# Epidemiology of the Perfect Stage of *Glomerella cingulata* on Apples

T. B. Sutton and W. W. Shane

Department of Plant Pathology, North Carolina State University, Raleigh 27650; and the Department of Plant Pathology, University of Minnesota, St. Paul 55121, respectively.

Journal Series Paper 8644 of the North Carolina Agricultural Research Service, Raleigh.

The use of trade names in this article does not imply either endorsement by the North Carolina Agricultural Research Service of the products named, or criticism of similar ones not mentioned.

Appreciation is extended to L. R. Pope for technical assistance.

Accepted for publication 24 March 1983.

## ABSTRACT

Sutton, T. B., and Shane, W. W. 1983. Epidemiology of the perfect stage of *Glomerella cingulata* on apples. Phytopathology 73:1179-1183.

Perithecial isolates of *Glomerella cingulata* grew more rapidly than chromogenic isolates at 28 and 32 C, induced more rot in wound-inoculated fruit, and were equally pathogenic when fruit were inoculated with a spore suspension. Perithecial and chromogenic isolates of *G. cingulata* produced conidia on potato-dextrose agar (PDA) from 8 to 32 C; from 24 to 28 C was the optimum range. Chromogenic isolates generally produced more conidia on PDA; however, one perithecial isolate produced conidia copiously. Ascospores, produced in perithecia in dead wood, were released during rainfall and were dispersed in the air. Ascospores were usually trapped within 2–3 hr after rainfall began. After the rainfall ended, ascospores often continued to be trapped until the relative humidity dropped and branches began to dry. Ascospore dispersal in rainwater was not an important dispersal mechanism. Airborne ascospores were trapped throughout the apple-growing season, indicating the potential for early season epidemic development. An extensive epidemic, initiated by airborne ascospores, was monitored in one orchard in 1979.

*Additional key words*: bitter rot, *Colletotrichum gloeosporioides*

Bitter rot, which is induced by *Glomerella cingulata* (Ston.) Spa. and v. Sch. (anamorph, *Colletotrichum gloeosporioides* Penz.), is an important apple (*Malus domestica* Borkh.) disease in many apple-growing areas in the southeastern United States. Perithecial and conidial strains of *G. cingulata* have been reported (4). Perithecial strains form gray-green colonies in culture on potato-dextrose agar (PDA) and produce both perithecia and conidia. Five mycelial types have been described (4,5). Conidial production usually is sparse on fruit infected with perithecial strains (2). Conidial strains are isolates that do not produce perithecia. In PDA cultures, conidial strains produce colonies that either are pink to red (termed chromogenic) or range from light cream to dark brown or green. Perithecial strains may revert to conidial strains. Because no chromogenic perithecial isolates have been reported, it seems unlikely that chromogenic strains are perithecial revertants.

Both chromogenic and nonchromogenic conidial strains are usually associated with bitter rot in the orchard; perithecial strains are not as common. Recently we reported that perithecial strains were present in approximately one-third of the orchards surveyed in North Carolina (2) and in some orchards, perithecial strains predominated. Low numbers of airborne ascospores were detected in one of these orchards during the evening and early morning on rainy days, suggesting that airborne ascospores could be an

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

important dispersal mechanism for *G. cingulata* (2). However, the relative pathogenicity of perithecial strains compared to conidial strains is unknown. Furthermore, the period of inoculum availability and factors favoring spore dispersal have not been determined.

The objectives of this study were to compare the cultural characteristics and pathogenicity of perithecial and conidial (chromogenic) strains of *G. cingulata* and to identify the factors that influence the spread of perithecial strains under orchard conditions.

## MATERIALS AND METHODS

**Temperature effect on growth of conidial and perithecial isolates.** Three tests were conducted to compare the effects of temperature on the growth of conidial and perithecial isolates. In each test, 20 ml of PDA (prepared from fresh potatoes) was stabilized in 9-cm-diameter petri dishes at 8, 12, 16, 20, 24, 28, 32, or 36 C for 12 hr. Mycelial plugs (5 mm in diameter) from the advancing edge of each isolate growing on PDA were transferred to the center of each of three plates for each isolate and temperature combination. Plates were maintained in plastic bags in the dark and two perpendicular colony-diameter measurements were made after 2, 6, and 8 days. In test 1, chromogenic isolates 10C and 44C and perithecial isolates 38P and 351P were used; in test 2, chromogenic isolates 381C and 367C and perithecial isolates 373P and 362P were used; in test 3, chromogenic isolates 497C and 500C and perithecial isolates 377P and 458P were used. Average daily radial growth of each isolate was calculated from the 6-day measurements only.

