Relationship of Apple Fruit Maturity and Inoculum Concentration to Infection by *Glomerella cingulata*

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ABSTRACT

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An inoculum concentration of 10^7 conidia per milliliter resulted in 65% infection of mature apple fruit by *Glomerella cingulata*. The rate of infection dropped off rapidly to only 25% at 10^6 conidia, and no infection occurred at 10^4 or 10^3 conidia per milliliter. Wounding did not affect the percentage of fruit developing lesions. Sucrose concentration and pH of extracted juice from apple fruit were not positively correlated with susceptibility to infection by *G. cingulata*. Apple fruit was susceptible at all stages of development.

Additional key words: bitter rot, epidemiology

Bitter rot of apples, caused by *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, has generally been considered to be a midsummer to late-summer disease. Reports from various areas have established the typically late appearance of this disease. Hopperstead et al (6) reported that the disease does not appear until the beginning of July in Delaware. In New South Wales (3) and Kentucky (7), the disease reportedly is not evident until the fruit is half-developed. The disease appears no earlier than June in Virginia (5). Roberts (10) reported that the disease appears no earlier than June in the eastern United States, but that different varieties of apple are susceptible at different stages of development. The apple spray guide for Georgia recommends that specific sprays for the control of bitter rot begin about 24 days after petal fall (mid-May in a typical year) (8).

It has generally been thought that immature fruit is resistant to infection by *G. cingulata* (1,2). Sitterly and Shay (11) reported that susceptibility of apple fruit to infection by *G. cingulata* was dependent on the sucrose content of the fruit and that susceptibility could be induced in immature fruit by sucrose infusion. Taylor (12), however, reported susceptibility of immature fruit during or shortly after bloom, although lesions did not appear until early to mid-May.

This study establishes the susceptibility of apple fruit to infection by *G. cingulata* at all developmental stages and reviews the relationship of sucrose content to susceptibility. More precise knowledge of the resistance or susceptibility of apple fruit as it matures may result in a more effective program of control. The number of control applications could be reduced or increased according to the susceptibility of the developing fruit.

To our knowledge, there have been no reports on the susceptibility of apple fruit to infection by *G. cingulata* at different inoculum concentrations. An inoculum concentration established for a consistently high rate of infection on mature fruit in the laboratory could be used as a standard to assess the resistance or susceptibility of immature apple fruit.

MATERIALS AND METHODS

A conidial suspension was prepared from 11-day-old cultures grown at 26 C on acidified potato-dextrose agar made from fresh potatoes. Sterile distilled water was poured into the petri dishes, and cultures were scraped with a rubber spatula. The resulting suspension was poured through four layers of cheesecloth, and conidia were counted with a hemacytometer and adjusted to a concentration of 10^7 conidia per milliliter. Serial dilutions were prepared of 10^6, 10^5, 5×10^4, 10^4, and 10^3 conidia per milliliter. Mature Delicious apples were washed, rinsed in deionized water, and allowed to dry. Twenty apples were sprayed with 20 ml of each of the six inoculum concentrations. The apples were placed in damp chambers lined with wet cotton and incubated for 21 days at 26 C. Twenty uninoculated apples were held in damp chambers as a control.

In a second experiment, four circles (1 cm diameter) were drawn on an apple with a wax pencil, and 0.01 ml of inoculum suspension was pipetted into each circle. Five apples were inoculated with each inoculum concentration for a total of 20 sites per concentration. Inoculated apples and uninoculated controls were incubated as described earlier. The drops were not allowed to dry before placement of apples in the damp chambers.

To assess the effect of wounding on susceptibility, mature Delicious apples

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were rubbed with cheesecloth soaked with suspensions of Celite and Carborundum (5 g/100 ml of water). Treated apples were examined microscopically to ensure that the epidermis had been penetrated in the wounding process. An inoculum at a concentration of 10^7 conidia per milliliter was prepared as previously described. Wounded and unwounded apples were spray inoculated and incubated in damp chambers as described earlier. Wounded and unwounded, uninoculated controls were also incubated. Ten apples were incubated for each treatment.

