

## Cultural Characteristics and Pathogenicity of *Glomerella cingulata* Isolates from Apples in Alabama

A. J. LATHAM, Department of Botany, Plant Pathology, and Microbiology, and J. C. WILLIAMS, Department of Research Data Analysis, Auburn University, AL 36849

### ABSTRACT

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Apples inoculated with the perithecial type of *Glomerella cingulata* produced orange conidial masses on apple lesions that became gray and then black after 10–12 days of incubation at 28 C. Apples inoculated with the asexual chromogenic type produced orange conidial masses on apple lesions that became reddish brown after 12 days of incubation at 28 C. Monoconidial isolates from both types were found that produced few or no conidia on apple lesions. Results similar to those found in the laboratory occurred on apples naturally infected by *G. cingulata* under orchard conditions. The overall growth rate of apple lesions caused by perithecial types was significantly greater than those of the asexual chromogenic types; however, conidial mass ratings of the asexual chromogenic type were significantly larger than those of the perithecial type.

Bitter rot, caused by *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, is a major disease of apples (*Malus sylvestris* Mill.) in the southeastern United States from mid-June into September if the weather is hot and moist (7,8). Several strain types of *G. cingulata* have been reported, but a clear description of their relationship with disease symptoms and signs has not been presented.

Clinton (2) reported that black acervuli (acervuli = pustules sensu 1–4,8,13) of *G. cingulata* appeared at the centers of apple lesions that were 6–12 mm in diameter. When apples were stored under a favorable environment, the epidermis ruptured and pinkish conidial masses formed in the acervuli. With age, the conidial masses changed to dark olive. Clinton observed that the dark color was due to germination of the *G. cingulata* conidia and growth of mycelium from the acervuli. According to von Schrenk and Spaulding (13), heavy dew and rain may wash away the pink conidial masses to expose the interior of the acervulus that appears sooty black. Roberts (8) reported that newly formed conidial masses were pink, but when dried, they became dark and had a hard, horny consistency. Later descriptions of conidial masses of the imperfect stage, *Colletotrichum gloeo-*

*sporioides* Penz. (12), indicate that they darken and weather-off, exposing dark brown to black underlying tissues (1,3,4,6).

In Edgerton's (5) study, nearly all *G. cingulata* isolates known to have perfect stages were of southern origin. On potato-glucose agar, they were characterized by very rapid growth and a very dark, greenish black color of the substrate and aerial hyphae; the northern forms grew more slowly, had little dark color, and were colored pink from the profuse development of conidia. Differences in pathogenicity between the two forms were not discussed. The cultural types of *G. cingulata* defined by Struble and Keitt (10) on potato-dextrose agar (PDA) were perithecial (plus = light, minus = dark); asexual chromogenic, conidial (pink to nearly red in color); and asexual nonchromogenic, conidial (light cream-colored). Studies on the cultural characteristics and pathogenicity of several isolates of *G. cingulata* from apples in Alabama are reported.

### MATERIALS AND METHODS

**Isolates.** Perithecial isolates of *G. cingulata* were obtained from apple fruit or leaves with yellow mottle (11) and necrotic lesions from the following locations: Coffee County (CC), Houston County (HC), North Alabama Horticulture Substation (NAHS), Piedmont Substation (PDS), and Wiregrass Substation (WES). In addition, asexual chromogenic isolates were obtained from CC, NAHS, PDS, and Lee County (LC).

Apple disease control test plots established on the NAHS in 1981 were surveyed to determine the frequency of different types of *G. cingulata*. Fifty

apples selected at random from each of 30 plots were stored 14 days at  $26.6 \pm 12.2$  C for rots to develop. Diseased apples were surface-sterilized in 0.525% sodium hypochlorite for 10 min, then segments of diseased tissue as large as 5 mm<sup>2</sup> were cut from small lesions and plated onto PDA and incubated 7 days at 28 C. Frequency of occurrence of the various types was recorded.

