Variation of Several Anthracnose Fungi in Virulence to Strawberry and Apple

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ABSTRACT

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Isolates of *Glomerella cingulata, Colletotrichum gloeosporioides, C. fragariae*, and *C. dematium* were evaluated for variation in pathogenicity and virulence in strawberry stolons and fruits and apple fruits. Isolates of two nonstrawberry pathogens, *C. trifolii* and *C. coccodes*, were also included as were subcultures of naturally occurring colony sectors of *C. gloeosporioides* and *G. cingulata* from culture. Isolates of *C. trifolii* were avirulent to moderately virulent in stolons. One isolate of *C. coccodes* and several isolates of *C. gloeosporioides* and *G. cingulata* were as virulent as *C. fragariae* in stolons.

Anthracnose diseases of strawberry are primarily fruit rots (2,16,19,21,23) or diseases of stolons, leaves, petioles, and crowns (3,4,10,12,13). The array of pathogens (17) includes *Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc. (teleomorph *Glomerella cingulata* (Stonem.) Spauld. & Schrenk), *C. fragariae* Brooks (= *C. gloeosporioides*? [15,22]), *C. dematium* (Pers. ex Fr.) Grove, and *C. acutatum* Simmonds. *C. fragariae* can cause serious fruit rot (11) and affect stolons, leaves, petioles, and crowns of strawberry. *G. cingulata* causes fruit rot of strawberry (14) and also may be involved in crown necrosis (C. M. Howard, *unpublished*). Most strawberry anthracnose fungi except *C. fragariae* have broad host ranges and (except possibly *C. acutatum*) occur worldwide. Recently, *C. acutatum*, which has not been recorded from the United States, was isolated from plants grown in California and shipped to Great Britain (5). In Australia, *C. acutatum* causes a serious strawberry fruit rot (19,21).

The purpose of this study was to examine the pathogenicity and variability in virulence of isolates representing several species of fungi involved in the strawberry anthracnose disease complex. Isolates of *C. trifolii* Bain & Essary and *C. coccodes* (Wallr.) Hughes were included as nonpathogens of strawberry.

MATERIALS AND METHODS

Fungal isolates were obtained from several sources (Table 1) including our own collections from various plant materials. Isolates originating from subcultures of naturally occurring colony sectors in culture are designated by the number of the parent isolate followed by "-S." Cultures were grown on potato-dextrose agar in petri plates at 22–24 C under continuous fluorescent light for 7 days to stimulate sporulation.

Strawberry plants (cultivar Blakemore, which is rated intermediate in resistance to *C. fragariae*) were grown in 10-cm plastic pots in the greenhouse with a 16-hr photoperiod using supplemental incandescent light to encourage stolon production.

Spraying plants with conidial suspensions of *C. fragariae* (8) or *G. cingulata* (C. M. Howard, *unpublished*) is effective for determining isolate pathogenicity. Our objective, however,

Table 1. Isolates of anthracnose fungi inoculated to punctures in strawberry stolons and fruit and apple fruit

Genus Species	Isolate	Source of isolate: Host	Source of isolate: Location
Colletotrichum trifolii	M505	Alfalfa	Argentina
	M517	Alfalfa	Argentina
	M531	Alfalfa	Argentina
	CT-Rel	Alfalfa	Maryland
	CT-5-3	Alfalfa	Maryland
	CT-271	Alfalfa	Maryland
coccodes	CP-8	Potato	Ohio
	CP-241	Potato	Ohio
	C-13	Tomato	New York
	C-115	Tomato	Maryland
dematium	CD-1	Strawberry	Maryland
fragariae	C-73	Strawberry	Florida
	C-73-S	Sector subculture	···
	C-131	Strawberry	Florida
	C-132	Strawberry	Florida
	SXE	Strawberry	Mississippi
	CF-4	Strawberry	N. Carolina
	CF-4-S	Sector subculture	···
	CF-1	Strawberry	Louisiana
	CF-14	Strawberry	Florida
	CF-6	Strawberry	Mississippi
	CF-6-S	Sector subculture	···
gloeosporiodes	C-66	Apple	Maryland
	C-85	Blueberry	New Jersey
	C-91	Tomato	Georgia
	C-101	Tomato	New Jersey
	GL-St	Strawberry	Maryland
	GL-Bb	Blackberry	Maryland
	GL-T-77	Tomato	Maryland
	GL-Bl	Blueberry	Michigan
	GL-D	Strawberry	Maryland
	GL-Ap-78	Apple	Maryland
	GL-T-78	Tomato	Maryland
	GL-G139	Blueberry	New Jersey
Glomerella cingulata	GC-4	Blueberry	N. Carolina
	GC-4-S	Sector subculture	···
	GC-8	Blueberry	N. Carolina
	GC-8-S	Sector subculture	···
	GC-9	Grape	N. Carolina
	GC-10	Strawberry	N. Carolina
	GC-10-S	Sector subculture	···

