Germination, Appressorium Formation, and Infection of Immature and Mature Apple Fruit by *Glomerella cingulata*

W. W. Shane and T. B. Sutton

Graduate research assistant and assistant professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27650.

We thank Ray Pope for technical assistance.

Journal Series Paper 6449 of the North Carolina Agricultural Research Service, Raleigh.

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

Accepted for publication 9 October 1980.

ABSTRACT

Shane, W. W., and Sutton, T. B. 1981. Germination, appressorium formation, and infection of immature and mature apple fruit by *Glomerella cingulata*. Phytopathology 71:454-457.

Conidial ability to germinate and produce appressoria was related to age of *Glomerella cingulata* culture on potato-dextrose agar. On the surface of detached apple fruit less germination and formation of appressoria occurred at 24 hr with conidia from 12- and 20-day-old cultures than from 6-day-old cultures. Conidia from 6-day-old cultures produced more infections of detached, immature, and mature Golden Delicious (GD) apples than did conidia from 12- and 20-day-old cultures. Inoculations and observations of fruit in orchard and laboratory studies with detached GD fruit showed that immature fruit were susceptible to infection by *G. cingulata* conidia. In the orchard, infections were observed within 35 days of petal fall and, in the laboratory, fruit were infected within 19 days of petal fall. No seasonal trend was evident in the percentage of fruit infected after 24 or 55 hr of incubation. A 20-hr incubation period at 28 C was required to establish appreciable infection of detached apple fruit.

Additional key words: Colletotrichum gloeosporioides.

Bitter rot of apple (*Malus sylvestris* Mill) which is caused by *Glomerella cingulata* (Ston.) Spa. and v. Sch. (imperfect stage, *Colletotrichum gloeosporioides* Clk.) is a serious although sporadic disease in North Carolina orchards. The disease is usually first observed during the latter part of June and may cause 100% fruit rot by mid-August. Resistance of immature fruit to bitter rot has been reported (5,6,8); however, observations of the disease on immature fruit suggest the contrary (1,5,7).

In the southeastern United States, knowledge of immature fruit susceptibility to *G. cingulata* is crucial in apple disease management programs that utilize reduced fungicide rates or extended spray intervals. The causal organism has a short incubation period and sporulates profusely on infected fruit. As a result, bitter rot epidemics can develop rapidly under favorable conditions. Once *G. cingulata* is established on fruit within an orchard, control becomes very difficult.

Studies on the susceptibility of immature fruit generally have not included the fact that younger fruit present a smaller surface for deposition of inoculum and that spore concentration (3) and inoculum age (9) may influence disease development. Also, spray inoculation is preferable over wound inoculations because *G. cingulata* infects apple fruit by direct penetration of the cuticle (2,4).

The objective of this investigation was to study the effect of fruit maturity on the susceptibility of Golden Delicious (GD) apples to *G. cingulata*.

MATERIALS AND METHODS

Inoculum preparation. *G. cingulata* isolates were obtained from diseased apples and were single-spore maintained on potato-dextrose agar (PDA) slants at 4 C. All isolates used were chromogenic; they produced a distinctive red pigment on PDA. Inoculum was prepared by shaking 5 ml of sterile distilled water in each culture tube and spreading 0.1 ml of the spore suspension evenly over the surface of freshly prepared PDA plates. The cultures were incubated at 25 C in plastic bags without light. Sparse mycelial growth and abundant sporulation occurred in 3 days. A conidial suspension was prepared by flooding plates with sterile distilled water, gently rubbing the agar surface with a sterile bent glass rod, and filtering the suspension through two layers of cheesecloth. Conidial inoculum suspensions for use in laboratory tests were centrifuged twice (20 min, 2,000 rpm) to remove nutrients supplied by the medium. Conidia used in orchard studies were not centrifuged. Hemacytometer counts were used to adjust spore concentrations to 5×10^5 conidia per milliliter for both orchard and laboratory studies. Times after inoculation reported in laboratory studies do not include the 2 hr the spores were in water before deposition on the test surfaces.

