Morphological, Cultural, and Pathogenic Variation Among *Colletotrichum* Species Isolated from Strawberry

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ABSTRACT

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Conidia, cultural characteristics, appressoria, and setae of 24 isolates of *Colletotrichum* species from strawberry were compared. The virulence of each isolate on plants of 14 strawberry cultivars and one breeding clone was evaluated. Thirteen isolates identified as *C. fragariae* produced cylindrical conidia; developed beige to olive to dark gray colonies, generally with dark olive to dark gray reverse colony colors; and did not form the ascigerous state in culture. Two isolates identified as *Glomerella cingulata* (anamorph: *C. gloeosporioides*) developed gray or olive-gray colonies with dark gray to dark olive reverse colony colors, produced cylindrical conidia, and formed the ascigerous state in culture. Nine isolates identified as *C. acutatum* produced fusiform conidia and developed pink, orange, rose, or beige colonies with predominantly cream, pink, or rose reverse colony colors; none formed an ascigerous stage in culture. *C. acutatum* isolates could be easily differentiated from *C. fragariae* and *C. gloeosporioides* isolates by their growth rate in plate culture on potato-dextrose agar. The greatest difference in growth rate occurred at 32 C, where the average diameter of 5-day-old *C. acutatum* cultures was 13 mm, compared with 69 mm for *C. fragariae* and 63 mm for *C. gloeosporioides*. All of the *C. fragariae* isolates induced disease symptoms when wound-inoculated into strawberry leaves and fruit, whereas all of the *C. acutatum* isolates caused fruit rot but none caused leaf lesions. All 13 of the *C. fragariae*, four of the five *C. acutatum*, and one of the two *C. gloeosporioides* isolates tested caused a crown rot of certain strawberry cultivars. Disease severity ratings after plant spray inoculations resulted in a highly significant isolate × cultivar interaction, suggesting that some isolates may represent different races among the tested isolates of *C. fragariae* and *C. acutatum*. Overall, *C. fragariae* isolates caused more severe petiole and crown symptoms than did *C. acutatum* isolates, which in turn caused more severe symptoms than did *C. gloeosporioides* isolates. However, some cultivars were more susceptible to certain *C. acutatum* isolates than to some *C. fragariae* isolates, e.g., the cultivar Sunrise was susceptible to *C. acutatum* isolates Goff and Mil-1 but resistant to *C. fragariae* isolate Fla-2.

Colletotrichum fragariae Brooks was identified as the causal organism of strawberry (*Fragaria* × *ananassa* Duch.) anthracnose in Florida in 1931 (3), and its role in crown rot and wilt was demonstrated in 1932 (4). Subsequently, *C. fragariae* was implicated as the cause of stolon, petiole, and fruit lesions in addition to crown rot and summer wilt of plants (5,6,13,14,16–18,31) throughout the southeastern United States. Recently, the name "anthracnose crown rot" was suggested to distinguish the disease caused by *C. fragariae* from those caused by other species of *Colletotrichum* (31).

Some uncertainty has been expressed about the taxonomic status of *C. fragariae*. *C. fragariae* fits within the group species *C. gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulata* (Stonem.) Spauld. & Schrenk) and was included in this group by von Arx (37). However, more recent research reports dealing with *Colletotrichum* species from strawberry have suggested that *C. fragariae* should be considered a separate species (17–19,23).

Fungi other than *C. fragariae* and *C. gloeosporioides* also have been reported to cause anthracnose diseases of strawberry. These include *C. acutatum* Simmonds (7,28,30), *C. dematium* (Pers. ex Fr.) Grove (2), and *Gloeosporium* species (21,33,34,38). *Gloeosporium* species have been reported to cause a ripe fruit rot as well as stolen, petiole, peduncle, and pedicel lesions of strawberries in Australia (33,34); a fruit rot of strawberries shipped from Louisiana to Chicago (38); and a fruit rot in fields in Maryland (21). Subsequently, Simmonds (28) included the Australian *Gloeosporium* sp. from strawberry in the recently established species *C. acutatum*, whose fusiform conidia distinguished it from other *Colletotrichum* species with cylindrical conidia (35). Since then, *C. acutatum* has been reported in England to cause petiole and runner lesions as well as a fruit rot on strawberry plants obtained from California (7). More recently, *C. acutatum* also has been shown to cause a crown rot and wilt of strawberry plants (30).

In his early work on *C. fragariae*, Brooks (3–5) did not mention the cultivars on which this disease occurred in Florida or if the degree of susceptibility varied among cultivars being grown at that time. Since then, however, there have been reports of variation among strawberry cultivars and breeding clones in their degree of susceptibility to *C. fragariae*. Horn et al (12) separated eight isolates of *C. fragariae* into three races using six breeding clones and two cultivars of strawberries as indicators. Delp and Milholland (9) found a wide range of disease responses among 16 cultivars and three breeding clones to infection by 10 isolates of *C. fragariae* but did not attempt to separate the isolates into races. Sudden shifts year to year in anthracnose crown rot severity on certain strawberry cultivars and disease reactions in field trials at Poplarville, Mississippi, (29) that are inconsistent with published reactions for certain cultivars (Table 1) provide additional evidence for the occurrence of races of *C. fragariae*.

As part of a screening program (32) to identify plants resistant to *C. fragariae*, many *Colletotrichum* isolates from strawberry plants and fruit were collected from various locations in the southeastern United States and California (Table 2). There was a wide range in the cultural and morphological characteristics among the isolates, and it became apparent that some isolates in the collection were not conspecific with *C. fragariae*. The present study was initiated: 1) to characterize the variations among these isolates and to identify them to species using criteria established in the current literature, 2) to examine the dis-

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ease responses of several strawberry clones and cultivars to some of these isolates, and 3) to provide a better basis for selection of isolates of these fungi to use as inoculum in a disease resistance breeding program.

MATERIALS AND METHODS

Fungal cultures. Twenty-four isolates of *Colletotrichum* from strawberry collected over a period of 16 yr from the southeastern United States (Table 2) were used in this study. Single-spore cultures were derived from each isolate, tested for pathogenicity, and stored at 4 C on silica gel (27). A fresh culture of each isolate was started before the beginning of each study. All culture media used were commercial preparations by Difco (Detroit, MI).

