

## A Necrotic Leaf Blotch and Fruit Rot of Apple Caused by a Strain of *Glomerella cingulata*

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### ABSTRACT

A strain of *Glomerella cingulata* morphologically similar to the apple bitter rot fungus causes a necrotic leaf blotch as well as a fruit rot of Golden Delicious apple and a fruit rot of other cultivars. It differs from the common isolates of the bitter rot organism culturally, by its capacity to cause a

leaf blotch, overwinter and reproduce on apple leaves, and cause characteristic fruit symptoms without sporulation in fruit lesions. Fungicides and resistant varieties appear to offer an economical means of control. *Phytopathology* 61:221-224.

A necrotic leaf blotch on the Golden Delicious cultivar of apple in southeastern USA is associated with severe defoliation during July and August, while other cultivars are not affected (3). In some orchards, a fruit rot was frequently associated with the leaf spot.

Surveys show that the disease is widespread in the Piedmont and Coastal Plains area of Georgia, causing extensive defoliation and fruit losses on Golden Delicious and less severe fruit losses on other cultivars. The rot was tentatively diagnosed as black rot, caused by *Phylospora obtusa*, on the basis of symptoms; however, platings from fruit and leaves gave cultures of *Glomerella cingulata* (Ston.) Spauld. & Schrenk differing from those commonly isolated from bitter rot of apple (2, 5).

The fungus has been found attacking leaves, fruit, and stems of Golden Delicious in orchards and causing fruit rot of Red Delicious and Jonathan. Symptoms resulting from artificial inoculations were similar to those observed in nature.

On the leaves, the first evidence of the disease is the appearance of small red specks within 3 days after artificial inoculation. Ordinarily these are first evident in early summer in nature. On immature leaves, necrosis begins to appear within 5 days, and irregular light tan spots measuring 3 to 12 mm in diam develop within 10 days (Fig. 1-A). If infection is severe the spots coalesce, sometimes involving the entire leaf. Such leaves turn brown and drop within 2 weeks. Less severely affected leaves usually yellow and drop within 2-4 weeks, depending on the severity of infection. Leaves that are fully grown at the time of infection show a wide range of symptoms, from dark brown necrotic spots to little evidence of necrosis. All such leaves usually develop a bright yellow mottle and drop. When weather conditions are suitable for disease development throughout the summer months, wave after wave of yellow leaves drop, resulting in almost complete defoliation. When developing leaves of resistant varieties are inoculated, the fungus becomes established in the host tissue, resulting in dwarfing and crinkling of leaves without necrosis (Fig. 1-C). But varying degrees of necrosis or yellow mottling may develop as the leaves age.

On the fruit, infections may be detected as the crop approaches maturity. The first sign of the disease on the fruit is a faint light brown speck on the ripening apple. As the spot enlarges, it becomes dark brown, and usually a series of alternating concentric rings form in the rotted tissue similar to the black rot caused by *P. obtusa*. The darker bands frequently become a deep mahogany brown. As the lesion reaches about 20 mm in diam, scattered black specks may appear beneath the cuticle, but these seldom rupture the cuticle and very few conidia are produced. This character serves to differentiate the rot from the common bitter rot. The affected tissue is somewhat firm and inclined to be leathery. The rotted area retains the original contour of the apple at first, thus further differentiating it from bitter rot lesions, which show a saucerlike depression. As the spot ages, it becomes somewhat flattened (Fig. 1-B). In general, the rotted internal tissue is light brown with a purplish brown pigment development near the center of the rotted area. This coloration is not detectable externally.

On the stem, infections appear as small red specks that fail to develop further in nature.

Since Golden Delicious is widely planted as a pollinator for other cultivars in the southeastern USA, studies were undertaken to identify the causal organism, determine the symptoms of the disease on fruit and leaves, study the disease cycle, and develop control measures.

