The *Glomerella cingulata* Perfect Stage and Apple Bitter Rot

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ABSTRACT

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Perithecial strains of *Glomerella cingulata* were detected in five apple orchards in North Carolina. The relative frequency of perithecial strains varied from orchard to orchard. Airborne *G. cingulata* ascospores were detected within an apple orchard and within 3 m of both naturally and artificially infected apple branches. Ascospore catches were maximal during the evening and early morning, especially on and after rainy days.

Additional key word: epidemiology

Bitter rot, induced by *Glomerella cingulata* (Ston.) Spa. and v. Sch., is one of the most important apple diseases in the southeastern United States. The conidia of the imperfect stage, *Colletotrichum gloeosporioides* Penz., are produced in mucilaginous masses on colonized necrotic tissue and diseased apples and are spread within the tree canopy by insects and splashing rain (5,8). Conidial (and chromogenic) strains produce only conidia; perithecia have not been observed in association with these strains (6).

Several perithecial strains have been described (6,9). They range from highly self-fertile homothallic strains to heterothallic strains (9). The self-fertile homothallic strain has been reported as the most common perithecial strain in orchards (6). The importance of perithecial strains in the epidemiology of bitter rot is not known. Clinton (1) reported that nearly every culture of *G. cingulata* isolated from apples produced ascospores; other researchers, with the possible exception of Taylor (7), have indicated that perithecial strains are uncommon.

The prevalence of perithecial strains in North Carolina orchards has not been recorded. The occurrence of airborne *G. cingulata* ascospores in apple orchards has not been studied, although forcible discharge of ascospores has been shown for *G. cingulata* on soybean (3). Laboratory studies with an undetermined species of *Glomerella* on wood showed that discharge of ascospores can be triggered by the onset of a dark period, a decrease in humidity, or a temperature drop of at least 5.5 C (4).

This investigation was conducted to determine the prevalence of *G. cingulata* perithecial strains in North Carolina apple orchards and to assess the role of ascospores in the epidemiology of bitter rot.

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MATERIALS AND METHODS

Frequency of perithecial isolates. Isolations from diseased apples from various North Carolina orchards were made to determine the frequency of isolates capable of producing perithecia. In 1977 and 1978, isolations were made using diseased fruit treated only with an insecticide from Central Crops Research Station (CC), Clayton, NC. Isolations from other orchards were made as diseased fruit became available. To avoid limiting the range of characteristics within the isolate collection, isolations were made from any fruit exhibiting a single necrotic lesion that could not be definitely classified as some disease other than bitter rot.

Sections of diseased tissue were placed on potato-dextrose agar (PDA) prepared from fresh potatoes and amended with streptomycin sulfate, 100 μg/ml. The plates were kept in polyethylene bags at room temperature (21–23 C) and light and were examined at 4-day intervals for 4 wk. Isolate identity was confirmed microscopically.

Forcible discharge of ascospores. Perithecial strains were used to determine if ascospore discharge could be induced. Water suspensions of conidia from PDA cultures were seeded on sterile filter paper placed on the surface of PDA. The filter papers were covered with abundant perithecia after about 2-wk incubation at laboratory temperature and light. The filter papers with perithecia were cut into small pieces and placed on glass slides. A second slide coated with petroleum jelly was inverted over each of these slides and maintained at a distance of 2 mm. Each two-slide unit was placed into a closed petri dish with a water-saturated filter paper. The greased slides were examined for ascospores after 24 hr in the dark at room temperature.

Apple limbs inoculated with perithecial strains of *G. cingulata* were also used to study ascospore discharge., Apple limbs, 1- to 3-yr-old, were steamed in a covered soil cart for 1 hr and allowed to cool overnight. A spore and mycelial suspen-

Table 1. Frequency of *Glomerella cingulata* colony types isolated for diseased apple fruit from North Carolina orchards