Production of the ascigerous state. An attempt was made to induce each *Colletotrichum* isolate to produce the ascigerous state. Four plate cultures of each isolate were grown on each of the following media: potato-dextrose agar (PDA), cornmeal agar, nutrient agar, Czapek solution agar, Sabouraud dextrose agar, PDA:oatmeal agar (1:1, v/v) (PDA:OMA), A-PDA (PDA acidified with 2 ml of 88% lactic acid per liter), and M-PDA (PDA amended with 5 g of powdered milk per liter). The cultures were grown under continuous fluorescent light and examined for perithecial formation after 3 wk. In a separate study, each isolate was paired with every other isolate on PDA plates. Four plate cultures of each isolate pair were grown under continuous fluorescent light at room temperature and examined weekly over a period of 6 wk for perithecial formation.

Conidial characteristics. Each isolate was grown on PDA for 9–12 days under continuous fluorescent light at room temperature (approximately 25 C). A conidial suspension was prepared in sterile distilled water, and the shape of conidia of each isolate was determined by examining 100 randomly chosen conidia and placing each in one of three shape categories: 1) fusiform, sides tapered to a point on both ends; 2) cylindrical, sides straight with conidia pointed on one end and rounded on the other; and 3) cylindrical, sides straight with conidia rounded on both ends. Conidial size of each isolate was determined by measuring the length and width of 25 randomly chosen conidia from each of four subcultures of the same isolate and was expressed as a range of means.

Conidial color of each isolate was determined by examination of a conidial mass against a white ceramic background. Conidia for this purpose were scraped from 14-day-old plate cultures grown on PDA:OMA under continuous fluorescent light at room temperature. The study was conducted four times.

Appresorial characteristics. Appressoria were studied using a slide culture method modified from Hawksworth (10). Slide cultures were made of each

Table 1. Strawberry cultivars and clones utilized in this study and their reported responses to *Colletotrichum fragariae*

Strawberry cultivar or clone	Origin	Reported disease response[a]		
		Field observations		Greenhouse tests
		Published[b]	USDA-MS[c]	
Albritton	North Carolina	VS	U	S[d]
Apollo	North Carolina	I	I	I[d]
Cardinal	Arkansas	U	I	U
Florida Belle	Florida	R	S	U
Florida 90	Florida	U	I	I[d]
MSUS 70	Mississippi	U	R	R[e]
Prelude	North Carolina	S	U	S[d]
Rosanne	North Carolina	R	U	I[d]
Sequoia	California	R	R	R[d]
Sunrise	Maryland	S	I	S[d]
Surecrop	Maryland	VS	U	U
Tangi	Louisiana	S	U	U
Tennessee Beauty	Tennessee	VS	U	I[d]
Tioga	California	VS	S	U
Titan	North Carolina	VS	U	I[d]

[a] VS = very susceptible, S = susceptible, I = intermediate, R = resistant, U = unknown.
[b] From Maas, J. L. (22).
[c] Unpublished data from 1981–1982 strawberry yield trials at USDA-ARS Small Fruit Research Station, Poplarville, Mississippi.
[d] From Delp, B. R., and Milholland, R. D. (9).
[e] Anthracnose-resistant selection from USDA-ARS greenhouse screening and field testing at Poplarville, Mississippi.

Table 2. Source and designation of *Colletotrichum* isolates obtained from strawberry plants

Isolate designation	Isolated by, at	State of origin[a]	Isolated from	Year isolated
CF-1	N. Horn, Louisiana State University	Louisiana	Crown	1968
CF-4	R. Milholland, North Carolina State University	North Carolina	Crown	1978
Fla-1	C. Howard, University of Florida	Florida	Crown	1978
Fla-2	C. Howard, University of Florida	Florida	Crown	1978
MS-9	B. Smith, USDA, Mississippi	Mississippi	Crown	1978
La-1	N. Horn, Louisiana State University	Louisiana	Crown	1979
La-2	N. Horn, Louisiana State University	Louisiana	Crown	1979
CF-card	B. Smith, USDA, Mississippi	Mississippi (North Carolina)	Crown	1980
CF-56	B. Smith, USDA, Mississippi	Mississippi	Crown	1981
CF-63	B. Smith, USDA, Mississippi	Mississippi	Crown	1981
CF-75	B. Smith, USDA, Mississippi	Mississippi	Crown	1981
Ark C-1	R. Sterne, University of Arkansas	Arkansas	Crown	1982
Ark P-1	R. Sterne, University of Arkansas	Arkansas	Petiole	1982
CF-167	C. Howard, University of Florida	Florida (Arkansas)	Fruit	1982
CG-162	C. Howard, University of Florida	Florida	Crown	1982
CG-163	C. Howard, University of Florida	Florida (Tennessee)	Crown	1982
CG-164	C. Howard, University of Florida	Florida (North Carolina)	Crown	1982
Goff	W. Goff, Southwest Missouri State University	Missouri (Arkansas)	Petiole	1982
Mil-1	B. Smith, USDA, Mississippi	Mississippi (Arkansas)	Crown	1983
Mil-2	B. Smith, USDA, Mississippi	Mississippi (Arkansas)	Fruit	1983
Cal A	S. Wilhelm, University of California	California	Fruit	1984
Cal B	S. Wilhelm, University of California	California	Fruit	1984
Cal C	S. Wilhelm, University of California	California	Fruit	1984
Cal D	S. Wilhelm, University of California	California	Fruit	1984

[a] Location of production field or nursery in which plant was growing at time isolate was obtained (location of nursery if different from production field).

isolate by placing a drop of cooled, molten water agar (25 g of agar per liter) on a sterilized slide and transferring a mycelial tip or a drop of conidial suspension onto the agar. A sterile coverslip was placed over the seeded water agar, and the slide culture was held in a petri dish that served as a moisture chamber for 6–8 days, after which appressorial formation against the coverslip was examined microscopically. A minimum of 25 appressoria were examined from each isolate.