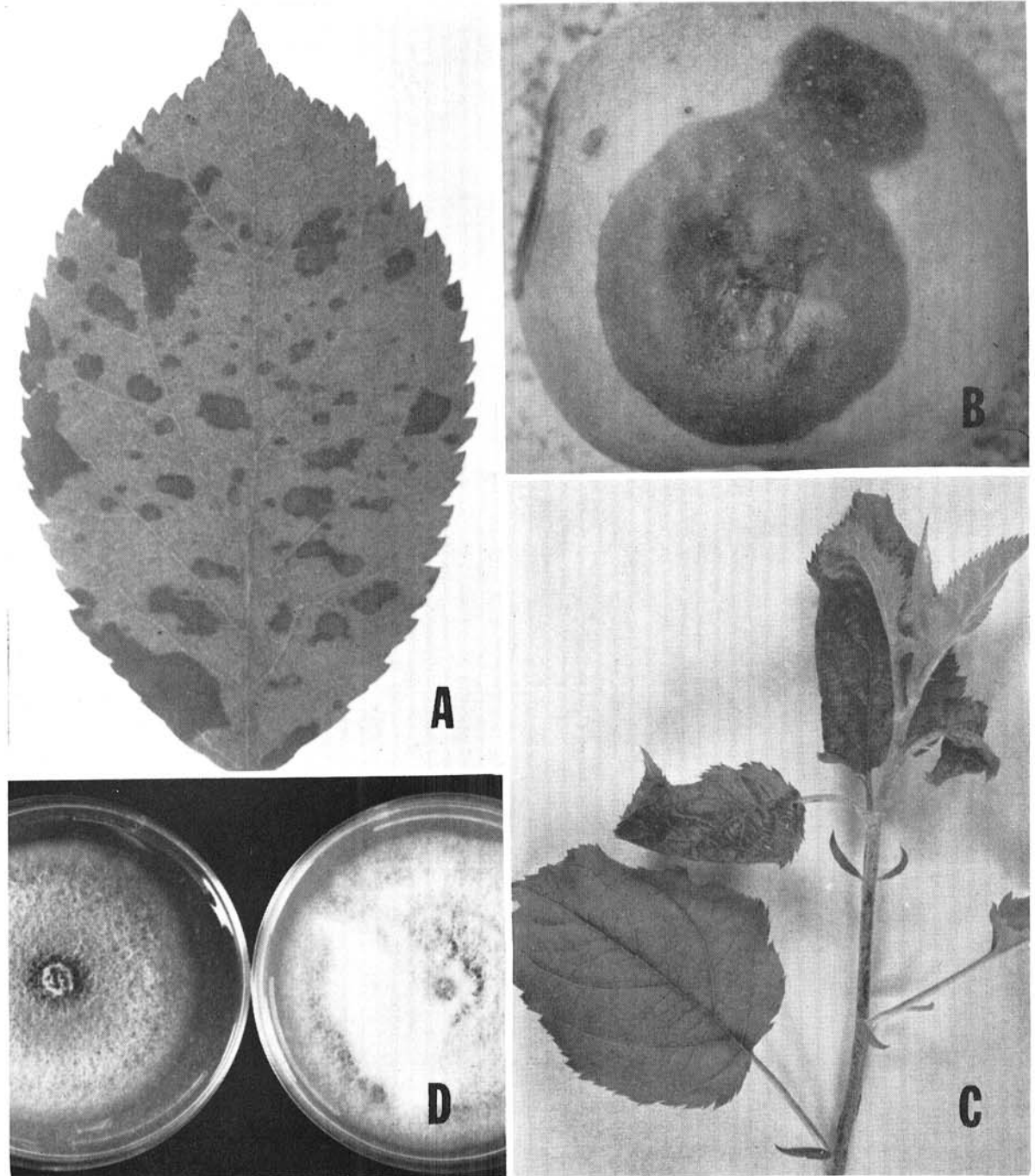
**MATERIALS AND METHODS.**—Cultures used in these studies were obtained from fruit and leaves of Golden Delicious and from fruit rots of other apple cultivars growing in middle Georgia orchards. Single spore isolates were compared in culture, tested for pathogenicity on leaves and fruit of Golden Delicious, and maintained on potato-dextrose agar (PDA) at 5 C.

