA was cut with restriction enzyme *Eco*RI or *Hin*dIII (Promega Co., Madison, Wis.), size-separated by electrophoresis through a 0.8% agarose gel, and transferred to a nylon membrane according to the manufacturer's instructions (Hybond, Amersham, Arlington Heights, Ill.) for Southern hybridization. Membranes were probed with DNA containing the 18-28S ribosomal transcriptional unit from *Aspergillus nidulans* (Eidam) G. Wint., radiolabeled with ^{32}P, and then exposed to X ray film overnight at –80°C (12).

RESULTS

Cultural and conidial morphology. Two distinct morphological colony types were observed. Fifty-four isolates were pink when viewed from above, or orange from the underside (pink strains). Colony diameters increased 7.2 to 10.4 mm per day (average 8.4 mm per day), when grown from 5-mm-diameter mycelial plugs (Table 2), and produced little aerial mycelium. Conidia en mass were orange, produced in older mycelium near the center of the colony. As individuals, 25 to 83% were fusiform, 11 to 44% oblong, and 4 to 37% obclavate. Size ranged from 8.1 to 15.3 µm × 3.5 to 5.2 µm (average 10.4 × 4.6 µm) (Table 2). Four pink isolates (PCN-5, STR-2, STR-3, and STR-5) had more aerial mycelium and a denser growth habit than other isolates, but growth rate and conidial morphology were the same.

Eighteen isolates were characterized as gray strains. When viewed from above, mycelium appeared gray and, from the underside, gray to olive green. Colony diameters increased 12.0 to 15.0 mm per day (average rate 13.5 mm per day) when grown from 5-mm-diameter mycelial plugs (Table 2). Aerial mycelium was prominent. Masses of conidia were orange, like those produced by pink isolates, and were produced from all but the edges of the colony. All gray strains conidiated profusely after 5 to 7 days except PCH-13 and APL-18, which produced few conidia. Conidia were 1 to 11% fusiform, 73 to 89% oblong, and 6 to 16% obclavate, and the size ranged from 12.3 to 14.8 µm × 4.6 to 5.5 µm (average 13.6 × 5.1 µm) (Table 2).

Isolates of *C. acutatum* used for comparison produced conidia that were 26 to 88% fusiform, 2 to 39% oblong and 7 to 63% obclavate, and the size ranged from 9.7 to 15.3 µm × 4.3 to 5.2 µm (average 12.0 × 4.6 µm) (Table 2).

Table 2. Colony color and morphological characteristics of conidia from 21 *Colletotrichum* isolates from peach, apple, pecan, myrtle, blueberry, grape, strawberry, camellia, and avocado, compared with seven *Colletotrichum acutatum* isolates from strawberry

Isolate	Colony color[a]	Conidial size (µm)[b]	Percentage of conidia[c]		
			Fusiform	Oblong	Obclavate
SF-3[d]	pink	8.1 × 3.8	44	16	29
SF-10	pink	8.1 × 4.0	44	16	29
SF-14	pink	11.1 × 5.0	66	16	14
SF-15	pink	7.5 × 3.5	44	24	25
PCH-8	pink	9.3 × 4.5	43	29	24
APL-2	pink	11.8 × 5.2	44	35	19
APL-33	pink	11.8 × 4.9	25	44	27
BLB-1	pink	11.5 × 5.1	73	11	16
GRP-1	pink	11.6 × 4.7	36	36	26
MYR-1	pink	11.6 × 4.8	36	25	37
PCN-5	pink	13.8 × 5.2	83	11	4
STR-2	pink	15.3 × 4.9	72	19	9
STR-3	pink	14.0 × 5.1	41	28	31
STR-5	pink	14.0 × 5.1	41	28	31
PCH-13	gray	13.8 × 4.6	1	86	13
APL-52	gray	13.4 × 5.5	10	73	16
APL-53	gray	14.8 × 5.3	1	89	10
PCN-3	gray	13.7 × 5.1	1	87	12
AVO-1	gray	12.3 × 5.4	9	79	11
AVO-5	gray	14.6 × 4.9	2	88	10
CAM-1	gray	13.8 × 4.8	11	83	6
Mil-1[e]	*C. acutatum*	10.5 × 5.2	44	33	21
Mil-2[e]	*C. acutatum*	10.2 × 3.3	26	11	63
Goff[e]	*C. acutatum*	10.6 × 4.5	42	24	29
Cal-A[e]	*C. acutatum*	13.4 × 4.6	62	15	22
Cal-B[e]	*C. acutatum*	12.5 × 5.0	62	17	21
Cal-C[e]	*C. acutatum*	14.5 × 4.8	83	2	15
Cal-D[e]	*C. acutatum*	15.3 × 4.7	88	5	7

[a] Colony color of 2-week-old isolates grown on potato-dextrose agar in petri plates incubated at 28°C in the dark.