Setae production. Production of setae in culture was determined by examining 7- to 17-day-old cultures of each isolate grown under continuous fluorescent light on either PDA or PDA:OMA. Presence or absence of setae in culture was confirmed by microscopic examination of colonies. Setae production on the host was determined by examination of epidermal strips from lesions formed on the petiole 10–20 days after inoculation of Albritton, Tangi, or Tioga strawberry plants. Plants were wound-inoculated by placing a drop of inoculum on the petiole and pricking the petiole three times through the drop with a No. 0 insect pin. If setae were present in the resulting lesion, 10 randomly chosen setae from different acervuli on Tioga plants were measured. Each study of setae production was repeated four times.

Cultural characteristics and temperature response. Single colonies of each isolate were initiated by inverting a 4-mm mycelial plug onto PDA in glass petri dishes at room temperature under continuous fluorescent light. Color was determined by examining the cultures against a white background after 8 days' growth.

The effect of temperature on the radial growth of eight of the isolates was determined. Four plate cultures of each isolate were grown on PDA in the dark at 8, 12, 16, 20, 24, 28, 32, and 36 C. Colonies were initiated with an inverted 4-mm mycelial plug from a PDA plate culture of each isolate. The test plates were incubated at room temperature for 1 day before being placed into incubators. The diameter of each resultant colony was measured after 5 days' growth at each temperature. The experiment was performed twice.

Inoculum and inoculation. Fungal isolates were grown on PDA:OMA in petri dishes for 7–14 days at room temperature under continuous fluorescent light. Conidia for inoculum were washed from the surface of the plates and suspended in sterile distilled water containing two drops of Tween 20 per liter. Inoculum was adjusted to 1.5×10^6 conidia per milliliter using a hemacytometer and applied to the plants as a spray to runoff (31). Immediately after inoculation, plants were placed in a dew chamber with near 100% RH at 32 ± 1 C for 48 hr, then returned to the greenhouse (31).

Strawberry plants. Plants of all strawberry cultivars except Tangi were purchased as dormant crowns from commercial nurseries, planted in 10-cm pots in a 1:1 (v/v) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand, and grown for a minimum of 6 wk before inoculation. During this period the plants were observed to be sure they remained free from symptoms of anthracnose crown rot. Runner plants of the breeding clones and Tangi were taken from stock mother plants in the greenhouse and grown for a minimum of 12 wk before inoculation. All plants were maintained in a greenhouse at about 28 C as previously described (31).

Plant tissue susceptibility. The capability of each of the isolates to cause a fruit rot was tested using fruit from a flat of strawberries purchased at a supermarket in Plant City, Florida. Unblemished fruit were surface-sterilized by immersing them first in 95% ethyl alcohol for 1 min, then in 0.525% sodium hypochlorite solution for 20 min, and rinsing in sterile distilled water four times, followed by air-drying (14). Four fruit were placed individually in sterile 100-ml beakers and inoculated with each fungal isolate by placing one drop of inoculum on the side of each fruit. Controls consisted of 12 fruit handled similarly but treated with a drop of sterile distilled water. The beakers containing the inoculated fruit were held in closed plastic refrigerator boxes containing moistened paper towels for 5 days at room temperature under continuous fluorescent light. Fruit rot development was rated positive if a firm, tan rot developed at the inoculation site on the fruit. Isolations were made from at least two of the lesions caused by each isolate to confirm the identity of the pathogen.

The capability of each isolate to infect Tangi and Tioga strawberry plants was first tested by wound inoculation of leaves and petioles of two plants of each cultivar by pricking through an inoculum drop (1.5×10^6 conidia per milliliter) with an insect pin. Plants were placed in an unlighted dew chamber at 30 C for 48 hr after inoculation and returned to the greenhouse at about 28 C for disease development. Disease responses were recorded 10 and 15 days after inoculation. A leaf spot ≥3 mm in diameter and a petiole lesion ≥3 mm long were considered positive responses to individual tests.

Three separate pathogenicity studies were conducted. The first included plants of 13 cultivars and three breeding clones inoculated with 12 of the *Colletotrichum* isolates, the second included plants of six cultivars and one breeding clone inoculated with 15 *Colletotrichum* isolates, and the third included plants of 14 cultivars and one breeding clone inoculated with 20 *Colletotrichum* isolates. Conidial suspensions were applied as a spray to the point of runoff using a hand pump sprayer. Each of the isolates was used to inoculate four plants of each cultivar or clone in each study to determine the disease reaction of each host/pathogen combination.

Disease severity rating (DSR). Inoculated plants were evaluated for severity of disease expression 30 days after inoculation on a scale ranging from 0 to 6 (31). Rating categories were: 0 = plant with no visible lesions, 1 = plant with petiole lesions <3 mm long, 2 = plant with petiole lesions 3–10 mm long, 3 = plant with petiole lesions >10–20 mm long, 4 = plant with petiole lesions >20 mm long, 5 = plant whose youngest leaf was wilted with or without petiole lesions, and 6 = dead plant with necrotic crown. Plants receiving an average DSR of 2.0 or less were considered resistant, those receiving an average rating of 4.0 or greater were considered susceptible, and those with an average rating between 2.1 and 3.9 were considered intermediate.

Statistical analyses of data. The SAS statistical package (11) was used to conduct analysis of variance tests. Separation of treatment means was by least significant difference.

RESULTS

Production of the ascigerous state. Of the *Colletotrichum* isolates studied for perithecial production, only Ark P-1 and CG-162 produced the ascigerous state. Both isolates produced perithecia containing asci and ascospores on all media except cornmeal agar. None of the other isolates produced perithecia either alone or in any of the paired combinations. Furthermore, most of the isolates have been observed in culture under various temperature and light conditions and on various media, including plant decoction media, for over 6 yr. During this time, only isolates Ark P-1 and CG-162 have been observed to routinely produce the teleomorph in culture. Isolate CG-162 also occasionally produced the teleomorph on the host. The perithecia, asci, and ascospores of both isolates fit the description of *G. cingulata* (26).

Conidial characteristics. Conidia of all *Colletotrichum* isolates in this study were pink to orange in mass. Because the range of colors among isolates overlapped, color of conidia could not be used as a means of separation.