**Cultural characteristics.** Growth and cultural characteristics of *G. cingulata* mycelium on PDA (Difco Laboratories, Detroit, MI 48232) were made using a perithecial and chromogenic type from the CC, NAHS, and PDS. Pure cultures were grown on 15 ml PDA in petri plates for 7 days at 28 C for inoculum. Thirty monoconidial isolates were made from a culture from each location, then replicated three times for each type and location. A 5-mm agar-mycelial disk cut from the advancing margin of the colony was placed in the center of each PDA plate and incubated for 120 hr at 28 C. Colony diameters were measured and evaluated by analysis of variance. Analysis of variance included a comparison of the means of the perithecial and chromogenic types.

**Pathogenicity.** Golden Delicious apples were washed gently in detergent, soaked 15 min in 1.13 g/L dicofol (Kelthane) to control mites, rinsed with sterile distilled water, and dried. Treatments consisted of 10 apples for each perithecial isolate from CC, HC, NAHS, PDS, and WES and 10 apples for each chromogenic isolate from CC, LC, NAHS, and PDS. Apples were wounded (6 mm deep) once with a sterile 1-mm-diameter nail and swabbed with a water suspension of  $10^5$  to  $10^7$  conidia per milliliter from each 7-day-old culture, using a Q-tip (Chesebrough-Ponds, Inc., Greenwich, CT 06830). Ten apples that were wounded and swabbed with a Q-tip saturated with sterile water served as controls. Apples were placed in open petri dishes on filter paper and incubated 20 days in a Percival I-60 DL Dew Chamber (Percival Mfg. Co., Boone, IA 50036) programmed for 100% relative humidity (RH) at 28 C; the RH decreased to  $67 \pm 3\%$  with a 12-hr photoperiod at 5.5 klux. Disease development and lesion diameters were determined every 2 days

for 12 days after inoculation, and the amount of sporulation was estimated after 20 days.

Lesion diameters and conidial mass incidence were evaluated on mature, crisp Golden Delicious, Jonathan, Red Delicious, and Rome apples in dew chambers programmed to a temperature regime of 20 and 28 C with 12-hr photoperiods. The HC perithecial isolate and the LC chromogenic isolate were used in these evaluations. Ten apples of each cultivar were prepared, inoculated, and incubated as described. Ten apples of each cultivar that were wounded and swabbed with a Q-tip saturated with sterile water served as controls.

Monoconidial isolates from the cultural study that developed different colony morphologies were selected for pathogenicity investigations. Conidia from an isolate showing a different colony morphology were tested in 10 Golden Delicious apples prepared, inoculated, and incubated as described. Ten apples wounded and swabbed with sterile water served as controls.

Sporulation after 20 days of incubation was estimated on a 0–5 scale, where 0 = no conidial masses, 1 = up to 1% of lesion area showing acervuli and some conidia (acervuli dark or hyaline and appearing as blisters), and 2 = 2–10% of lesion area showing erumpent acervuli and orange or black conidial masses, 3 = 11–30%, 4 = 31–70%, and 5 = 71–100%. Means of isolates within types were compared by Duncan's multiple range test.

## RESULTS

**Isolates.** Fifty-eight percent of 59 *G. cingulata*-infected apples from the NAHS disease control tests had orange conidial masses and were of the chromogenic type. Of the 35% infected apples not showing conidial masses, 32% were identified as perithecial types from asci and ascospores and the other 3% as nonchromogenic types. The remaining 7% diseased apples had a few black conidial masses in the centers of the orange conidial masses and were identified as perithecial types. Conidia from two isolates of the asexual nonchromogenic type were inoculated into 10 Golden Delicious apples per isolate. The HC and LC isolates were included in the experiment. No conidial masses formed on apples inoculated with conidia from the asexual nonchromogenic type of *G. cingulata* incubated 20 days but they did on apples inoculated with conidia from either the HC or LC isolates.

Some apples with bitter rot symptoms collected from the PDS apple orchard in 1980 bore no orange or black conidial masses on the lesions. In that orchard, we also found the chromogenic type that produced orange conidial masses and the perithecial type that produced orange conidial masses that became black after nearly 2 wk of incubation.

