Histopathology of Fleck and Lesion Symptoms on Blueberry Infected with Gloeosporium minus

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ABSTRACT

Host-pathogen relationships of *Gloeosporium minus* were studied histologically in infected blueberry leaves. Penetration of the leaf tissue was directly through the cuticle. Appressoria were formed 48 hr after inoculation and were unicellular, generally spherical to obovate, and measured 2 to 4 μ in size. Penetration hyphae enlarged within the epidermal cell into broad primary hyphae ca. 2 μ in diam which invaded adjacent cells intracellularly. Addition of glucose to the inoculum increased spore germination, appressoria formation, and hyphal growth prior to penetration. Infected epidermal cells located near the center of the lesion were filled with pseudoparenchyma. In several instances, infection of the hydathode tissue resulted from germ tube growth through the broken cuticle without appressoria formation. All leaf tissues from the lower epidermis to the upper epidermis, including the vascular tissue, were invaded by the fungus. Twenty-four days after inoculation, the hydathode and infected leaf tissues were completely disorganized and necrotic, and mature acervuli had developed. The resistant fleck symptom apparently is a hypersensitive reaction.

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Additional key words: inoculum potential, resistant, susceptible.

Gloeosporium leaf spot caused by *Gloeosporium minus* Shear is a serious disease of highbush blueberry (*Vaccinium corymbosum* L.) in southeastern North Carolina, and frequently results in severe defoliation in blueberry plantings. Two distinct types of pathogenic reactions that occur on blueberry leaves are flecks and lesions. The first symptoms of the disease are small reddish flecks on young succulent leaves and stems of succulent shoots (8, 9). Leaf flecks do not develop further, but leaves that are heavily infected become puckered and malformed. Development of large, brown, circular to irregular-shaped lesions which are 5 to 10 mm in diam occurs near leaf hydathodes or glands where high concentrations of carbohydrates are exuded, and from wound inoculations (5). Large lesions were induced anywhere on the leaves by the addition of high concentrations of glucose to the inoculum. The research reported herein was undertaken to determine the mode of infection and the histological effects of the pathogen on various leaf tissues.

MATERIALS AND METHODS.—Cultures of *G. minus* were obtained from large lesions on blueberry leaves collected in southeastern North Carolina. Monoconidial isolates were obtained from these cultures and used in all inoculation tests.

I collected conidia by scraping with a razor blade the surface of a 2-week-old culture grown on potato-dextrose agar (PDA) in a petri dish, then flooding the culture with sterile distilled water. I suspended conidia uniformly in either distilled water or 50% glucose solution, and made inoculations by spraying the inoculum (10^6 conidia/ml) onto young succulent leaves of the cultivar Croatan or by applying the inoculum to the lower leaf surface and hydathodes with a camel's-hair brush. Leaves to be examined for germination and penetration by the pathogen were excised, placed in moist petri dishes, and incubated at 27 C. Inoculated leaves were not excised from other plants that were placed in a moist chamber at 25 to 30 C for 48 hr, then transferred to a greenhouse bench.

Spore germination and appressoria formation were observed 24, 48, and 72 hr after inoculation. Leaves were cut into 5-mm sections, cleared, and stained with cotton blue in lactophenol (7). Fungal penetration and disease development were determined 3, 6, 12, and 24 days after inoculation. Leaves were cut into 5-mm sections, cleared, and fixed in Carnoy's solution, dehydrated in tertiary butyl alcohol, and embedded in paraffin. Sections 10 μ thick were mounted on slides with Haupt's adhesive and stained with safranin and fast green (3).

RESULTS.—*Lesion development.*—Germination of conidia suspended in a glucose solution and applied to the lower leaf surface was ca. 88% after 72 hr. Germ tubes ranged in length from 5 to 300 μ (average 90 μ) and were extensively branched. Seventy-two percent of the germinated conidia produced appressoria. This resulted in 63% of the conidia being potentially infective propagules. Occasionally, hyphae from germ tubes were observed penetrating open stomates without forming appressoria. Appressoria were unicellular, generally spherical to obovate, sometimes clavate in shape, and measured 2 to 4 μ in diam.

Fifteen percent of the appressoria formed after inoculations with conidia suspended in glucose penetrated the epidermal cells. Although penetration was usually direct, penetration from an appressorium through a stomate occasionally was observed. A short penetration hypha from the lower surface of the appressorium penetrated through the cuticle and the epidermal cell wall. The hypha enlarged into a broad primary hypha ca. 2 μ in diam within the cell (Fig. 1-A). Secondary hyphae grew from the primary

hyphae and invaded adjacent cells. The host cells and fungus were apparently compatible, since no physical or staining abnormalities were observed during the early period of infection.