Effect of culture age on inoculum effectiveness. The effect of culture age on spore germination and formation of appressoria was studied on 5-mm-diameter cellophane disks punched from sheets of du Pont PUDO-193 cellophane (E. I. du Pont de Nemours & Co., Wilmington, DE). Disks were placed on 12.7-mm-diameter antibiotic assay disks (Schliecher and Schuell, No. 740-E) in Coors porcelain spot plates (112 mm long, 92 mm wide, with 5 mm depressions, Coors Co., Golden, CO). The sterile assay disks had previously been saturated with sufficient distilled water to form a slight meniscus between the edge of the pad and the porcelain plate. Droplets of spore suspension were transferred with capillary tubes to the surface of the cellophane disks. The plates were stacked in a closed plastic box lined with wet paper towels and evaluated after incubation for 24 hr at 27 C. Conidia were harvested from cultures incubated for 4, 12, and 20 days. Two hundred conidia selected at random were examined on each cellophane disk and 20 disks were examined for each culture age. A spore was considered to have germinated when the germ tube length exceeded the width of the spore.

The effect of culture age on spore germinability and appressoria formation also was examined on the cuticles of immature GD apples picked on 25 June 1979 from an orchard at the Central Crops Research Station, Clayton, NC (CC). Apples were carefully picked to avoid bruising and immediately placed in round molded plastic containers (89 mm deep, 254 mm in diameter) with their stems inserted into 2-cm-thick florist's Oasis blocks (Smithers-Oasis,

0031-949X/81/04045404/$03.00/0

Kent, OH) saturated with tap water. In the laboratory, apple fruits were inoculated by atomization to the point of runoff with spore suspensions and incubated in the same closed containers at 28 C. The number of spores deposited was approximately 1.5×10^6 conidia per 250 mm^2 of fruit cuticle. Spores from 6-, 12-, and 20-day-old cultures were used. Thin strips of apple cuticle were cut with a razor blade from the surfaces of inoculated apples after 24 hr of incubation, mounted on glass slides, and stained with cotton blue in lactophenol. Two hundred conidia were observed at random per cuticle strip on each of 10 apples per culture age used.

To monitor infection by *G. cingulata,* spray-inoculated apples were surface disinfested at various times after inoculation by agitation for 1 min in freshly prepared 1% NaOCl, washed in tap water, and swabbed with 95% ethyl alcohol. Ten squares of cuticle (each 5×5 mm with approximately 2 mm of underlying tissue) were cut from each apple and plated on water agar plus 100 μg/ml streptomycin sulfate to inhibit bacterial growth. Preliminary experiments showed that the surface disinfection method effectively eradicated *G. cingulata* from detached GD apples inoculated with a conidial suspension and incubated for 5–16 hr at 27 C. Incubation periods of 20 hr or more resulted in fungal invasions that were not eradicated by surface disinfestation (Fig. 1). It was necessary to culture the apple squares for at least 20 days to reveal all infected pieces. Identification of *G. cingulata*-infected apple pieces was aided by the presence of abundant sporulation on the cuticles (Fig. 2).

Studies were made on the relationship of culture age to infection of detached apple fruit with the method described above. GD fruit picked on 25 June 1979 were inoculated and incubated at 27 C as previously described. Thirty apples were sampled per culture age (6-, 12-, and 20-day-old) after 24 and 55 hr of incubation. A total of 250 mm^2 was sampled from each apple surface. The plates were examined weekly for fungal growth during incubation at 27 C for 4 wk.

The experiment was repeated with mature GD apples harvested August 1978 and stored at 1 C until March 1979. The fruit was disinfested for 1 min in 1% NaOCl, washed for 5 min under running tap water, and air-dried overnight. Inoculation and reisolation procedures were similar to those used with immature fruit, except that apples from storage were incubated at 27 C on metal trays lined with wet paper towels and placed inside large plastic bags. Forty apples were inoculated per culture age (6-, 12-, and 20-day-old) and incubation time (24 and 38 hr) combination.