**Cultural characteristics.** Perithecial isolates grew more rapidly than chromogenic isolates when cultured on PDA (Table 1). Colony characteristics of

chromogenic monoconidial isolates according to the PDA substrate after 5 days of incubation were as follows: A-1 = purple-red colony, A-2 = reddish brown colony, and A-3 = dark green center and green mycelial rings interspersed with orange rings of conidia. The six subcultures found from the perithecial types of *G. cingulata* were: B-1 = dark greenish black colony, B-2 = large olive-gray center surrounded by one or more white mycelial rings interspersed with olive-gray rings, B-3 = small olive-gray center with broad white margin, B-4 = pale orange and white in a zonate pattern with occasional pale greenish mottle, small dark spots throughout colony, B-5 = dark olive-green in a zonate pattern, and B-6 = orange with dark greenish black mottle, occasionally in a zonate pattern. All colonies except B-3 had narrow white margins at the colony perimeter.

**Pathogenicity.** After 4 days of incubation, Golden Delicious apples inoculated with a perithecial or chromogenic isolate of *G. cingulata* developed lesions that were caramel-colored to dark brown. Dark acervuli were present under the lesion epidermis of both perithecial or chromogenic types. Lesions averaged 14 mm in diameter for the perithecial type (HC) and 8 mm for the chromogenic type (LC) at 28 C. A yellow halo up to 4 mm wide surrounded lesions caused by either type on GD apples that were still green. After 6 days of incubation, the epidermis had ruptured over the acervuli and orange conidia were visible (Figs. 1A and 2A). After 8 days of incubation, orange conidial masses were evident on some apples of both types except the perithecial type from the PDS.

The main difference between types on day 8 was in lesion diameters. In 10-day-old lesions caused by the perithecial type, conidial masses near the wound were beginning to change from orange to gray and then black (Figs. 1B and 2B). No changes were observed in the appearance of lesions caused by the chromogenic type.

After apples inoculated with a perithecial type were incubated for 12 days, a black conidial mass developed in an area 2–3 cm in diameter centered on

**Table 1.** Growth and pathogenicity of two types of *Glomerella cingulata* isolated from apples in Alabama

Type	Colony diameters* (mm)	Pathogenicity	
		Lesion diameters (mm) <sup>x</sup>	Conidial mass ratings <sup>y</sup>
Asexual chromogenic	43.2	35.0	4.6
Perithecial	72.3 <sup>z</sup>	45.0 <sup>z</sup>	2.9 <sup>z</sup>

\* After 5 days on PDA plates; data are averages for 90 plates for each of three isolates of the asexual chromogenic type and perithecial type.

<sup>x</sup> At 12 days after inoculation; data are averages for 10 apples for each of four isolates of the asexual chromogenic type and perithecial type.

<sup>y</sup> At 20 days after inoculation; data based on a 0–5 scale, where 0 = no conidial masses, 1 = up to 1% of lesion area showing acervuli and some conidia, and 2 = 2–10% of lesion area showing erumpent acervuli and orange or black conidial masses, 3 = 11–30%, 4 = 31–70%, and 5 = 71–100%.

<sup>z</sup> Significantly different ( $P = 0.01$ ) from the chromogenic type according to analysis of variance tests.

**Table 2.** Pathogenicity of a perithecial and asexual chromogenic type of *Glomerella cingulata* on four apple cultivars at two temperatures

Cultivar	Lesion diameters (mm) at 12 days				Conidial mass ratings at 20 days <sup>w</sup>			
	20 C		28 C		20 C		28 C	
	Perithecial <sup>x</sup>	Chromogenic <sup>y</sup>	Perithecial	Chromogenic	Perithecial	Chromogenic	Perithecial	Chromogenic
Golden Delicious	39.9 a <sup>z</sup>	27.6 b	65.1 a	41.1 b	2.2 a	2.7 a	3.4 b	4.3 a
Jonathan	21.2 a	20.5 a	54.4 a	35.7 b	2.6 a	2.9 a	4.1 a	4.4 a
Red Delicious	29.4 a	25.1 a	66.0 a	42.5 b	1.8 b	2.7 a	3.8 a	3.7 a
Rome	47.2 a	31.8 b	70.2 a	34.9 b	2.9 a	3.1 a	3.1 a	2.9 a