Source (orchard)	Year sample taken	Total no. of fruit sampled	Percent of isolate types[a]		
			Perithecial	Chromogenic[b]	Other[c]
1	1977	225	1.3	42.7	56.0
	1978	146	0.7	38.7	60.6
2	1978	21	42.9	0.0	57.1
	1979	83	80.7	16.9	2.4
3–9[d]	1978	22	0.0	86.4	13.6
3	1979	48	0.0	62.5	37.5
4	1979	48	0.0	91.7	8.3
10	1979	30	0.0	83.3	16.7
11	1979	20	50.0	50.0	0.0
12	1979	44	18.2	61.4	20.4
13	1979	16	18.7	56.3	25.0
14	1979	57	0.0	68.4	31.6
15	1979	45	0.0	73.3	26.7

[a] Isolates were grown on potato-dextrose agar and examined for mycelial color and perithecia formation for 30 days.
[b] Chromogenic isolates produce red pigment in vitro and have never been known to produce perithecia.
[c] *G. cingulata* isolates that were nonchromogenic and nonperithecial.
[d] Results from seven orchards with low incidence of bitter rot in 1978 are combined.

sion from four *G. cingulata* perithecial isolates was introduced under the bark of the limbs at approximately 10-cm intervals by using a hypodermic syringe. The limbs were incubated in the cart under a plastic sheet for approximately 2 wk and were then placed in wire cages in the field until perithecia developed. A few selected limbs with perithecia were cut into 2- to 3-cm pieces and taped to the inner sides of petri dish lids. The lids with limb pieces were flooded with tap water for 1 min, and the petri dish bottoms with PDA were inverted over each lid for 24 hr at room temperature and light.

Airborne dispersal. The occurrence of airborne *G. cingulata* ascospores in an orchard and in the air surrounding naturally infected and artificially inoculated apple limbs was studied at CC. On 22 April 1977, a Burkard Volumetric Spore Trap (Burkard Scientific Sales Ltd., Rickmansworth, Hertfordshire, England) was placed in an open field approximately 200 m from the orchard. The trap was surrounded at distances of 3 m by four 2.54-cm mesh wire cages (0.6 ×1.2 × 0.6 m), supported 0.3 m above the ground, and filled with apple limbs.

The 1- to 3-yr-old limbs had been kept near the orchard for 1–2 yr before being placed in the wire cages in the spring of 1977. Inoculum concentration in these limbs was apparently low because several attempts to find *G. cingulata* in them during the summer of 1977 were unsuccessful.

On 15 June 1977, a second Burkard trap was mounted approximately 2 m from the ground in the scaffold limbs of a Stayman apple tree surrounded by Delicious, Golden Delicious, and Stayman trees. These trees were sprayed only with insecticides during 1977. Bitter rot was present on these trees in 1976.

In 1978, ascospore discharge from artificially inoculated apple limbs was studied. On 7 November 1977, 1- to 3-yr-old apparently healthy Golden Delicious apple limbs were cut and placed in four wire cages in an open field at CC. A spore and mycelial suspension was prepared from 3- to 4-wk-old cultures of two *G. cingulata* perithecial isolates grown on PDA in petri plates at room temperature and light. The spores and mycelia of the cultures were scraped with a scalpel from the surface of the medium into sterile tap water. The suspension was poured over the apple limbs and a hypodermic syringe was used to inoculate the ends of the limbs with the suspension.

To further insure colonization, a second set of limbs, 1- to 3-yr-old, pruned from the same orchard on 9 February 1978 were air-dried in the laboratory for 9 days and autoclaved at 15 psi and 110 C for 10 min to reduce competition from saprophytic fungi. After cooling to room temperature, the limbs were inoculated, as described, with the *G. cingulata* perithecial isolates. The limbs were incubated under a greenhouse table in large polyethylene bags with wet paper towels for 25 days and then were placed in the four wire cages. Conidia and mycelia were evident on the limbs. The wire cages were placed in a circle approximately 3 m from a Burkard trap. On 9 June 1978, *G. cingulata* perithecia with mature ascospores were present in dark necrotic areas on inoculated limbs.