**Temperature effects on growth and sporulation of conidial and perithecial isolates.** Two tests were conducted to compare the effects of temperature on the sporulation of conidial and perithecial isolates. In the first test, chromogenic isolates 10C and 44C were compared to perithecial isolates 38P and 351P. In the second test, chromogenic isolates 301C, 367C, and 378C were compared to perithecial isolates 362P and 373P. Plugs 5 mm in diameter from the margins of actively growing cultures of each isolate were transferred to PDA plates. Cultures were grown at 24 C for 8 days. Three plates of each isolate were then transferred to 8, 12, 16, 20, 24, 28, 32, or 36 C for 8 days in the dark. Spores were removed from these plates by flooding the agar surface with 10 ml of water, scraping the surface with a razor blade, and filtering the resulting suspension through two layers of cheesecloth into 80 ml of water. Plates were rinsed again with 10 ml of water. Spore concentrations were determined by using a hemacytometer. Conidia and ascospores were not differentiated when counting spores produced by perithecial isolates.

**Pathogenicity studies on unwounded fruit.** Pathogenicity tests were conducted with perithecial and chromogenic isolates on immature and mature Golden Delicious fruit. The inoculation technique previously described by Shane and Sutton (3) was used in all tests. Chromogenic and perithecial isolates were grown on PDA. Chromogenic isolates used in the tests were 6 days old; perithecial isolates were 21 days old. Spores were harvested from the cultures by flooding the plates with 10 ml of distilled water and then scraping the agar surface with a scalpel. The suspension was filtered through two layers of cheesecloth. To obtain sufficient inoculum from the perithecial isolates, plates were flooded and scraped three times. Spore suspensions were centrifuged twice (10 min, 4,000 rpm) in 40 ml of water to remove nutrients supplied by the medium. Inoculum concentrations were adjusted to $2 \times 10^5$ spores per milliliter of water. The inoculum from the perithecial isolates consisted of ascospores and conidia. In each test, 18–20 fruit were sprayed (with an atomizer) to runoff with aqueous spore suspensions from either chromogenic or perithecial isolates. Eighteen to 20 fruit comparably sprayed with distilled water served as checks.

Apples to be inoculated were carefully picked to avoid bruising and were placed in plastic containers with their stems inserted into 2-cm-thick Oasis blocks (Smithers-Oasis, Kent, OH 44240), saturated with tap water. Following inoculation, fruit were incubated in the closed plastic containers for 48 hr at 28 C in the dark.

Inoculated fruit were disinfested after 48 hr by immersion for 1 min in freshly prepared 1% NaOCl, washing them in tap water, and swabbing their surfaces with 95% ethyl alcohol. Ten squares of cuticle (each 5 × 5 mm with approximately 2 mm of underlying tissue) were placed on water agar amended with 200 μg of vancomycin per milliliter. Plates were incubated at room temperature (20–23 C) and normal fluorescent light. Identification of apple pieces infected with *G. cingulata* was made after 21–30 days. Immature fruit were collected and inoculated on 1 July 1982, approximately 70 days after full bloom. Inoculations on mature fruit were made on 28 August 1981, approximately 142 days after full bloom, and on 5 September 1982, 135 days after full bloom.

**Pathogenicity studies on wounded fruit.** Two perithecial isolates (7P and 21P) and two chromogenic isolates (2C and 18C) were used to compare the pathogenicity of perithecial and conidial isolates in mature fruit. A 5-mm-diameter well, ~5 mm deep, was made into the side of Golden Delicious fruit of uniform ripeness. A 5-mm-diameter plug of mycelium from actively growing cultures of the isolates on PDA was inserted (mycelial surface downward) into the well. The wound was covered with a small piece of moist sterile cotton and sealed with a piece of masking tape. Inoculated fruit were placed in sealed plastic bags, such that fruit did not touch one another, and were incubated at 12, 16, 20, 24, 28, or 32 C. Three replications were used for each isolate × temperature combination. After 6 days, fruit were cut radially at the point of inoculation. The diameter and depth of the rotten area was measured. Because lesions in fruit infected with *G. cingulata* are "V"-shaped in cross section, the volume of the rotten area in each fruit was determined utilizing the formula for the volume of a cone. The test was repeated once.