Apple maturity and bitter rot susceptibility. The relationship between apple maturity and susceptibility of GD apples to infection was studied in the orchard and laboratory. GD trees 23 yr old and 6 m high were used in the orchard studies conducted at CC. Before and during bloom (10 April 1978) the trees were pruned to remove necrotic tissue that might harbor the bitter rot fungus. At 90% petal fall (15 April) a grafting compound was applied to any remaining necrotic areas on the test trees. Fruits (>100/tree) on non-(fungicide) treated trees (two trees at each inoculation date) were inoculated on 17 May, 3 June, 7 June, and 25 June. Shortly before nightfall, all fruit clusters were spray inoculated until runoff with a conidial suspension prepared from chromogenic isolates as described previously. Fruit on inoculated trees and four uninoculated trees were examined at intervals of 7 days or less. Isolations were made from suspect fruit picked on the first day that symptoms were observed.

Laboratory studies were conducted using GD apples picked periodically during 1978 and 1979 from non-(fungicide) treated trees at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, NC, and CC. Apples were carefully picked to avoid bruising, immediately placed in the round plastic containers with stems inserted downward into 2-cm-thick florist's Oasis blocks saturated with tap water, and transported to the laboratory. Fruit were atomized to runoff with 5×10^5 conidia/ml suspensions prepared as previously described. Culture ages varied and are noted in the results. The apples were incubated at 28 C in closed plastic containers. Germination and appressoria formation on apple cuticle and infection establishment were examined after 24 and 55 hr incubation.

In 1978 at CC, the first fruit samples for laboratory studies were taken on 1 June, 45 days after petal fall (15 April); at MHCRS the first samples were taken on 11 June, 47 days after petal fall (25 April). In 1979, the first samples at CC were taken on 7 May, 19 days after petal fall (18 April).

RESULTS AND DISCUSSION

Effect of culture age. Culture age affected conidial germination, appressoria formation, and fruit infection. No significant differences were observed in percent germination between 4-, 12-, and 20-day-old cultures using the cellophane disk technique. However, on the cuticle of detached apple fruit significantly less germination occurred with 12- and 20-day-old cultures than with 6-day-old cultures (Table 1).

Conidia from 12- and 20-day-old PDA cultures developed fewer appressoria than 4-day-old cultures on cellophane disks after 24 hr incubation. On the apple cuticle, appressoria formation was significantly less with 12- and 20-day-old cultures than with 6-day-old cultures (Table 1). With longer incubation periods, the differences observed were not as great. Appressoria development was less on cellophane disks than on the apple cuticle with all ages of inoculum. Germ tube growth was more extensive on cellophane disks than on apple cuticle. Prolonged *G. cingulata* conidia germ tube growth was also observed in preliminary tests on glass slides.

Greater infection in both immature and fruit from storage occurred with 6-day-old cultures than 12- and 20-day-old cultures

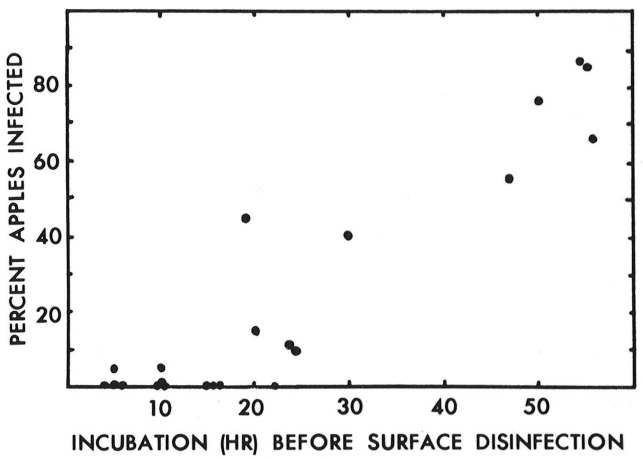

Fig. 1. Relationship between incubation time (hr) following inoculation and before surface disinfection and the percent Golden Delicious apples infected.