[b] Average measurements of 25 conidia taken from 2-week-old cultures grown on potato-dextrose agar.

[c] Percentage of conidia appearing fusiform, oblong or obclavate from observations of 100 conidia per isolate. Percentages may not add up to 100% because some conidia were round or oddly-shaped rather than distinctly fusiform, oblong, or obclavate.

[d] SF-isolates designate isolates collected from peach at one location in South Carolina.

[e] Isolates of *C. acutatum* were supplied by Barbara Smith, USDA-ARS, Poplarville, Miss.

Benomyl sensitivity. At 2 µg per ml of benomyl, growth of pink isolates ranged between 17 and 47% (average 33%) of growth on nonamended medium. Gray isolates did not grow on the amended medium, except APL-8, which grew at 11% of the rate on nonamended medium (Table 1). Growth of *C. acutatum* isolates on benomyl-amended medium ranged between 33 and 45% (average 40%) of growth on nonamended medium (Table 1).

Inoculation tests. Thirteen pink, seven gray, and five *C. acutatum* isolates were used to inoculate firm-ripe, unwounded peaches. All isolates produced lesions by 7 days postinoculation. By the seventh day, average lesion diameters for the pink isolates ranged from 4 to 15 mm. Nineteen to 100% of the sites inoculated developed typical symptoms of anthracnose. For the gray isolates, average lesion diameters ranged from 1 to 15 mm; 7 to 96% of the sites inoculated developed lesions. Rate of lesion development for both types of isolates was slower on fruit that were less mature than on those that were approaching softness. Source of the isolate was not related to lesion diameter. *Colletotrichum acutatum* isolates caused lesions that were indistinguishable from those caused by other isolates, except for isolate Cal-C, which did not produce lesions on detached fruit.

Ribosomal DNA polymorphism. Polymorphisms in DNA corresponding to the genes for ribosomal RNAs also corresponded to morphological types. Southern hybridizations of *Eco*RI- and *Hin*dIII-digested DNA from all types detected polymorphic fragments hybridizing to ^{32}P-labeled *Aspergillus nidulans* 18-28S ribosomal DNA.

DNA from the pink isolates digested with *Eco*RI produced two major bands at 5.1 kb and 2.1 kb (Fig. 1A). The ribosomal DNA probe hybridized to three fragments in DNA digested with *Hin*dIII, one at 10.5 kb and two more intense bands at 5.0 and 4.6 kb (Fig. 1C). All pink isolates except four produced indistinguishable banding patterns; polymorphisms were present in isolates PCN-5, STR-2, STR-3, and STR-5. The *Eco*RI hybridization pattern of PCN-5 had one band at 5.5 kb and one band at 3.2 kb (Fig. 1A). The *Hin*dIII hybridization pattern of PCN-5 had one intense band at 12.5 kb and no other bands (Fig. 1C). *Eco*RI hybridization signals from STR-2, STR-3 and STR-5 were essentially the same as other pink isolates, but the *Hin*dIII digests produced intense signals at about 5.0 kb and no other bands (Fig. 1C).

DNA from most of the gray isolates digested with *Eco*RI produced two hybridization bands, one at 5.4 kb and the other at 3.2 kb (Fig. 1B). The *Hin*dIII hybridization pattern had one band at 12.5 kb and a second more intense band at 5.1 kb (Fig. 1D). All gray isolates produced similar banding patterns except APL-18, PCN-2, and PCH-13, which produced *Eco*RI bands at about 5.2 kb and 2.9 kb. One isolate, AVO-5, had one *Eco*RI band at about 5.5 kb and a second band at 2.9 kb. APL-18 and PCH-13 had two *Hin*dIII bands, one at about 4.8 kb and a much smaller band at about 2.4 kb. PCN-2 had bands at about 9.8 kb and 4.8 kb. AVO-5 had only one *Hin*dIII band at 12.5 kb.