Isolate 669 of the necrotic leaf blotch fungus was selected as representative of all isolates for comparison with the bitter rot fungus. Growth rates were determined on 20-ml portions of PDA in 90 × 15 mm culture dishes. Discs 4 mm in diam were cut from the peripheries of 6-day-old cultures and inverted onto the agar medium. The seeded dishes were placed in temperature control chambers at 0, 5, 10, 15, 20, 25, 30, and 35 C.

Colony diam measurements were made after 6 days of each isolate replicated 5 times at each temp.

The necrotic leaf blotch fungus (NLB) was grown on PDA under continuous fluorescent light to produce spores for inoculation studies. For spore production, 1 ml of a suspension of conidia from a 5-day-old colony

was distributed over the surface of 20 ml of PDA in culture dishes. The inoculated plates were maintained under fluorescent light at 20 C for 5 days. Spore suspensions for inoculations were prepared by submerging the 5-day-old cultures in water and brushing the surface of the colony with a camel's hair brush. The resulting



**Fig. 1.** Leaf and fruit symptoms of necrotic leaf blotch and fruit rot caused by the necrotic leaf blotch (NLB) strain of *Glomerella cingulata* and a cultural comparison with the apple bitter rot fungus. **A)** Necrotic leaf blotch on Golden Delicious; **B)** fruit rot of Golden Delicious caused by the NLB strain of *G. cingulata*; **C)** leaf malformation on seedling of Golden Delicious × Red Delicious resulting from inoculation with the NLB fungus; **D)** PDA cultures of apple bitter rot organism (left) and the NLB fungus (right) grown in dark 6 days at 25 C.

suspension was agitated for 2-3 min in a blender, diluted to approx 6,000 spores/ml, and sprayed on leaves and stems of plants in the greenhouse. After the plants were inoculated, they were placed in a moist chamber at 20-25 C for 48 hr, then maintained on greenhouse benches.

Tests for host resistance were made with fruit and leaves of the Golden Delicious, Red Delicious, Winesap, Yates, and Detroit cultivars. Leaves and stems of three plants of each cultivar were sprayed with aq suspensions of conidia. One branch of each plant was protected from inoculum to serve as a control. Fruit inoculations were through wounds of ripe detached apples.

Isolates from lupine and camellia were compared with the apple bitter rot fungus and an isolate of the NLB fungus on leaves of Golden Delicious growing in the greenhouse. Spore suspensions of each were sprayed on separate branches of each plant. Leaves on one branch of each plant were protected from inoculum to serve as a check. Subsequent treatment of the host was similar to previous infection studies.

To determine a source of inoculum, specimens were collected from the affected orchard each month from January through 15 June 1969. Leaves showing evidence of leaf spot were examined for development of fruiting structures of the pathogen. Apples were stuffed with leaf material from each collection by removing a 15-mm plug from the fruit, placing the leaf material in the cavity, replacing the apple section, and incubating in a moist chamber at 25 C. Platings were made from margins of developing lesions.

The NLB pathogen was tested against fungicides in a depression slide bioassay and also in the greenhouse on leaves of Golden Delicious. Chemicals tested were captan [*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide], ferbam (ferric dimethylthiocarbamate), maneb [manganous ethylenebis (dithiocarbamate)], benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate], and Dikar [a formulation of coordination products of zinc ion and maneb (74%) plus a mixture of 2-(1-methylheptyl)-4,6-dinitrophenol and 2-(1-methylheptyl)-4,6-dinitrophenol crotonate (6%)]. In the depression slide tests, the fungicides were compared in three replications at 50, 5, and 0.5 ppm concn, and spore germination counts were made after 24 and 48 hr.