<sup>w</sup> Ratings based on: 0 = no conidial masses, 1 = up to 1% of lesion area showing acervuli and some conidia, and 2 = 2–10% of lesion area showing erumpent acervuli and orange or black conidial masses, 3 = 11–30%, 4 = 31–70%, and 5 = 71–100%.

<sup>x</sup> Perithecial type from Houston County.

<sup>y</sup> Asexual chromogenic type from Lee County.

<sup>z</sup> Means between fungal types within a temperature and cultivar of apple followed by the same letter do not differ ( $P = 0.05$ ) according to Duncan's multiple range test.

the wound, surrounded by orange conidial masses to about 4 mm of the lesion margin. After this time, changes of the conidial masses from orange to black continued as the lesion increased in diameter and rot progressed (Fig. 1B). Mycelial growth from the acervulus sometimes changed the smooth, charcoal-black appearance of the conidial masses to a pilose, dark, olive-green. The pilose masses occurred in wrinkles of rotting apple tissue or between the apple and filter paper in the petri dish. Perithecia with asci and ascospores were found around the stem-ends of apples inoculated with isolates that did not yield many black conidial masses. Margins of lesions caused by the perithecial isolates frequently were irregular, whereas margins of lesions caused by the chromogenic isolates usually were entire during the 12- to 20-day incubation

period. An intensification of black conidial mass formation occurred in the center of the lesion that contrasted with the chromogenic type where distinct conidial mass formation did not appear to continue.

The centers of lesions caused by the chromogenic type became reddish brown from drying spore masses surrounded by the freshly developed orange conidial masses (Fig. 1A). After 20 days of incubation, the orange and black conidial masses were washed off half of the apples of both types and incubated an additional 10 days. Lesions caused by the perithecial type did not appear to become darker but remained caramel-brown. Lesions caused by the chromogenic type became dark brown to black over the oldest lesion area where conidial masses had occurred.