Conidia suspended in glucose and inoculated onto blueberry leaves produced small, circular to irregular-shaped lesions after 6 days. Hyphal penetration had extended intracellularly from the lower epidermis through three layers of mesophyll cells. Hyphae were slightly swollen when contact was made with cell walls, and became somewhat constricted within the wall. Hyphae measuring 1 to 2 μ in diam occurred singly or in masses within infected cells. Many epidermal cells were enlarged and filled with pseudoparenchyma (Fig. 1-B). Some parenchyma cells in the mesophyll tissue had collapsed and a few cell walls were ruptured.

Specialized secretory hydathodes or glands of blueberry leaves consist of an outer layer of small, closely compacted parenchyma cells (epithem) ca. five to six cells deep (Fig. 1-C). Adjacent to these are the larger, more loosely arranged mesophyll cells that often surround the end of a vascular bundle. Penetration through the hydathode was generally by an appressorium (Fig. 1-D). In several instances, where the cuticle had been broken, the germ tubes without forming appressoria penetrated into the hydathode tissue and eventually infected the parenchyma cells.

Six days after inoculation, the fungus had invaded the modified parenchyma cells of the epithem. The outer layer of heavily infected cells had collapsed and were completely disorganized (Fig. 1-E). The fungus had penetrated through the hydathode into the mesophyll tissue of the leaf by the 12th day. All leaf tissue from the lower epidermis to the upper epidermis, including the vascular tissue, was invaded by the fungus. Many cell walls had ruptured, and invaded cells stained rapidly with safranin. Twenty-four days after inoculation, the hydathode and infected leaf tissue were completely disorganized and necrotic.

Development of the acervulus of *G. minus* occurred after the fungus invaded the palisade cells and upper epidermis. The fungus colonizes the tissue below the epidermal cells, and as the acervulus develops, epidermal cells are pushed upward, finally rupturing the epidermis and extruding a mass of hyaline conidia measuring 6-9 X 3-4 μ (Fig. 1-F).

Dark brown, irregular-shaped lesions 2 to 5 mm in diam were produced on leaves where infection had taken place at the hydathodes.

Fleck development.—*G. minus* conidia suspended in distilled water on blueberry leaves germinated 10, 40, and 60% after 24, 48, and 72 hr, respectively. Germ tube length ranged from 2 to 125 μ (average 65 μ) after 72 hr. Average width of germ tubes was 1.6 μ. Forty-two percent of the germinated conidia produced appressoria 72 hr after inoculation. This resulted in 25% of the spores being potentially infective propagules. Appressoria generally were distributed randomly over the lower leaf surface. The length of a germ tube from a conidium to an appressorium varied greatly. Some appressoria were produced with little or no measurable germ tube, whereas some germ tubes grew as much as 60 μ before forming an appressorium. Although some germ tube branching was observed, the majority of the germ tubes were unbranched.

Direct penetration of the epidermal cells resulted from hyphae (ca. 1.0 μ in diam) that grew from appressoria. Occasionally, the hypha that penetrated the cell wall became constricted and measured 0.2 to 0.4 μ in diam. After ingress through the cell wall, the hyphae widened into larger primary hyphae, causing the cell membrane to collapse. Immediately after penetration, the infected cell and those adjacent to it became impregnated with a dark-staining material.

Small red flecks were observed on the lower leaf surface 3 to 4 days after inoculation. The flecks were ca. 150 μ in diam and encompassed some 15 to 20 cells. Epidermal cells at the infection site had collapsed and were necrotic. Twelve days after inoculation, spongy mesophyll cells between the affected tissue and the upper epidermis had undergone cell division, giving rise to numerous closely compacted cells (Fig. 1-G). The layer of loosely arranged mesophyll cells between the palisade layer and the lower epidermis in healthy leaves ranges from five to six cells, whereas some 10 to 12 parenchyma cells were formed in an infected leaf (Fig. 1-H). Enlargement of the mesophyll tissue resulted from hyperplasia and not from hypertrophy. The extent of hyphal penetration was limited to the infected epidermal cells or the parenchyma cells just below the infection site. Twenty-four days after inoculation, the first three rows of cells, including the epidermis, had collapsed and were necrotic. Approximately two-thirds of the cells between the infected site and the upper epidermis had taken up the cotton blue stain, but were not necrotic. Hyphal penetration was still restricted to the epidermal cells or those adjacent to the infection site. Diameter of the fleck after 24 days was 300 to 400 μ. No fruiting structures were produced. Isolations both from small flecks and large lesions produced typical cultures of the pathogen.