**Airborne dispersal.** Airborne dispersal of ascospores of *G. cingulata* was studied in a research orchard at Central Crops Research Station (CCRS), Clayton, NC, from 24 April to 31 August 1979, and from 29 April to 23 August 1980, and at a grower's orchard in Cleveland County, NC, from 4 April to 15 August 1979. Spores were trapped at each location with a Burkard volumetric spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Hertfordshire, England). Traps were adjusted to sample 10 L of air per minute. In both years traps at CCRS were surrounded at distances of 3 m by four 2.54-cm wire mesh cages (0.6 × 1.2 × 0.6 m), supported 0.5 m above the ground and filled with 1- to 3-yr-old branches previously inoculated with perithecial isolates of *G. cingulata*. Branches to be inoculated were steamed for 2 hr in a soil cart. After cooling, a mycelial and spore suspension composed of four perithecial isolates was injected beneath the bark at 15-cm intervals along each branch. Inoculated branches were placed in the soil cart, covered with plastic, and incubated at room temperature (18–22 C) for 3 wk. At the end of 3 wk, dark lesions were visible around each point of inoculation and conidial sporulation was visible on some lesions. Branches were then placed in the cages. At CCRS, Burkard traps were supported such that the trap orifice was ~40 cm above the ground. At the Cleveland County orchard the trap was placed in the lower portion of the crown of an apple tree located in an area of the orchard where bitter rot was a severe problem the previous year. Tapes from the Burkard trap were cut into sections, mounted on glass slides, stained with cotton blue in lactophenol, and ascospores were counted under ×250 by making one traverse through the center of each hourly exposure.

The intraorchard spread of *G. cingulata* by ascospores was studied in 1979 in a nonsprayed section of the orchard just north of the spore trapping site used at CCRS. The spore trap and cages of brush were located approximately 25 m from the first tree. Rows in the orchards were oriented north-south and trees were spaced 8 m apart in rows 10 m apart. Fruit infection was determined on 29 May when most lesions were 2–3 mm in diameter. All diseased fruit were sampled on trees with low infestation and a subsample of 40–80 fruit was taken on trees with a large number of infected fruit. The total number of fruit per tree ranged from 33 to about 600. Diseased tissue was placed on water agar amended with 200 μg of penicillin per milliliter. Plates were incubated at room temperature (20–24 C)

and normal fluorescent light and strains were identified after 2–3 wk. A survey of the orchard in 1977 and 1978 indicated that less than 1% of the fruit each season were infected with perithecial strains.

Rainwater dispersal of ascospores and conidia was determined at CCRS by placing funnel traps beneath each cage of brush. Funnels were 10.5 cm in diameter and were inserted in 400-ml plastic bottles. Ten milliliters of a 5% copper sulfate solution was placed in each bottle to prevent spore germination. Bottles were changed weekly if it rained. The number of spores in each bottle was determined by filtering a 1- or 5-ml sample through a 25-mm-diameter (1.2-$\mu$m pore size) gridded filter and counting three grids at random. At the Cleveland County orchard, six funnel traps were placed on 1.5-m-high posts beneath the canopies of six trees chosen at random. Traps were maintained similar to those at CCRS.