The isolates were separated on the basis of conidial morphology into two groups: cylindrical-spored and fusiform-spored (Table 3). Fifteen isolates were classified as cylindrical-spored and nine as fusiform-spored. Among the cylindrical-spored isolates were the two that had produced the ascigerous state, Ark P-1 and CG-162. The conidial size and shape of these isolates fit the description for conidia of *C. gloeosporioides*, the anamorph of *G. cingulata* (26,37). The

conidia of these isolates were cylindrical, and 92% were pointed on one end (Fig. 1C). Conidial size of the two isolates, expressed as a mean for each isolate, was 12.9–16.1 μm long and 4.4–5.4 μm wide. These two isolates will be referred to as *C. gloeosporioides* in the remainder of this paper.

Conidial size and shape of the remaining 13 cylindrical-spored isolates fit the description for conidia of *C. fragariae* (3). Conidia with one end pointed predominated in all 13 isolates, constituting 80–100% of the conidia in each isolate, for an average of 89% for all isolates (Fig. 1B). The remaining conidia were rounded on both ends. Conidial size, expressed as a mean for each isolate, was 12.4–15.0 μm long and 4.4–5.2 μm wide. These 13 isolates will be referred to as *C. fragariae* in the remainder of this paper. Conidia of the *C. fragariae* isolates used in this study were compared with conidia of two *C. fragariae* isolates obtained from the American Type Culture Collection (ATCC, Catalogue of Strains I, 15th ed., 1982, Rockville, MD). The conidia of ATCC isolates 21313 and 21315 were generally of the same shape and size as those of the *C. fragariae* cultures in this collection. Conidia of ATCC isolate 21313 averaged 16.6 μm long and 5.0 μm wide, and those of ATCC isolate 21315 averaged 16.7 μm long and 5.1 μm wide.

Conidial size and shape of the remaining nine isolates fit the description for conidia of *C. acutatum*. Fusiform conidia, i.e., conidia tapered to a point on both ends, were predominant in all nine isolates, constituting 64–100% of the conidia of each isolate, with an average of 87% for all isolates (Fig. 1A). The remaining conidia were either rounded on one end and tapered to a point on the other or rounded on both ends. Conidial size, expressed as a mean for each isolate, was 12.3–14.7 μm long and 4.6–5.3 μm wide. These nine fusiform-spored isolates will be referred to as *C. acutatum* in the remainder of this paper. Conidia of these isolates were compared with conidia of ATCC isolate 26255 of *C. acutatum* and were found to be of similar size and shape. Conidia of isolate 26255 had an average length of 12.3 μm and an average width of 4.4 μm.

Appressorial characteristics. Because only a few isolates produced mycelial appressoria, shapes of appressoria formed by germinating conidia were compared. Generally, appressoria of *C. fragariae* and *C. gloeosporioides* isolates were similar and slightly more lobed or clavate than those of *C. acutatum* isolates. However, there was considerable overlapping of appressorial types among the three species. The *C. fragariae* and *C. gloeosporioides* isolates usually produced appressoria 2–3 days sooner than did the *C. acutatum* isolates.

Setae production. All nine *C. acutatum* isolates and the two *C. gloeosporioides* isolates failed to produce setae in culture or on the host, whereas all 13 *C. fragariae* isolates produced setae on the host and seven produced them in culture (Table 3). The setae were one- to two-septate and sparse to abundant on the host, depending on the isolate. The mean length of setae produced on Tioga was 72 μm for the *C. fragariae* isolates, and average length ranged from 45 μm for isolate CF-card to 107 μm for isolate La-1. The width of setae of the 13 isolates ranged from 3.7 μm for isolate CF-4 to 5.6 μm for isolate CF-1, and the mean was 4.3 μm.

Setae of several *C. fragariae* isolates formed conidia under certain conditions (Fig. 1D and E). Within the same acervulus, conidial production occurred both at the tips of setae and on short, hyaline conidiophores. Sporulation at the tips of setae was enhanced by incubating excised infected petioles in a moisture chamber for 24–48 hr. During this incubation period, setae would become hyaline at the tip and begin producing conidia.

Cultural characteristics and temperature response. Colony color of the *C. fragariae* isolates varied from beige to olive to dark gray when plate cultures were viewed from above, and those of the *C. gloeosporioides* isolates were gray

Table 3. Morphological and cultural characteristics of *Colletotrichum* isolates from strawberry plants and disease reaction of different strawberry tissues after inoculation with conidial suspension of these isolates

Species / Isolate	Conidial shape	Setae		Colony color[a]	Strawberry tissue		
		In culture	On plant		Fruit[b]	Petiole[c]	Leaf[c]
C. fragariae							
CF-56	Cylindrical	+[d]	+[e]	Olive, dark gray	+	+	+
La-1	Cylindrical	+	+	Olive, dark gray	+	+	+
La-2	Cylindrical	−	+	Olive, dark gray	+	+	+
CF-75	Cylindrical	+	+	Olive, dark gray	+	+	+
CF-63	Cylindrical	−	+	Brown, gray	+	+	+
MS-9	Cylindrical	+	+	Olive, dark gray	+	+	+
CG-163	Cylindrical	−	+	Olive, gray, black	+	+	+
CF-card	Cylindrical	+	+	Olive, gray, white	+	+	+
CG-164	Cylindrical	−	+	Olive, dark gray	+	+	+
CF-4	Cylindrical	+	+	Beige	+	+	+
Fla-1	Cylindrical	+	+	Olive, dark gray	+	+	+
Fla-2	Cylindrical	−	+	Olive, gray	+	+	+
CF-1	Cylindrical	−	+	Olive, gray	+	+	+
C. gloeosporioides							
Ark P-1	Cylindrical	−	−	Gray	+	−	+
CG-162	Cylindrical	−	−	Olive, gray	+	+	+
C. acutatum							
Cal B	Fusiform	−	−	Orange, dark brown	+	−	−
Cal A	Fusiform	−	−	Orange, brown, white	+	−	−
Mil-2	Fusiform	−	−	Pink, orange, brown	+	+	−
Goff	Fusiform	−	−	Orange, brown	+	+	−
Ark C-1	Fusiform	−	−	Beige, orange, brown	+	−	−
Cal D	Fusiform	−	−	Beige, orange, brown	+	−	−
Cal C	Fusiform	−	−	Beige, pink, gray	+	+	−
Mil-1	Fusiform	−	−	Pink, orange, brown	+	+	−
CF-167	Fusiform	−	−	Rose, olive, gray	+	−	−