DNA from the *C. acutatum* isolates Goff and Mil-1 used for comparison had *Eco*RI hybridization patterns essentially the same as those produced by the pink isolates, with bands at 5.1 and 2.1 kb (Fig. 1A), and *Hin*dIII hybridization patterns the same as those produced by STR-2, STR-3, and STR-5, with only one band at 5.0 kb (Fig. 1C).

IMI identification. Two representative gray and three representative pink isolates were sent to the International Mycological Institute (IMI) for independent identification based on morphological characteristics. PCN-4 and APL-7 (gray strains) were identified as *Glomerella cingulata*, anamorph *Colletotrichum gloeosporioides* and STR-3, PCN-5, and APL-2 (pink strains) were identified as *Colletotrichum acutatum* (P. F. Cannon, *personal communication*). Isolates PCN-4, PCN-5, STR-3, and APL-2 have been placed in the IMI collection as IMI 354569, IMI 354571, IMI 354568, and 354572, respectively.

DISCUSSION

Spore morphology, cultural characteristics, reaction to benomyl-amended medium, and RFLP analyses clearly separated two types of *Colletotrichum* found infecting fruits of apple, peach, pecan, and other hosts. Pink strains grew slowly with pink mycelium on PDA, with limited aerial mycelium and orange spores, whereas the gray type grew faster and was gray on PDA with profuse aerial mycelium. Conidia of pink strains were smaller and more frequently fusiform than those of the gray type, which had conidia that were more