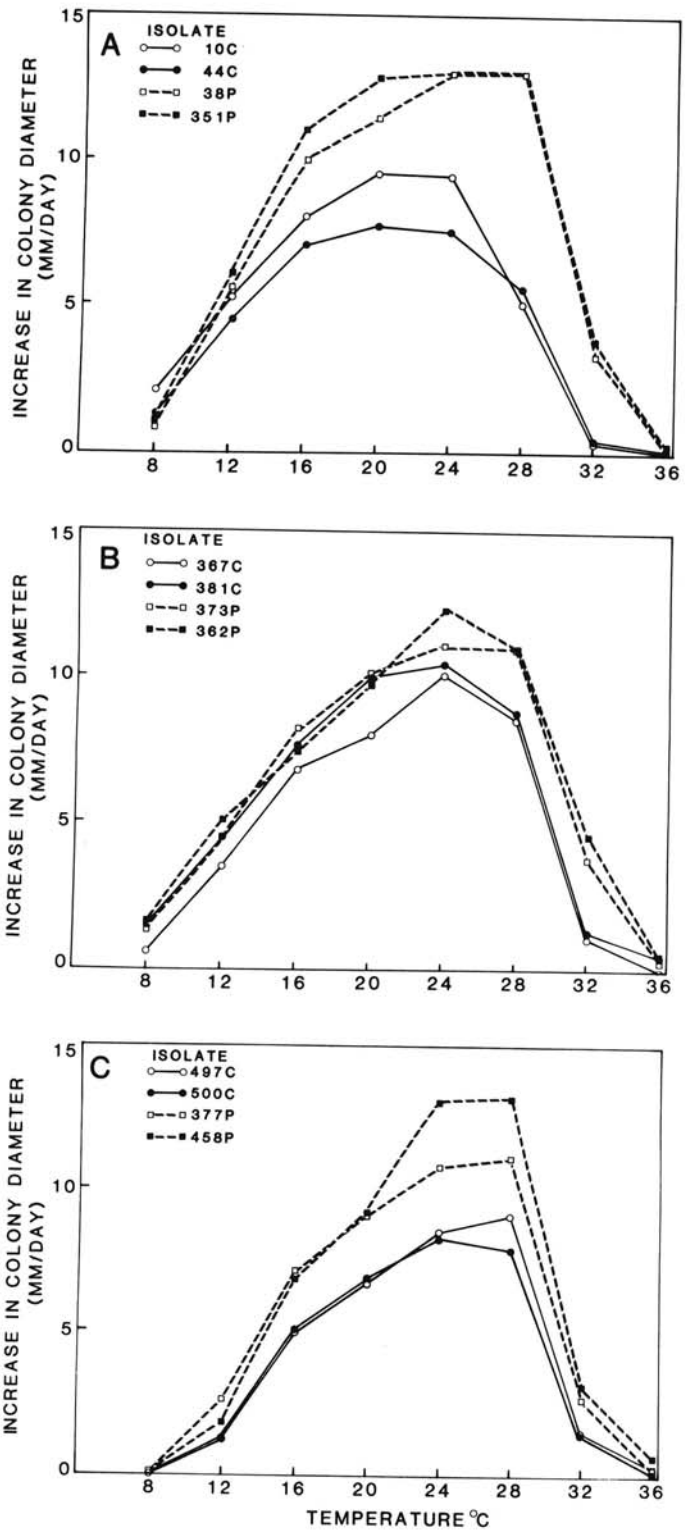


**Fig. 1.** Growth rates of chromogenic and perithecial isolates of *Glomerella cingulata* on potato-dextrose agar at eight temperatures. **A,** Test 1, isolates 10C, 44C, 38P, 351P; **B,** Test 2, isolates 367C, 381C, 373P, 362P; and **C,** Test 3, isolates 297C, 500C, 377P, 358P.

## RESULTS

**Effect of temperature on growth and sporulation.** In tests 1 and 3 (Fig. 1A and C) the perithecial isolates grew more rapidly at 12, 16, 20, 24, 28, and 32 C ($P = 0.05$) than the chromogenic isolates. In test 2 (Fig. 1B), the perithecial isolates grew more rapidly at 28 and 32 C ($P = 0.05$). Perithecial isolates grew most rapidly at 24, 28, and 32 C; 20 to 28 C was optimum for the chromogenic isolates. None of the isolates grew well at 36 C.

The chromogenic isolates generally produced more spores than the perithecial isolates (Table 1). Three of the four perithecial isolates produced very few spores; isolate 373P produced abundant conidia. Spores were produced over the range of temperatures tested. Sporulation was greater from 20 to 28 C; 24 C was optimum for five of the nine isolates tested. Sporulation was least at 36 C.