The fungicides assayed on depression slides were also evaluated on the host. Six branches were grown on each of five Golden Delicious trees in the greenhouse. Leaves and stems of one branch on each plant were sprayed with a water suspension of each fungicide, one branch of each plant was sprayed with water alone, and one branch was protected from fungicides and from subsequent inoculation with the pathogen. After the fungicides had dried, the leaves of the six treated branches were sprayed with a water suspension of 1,000 spores/ml. The plants were placed in a moist chamber for 48 hr, then maintained on greenhouse benches. After 7 days, infection ratings were made.

**RESULTS.—Morphology of the fungus.**—Both acervuli and perithecia are produced in culture and on

apple leaves in nature, but in contrast to the bitter rot fungus, sporulation is seldom observed on the fruit.

Mycelium is branched, septate, originally hyaline, later taking on an olive tint. In leaf and fruit tissue, the mycelium produces dark-walled stromata. They seldom develop into sporulating structures on the fruit, but the dark stromata formed beneath the leaf epidermis later rupture the epidermis, producing acervuli which usually exhibit irregular setae.

Conidial morphology of the leaf blotch fungus is generally more uniform than the literature shows for *G. cingulata* (5). Conidia are subhyaline, pinkish in mass, oblong-cylindrical, and  $3.9\text{--}5.2 \times 13\text{--}18.2 \mu$ , mostly  $5 \times 15.5 \mu$ . In culture, conidia may be produced individually or in small clusters along the aerial mycelium, or in pinkish masses on stromatic cushions.

Perithecia are subspherical, immersed to erumpent in leaf tissue, and single or grouped in stromata. Asci are clavate, pedicellate, evanescent, and  $10.4 \times 68 \mu$ . Ascospores are hyaline, continuous, allantoid, mostly  $5.2 \times 18.2 \mu$ , and when mature, are readily distinguishable from conidia. Perithecia with mature spores are produced in 15-20 days on PDA under fluorescent light at 25 C.

The NLB fungus consistently differed from the apple bitter rot organism in cultural studies. Isolates almost invariably produced perithecia, asci, and viable ascospores on PDA under fluorescent light at 15-30 C, whereas those of the bitter rot fungus did not. Colonies were white and cottony at first (Fig. 1-D), later becoming light to medium-gray-and-white borders. Cultures became dark gray as perithecia developed. Those of the bitter rot fungus usually produced a pinkish color in PDA.

Development of conidia was profuse in most NLB isolates, but sporulation became less luxuriant as the fungus was maintained in culture.

Growth rates of the NLB fungus in vitro at various temp were similar to those of bitter rot isolates, and fell within limits established for *G. cingulata* (4). Some growth occurred in 6 days on PDA at temp ranging from 5-35 C. The fungus grew well at temp ranging from 15-30 C, with max growth occurring between 25-30 C.

**Pathogenicity.**—The NLB fungus was pathogenic on leaves of Golden Delicious and on ripe fruit of all cultivars tested. *Glomerella cingulata* isolates from apple bitter rot, lupine, and camellia failed to cause symptoms on Golden Delicious leaves.

Inoculations with an NLB isolate developed red flecks on Golden Delicious leaves and stems in 3 days. Necrosis was first observed on leaves in 4 days, with spots attaining diam of 1-7 mm in 10 days. Infected leaves developed chlorosis and dropped in 10-20 days after inoculation. Symptoms were similar to those observed in the orchard. Necrosis failed to develop in stem infections. The fungus was reisolated in every case.

The pathogen became established in developing leaves of Red Delicious, Yates, Winesap, and Detroit, resulting in severe malformation without necrosis. Invasion was confirmed by reisolation.