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was to determine isolate virulence by observing lesion development. For this reason, wound inoculations were used. Strawberry fruits and stolons with developing daughter plants were inoculated by puncturing the surface with a sterile transfer needle dipped into a conidial mass in culture. Stolons were wiped with 70% ethanol, inoculated at the midpoint between the mother and daughter plants, and the inoculation site was wrapped with paraffin film for 4 days. Plants were incubated on a greenhouse bench and spaced to allow watering without cross-contamination of isolates. Strawberry fruits were dipped into 70% ethanol, rinsed once in sterile distilled water, inoculated at a single point each, and incubated individually in compartments of plastic boxes at 16 C. Four replicates of fruits and stolons were made for each isolate. Fruits were examined 3 and 5 days, and stolons, 7 and 14 days after inoculation.

Infectivity of anthracnose fungi in apple fruits has been used (1,18) as a comparative measure of isolate virulence and was included in this study to determine the ability of each isolate to infect apple fruits and to sporulate in the lesions formed. Four mature fruits each of McIntosh, Delicious, Yellow Delicious, and Jonathan were wiped with 70% ethanol, wound-inoculated as described, and incubated at 16 C. Lesion size and amount of sporulation were rated after 12 and 20 days.

Lesion development was evaluated with a modified scale of Delp and Milholland (6,7), where 1 = no lesions, 2 = streaking only, 3 = lesions less than 5 mm long, 4 = lesions 5 mm long × 1 mm wide, 5 = lesions 10 mm long × 2 mm wide, 6 = lesions 15–20 mm long × 2–3 mm wide, 7 = lesions 25 mm long and nearly girdling stolon, 8 = lesions more than 25 mm long and stolon girdled, and 9 = lesions more than 25 mm long, stolon girdled, and daughter plant distal to the lesion dead.

Fruit infection was rated either as present (+) or absent (−) because of variability in lesion development resulting from variation in size and maturity of fruits. Diameters of lesions on apple fruits were measured. Sporulation on lesions was rated on a scale of 1–5, where 1 = no sporulation, 2 = about 25% of lesion area covered with masses of conidia, 3 = about 50%, 4 = about 75%, and 5 = about 100% of lesion area covered. Scaled values are presented as a sporulation index (SI).

RESULTS

Isolates of *C. trifolii* ranged from avirulent to slightly virulent in strawberry stolons (Table 2) but failed to cause lesions in strawberry and apple fruits. Maximum mean lesion length was less than 5 mm, and no lesions girdled stolons. Isolates of *C. coccodes* were slightly (CP-241 from potato) to highly (C-115 from tomato) virulent in stolons. Isolate C-115 caused lesions that completely girdled stolons and caused the daughter plants to wilt and die. Isolate C-115 was also virulent in apple fruits and sporulated profusely (SI 2.5), whereas the other three isolates did not. Lesions that developed in strawberry fruits were similar in appearance to lesions caused by strawberry anthracnose isolates. Although *C. dematium* caused lesions to form in strawberry and apple fruits, no stolon lesions developed and no sporulation occured on apple lesions.