[a] Colony color of 8-day-old cultures on PDA in petri plates incubated under continuous fluorescent light.
[b] + = Fruit rot and − = no fruit rot 5 days after inoculation.
[c] Disease response of two plants each of Tangi and Tioga recorded 15 days after wound inoculation of petioles and leaf lamina; + = lesions ≥3 mm and − = lesions <3 mm.
[d] Presence (+) or absence (−) of setae on 5- to 20-day-old cultures on PDA or PDA:OMA.
[e] Presence (+) or absence (−) of setae in anthracnose petiole lesions on Albritton, Tangi, or Tioga plants, 10–20 days after wound inoculation.

to olive-gray. The reverse of the cultures of both these species was dark olive to dark gray (rarely orange and never pink or rose). Colonies of *C. acutatum* isolates usually were white during the first few days of growth and later became beige, orange, pink, or rose with a cream, pink, or rose (rarely olive) reverse (Fig. 2, Table 3).

Three isolates of *C. fragariae*, two isolates of *C. gloeosporioides*, and three isolates of *C. acutatum* were included in the study of the temperature effect on radial growth. There were no significant differences in the growth rate among isolates within any species at any temperature. The mean growth rate of the *C. acutatum* isolates was significantly less than that of the *C. fragariae* and *C. gloeosporioides* isolates at all temperatures, but the differences were greatest at 28 and 32 C (Fig. 3). The greatest radial growth for all isolates occurred at 28 C (Fig. 3). The study was repeated once, but because results were very similar, data from only one of the studies are presented.

Plant tissue susceptibility. All isolates tested of the three species included in this study caused similar anthracnose-like fruit rots (Table 3), and reisolation from lesions always yielded an isolate with characteristics similar to the one used to inoculate the fruit. All of the *C. fragariae* and both *C. gloeosporioides* isolates caused leaf spots on the cultivars Tangi and Tioga, but none of the *C. acutatum* isolates caused leaf spots (Table 3). All of the *C. fragariae* isolates caused petiole lesions after wound inoculation, whereas four of the nine *C. acutatum* and one of the two *C. gloeosporioides* isolates caused petiole lesions (Table 3).

Analysis of variance of each of the three pathogenicity studies showed significant main effects in DSR due to cultivar and to isolate. Additionally, there was a significant cultivar × isolate interaction indicating the presence of races within each of the *Colletotrichum* species. Detailed data are presented only for the third and most comprehensive of the three studies (Table 4). The *C. fragariae* isolates caused a higher overall mean disease severity rating (DSR) than did the *C. gloeosporioides* or *C. acutatum* isolates. Isolate CF-63 of *C. fragariae* caused the highest overall DSR. Of the 14 strawberry cultivars and the single breeding clone tested, Surecrop received the highest overall DSR in response to both the *C. fragariae* and *C. acutatum* isolates, and Albritton received the highest DSR to the *C. gloeosporioides* isolates. MSUS 70 received the lowest overall DSR to the *C. fragariae* isolates, and Apollo received the lowest DSR to the *C. acutatum* isolates. Both MSUS 70 and Apollo were highly resistant to the *C. gloeosporioides* isolates.

Isolates within each fungal species varied greatly in virulence to the various strawberry cultivars on which they were tested (Table 4), and there was a significant cultivar × isolate interaction within each species. No cultivar was susceptible (DSR ≥4.0) to all isolates. Isolate LA-1 of *C. fragariae* caused a susceptible response on the most (nine of 15), and isolate CG-164 on the fewest (three of 15), cultivars tested. Among the five *C. acutatum* isolates, Goff, Mil-1, and Mil-2 each caused susceptible reactions on four cultivars, whereas isolate CF-167 failed to cause a susceptible reaction on any cultivar. Isolate CG-162 of *C. gloeosporioides* caused susceptible reactions on five cultivars, but isolate Ark P-1 produced a susceptible reaction on only a single cultivar. All *C. fragariae* isolates, all *C. acutatum* isolates except CF-167, and *C. gloeosporioides* isolate CG-162 caused a crown rot and wilt of plants of at least one of the cultivars tested.

DISCUSSION

On the basis of a combination of characteristics, including conidial morphology, colony color, growth rate in culture, and presence or absence of setae, we separated the 24 *Colletotrichum* isolates from strawberry into three species: *C. fragariae*, *C. gloeosporioides*, and *C. acutatum*. There is a debate among scientists as to whether *C. fragariae* should be retained as a distinct species or be combined with *C. gloeosporioides* (15–19,23,37). Cylindrical-spored *Colletotrichum* isolates that cause strawberry anthracnose crown rot have been separated by some authors (17,18) into *C. gloeosporioides* and *C. fragariae* on the basis of presence or absence, respectively, of a perithecial state. In another study (23), these two species were distinguished on the basis of virulence in apple and strawberry fruit. Two of the 15 cylindrical isolates in our tests produced the perithecial state and were designated *C. gloeosporioides* (teleomorph: *G. cingulata*), although we found no other reliable morphological characteristic to separate these two isolates from the 13 isolates designated *C. fragariae*. The *C. gloeosporioides* isolates were much less virulent to strawberry cultivars than the *C. fragariae* isolates and failed to produce setae on inoculated tissue, whereas all *C. fragariae* isolates did.