Perithecial and chromogenic isolates differed in lesion size and conidial mass

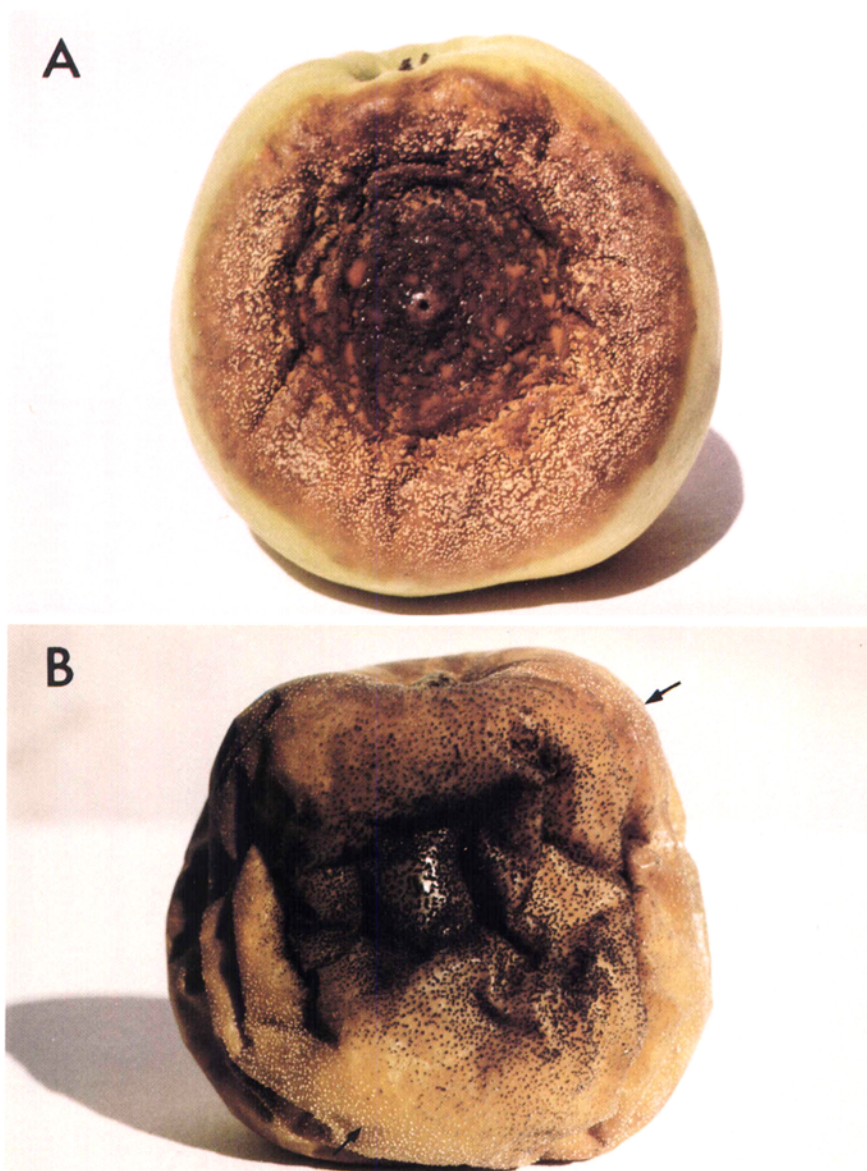
**Table 3.** Lesion diameters and conidial mass ratings of *Glomerella cingulata* on Golden Delicious apples inoculated with conidia of a different colony morphology, then incubated at 28 C

Colony morphology type	Lesion diameters (mm) at 12 days	Conidial mass ratings <sup>a</sup> at 20 days
Asexual chromogenic		
A-3	23.0 a <sup>b</sup>	0.0 a
A-2	24.2 a	3.8 cd
A-1	33.2 bc	4.6 d
Mean	26.8 <sup>c</sup>	...
Perithecial		
B-6	19.2 a	0.5 a
B-4	25.2 ab	1.8 b
B-3	38.8 cd	3.1 c
B-2	42.8 de	3.3 c
B-5	45.0 de	3.9 cd
B-1	50.1 e	3.6 cd
Mean	36.9 <sup>c</sup>	...

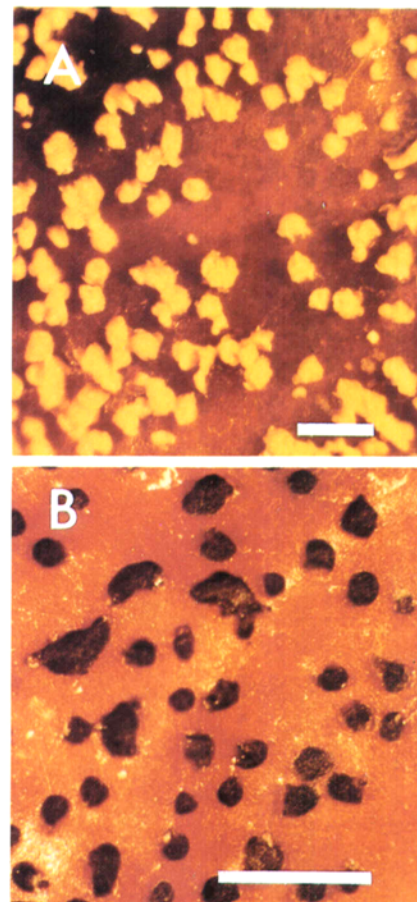
<sup>a</sup>Index ratings based on: 0 = no conidial masses, 1 = up to 1% of lesion area showing acervuli and some conidia, and 2 = 2-10% of lesion showing erumpent acervuli and orange or black conidial masses, 3 = 11-30%, 4 = 31-70%, and 5 = 71-100%.