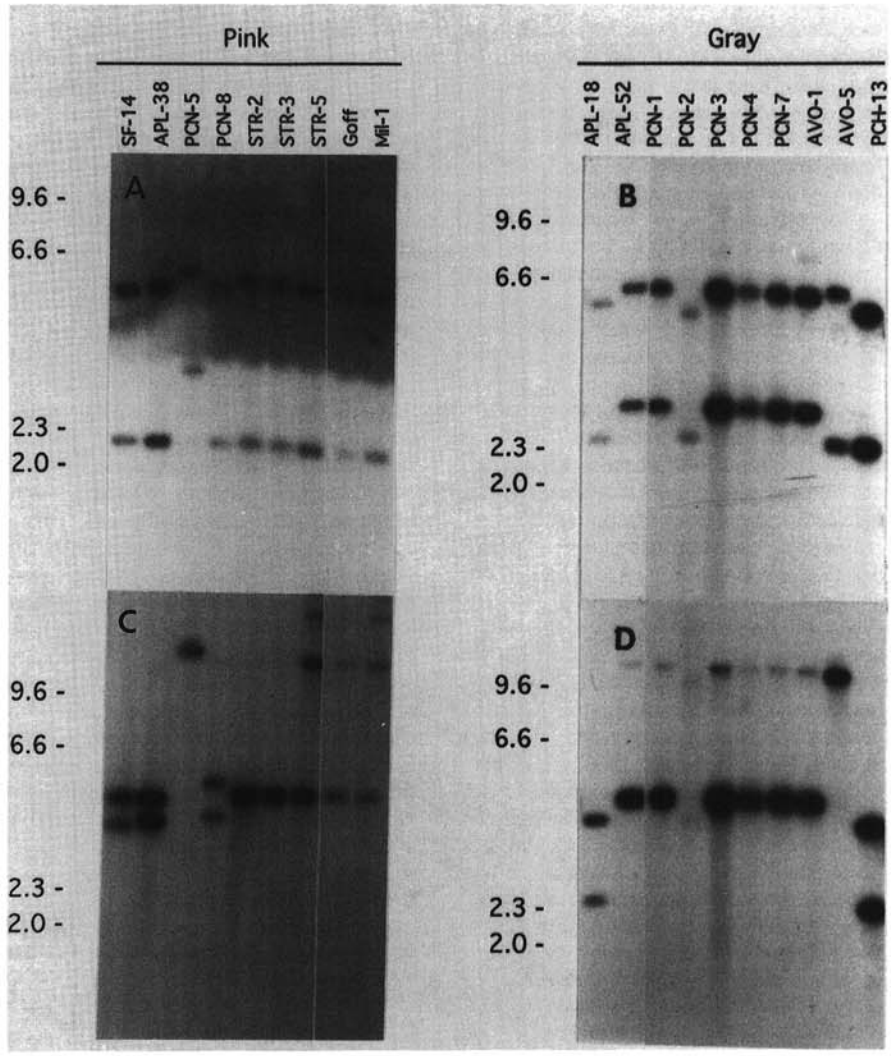

Fig. 1. Restriction fragment length polymorphism analysis of genomic DNA of *Colletotrichum* from apple (APL), pecan (PCN), strawberry (STR), avocado (AVO) and peach (PCH). Two *C. acutatum* isolates included for comparison are labeled Mil-1 and Goff. Fragments were cut with restriction endonucleases *Eco*RI **(A, B)** or *Hin*dIII **(C, D)** and hybridized to ^{32}P-labeled 18-28S ribosomal DNA from *Aspergillus nidulans*. Polymorphisms between pink isolates were present in PCN-5, STR-2, STR-3, and STR-5 when cut with *Eco*RI and in PCN-5 only when cut with *Hin*dIII.

frequently oblong. Pink strains were not sensitive to 2 µg per ml of benomyl, whereas the gray type, except for one isolate, was highly sensitive. RFLP analyses also showed clear differences between the pink and gray types with both restriction enzymes *Eco*RI and *Hin*d III.

Pink and gray strains in peach and apple have been reported previously (9,18,19). Pink strains were reported to grow more slowly on cornmeal agar and to have smaller conidia with more pointed ends than gray strains. The two types were not separated as to species, but both have been referred to as *C. gloeosporioides*.

Gunnell and Gubler (8), Bonde et al. (2), and Smith and Black (18) compared species of *Colletotrichum* occurring on strawberry. They reported that conidia of *C. acutatum* were smaller than those of *C. gloeosporioides* and elliptic-fusiform in shape, whereas conidia of *C. gloeosporioides* were oblong with obtuse ends (8,16). These descriptions roughly correspond to the pink and gray types reported here. Based on conidial, setal, and general morphological characteristics, Smith and Black (16) concluded that *C. acutatum* from strawberry is distinctly different from *C. gloeosporioides* from strawberry. Bonde et al. (2) accepted *C. acutatum* from strawberry as a species separate from *C. fragariae* and *C. gloeosporioides* based on conidial morphology, but indicated that *C. acutatum* and *C. gloeosporioides* from strawberry were closely related based on isozyme analyses.

Sutton (19) suggested that bitter rot of apples is caused by *C. gloeosporioides* and *C. acutatum*. Our results strongly support this conclusion. Tolerance of benomyl by pink isolates does not appear to be a result of selection pressure for benomyl resistance on the pink population. Gray strains, often obtained under similar circumstances, were sensitive to benomyl, except for one gray isolate, APL-8, which grew to a limited extent on 2 µg per ml of benomyl.

Several isolates found in this study did not correspond clearly to either pink or gray types. PCN-5 grew slowly, had 83% fusiform spores, and was not sensitive to benomyl, and so was grouped with the pink isolates. However, this isolate was whitish-gray in culture, produced aerial mycelium, and displayed an RFLP pattern similar to the gray isolates but lacking the lower band in the *Hin*dIII digest.

Cultural characteristics and spore morphology of PCH-13, APL-18, and AVO-5 resembled *C. gloeosporioides*, and these isolates were highly sensitive to benomyl. However, these isolates displayed 18-28S ribosomal DNA polymorphisms different from other gray isolates. AVO-5 lacked the 5.4-kb *Hin*dIII band present in other gray isolates. The polymorphisms displayed by these three isolates could indicate a variant of the gray strain, unrelated to host, or could merely be a phenotypically insignificant sequence difference that is not important in classification of these strains.

STR-2, STR-3, and STR-5 (all from strawberry) lacked a 4.6-kb band comparable to that of the other pink isolates in the *Hin*dIII digest and had a slightly larger upper band in the *Eco*RI digest. These banding patterns corresponded exactly with the banding patterns of the *C. acutatum* isolates provided by Smith. Since all isolates from strawberry displayed this polymorphism and no other isolates did, this may be an indication of host specificity, or might serve as a useful marker for strawberry isolates. However, strawberries are propagated asexually, often with large numbers of transplants coming from one or two areas. This strain of *C. acutatum* might have been propagated inadvertently with the strawberries.

Pathogenicity tests on detached peach fruits were adequate for determining if isolates produced characteristic anthracnose-type lesions on detached fruit, but the results were too variable to determine if there were differences in virulence on peach fruit. Isolate Cal-C, a *C. acutatum* isolate, did not produce lesions on detached fruit. This inability may have resulted from loss of virulence in storage, or may be an indication of host specificity which was not detectable in any of these tests. More research will be needed to ascertain host range.