Burkard traps were adjusted to sample 10 L of air per minute. Very few waterborne spores are caught by the Burkard trap (2); therefore, catches represent windblown dissemination. Spore trapping was discontinued at the end of August. The Melinex tapes from the Burkard traps were cut into sections, placed

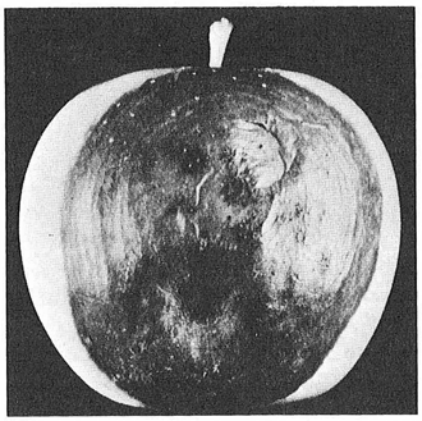

Fig. 1. Sparse sporulation on Golden Delicious apple infected with *Glomerella cingulata* perithecial isolate.

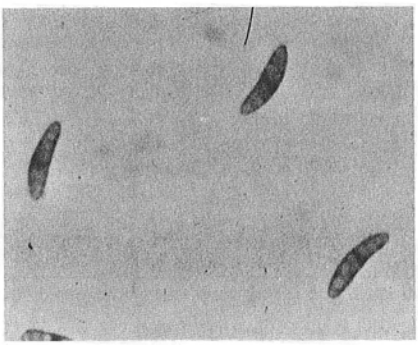

Fig. 2. Appearance of *Glomerella cingulata* ascospores after staining with cotton blue in lactophenol.

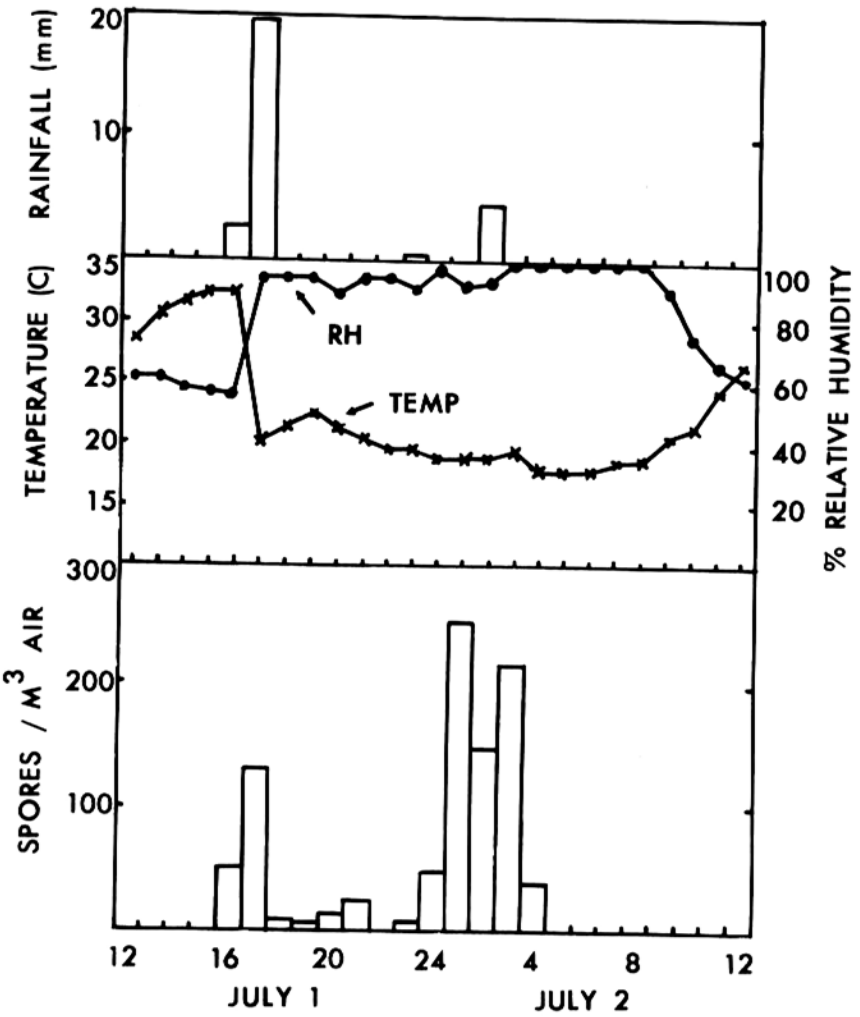

Fig. 3. Hourly ascospore catches from naturally infected apple limbs at Central Crops Research Station during 1 and 2 July 1977. Example of spore discharge associated with rainfall.

on glass slides, stained with cotton blue in lactophenol, and counted under ×250 magnification by making a single traverse through the middle of each hourly exposure. Counts were corrected for the area sampled but not for trap efficiency. Approximately one-third of the season's catches in 1977 were counted as a representative sample. In 1978, counts were made on days with rain or following a rainy period as we found that *G. cingulata* ascospores were caught only under these conditions.