**Pathogenicity tests.** Perithecial isolates were as virulent as the chromogenic isolates when fruit were inoculated with a spore suspension (Table 2). Greater infection was obtained with the perithecial isolate in test 1 (mature fruit). In 1982, there were no differences in pathogenicity when immature (test 2) or mature (test 3) fruit were used.

More rot was induced by perithecial isolates than chromogenic isolates at all temperatures except 12 and 32 C ($P = 0.05$) following inoculation in a wound with a mycelial plug (Fig. 2). Perithecial isolate 21P generally induced more rot than 7P.

TABLE 1. Number of spores produced on potato-dextrose agar by chromogenic (C) and perithecial (P) isolates of *Glomerella cingulata* at various temperatures

| | Temperature (C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Isolate | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| Test 1 | | | | | | | | |
| 10C | 65[a] | 57 | 57 | 164 | 334 | 180 | 63 | 25 |
| 44C | 14 | 8 | 18 | 13 | 79 | 63 | 36 | 0 |
| 38P | 1[b] | 1 | 0 | 1 | 2 | 2 | 2 | 0 |
| 351P | 6 | 4 | 8 | 5 | 12 | 7 | 8 | 0 |
| Test 2 | | | | | | | | |
| 301C | 65 | 122 | 31 | 31 | 84 | 76 | 416 | 8 |
| 367C | 56 | 27 | 26 | 41 | 93 | 91 | 27 | 3 |
| 378C | 208 | 353 | 308 | 519 | 164 | 381 | 139 | 167 |
| 362P | 0 | 1 | 4 | 4 | 8 | 31 | 29 | 0 |
| 373P | 94 | 109 | 451 | 171 | 1,234 | 179 | 118 | 66 |

[a] Number of spores per plate $\times 10^4$. Mean of three replications.
[b] Ascospores and conidia counted for perithecial isolates.

TABLE 2. Comparison of pathogenicity of chromogenic and perithecial isolates of *Glomerella cingulata*

| | Isolate type | | |
|---|---|---|---|
| | Perithecial | Chromogenic | $\chi^2$[d] |
| Test 1[a,b] | 56[c] | 17 | 5.90* (1 d.f.)[e] |
| Test 2 | 90 | 90 | 0.00 NS |
| Test 3 | 95 | 90 | 0.36 NS |

[a] Test 1 based on 18 fruit; test 2 and 3 each based on 20 fruit. Fruit in test 1 and 3 were mature; fruit in test 2 were immature.
[b] Fruit were spray-inoculated with a spore suspension of $2.5 \times 10^5$ spores per milliliter and were incubated for 48 hr at 28 C before surface disinfestation. Ten apple pieces with a total surface area of 250 mm$^2$ were cut from each apple. The check was sprayed with distilled water.
[c] Percentage of fruit infected.
[d] $H_0$: no difference between percent infection and isolate type.
[e] Significant at $P = 0.05$ with one degree of freedom. NS = nonsignificant.

**Spore dispersal.** The first ascospores were trapped at CCRS on 12 May 1979. Large numbers of spores were trapped during and after rains from late May through July (Fig. 3). Rain was frequent, occurring on 28 days during this period. Most ascospores were trapped during and just after rainfall. Very few ascospores were trapped on days without rainfall. During rain periods, the first ascospores were usually trapped within 2–3 hr after the rainfall began. After rainfall ended, ascospores were usually trapped until the relative humidity lowered and the branches began to dry. The ascospore catch on 21 and 22 June 1979 at CCRS is representative of this dispersal pattern (Fig. 4).

Fewer airborne ascospores were trapped in 1979 in the grower's orchard in Cleveland County than at CCRS (Fig. 5). Ascospores were trapped during and after rain from 10 April to 13 August. Ascospore discharge was greatest from mid-May through June. By mid-August most fruit in the orchard were infected with bitter rot; approximately 85% of the fruit were infected with the perithecial strain.