Apart from the differences shown by several isolates, two distinct groups were identified. The consistent and marked differences in conidial and cultural morphology, benomyl sensitivity, and genetic make-up indicate that the pink strains studied here are *C. acutatum* and the gray strains are *C. gloeosporioides*. Both *C. gloeosporioides* and *C. acutatum* were found on peach, apple, and pecan. This is the first report of *C. acutatum* infecting peach fruit. No host-specific traits were found in either species, and isolates from the different hosts were able to cause typical anthracnose symptoms on detached peach fruit. This finding implies that dissemination between peach and apple orchards, and perhaps between other hosts, is not regulated by host specificity but by factors such as overwintering sites, means of dissemination, or other undiscovered aspects of pathogen behavior.

ACKNOWLEDGMENTS

Funding for this study was provided in part by Gerber Products Co., Freemont, Mich. We thank the persons who contributed to this research by providing cultures of *C. gloeosporioides* and *C. acutatum* for our study. We also thank P. F. Cannon, International Mycological Institute, Kew, U.K., for the identification of selected isolates.

LITERATURE CITED

1. Agostini, J. P., Timmer, L. W., and Mitchell, D. J. 1992. Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. Phytopathology 82:1377-1382.
2. Bonde, M. R., Peterson, G. L., and Maas, J. L. 1991. Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. Phytopathology 81:1523-1528.
3. Brenneman, T. B. 1989. Recent occurrence of pecan anthracnose caused by *Glomerella cingulata*. Plant Dis. 73:775.
4. Dyko, B. J., and Mordue, J E. M. 1979. CMI Descriptions of Pathogenic Fungi and Bacteria, No. 630. Commonw. Mycol. Inst., Kew, U.K.
5. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossmann, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, Minn.
6. Freeman, S., Pham, M., and Rodriguez, R.J. 1993. Molecular genotyping of *Colletotrichum* species based on arbitrarily primed PCR, A + T rich DNA, and Nuclear DNA analyses. Exper. Mycol. 17:309-322.
7. Garman, H. 1883. *Gloeosporium versicolor*. Ky. Agric. Exp. Stn. Bull. 44:3-31.
8. Gunnell, P. S., and Gubler, W. D. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. Mycologia 84:157-165.
9. Latham, A. J., and Williams, J. C. 1983. Cultural characteristics and pathogenicity of *Glomerella cingulata* isolates from apples in Alabama. Plant Dis. 67:1065-1068.
10. Liyange, H. D., McMillan, R. T., Jr., and Kistler, H. C. 1992. Two genetically distinct populations of *Colletotrichum gloeosporioides* from citrus. Phytopathology 82:1371-1376.
11. Ramsey, G. B, Smith, M. A., and Heiberg, B. C. 1951. Anthracnose of peaches. Phytopathology 41: 447-455.
12. Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. Shabi, E. and Katan, T. 1983. Occurrence and control of anthracnose of almond in Israel. Plant Dis. 67:1364-1366.
14. Shabi, E., Katan, T., Gera, H. and Elisha, S. 1993. Taxonomical determination of pathogenic *Colletotrichum gloeosporioides* of almond, anemone and avocado according to fungicide sensitivity. Phytoparasitica 21:2.
15. Sonoda, R. M., and Pelosi, R. R. 1988. Characteristics of *Colletotrichum gloeosporioides* from lesions on citrus blossoms in the Indian River area of Florida. Proc. Fla. State Hortic. Soc. 101:36-38.
16. Smith, B. J., and Black, L. L. 1990. Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant Dis. 74:69-76.
17. Sreenivasaprasad, S., Brown, A. E., and Mills, P. R. 1992. DNA sequence variation and interrelationships among *Colletotrichum* species causing strawberry anthracnose. Physiol. Mol. Plant Pathol. 41:265-281.
18. Struble, F. B., and Keitt, G. W. 1950. Variability and inheritance in *Glomerella cingulata* (Stonem.) S. and V.S. from apple. Am. J. Bot. 37:563-576.
19. Sutton, T. B. 1991. Bitter Rot. Page 15-16 in: Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, Minn.
20. Sutton, T. B., and Shane, W. W. 1983. Epidemiology of the perfect stage of *Glomerella cingulata* on apples. Phytopathology 73:1179-1183.
21. von Arx, J. A. 1957. Die Arten der Gattung *Colletotrichum* Cda. Phytopathol. Z. 29:413-468.