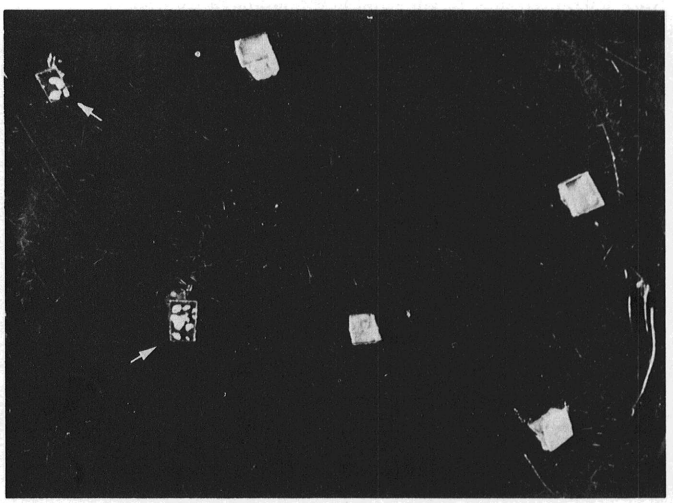

Fig. 2. Sporulation on tissue pieces taken from a Golden Delicious apple inoculated with *Glomerella cingulata.* Arrows indicate infected pieces.

(Table 2). The low infection of the mature fruit from storage may have been due to poor germination and appressoria formation on the fruit surface as well as to difficulties in detection of *G. cingulata* in apple pieces caused by overgrowth of saprophytic fungi.

Apple maturity and bitter rot susceptibility. Studies and observations in the orchard revealed that immature GD fruit are susceptible to *G. cingulata*. Infections occurred as early as 7 June in 1978 (Table 3). Infected fruit were observed by 17 June in trees inoculated on 7 June 1978. The number of infected fruit continued to increase, and by late July most fruit were rotten. Significant natural infection did not begin to develop in uninoculated trees until mid-July. Infections did not show up in trees inoculated prior to 7 June until natural infection was common on uninoculated trees. By 23 July, the chromogenic strain had been isolated from 91% of the rotted fruits in trees inoculated on 7 June as opposed to 54% of those in uninoculated trees.

In 1979, bitter rot symptoms appeared on fruit in the same orchard before 26 May. The percentage of diseased fruit per tree on 26 May ranged from 0 to 80. Isolations from a sample of 300 diseased fruit showed that six fruit from four trees were infected with conidial chromogenic *G. cingulata* strains and 294 fruits from nine trees were infected with nonchromogenic perithecial strains. Apple limbs previously inoculated with perithecial isolates and placed on the perimeter of the orchard provided the inoculum source for the perithecial isolates.

Environmental conditions were generally unfavorable for bitter rot development during the last week of May and first week of June in 1978. However, in 1979 warm temperatures and abundant rainfall occurred during May. This may help to explain why infection of fruit did not occur as early in 1978 as was observed in 1979.

Laboratory studies with detached fruit showed that immature GD apples were susceptible to infection by *G. cingulata* (Table 4). In both years of the study, infections occurred on inoculated fruit at the first sample date. The percent of fruit infected after 55 hr incubation varied from 30 to 95 while infection after 24 hr incubation ranged from 0 to 45. No seasonal trend was apparent in the percent fruit infected after 24 or 55 hr incubation. Variations in culture ages used may have hidden a seasonal trend in change of susceptibility because a trend for reduced spore germination was observed when older cultures were used for inoculations (Fig. 3). However, no relationship was found between the percent spore germination or appressoria formation and the percent of infected fruit after 24 or 55 hr incubation. Apparently, sufficient numbers of spores were deposited on the surface area sampled so that the percent germination and appressoria formation did not affect the percent apples infected.

TABLE 1. Relationship of culture age to conidial germination and appressorium formation by *Glomerella cingulata*[a]

Culture age (days)	Cellophane disks		Apple cuticle	
	Spore germination (%)	Appressoria formation (%)	Spore germination (%)	Appressoria formation (%)
4	97.5 a[b]	14.5 a[b]	...	...
6	...	...	82.1 a[c]	75.0 a[c]
12	98.6 a	4.2 b	63.4 b	53.1 b
20	94.4 a	1.0 c	67.5 b	46.4 b

[a] Conidia were examined after 24 hr of incubation at 27 C. Each value is the mean of 20 and 10 replications for the cellophane disks and apple cuticle, respectively. Replications consisted of 200 randomly selected conidia.
[b] Numbers followed by the same letter are not significantly different ($\alpha = 0.05$) according to Student's *t*-test of means with unequal variances.
[c] Numbers followed by the same letter are not significantly different ($\alpha = 0.05$) according to Duncan's new multiple range test.