Brooks (3) described *C. fragariae* conidia as spindle- to boat-shaped, but in his photomicrograph and drawing the conidia appear to be of two types: 1) cylindrical with one end rounded and the

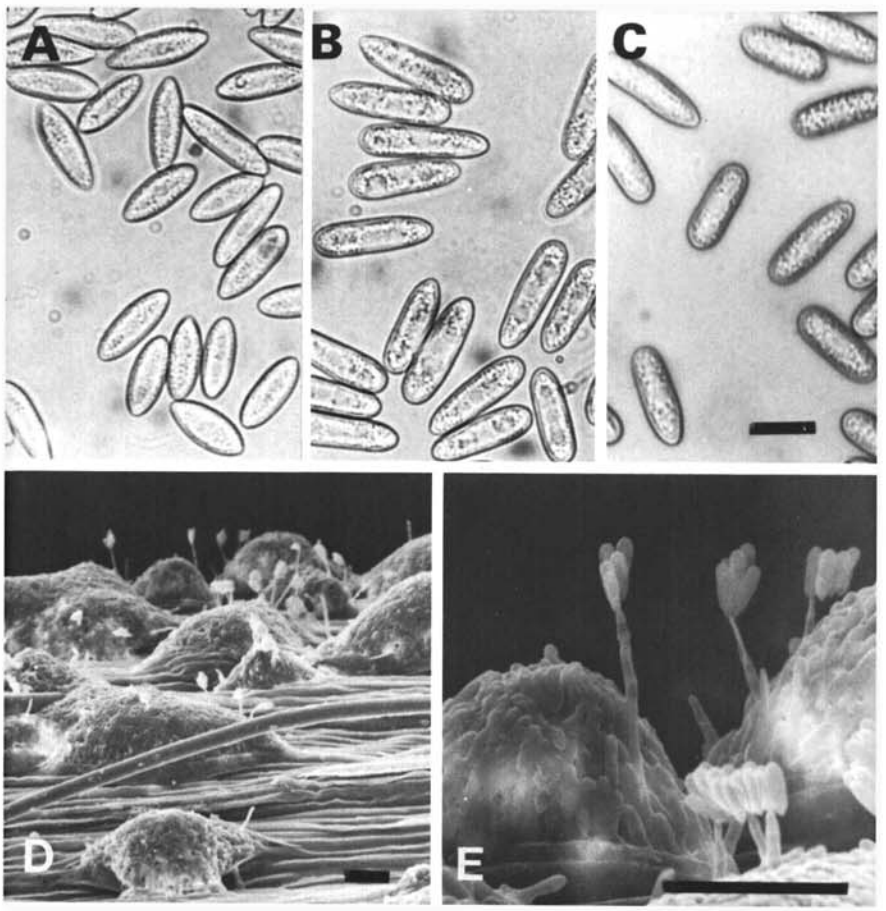

Fig. 1. Conidia of **(A)** *Colletotrichum acutatum* isolate CF-167, **(B)** *C. fragariae* isolate CF-63, and **(C)** *C. gloeosporioides* isolate Ark P-1; scale bar = 10 μm. **(D)** Scanning electron micrograph of acervuli of *C. fragariae* isolate CF-card on strawberry stolon with erumpant masses of conidia and setae bearing clusters of conidia at the tips, and **(E)** setae of *C. fragariae* isolate CF-card producing conidia; scale bars = 50 μm.

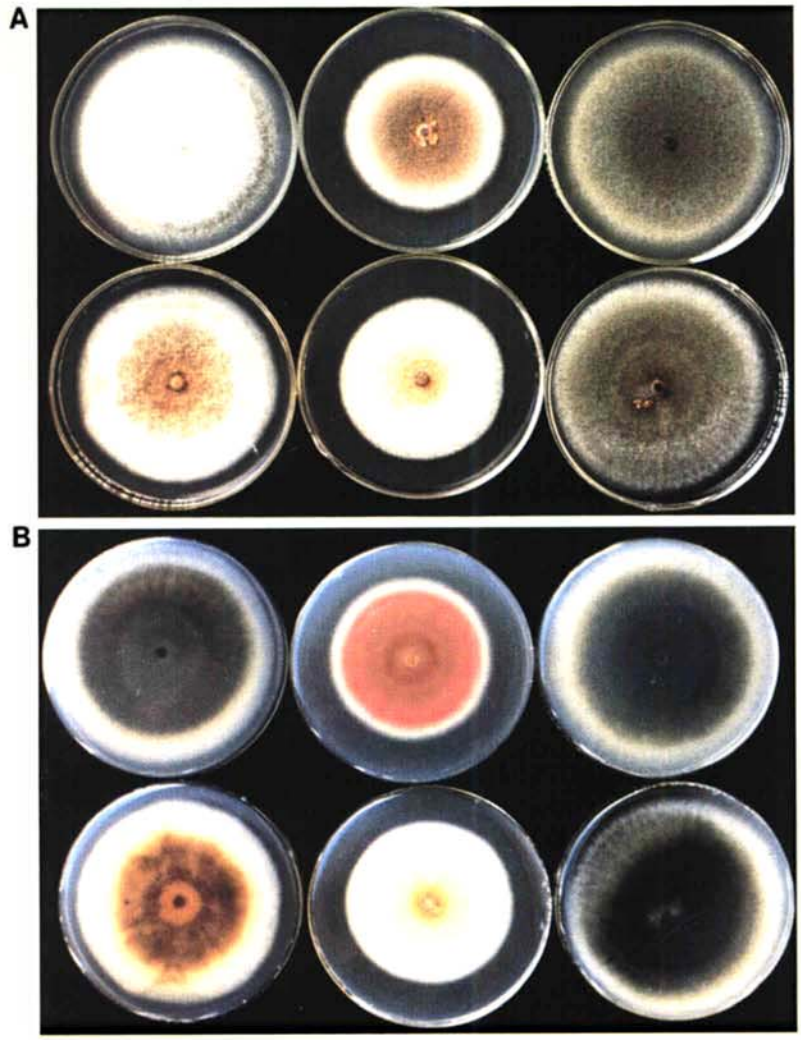

Fig. 2. (A) Upper colony surface and **(B)** lower colony surface of cultures of *Colletotrichum fragariae* isolates CF-1 (top left) and CF-63 (bottom left), *C. acutatum* isolates CF-167 (top middle) and Mil-2 (bottom middle), and *C. gloeosporioides* isolate CG-162 (top right) and Ark P-1 (bottom right) grown at room temperature (about 24 C) for 7 days under continuous fluorescent light on PDA following a 5-mm mycelial plug transfer.