To assess the effect of developmental stage on susceptibility of apple fruit to infection with *G. cingulata*, 20 Delicious apples were collected weekly throughout the growing season from each of two locations: Gray, in middle Georgia, and Blairsville, in north Georgia. Bloom and harvest dates differed by about 4 wk at these locations. Apples were washed, rinsed in deionized water, allowed to dry, and sprayed with a conidial suspension (10^7/ml) at the rate of 20 ml/20 apples. Apples were incubated as described earlier.

Twenty apples were also collected weekly at each of the two locations for analysis of the sucrose content and pH of the extracted juice. Sections of tissue were cut from each apple and squeezed, and the juice was collected in a 50-ml flask on ice. Sucrose concentration was determined with a hand-held refractometer. The pH of the extracted juice was determined with an electrode pH meter.

RESULTS

Apple fruit did not develop bitter rot lesions when inoculated with 10^3 and 10^4 conidia per milliliter (Table 1). Only 15% of the spray-inoculated fruit and 3–5% of the drop-inoculated sites developed lesions at 5×10^4 conidia per milliliter. The proportion of fruit developing lesions greatly increased between 10^6 (25%) and 10^7 conidia per milliliter (65%). The percentage of drop-inoculated sites that developed lesions also increased, but only to 35% at 10^7 conidia per milliliter. Thus, only about one-fifth of the sites developed lesions when 10^5 conidia were placed on each site.

Table 1. Effect of inoculum concentration applied as spray or pipetted drops on infection of apple fruit by *Glomerella cingulata*

Conidia ($\times 10^3$/ml)	Fruit developing lesions (%)[x]	
	Spray[y]	Drop[z]
10	0 c	0 b
50	15 bc	5 b
100	17 b	15 ab
1,000	25 b	15 ab
10,000	65 a	35 a

[x]Data with a common letter are not significantly different ($\alpha = 0.05$) based on chi-square test of proportions.

[y]Data are averages from 40 apples in two experiments.

[z]Data are averages from 40 sites in two experiments.

Wounding apple fruit by rubbing with Celite and Carborundum suspensions did not increase the percentage of fruit developing bitter rot lesions. Combined results of two experiments showed 60 and 55% lesion development for unwounded and wounded fruit, respectively. The epidermis was abraded and ruptured on all the wounded fruit. Lesions were not present in any of the uninoculated, wounded or unwounded control apples.

Susceptibility of apple fruit to infection by *G. cingulata* did not increase as the fruit approached maturity. All fruit in the early stages of development became infected after inoculation with the fungus, and 80–90% of those approaching maturity became infected. The sucrose concentration in extracted juice increased from 7.0 to 13.8% in apples collected at Gray and from 7.6 to 10.5% in those from Blairsville. This increase in sucrose concentration did not increase susceptibility to infection with *G. cingulata*. The pH of extracted juice increased from 3.5 to 3.8 at both locations. Although there was fluctuation in sucrose concentration and pH throughout the growing season, there was no positive correlation with the frequency of infected fruit. All correlation coefficients were negative. The correlation between sucrose concentration and infection frequency, $r = -0.52$, was significant at $\alpha = 0.05$.

DISCUSSION

Wallace et al (14) reported that *G. cingulata* was relatively low in virulence when compared with other fungal pathogens of apple fruit. They further stated that this relatively low degree of virulence was the result of low production levels of extracellular pectolytic enzymes by the fungus. A high inoculum level was required to achieve a high rate of lesion development on mature fruit in the laboratory. This high inoculum concentration is conceivable in view of the capacity of this organism to produce conidia. Conidial production typically ranges from 3 to 6×10^9 conidia on a 100-mm petri dish of potato-dextrose agar after 11 days. Miller and Baxter (9) reported conidial production in the range of 2.7–5.7×10^9 conidia on a 100-mm petri dish of carrot juice agar after 14 days for an isolate of *G. cingulata* pathogenic to camellias. With raindrop dispersal of conidia in gelatinous spore masses, such high inoculum concentrations can be expected under field conditions. Fruit losses as high as 80% have been reported in unsprayed orchards (13). The infection rates obtained at lower inoculum concentrations were sufficient to cause considerable economic losses because one lesion on an apple results in total loss.