<sup>b</sup>Means within a column not followed by the same letter differ significantly ( $P = 0.01$ ) according to Duncan's multiple range test.

<sup>c</sup>Differences between asexual chromogenic and perithecial isolates significant ( $P = 0.01$ ) for lesion diameters.



**Fig. 1.** Bitter rot of apples caused by *Glomerella cingulata*: (A) asexual chromogenic type showing orange conidial masses on lesion periphery and reddish brown coalesced conidia over dark tissue of lesion central area and (B) perithecial type showing black conidial masses over lesion central area, surrounded by orange conidial masses (arrow).



**Fig. 2.** Stereoscopic view of *Glomerella cingulata* conidial masses: (A) asexual chromogenic type and (B) perithecial type. Scale bar = 2 and 4 mm, respectively.

ratings (Table 1). Lesions in apples infected with the perithecial type were significantly larger than those of the chromogenic type, but conidial mass ratings for the chromogenic type were larger. Growth of the HC perithecial type of *G. cingulata* was significantly larger on Golden Delicious and Rome apples at 20 C and significantly larger for each cultivar at 28 C. Conidial mass ratings for the LC chromogenic type were significantly larger on Red Delicious at 20 C and Golden Delicious at 28 C (Table 2).

Significant differences in lesion diameters of infected apples occurred among perithecial and chromogenic colony morphological types (Table 3). Conidial mass ratings also differed significantly among A colony morphology types and among B colony morphology types but not between the A and the B types. The A-3 colony morphology type did not produce any conidial masses on the lesion surface. Also, the B-6 colony morphology type developed conidial masses on only three of the 10 test apples. No growth occurred on any of the apples inoculated with sterile water to serve as controls (Tables 1-3); therefore, the 0-mm growth or 0 ratings were not included in the analysis of variance tests.

## DISCUSSION

The occasional development of olive tufts of mycelium among the black conidial masses corresponds with the observations of Clinton (2), who implied that high RH was the cause of such mycelial growth; however, he did not report a change of color in the conidial masses from orange to black. Robert's (8) observation about pink conidial masses: "Later, however, upon drying they became dark colored..." is confusing. In our studies, when fresh conidial masses

dried, they became pale orange; when 10-12 days old, the perithecial type of *G. cingulata* became black.

Development of black conidial masses on Jonathan, Red Delicious, and Rome apples inoculated with the perithecial isolate demonstrated that black conidial mass occurrence was not a phenomenon peculiar to Golden Delicious apples. Also, development of black conidial masses appeared to occur at or near maximum proportions on Jonathan and Rome apples. This is substantiated by a comparison of conidial mass ratings among the four cultivars at 28 C. The most rapid growth of lesions occurred on Rome apples, with the perithecial isolate growing twice as fast as the chromogenic isolate. These differences among the cultivars tested agree with earlier reports (2,13) that bitter rot progresses at different rates on different cultivars of apples.

Taylor (11) reported an atypical strain of *G. cingulata* from Georgia that sporulated sparingly or not at all in fruit lesions and produced olive-tinted mycelium and a gray pigment in PDA. According to cultural studies reported by Edgerton (5), Taylor's fungus apparently was a perithecial isolate of *G. cingulata*. Our investigations have shown that monoconidial isolates from both the perithecial and chromogenic types may produce lesions on which no conidial masses occur. These observations with the perithecial type also agree with the report of Shane and Sutton (9), who found conidial production was often sparse on the surface of apples infected with perithecial strains.

From this study, it appears that a more complete description of signs and symptoms of apple bitter rot should include 1) perithecial type, orange

conidial masses turning black with age (when conidial masses weather-off, underlying apple epidermis remains brown); 2) asexual chromogenic type, orange conidial masses that coalesce to a reddish brown with age (when conidial masses weather-off, underlying apple epidermis becomes dark brown to black); and 3) perithecial, asexual chromogenic, or asexual nonchromogenic types that produce no prominent conidial masses on apple lesions.

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