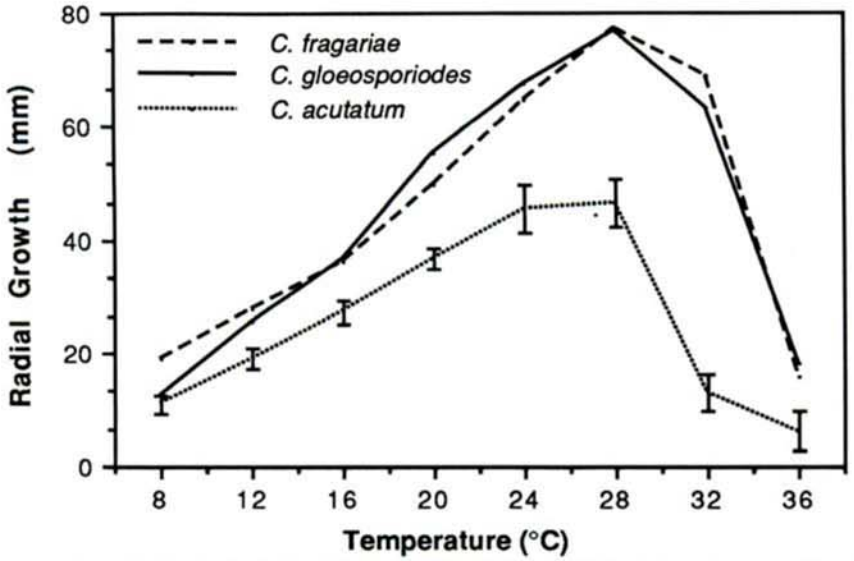

Fig. 3. Average colony diameter of 5-day-old petri plate cultures of three *C. acutatum* isolates (Goff, Mil-1, Mil-2), three *C. fragariae* isolates (CF-1, CF-63, CF-75), and two *C. gloeosporioides* isolates (Ark P-1, CG-162) grown on PDA in the dark.

other pointed and 2) cylindrical with both ends rounded. The *C. fragariae* isolates in this study were predominantly of the first shape. About half the *C. fragariae* isolates produced conidia that were shorter on the average than the lower end of the length range reported in the original description (3), but the width of the conidia of these isolates fell within the reported range.

Conidial shape is a diagnostic feature of *C. acutatum* and is described as fusiform (35). All isolates with fusiform conidia in this study were placed in *C. acutatum* even though all produced conidia wider than those in the type description. Simmonds (28) included a photomicrograph of a "larger spored form" of *C. acutatum* but did not report its size. The appressoria of the *C. acutatum* isolates in this study fit Simmonds's (28) description of the appressoria of *C. acutatum* as sparse, obovate, and rarely lobed.

The lack of setae within the acervuli of *C. acutatum* isolates agrees with Simmonds's (28) description of the species. However, Baxter et al (1) reported sparse setae production by *C. acutatum* on malt salts agar. The acervuli of *C. gloeosporioides* may be setose or glabrous (26); both of the *C. gloeosporioides* isolates in our study were glabrous. All of our *C. fragariae* isolates were setose on the host and about half were setose in culture. All *C. fragariae* isolates in Lenné's (19) studies were setose on the plant and glabrous in culture. In the original description of *C. fragariae*, Brooks (3) described the setae as sometimes having a constricted apical cell. His drawing appears somewhat like some stages of the sporulating setae observed on several of the isolates in this collection. Sporulating setae were typical of most of the *C. fragariae* isolates in our collection; few references were found to this characteristic, however. A photomicrograph by Milholland (25) and a drawing by Mena et al (24) both show what appear to be sporulating setae. In his description of *G. cingulata*, Mordue (26) indicates that conidia are occasionally borne on the setae. Lenné (19) and Lenné et al (20) found conidial production by setae to be common among several *Colletotrichum* species collected in Florida from a wide range of hosts and speculated that it might be a survival mechanism that allowed the fungus to survive cold, dry conditions (19) or it might be a spore dispersal mechanism during humid, windy conditions (20). Baxter et al (1) also reported fertile setae occurring on an isolate of *C. gloeosporioides* and speculated that this might indicate an homologous origin of setae and conidiogenous cells.

C. acutatum isolates can easily be separated from *C. fragariae* and *C. gloeosporioides* isolates by their slower

radial growth on PDA in plate culture. At 28 and 32 C, the differential growth rates of *C. acutatum* compared with those of *C. fragariae* and *C. gloeosporioides* are most obvious and provide a very useful method of separating *C. acutatum* from the other two species.

All isolates of the three species tested caused similar lesions on wound-inoculated fruit. Whereas all *C. fragariae* and *C. gloeosporioides* isolates caused lesions on wound-inoculated leaves, none of the *C. acutatum* isolates caused leaf lesions.

After spray inoculation of plants, all isolates of each *Colletotrichum* species caused severe disease symptoms on one or more of the strawberry cultivars tested. The diverse DSRs among the cultivars to individual *C. fragariae* isolates, the distinct DSRs among isolates on individual cultivars, the significant main effects due to cultivar and to isolate, and the highly significant cultivar × isolate interaction suggest that both vertical and horizontal resistance to *C. fragariae* may occur in the strawberry host lines and that races may exist among the isolates used in this study. Vanderplank (36) suggested ranking hosts by the order of their DSRs to the various isolates of a pathogen as a test for the occurrence of horizontal and/or vertical resistance. In this study, the rank of the 13 host lines based on their DSRs to individual *C. fragariae* isolates varied dramatically among the isolates tested (Table 4), suggesting the occurrence of vertical resistance within the lines. Many of the host lines also appear to possess horizontal resistance, however, because the mean DSR of the 13 host lines to all *C. fragariae* isolates ranged from 4.96 for Surecrop to 1.98 for MSUS 70. Host lines listed first in Table 4 generally received higher DSRs to most isolates, whereas the lines listed last generally received lower DSRs, suggesting the occurrence of horizontal resistance in the latter.

In view of strong evidence for the existence of races in *C. fragariae* in this study and others (9,12), it is not surprising that some of the cultivars responded differently to *C. fragariae* than has been reported in previous studies. It was surprising that Sequoia, which has been reported to be resistant to anthracnose crown rot in both field (22) and greenhouse (9) trials, was found to be susceptible to most *C. fragariae* isolates in this trial. The susceptible response of Sequoia to so many isolates in this study is most likely due to the high incubation temperature (32 C) immediately following inoculation used in this study. Earlier reports have shown that the disease response of Sequoia in particular to anthracnose crown rot is affected by incubation temperature after inoculation (8,31).