Fewer spores were trapped around the brush cages at CCRS in 1980 than in 1979 (Figs. 3 and 6). The greatest discharge occurred on 28 July. Rainfall was much less frequent than 1979. Conidia

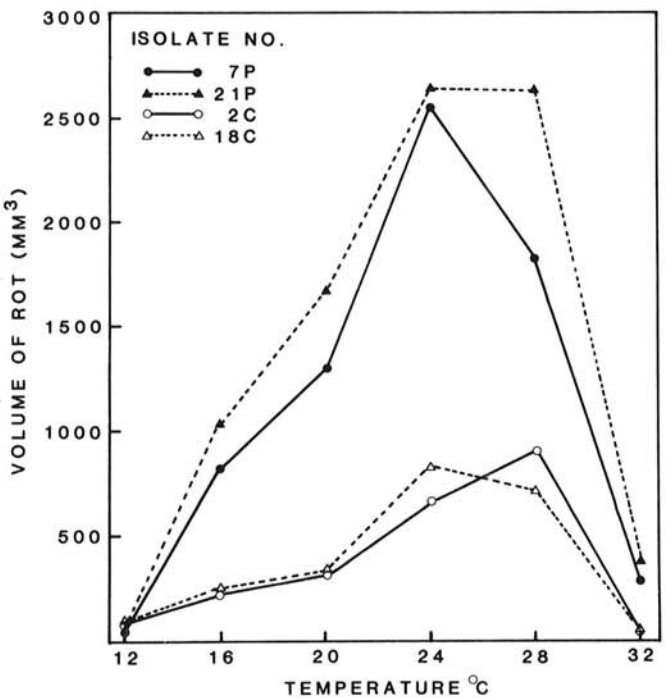


**Fig. 2.** Pathogenicity of chromogenic (C) and perithecial (P) isolates of *Glomerella cingulata* in wound-inoculated apple fruits. Volume of rot based on the volume of a cone. Data are the mean of two tests, three replications in each test.

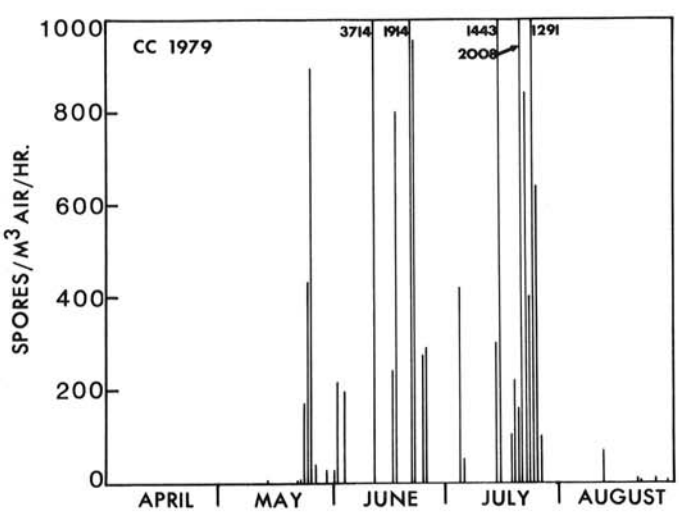


**Fig. 3.** The average number of airborne ascospores of *Glomerella cingulata* trapped per hour per day at Central Crops Research Station in Clayton, NC, from 21 April to 31 August 1979.

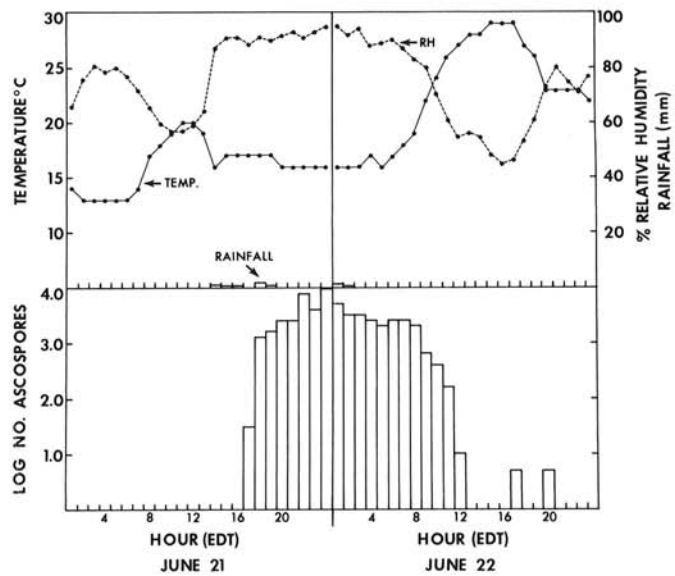


**Fig. 4.** The number of airborne ascospores of *Gomerella cingulata* trapped per hour, and temperature, relative humidity, and rainfall at Central Crops Research Station in Clayton, NC, on 21 and 22 June 1979.