All isolates of *C. fragariae* were highly virulent in stolons and all caused lesions in strawberry and apple fruits, but only isolates SXE and CF-4 sporulated profusely (SI 3.0 and 2.9, respectively) on apple lesions. Isolates designated as *G. cingulata* ranged from slightly to highly virulent (except isolate GC-4-S, a subculture of a GC-4 colony sector) in stolons and caused lesions in strawberry and apple fruits. Sporulation on apple lesions varied from none (isolates GC-4 and GC-8-S, a subculture of a GC-8 colony sector) to moderately profuse with isolates GC-8 and GC-10 (SI 2.9 and 2.2, respectively).

Isolates of *C. gloeosporioides* were moderately to highly virulent in stolons and caused lesions to develop in strawberry and apple fruits, except isolate GL-T-78 did not produce lesions

Table 2. Infection of strawberry stolons and fruit and apple fruit by isolates of several anthracnose fungi

Genus Species	Isolate	Strawberry infection		Apple fruit infection	
		Stolon disease index	Fruit lesions (+ or −)	Lesion diameter (mm)	Sporulation index
Colletotrichum trifolii	M505	2.0[a]	−	0	0.0[b]
	M517	1.0	−	0	0.0
	M531	1.0	−	0	0.0
	CT-Rel	2.3	−	0	0.0
	CT-5-3	3.0	−	0	0.0
	CT-271	3.0	−	0	0.0
coccodes	CP-8	4.5	+	10	0.0
	CP-241	3.0	+	5	0.0
	C-13	5.0	+	13	0.0
	C-115	9.0	+	46	2.5
dematium	CD-1	1.0	+	44	0.0
fragariae	C-73	9.0	+	39	0.0
	C-73-S	9.0	+	43	0.0
	C-131	9.0	+	53	0.0
	C-132	9.0	+	47	0.0
	SXE	9.0	+	63	3.0
	CF-4	9.0	+	57	2.9
	CF-4-S	9.0	+	55	0.8
	CF-1	9.0	+	55	0.0
	CF-14	9.0	+	45	0.0
	CF-6	9.0	+	38	0.2
	CF-6-S	9.0	+	28	0.0
gloeosporioides	C-66	4.0	+	40	0.0
	C-85	5.0	+	31	0.0
	C-91	7.5	+	46	2.0
	C-101	9.0	+	41	1.6
	GL-St	5.0	+	36	1.7
	GL-Bb	7.5	+	46	2.9
	GL-T-77	9.0	+	38	1.4
	GL-B1	9.0	+	38	1.7
	GL-D	8.2	+	39	1.1
	GL-Ap-78	9.0	+	40	1.6
	GL-T-78	5.7	+	0	0.0
	GL-G139	9.0	+	39	2.0
Glomerella cingulata	GC-4	6.0	+	24	0.0
	GC-4-S	1.0	+	...	...
	GC-8	3.0	+	45	2.9
	GC-8-S	6.0	+	22	0.0
	GC-9	9.0	+	43	2.8
	GC-10	6.0	+	44	2.2
	GC-10-S	9.0	+	39	1.0

[a] Stolon lesion development indexed on a scale of 1–9, where 1 = no lesions, 2 = streaking only, 3 = lesions less than 5 mm long, 4 = lesions 5 mm long × 1 mm wide, 5 = lesions 10 mm long × 2 mm wide, 6 = lesions 15–20 mm long × 2–3 mm wide, 7 = lesions 25 mm long and nearly girdling stolon, 8 = lesions more than 25 mm long and stolon girdled, and 9 = lesions more than 25 mm long, stolon girdled, and daughter plant distal to the lesion dead.

[b] Area of sporulation on lesions was indexed on a scale of 1–5, where 1 = no sporulation, 2 = about 25% of lesion area covered with masses of conidia, 3 = about 50%, 4 = about 75%, and 5 = about 100% of lesion area covered.

on apple fruits. Sporulation on apple fruits was moderate (SI 1.1) to profuse (SI 2.9), except isolates C-66 and C-85 did not sporulate.