The extreme pathogenic variation among isolates of *C. fragariae* must be considered when choosing isolates to use in an anthracnose crown rot screening program. It is obvious that no single isolate should be used to do all the resistance screening. Isolates such as La-1, CF-63, Fla-1, and CF-card, which caused susceptible reactions on eight or nine of the host lines, would be reasonable choices from isolates in this collection for use in an initial screening.

Ranking the host lines according to their DSRs to the various isolates of *C. acutatum* also suggests that both vertical and horizontal resistance to this species occurs among the host lines tested. The mean DSR of the host lines to the various isolates of *C. acutatum* closely paralleled the mean DSR of the same lines to *C. fragariae*. It is evident that much of the

Table 4. Disease severity ratings[a] of plants of 14 strawberry cultivars and one breeding clone 30 days after spray inoculation with conidia from isolates of three *Colletotrichum* species

Species	Cultivar or line[b]															
Isolate	SUR	TIO	ALB	TAN	SEQ	FBL	TIT	SUN	CAR	F90	PRE	ROS	TNB	APO	M70	Mean
C. fragariae																
CF-63	5.0	4.8	5.3	4.8	4.5	3.0	4.3	4.5	3.5	3.8	3.0	4.0	3.8	3.8	1.0	3.9
La-1	5.3	5.8	5.3	4.0	4.0	3.8	4.0	5.0	2.8	3.3	2.5	4.0	1.5	2.3	4.0	3.8
Fla-1	5.5	4.0	5.0	5.5	5.5	3.8	3.5	3.5	4.0	2.5	2.5	5.3	1.8	2.3	2.5	3.8
CF-card	5.3	4.5	4.8	5.0	2.5	4.5	4.5	4.3	3.3	2.0	2.5	3.8	4.5	2.5	1.5	3.7
CF-4	5.3	4.8	4.8	3.8	3.8	3.8	4.5	3.5	3.0	3.8	3.3	3.3	2.3	3.3	1.5	3.6
La-2	5.5	5.3	4.3	3.5	3.5	4.5	3.8	4.3	3.8	2.3	4.3	3.3	1.5	1.3	2.0	3.5
CF-75	4.3	4.0	4.5	3.8	4.0	4.3	5.0	3.8	2.3	2.5	4.0	2.5	2.0	2.3	1.5	3.4
MS-9	4.3	4.5	4.3	3.8	4.3	4.0	3.3	2.8	4.5	2.0	3.5	2.3	1.8	2.0	2.8	3.3
CF-56	5.5	4.5	4.5	4.0	3.3	3.8	3.0	4.0	2.5	3.5	4.0	1.0	3.3	1.3	1.3	3.3
CF-1	4.0	4.5	4.0	4.0	3.8	3.3	2.8	3.3	2.5	4.3	3.3	2.0	2.8	2.0	1.5	3.2
CG-164	4.5	5.8	2.0	3.5	3.5	3.3	2.0	3.8	2.0	3.5	2.3	0.5	5.5	2.5	2.0	3.1
Fla-2	6.0	4.0	4.3	4.5	3.5	2.8	4.3	1.5	2.0	2.3	1.5	3.0	2.0	2.0	2.3	3.1
CG-163	4.3	4.5	1.8	4.5	4.3	2.5	2.8	3.5	4.5	3.5	3.0	0.5	1.5	2.5	2.0	3.0
LSD ($P = 0.05$) for host/pathogen combination = 1.76																
Mean	5.0	4.7	4.2	4.2	3.9	3.6	3.7	3.7	3.1	3.0	3.0	2.7	2.6	2.3	2.0	3.4
C. gloeosporioides																
CG-162	4.8	4.0	5.0	2.3	4.3	3.3	4.0	2.3	2.0	2.3	2.3	1.3	2.5	1.3	1.0	2.8
Ark P-1	2.5	2.0	3.3	1.3	1.0	4.0	1.3	2.0	1.5	2.5	1.5	3.0	2.8	0.5	0.8	2.0
LSD ($P = 0.05$) for host/pathogen combination = 2.00																
Mean	3.6	3.0	4.1	1.8	2.6	2.6	2.6	2.1	1.8	2.4	1.9	2.1	2.6	0.9	0.9	2.4
C. acutatum																
Goff	5.0	4.0	4.0	3.3	3.0	3.8	3.5	4.8	2.5	3.5	3.0	2.8	3.8	2.8	2.8	3.5
Mil-1	5.0	3.5	3.5	5.0	3.5	3.5	2.8	5.0	2.3	1.5	2.8	2.3	4.0	1.3	2.8	3.2
Mil-2	4.8	4.0	4.0	2.0	3.5	3.0	4.5	3.8	3.5	1.8	2.8	2.8	2.5	1.5	2.3	3.1
Ark C-1	4.8	1.8	3.8	1.0	1.8	4.0	3.3	3.8	3.0	0.3	2.3	3.5	3.3	0.3	0.5	2.5
CF-167	3.8	2.3	2.0	1.8	3.0	0.0	1.0	1.8	0.5	0.8	0.8	0.0	0.8	1.0	1.3	1.4
LSD ($P = 0.05$) for host/pathogen combination = 1.88																
Mean	4.7	3.1	3.5	2.6	3.0	2.9	3.0	3.8	2.4	1.6	2.3	2.3	2.9	1.4	1.9	2.7

[a] 0 = No visible lesions, 1 = petiole lesions <3 mm long, 2 = petiole lesions 3–10 mm long, 3 = petiole lesions >10–20 mm long, 4 = petiole lesions >20 mm long, 5 = youngest leaf wilted, and 6 = dead plant with necrotic crown. Average DSRs of four plants are listed.

[b] SUR = Surecrop, TIO = Tioga, ALB = Albritton, TAN = Tangi, SEQ = Sequoia, FBL = Florida Belle, TIT = Titan, SUN = Sunrise, CAR = Cardinal, F90 = Florida 90, PRE = Prelude, ROS = Rosanne, TNB = Tennessee Beauty, APO = Apollo, M70 = MSUS 70 (breeding clone).

anthracnose crown rot resistance observed among the host lines is expressed against isolates of both *Colletotrichum* species.

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