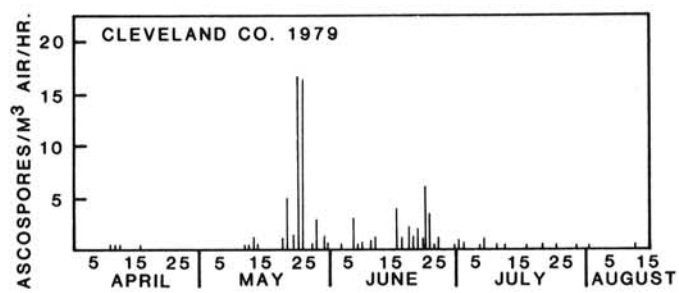


**Fig. 5.** The average number of airborne ascospores of *Glomerella cingulata* trapped per hour per day at a grower's orchard in Cleveland County, NC, from 4 April to 15 August 1979.

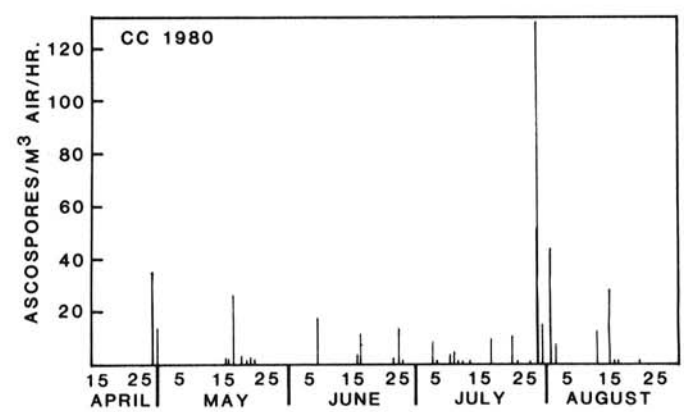


**Fig. 6.** The average number of airborne ascospores of *Glomerella cingulata* trapped per hour per day at Central Crops Research Station in Clayton, NC, from 29 April to 23 August 1980.

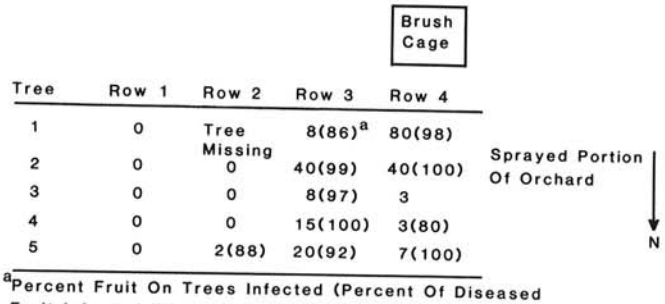


**Fig. 7.** Map of apple planting at Central Crops Research Station in Clayton, NC, showing percent fruit affected on each tree with bitter rot and percent diseased fruit infected with a perithecial strain on 29 May 1979.

were observed rarely on the spore trap tapes.

Very few conidia were trapped in rainwater either beneath the brush cages at CCRS or at the Cleveland County orchard in 1979. At CCRS, the greatest number of spores were trapped during the period 28 May to 4 June; at the Cleveland County orchard, the greatest number of conidia were trapped from 28 March to 4 April and from 9 May to 23 May. During these periods the concentration of conidia ranged from 300 to 400 per milliliter of rainwater collected. Similar results were obtained at CCRS in 1980; the greatest catch occurred during the period 29 July to 5 August. We did not identify any ascospores in the rainwater sampled during either 1979 or 1980.

**Disease development in the orchard.** On 29 May, fruit infection was greatest in trees in rows north of the spore trap and brush cages (Fig. 7, rows 3 and 4). Very few fruit were infected on trees in rows northeast of the trap (rows 1 and 2). Forty to 80% of the fruit were infected on the first two trees in row 4 and the second tree in row 3. Most fruits on these trees had multiple infections. The frequency of perithecial strains isolated from infected fruits on each tree ranged from 76 to 100%.