Results among apple cultivars were pooled for each isolate because no significant differences occurred among cultivars.

DISCUSSION

Morphological and pathological variation occurs among isolates of *G. cingulata* (18–20), *C. gloeosporioides* (8,15,22), *C. fragariae* (6,7,9,15), *C. acutatum* (15,19,21), and *C. coccodes* (1,15). Other than for *C. fragariae* (6,7), little attention has been given to variation in pathogenicity and virulence of anthracnose fungi to strawberry. In this study, isolates of *G. cingulata* and several *Colletotrichum* spp. varied in pathogenicity and virulence in strawberry stolons and fruits and apple fruits. Isolates of *C. trifolii* were not pathogenic to strawberry or apple. The single isolate of *C. dematium* (from strawberry fruit) was not pathogenic to stolons but did cause lesions in strawberry and apple fruits. This varies from an earlier report (1) that an isolate of *C. dematium* from tomato did not produce lesions in apple fruits. *C. coccodes* is not a pathogen of strawberry in nature; however, isolates ranged from moderately to highly virulent in stolons and caused lesions in strawberry and apple fruits. Barksdale (1) reported that one of several isolates of *C. coccodes* failed to cause lesions on strawberry fruits but lesions were produced in apple by all isolates. In stolons, isolate C-115 of *C. coccodes* produced symptoms very similar in appearance and development to those caused by *C. fragariae*. Individual isolates of *G. cingulata* and *C. gloeosporioides* reacted similarly in that isolates GC-9, GC-10-S, C-101, GL-T-77, GL-B1, GL-Ap-78, and GL-G139 were highly virulent in stolons and strawberry and apple fruits. Sporulation on apple lesions varied among isolates of *G. cingulata* and *C. gloeosporioides* from none to profuse.

Some authors have treated *C. fragariae* as a form of *C. gloeosporioides* (15,22). Although it is not our purpose to challenge these viewpoints, it is apparent that if a high degree of virulence is used as a criterion for classification, the isolates of *C. fragariae* represent a distinct group. Evidence that isolates GC-9 (from grape), GC-10-S (a sector subculture from a less virulent parent colony of GC-10 from strawberry fruit), C-101 and GL-T-77 (from tomato), GL-B1 and GL-G139 (from blueberry), and GL-Ap-78 (from apple) were apparently as virulent in strawberry as the *C. fragariae* isolates may reflect the naturally existing variability among isolates of *Colletotrichum* and *Glomerella* species. Many of these isolates may be pathogenic to strawberry under favorable environmental conditions. Isolates of *G. cingulata* cause strawberry fruit rot (14) and crown death (C. M. Howard, *unpublished*) in Florida.

Delp and Milholland (6,7) considered plants susceptible to *C. fragariae* if lesions in petioles were longer than 3–10 mm 14–21 days after inoculation. If lesion development in petioles corresponds to that in stolons, plants in our study receiving a lesion rating of 4 or greater are susceptible. Correspondingly, any isolate eliciting lesions with severity scores of 4 or greater is considered virulent. This would include three of four isolates of *C. coccodes*, all of *C. fragariae* and *C. gloeosporioides*, and five of seven isolates of *Glomerella cingulata*.

Subculturing naturally occurring sectors from colonies in culture often resulted in isolates equally as virulent or more virulent than the parent isolate. Although isolate GC-4-S did not cause stolon lesions, sector subcultures of C-73, CF-4, and CF-6 retained their pathogenicity and high level of virulence and GC-8-S and GC-10-S were more virulent than their parent isolates. These data may have an impact on results obtained after repeated maintenance subculturing of isolates for evaluation of germ plasm or cultivar resistance (6,7) and establishment of pathogen races (9). Although these sector subcultures have been tested only under artificial conditions, they do illustrate the high degree of variability that may be encountered in anthracnose fungi.

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