RESULTS AND DISCUSSION

Perithecial strains of *G. cingulata* were isolated from five of 15 orchards sampled in North Carolina (Table 1). Perithecia on PDA were observed after 12–16 days at room temperature. We did not try to identify the strains isolated, although in culture on PDA, most isolates resembled the clumped perithecial (Plus A) homothallic strain (6,9). The frequency of the perithecial isolates varied from orchard to orchard. Some nonperithecial *G. cingulata* strains from orchard 2 were very similar in colony morphology and color to strains that produced perithecia. It is possible that different culturing methods could induce such strains to produce ascospores. Samples from orchards 3–15 may have been biased toward collection of apples exhibiting conidial sporulation. Conidial production was often sparse on the surface of apples infected with perithecial strains, compared with nonperithecial strains (Fig. 1). Thus, the frequency of perithecial strains isolated is perhaps a conservative estimate (Table 1).

Forcible discharge of *G. cingulata* ascospores was observed in the laboratory from perithecia formed both on filter paper and apple limbs. More than 100 ascospores per slide from the perithecia on the filter paper and thousands of ascospores per petri dish from the limb pieces were collected in 24 hr. This demonstrates that the perithecial strains found at CC on apple are capable of forcible ascospore discharge.

Mature perithecia sometimes developed a mucilaginous drop at the ostioles, containing many free ascospores when grown on moist filter paper in closed petri dishes. Thus, rainfall may be important in the splash dispersal of ascospores formed under high humidity.

Very few airborne *G. cingulata* conidia were detected at the CC orchard, although more than 20% of the apples were infected on trees that were not sprayed. Few airborne conidia were detected from naturally infected or artificially inoculated apple limbs. Airborne *G. cingulata* ascospores were detected in the CC orchard and from both naturally and artificially infected apple branches. Identification of trapped ascospores was aided by the presence of guttules and a dark nucleus in the center of most spores when stained with cotton blue in lactophenol (Fig. 2). The ascospores ranged from a distinct curved shape to a straight form indistinguishable from the conidia. Occasionally a traverse septum was seen. Ascospores often occurred in clusters of up to eight spores on the trapping surface, which helped to distinguish them from conidia.

In 1977 at the CC orchard, the maximum number of ascospores detected was 15 per cubic meter of air per hour on 3 August, while the peak from the naturally infected limbs was 254 ascospores per cubic meter of air per hour on 2 July. The maximum number of ascospores detected from the inoculated apple limbs in 1978 was 79 per cubic meter of air per hour. Qualitatively, this indicated that *G. cingulata* ascospores were present in the orchard and that naturally infected apple limbs were one possible source. Although many perithecia with mature ascospores were observed on 9 June 1978 in the inoculated limbs, only a few airborne ascospores were trapped near these limbs compared with the number trapped around the naturally infected limbs in which the number of perithecia was apparently very low. The reasons for this are not known although differences in environmental conditions favoring spore discharge and dispersal between 1977 and 1978 are suspect. Maximal numbers of ascospores were detected during the evening and early morning, especially on rainy days (Fig. 3). Ascospores were occasionally trapped in low numbers on days with no rainfall. This corroborates the laboratory work of Pady and Kramer (4); however, not enough ascospores were caught to allow detailed analysis of the effect of each environmental factor.

The importance of perithecial strains of *G. cingulata* in the epidemiology of apple bitter rot in North Carolina is suggested by the presence of perithecial strains in approximately one-third of the orchards surveyed and their relative frequency in several orchards. Ascospores are forcibly discharged and transported by the wind and could be important in tree-to-tree spread. Further studies are needed for more complete understanding of the role of the perithecial strains in bitter rot epidemiology.

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