## DISCUSSION

Results of this study confirm that perithecial isolates of *G. cingulata* have the potential to contribute significantly to bitter rot epidemics in the orchard. The perithecial isolates tested grew more rapidly than chromogenic isolates in culture, induced more rot in inoculated fruit, and were equally pathogenic when fruit were inoculated with a spore suspension. Epidemics, initiated by airborne ascospores, occurred at CCRS and Cleveland County in 1979.

There were no clear differences in temperature optima for growth and sporulation between perithecial and chromogenic strains. Sporulation occurred over the entire range of temperatures tested, although 24–28 C was optimum. Growth of both strains was greatest at 24–28 C on PDA and in inoculated fruit. These results are consistent with those of Struble and Keitt (4).

In the orchard, conidial sporulation is often sparse on fruit infected with perithecial strains. Sporulation on PDA was low on three of the four isolates tested; culture age may have influenced these results. During this study, we observed that chromogenic and perithecial isolates lost the ability to sporulate when repeatedly transferred. Isolates used in tests 1 and 2 had been cultured for 2 yr and 1 mo, respectively. Low conidial sporulation by perithecial isolates would reduce the probability of secondary spread during the summer.

Spread of the perithecial strain in the orchard from overwintering sites is primarily by wind-blown ascospores that are liberated by rainfall. Although ascospores can be discharged in the absence of rainfall (1,2), spore trap catches on days without rainfall were very low and such discharge is probably not important in epidemic development. Rainwater is not an important mechanism for dispersing ascospores. During the course of this study, we did not detect any ascospores in the rainwater traps; however, it is likely that some ascospores are removed from the air by rainwater and that some are trapped in water droplets on the surface of dead wood while they are being discharged. Rainwater is important in dispersing conidia produced by perithecial strains in acervuli in dead wood or in infected fruit.

Ascospores were trapped in a grower's orchard and at CCRS around inoculated brush as early as petal fall. Thus, if weather conditions are favorable for infection, epidemics initiated by perithecial strains could begin very early in the growing season. This is evidenced by the extensive epidemic at CCRS in 1979. Although airborne ascospores were not detected until 12 May, by 29 May as many as 80% of the fruit were infected by perithecial strains on some trees. It is important to note that at CCRS ascospores traveled over 60 m. At the Cleveland County monitoring site, 85% of the fruit were infected by the perithecial strain by mid-August.

Surveys conducted in 1978 and 1979 (2) and subsequent isolation from orchards with bitter rot (T. B. Sutton, *unpublished*) indicate that perithecial strains of *G. cingulata* are widespread in North Carolina and in some instances are the predominant strain present. Macroscopic symptoms of fruit infected with perithecial strains resemble those of black rot incited by *Physalospora obtusa* (Schw.) Cke. or white rot incited by *Botryosphaeria dothidea* (Moug. ex. Fr.) Ces. & de Not. Thus, epidemics initiated by perithecial strains often may be misidentified. Because the potential for secondary spread is much greater with *G. cingulata* than *P. obtusa* or *B. dothidea*, extensive epidemic development could occur before the problem was accurately diagnosed.

## LITERATURE CITED

1. Patty, S. M., and Kramer, C. L. 1971. Spore discharge in *Glomerella*. Trans. Br. Mycol. Soc. 56:81-87.
2. Shane, W. W., and Sutton, T. B. 1981. The *Glomerella cingulata* perfect stage and apple bitter rot. Plant Dis. 65:135-137.
3. Shane, W. W., and Sutton, T. B. 1981. Germination, appressoria formation, and infection of immature and mature apple fruit by *Glomerella cingulata*. Phytopathology 71:454-457.
4. Struble, F. G., and Keitt, G. W. 1950. Variability and inheritance in *Glomerella cingulata* (Stonem.) S., and V.S. from apple. Am. J. Bot. 37:563-576.
5. Wheeler, H. E., and McGahen, J. W. 1952. Genetics of *Glomerella cingulata*. X. Genes affecting sexual reproduction. Am. J. Bot. 39:110-119.