Wounding did not affect the susceptibility of apple fruit to infection by *G. cingulata*. This would be expected according to previous reports of direct penetration of apple fruit by conidia of *G. cingulata* (2,5). Brook (4) has reported hyphal penetration through the cuticle and epidermal cells of uninjured apple fruit.

Sucrose content and pH of the extracted juice had no positive relationship to the susceptibility of apple fruit to infection by *G. cingulata*. The sucrose content increased only slightly, and pH was relatively stable during the growing season. Sitterly and Shay (11) did not report the percentage of sucrose of treated apples that resulted in increased susceptibility to *G. cingulata*. The percentage of sucrose as a result of the infusion treatments may have been much higher than occurs naturally. The infusion process may also have increased susceptibility to some degree by altering the internal metabolism of the apple fruit.

Apple fruit was susceptible to infection by *G. cingulata* at all stages of development. Taylor (12) reported that 14% of Detroit apple fruit was infected when sprayed with 10^4 conidia per milliliter at petal fall and 2 wk after petal fall. Our results, which showed that apple fruit was susceptible from 3 wk after petal fall until harvest, could affect current programs for the control of bitter rot. The apple fruit may become infected at any time from petal fall to harvest if there is enough inoculum and if environmental conditions are favorable. The typical appearance of this disease only in midsummer to late summer could be a function of the time necessary for the fungus to produce enough inoculum from overwintering sources for infection of the apple fruit. Environmental conditions as the season progresses would also play a part in the production and dissemination of conidia.

LITERATURE CITED

1. Anderson, H. N. 1956. Diseases of Fruit Crops. McGraw-Hill, New York. 501 pp.
2. Anonymous. 1956. Diseases of apples and pears. Ill. Univ. Coop. Ext. Serv. Publ. 16. 18 pp.
3. Biological and Chemical Research Institute. 1970. Bitter rot of apples. Pages 647-650 in: The Agricultural Gazette of New South Wales, Department of Agriculture, December.
4. Brook, P. J. 1977. *Glomerella cingulata* and bitter rot of apples. N.Z. J. Agric. Res. 20:547-555.
5. Drake, C. R., Hickey, K. D., and Shear, G. M. 1970. Apple diseases and their control in Virginia. Va. Polytech. Inst. Coop. Ext. Serv. Publ. 374. 20 pp.
6. Hopperstead, S. L., Goodwin, M. W., and Kadow, K. J. 1943. Bitter rot of apples and its control in Delaware. Del. Univ. Agric. Exp. Stn. Bull. 241. 23 pp.
7. Johnson, E. M., and Stacey, J. R. 1966. Bitter rot of apple. Plant Disease in Kentucky. Univ. Ky. Coll. Agric. Coop. Ext. Serv. Publ. 83. 2 pp.
8. McGlohon, N. E. 1978. Apple spray guide for middle Georgia. Univ. Ga. Coop. Ext. Serv. Bull. 740. 11 pp.
9. Miller, S. B., and Baxter, L. W., Jr. 1970. Some factors influencing asexual sporulation in a strain of *Glomerella cingulata* pathogenic to Camellias. Phytopathology 60:743-744.
10. Roberts, J. W. 1918. The sources of apple bitter

rot infections. U.S. Dep. Agric. Bull. 684. 25 pp.
11. Sitterly, W. R., and Shay, J. R. 1960. Physiological factors affecting the onset of susceptibility of apple fruit to rotting by fungus pathogens. Phytopathology 50:91-93.
12. Taylor, J. 1971. Epidemiology and symptomatology of apple bitter rot. Phytopathology 61:1028-1029.
13. Taylor, J., and McGlohon, N. E. 1975. Apple diseases and their control. Ga. Agric. Res. Spring 1975. 8-11.
14. Wallace, J., Kuc, J., and Williams, E. B. 1962. Production of extracellular enzymes by four pathogens of apple fruit. Phytopathology 52:1004-1009.