TABLE 1. Depression slide bioassay of fungicides against conidia of the necrotic leaf blotch fungus

Fungicide concentration — ppm active ingredient	% Germination				
	Benomyl	Captan	Ferbam	Maneb	Dikar <sup>b</sup>
Water control	41	36	53	38	44
0.5	37	31	T	0	0
5.0	7	T <sup>a</sup>	0	0	0
50.0	35	T	0	0	0

<sup>a</sup> Less than 1% germination.

<sup>b</sup> Formulation of coordination products of zinc ion and maneb (74%) plus a mixture of 2-(1-methylheptyl)-4,6-dinitrophenol and 2-(1-methylheptyl)-4,6-dinitrophenol crotonate (6%).

Seedlings of Golden Delicious × Red Delicious when inoculated showed three types of responses: (i) stem and leaf blight; (ii) necrotic leaf spots similar to those on the Golden Delicious parent; and (iii) leaf crinkle similar to symptoms on the Red Delicious parent (Fig. 1-C).

*Disease cycle and control.*—Leaf and fruit specimens collected from January through May produced cultures of the NLB fungus. Perithecia were found developing in overwintered leaves during May and June, with mature ascospores present in 15 June collections. Leaves collected from greenhouse inoculations and incubated in a moist chamber for 20 days at 25 C showed profuse development of mature setose acervuli and perithecia with viable ascospores.

The NLB fungus failed to sporulate in fruit lesions both in the laboratory and in the field. It sporulated in leaf spots in the orchard, however, and leaf lesions appeared to be the primary source of secondary spread. Field collections of diseased leaves showed abundant sporulation after 3 days in a moist chamber.

Depression slide bioassays indicated that the recommended captan spray program may not be adequate and that other materials may be more effective (Table 1). The greenhouse fungicide tests and depression slide results showed that maneb materials were consistently effective (Table 2). Benomyl was inconsistent in all tests.

DISCUSSION.—The NLB fungus is morphologically

TABLE 2. Fungicide tests against conidia of the necrotic leaf blotch fungus on leaves of Golden Delicious in the greenhouse

Fungicide and active ingredient concentration in g/liter	Avg no. spots/leaf
Noninoculated control	0
Inoculated, water control	63
Benomyl, .3	11
Captan, 1.2	2
Ferbam, 1.2	0
Dikar, <sup>a</sup> 1.2	0
Maneb, 1.2	0

<sup>a</sup> Formulation of coordination products of zinc ion and maneb (74%) plus a mixture of 2-(1-methylheptyl)-4,6-dinitrophenol and 2-(1-methylheptyl)-4,6-dinitrophenol crotonate.

similar to the bitter rot organism (5); however, the bitter rot *Glomerella* has not been reported to cause leaf spot on apple. Nor has it been found to overwinter and sporulate in leaves. Fruit symptoms resulting from infections of the two fungi are distinctly different, and the NLB fungus sporulates sparingly if at all in fruit lesions. In the bitter rot disease, fruit infections serve as a source of secondary spread, while fruit infections by the NLB fungus do not appear to be an important source of secondary inoculum. Since the conidial and perithecial stages of the new fungus are morphologically similar to *G. cingulata*, and since the species concept is considered to be sufficiently broad to encompass fungi causing fruit rots, leaf spots, and necroses of numerous plants (1), there is insufficient basis for describing the new fungus as a species or even as a variety of *G. cingulata*. Therefore, the apple necrotic leaf blotch and fruit rot fungus must be considered to be a specialized strain of the species.

Although Golden Delicious is the only apple cultivar found to be affected by the leaf blotch phase of the disease, the fact that some of the seedlings from Golden Delicious × Red Delicious are more susceptible than either parent indicates that susceptibility is conditioned by more than a single gene, and that susceptible genetic material may be widespread in cultivated varieties.

The NLB strain of *G. cingulata* reacted to fungicides essentially the same as the bitter rot fungus in laboratory and greenhouse tests. Reports of field tests for control of bitter rot support these studies (3, 6). Therefore, fungicidal programs for control of bitter rot may be expected to give satisfactory control of the new disease.

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