TABLE 2. Relationship of culture age to infection of immature and mature fruit from storage[a,b] by *Glomerella cingulata* conidia

Age of culture (day)	Time before surface disinfection			
	Immature fruit		Mature fruit	
	24 hr	55 hr	24 hr	38 hr
6	10[c]	67[c]	10[c]	5[c]
12	10	50	0	0
20	0	50	0	0

[a] Mature Golden Delicious fruit were harvested August 1978 and stored at 1 C until use in March 1979.
[b] All fruit were spray inoculated with a suspension containing 5×10^5 conidia per milliliter and incubated at 27 C before surface disinfection. Ten apple pieces with total surface area of 250 mm^2 were cut from each apple and plated on water agar amended with 100 μg/ml streptomycin sulfate.
[c] Percent petri plates of 30 or 40 for the immature and mature fruit, respectively, with at least one apple square infected with *G. cingulata*. Each plate represents a sample from one apple.

TABLE 3. Relationship between date of inoculation and incidence of bitter rot on Golden Delicious apples in 1978[a]

Inspection dates	Tree designation and number of symptomatic fruit infected by chromogenic *Glomerella cingulata* isolates											
	A	B	C	D	E	F	G	H	I	J	K	L
May 17	*[b]	*	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
June 3	0	0	*	*	0	0	0	0	0	0	0	0
7	0	0	0	0	*	*	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	1	1	0	0	0	0	0	0
20	0	0	0	2	0	2	0	0	0	0	0	0
25	0	0	0	0	2	2	0	0	0	0	0	0
28	0	0	0	0	2	0	*	*	1	0	0	0
July 1	0	0	0	4	1	7	0	1	0	1	0	0
6	0	1	0	2	0	0	3	2	0	0	0	1
17	3	0	2	15	27	9	0	3	4	0	0	0
23	0	1	0	0	5	13	0	0	6	2	0	2

[a] All fruit (>100/tree) on inoculated trees were sprayed until runoff with 5×10^6 conidia per milliliter prepared from chromogenic *G. cingulata* isolates.
[b] Asterisk (*) indicates day of inoculation for tree. Trees I, J, K, and L were not inoculated. All fruit exhibiting rot symptoms were picked on the first day symptoms were noticed.

TABLE 4. Relationship of Golden Delicious apple maturity to germination, appressoria formation, and susceptibility to infection by *Glomerella cingulata* in the laboratory

Source of fruit and date of inoculation	Culture age (days)	Percent spore germination	Percent appressoria formation	Percent infected apples[a] after incubation for:	
				24 hr	55 hr
Central Crops 1978					
1 June	7	78.5[b]	33.0[b]	45.0[c]	55.0[c]
8 July	8	33.4	20.4	10.0	75.0
29 July	12	27.0	13.0	0.0	30.0
27 August	18	27.8	16.8	25.0	90.0
MHCRS[d] 1978					
11 June	...	69.1	45.9	45.0	55.0
21 June	7	65.3	32.4	0.0	85.0
12 July	12	22.8	12.2	10.0	65.0
26 July	9	33.0	20.0	35.0	95.0
23 August	14	17.5	7.8	5.0	45.0
Central Crops 1979					
7 May	7	...	...	3.3	36.7
28 May	7	82.0	75.0	10.0	66.7

[a] Ten apple pieces with total surface area of 250 mm^2 were sampled from each apple.
[b] Percent of 300 conidia chosen at random that had germinated or formed appressoria after 24 hr of incubation on surfaces of detached apples. The values are the means of 10 replications.
[c] Percent of petri plates (each representing one apple) with at least one apple piece infected with *G. cingulata*. Twenty apples per treatment were used in 1978, 30 in 1979.
[d] Mountain Horticultural Crops Research Station, Fletcher, NC.

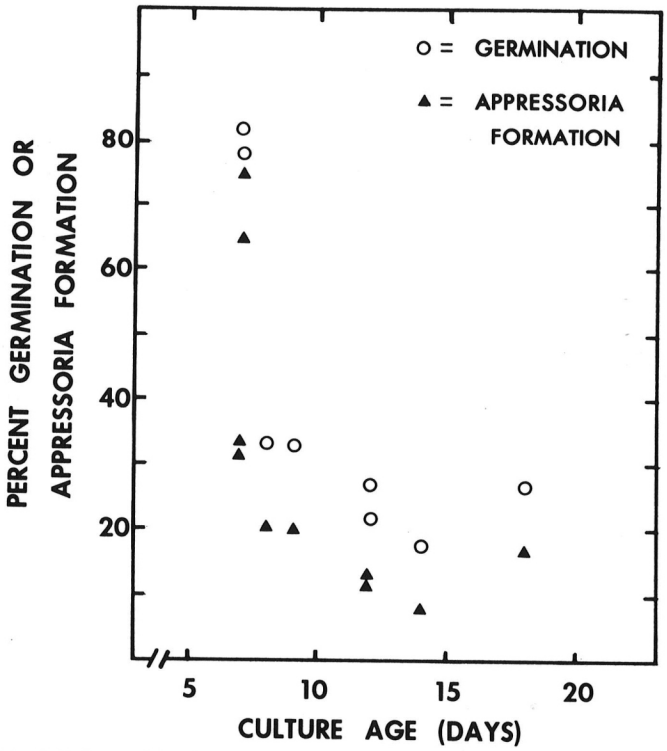

Fig. 3. Relationship of culture age to germination and percent appressorial formation on cuticle of Golden Delicious fruit.

The delayed appearance of bitter rot in North Carolina orchards may be attributed in part to size difference between immature and mature fruit. In 1978 at CC, GD apples (assuming a spherical shape) on 16 May, 1 June, 17 June, and 15 August had an average surface area of 5,900, 19,000, 25,000, and 101,700 mm^2, respectively. Thus, mature apples present more than 17 times the surface area offered by an apple 1 mo after petal fall. The smaller surface areas presented by immature apples as compared to mature were not factors in these experiments because constant surface areas were sampled. Other factors are inoculum scarcity, unsuitable temperatures for infection early in the season, and/or latent infection in young fruit. Taylor (7) reported that early infection by *G. cingulata* appeared as small gray-brown flecks that generally did not develop until the fruit began to mature. Similar brown flecks were seen in our field studies; however, many of these spots quickly developed into large lesions in May and June (Fig. 4).

The potential for bitter rot epidemics exists as early as May or June in North Carolina if inoculum is available and environmental conditions are optimal. The reasons for the intraseasonal fluctuations in susceptibility shown by this study are not known; additional experiments are needed to see if this phenomenon occurs in the field.

Fig. 4. Well-developed bitter rot lesions on naturally infected immature Golden Delicious apples 40 days after petal fall.

LITERATURE CITED

1. Garman, H. 1883. *Gloeosporium versicolor.* Kentucky Agric. Exp. Stn. Bull. 44:3-31.
2. Hasselbring, H. 1906. The appressoria of the anthracnoses. Bot. Gaz. 42:135-142.
3. Lingappa, B. T., and Lingappa, Y. 1966. Effect of nutrients on self-inhibition of germination of conidia of *Glomerella cingulata.* J. Gen. Microbiol. 41:67-75.
4. Müller, H. J. 1962. Beiträge zur Kenntnis von *Gloeosporium fructigenum* Berk. und *Gloeosporium musarum* Cks. et Mass. Phytopathol. Z. 44:381-412.
5. Roberts, J. W. 1918. The sources of apple bitter-rot infections. U.S. Dep. Agric. Bull. 684. 24 pp.
6. Sitterly, W. R., and Shay, J. R. 1960. Physiological factors affecting the onset of susceptibility of apple fruit by fungus pathogens. Phytopathology 50:91-93.
7. Taylor, J. 1971. Epidemiology and symptomatology of apple bitter rot. Phytopathology 61:1028-1029.
8. Wallace, J., Kuć, J., and Draudt, H. N. 1962. Biochemical changes in the water insoluble material of maturing apple fruit and their possible relationship to disease resistance. Phytopathology 52:1023-1027.
9. Welty, R. E., and Collins, H. B. 1979. Loss of viability of spores of *Colletotrichum trifolii* produced on lima bean agar. Plant Dis. Rep. 63:486-489.