DISCUSSION.—Conditions existing at the leaf surface prior to infection by a pathogen play an important role in the incidence and severity of subsequent disease (1, 2, 4, 6). Large anthracnose-type lesions produced by *G. minus* on blueberry leaves in the field develop when infection takes place through the hydathodes or wounds. In previous studies (5), it was noted that the severity of infection depended upon number of spores and glucose concentrations. A relatively small percentage of the spores that were applied to the leaf surface in the present studies actually completed all phases of the infection process. When suspended in distilled water, 60% of the conidia applied to the leaf surface germinated after 72 hr, but only 42% of the germinated conidia produced appressoria. This means that only 25% of the inoculum applied to the leaf surface was capable of producing penetration hyphae and thereby causing infection. This is in contrast to

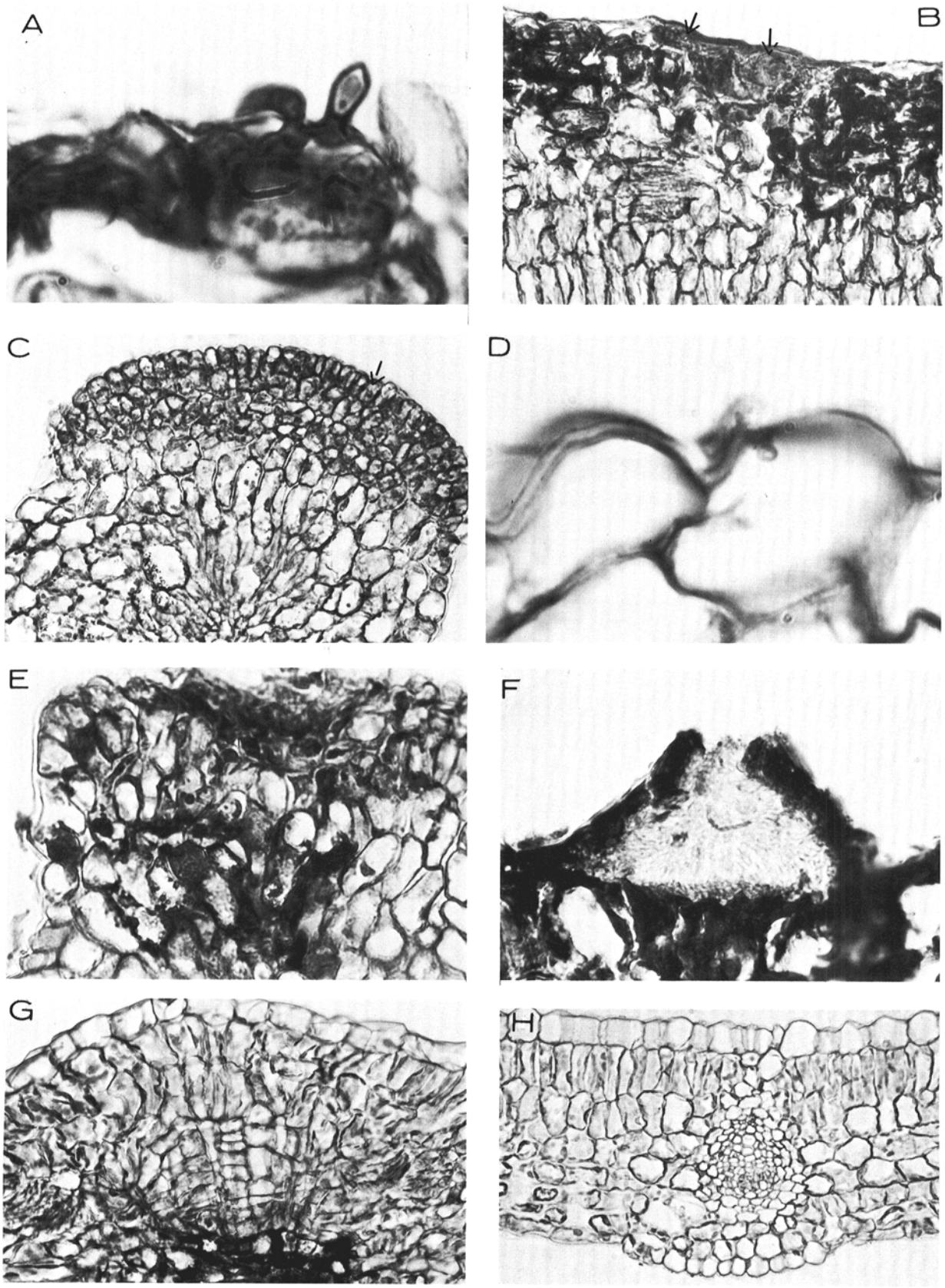
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Fig. 1. Histological relationships of blueberry (*Vaccinium corymbosum* L.) and *Gloeosporium minus*. **A)** Transverse section of lower epidermis showing appressoria and penetration. Secondary hypha growing out from enlarged primary hypha (× 800). **B)** Transverse section of a 6-day-old lesion on blueberry leaf caused by *G. minus* showing pseudoparenchyma (arrow) in epidermal cells (× 320). **C)** Transverse section of a hydathode showing modified parenchyma cells of the epithem (arrow) (× 80). **D)** Appressorium and penetration of hydathode, showing enlarged primary hypha (× 800). **E)** Transverse section of a 6-day-old infected hydathode. Parenchyma cells have collapsed and the epithem is completely disorganized (× 320). **F)** Mature acervulus of *G. minus* 24 days after infection in blueberry leaf (× 320). **G)** Transverse section of fleck on blueberry leaf 12 days after inoculation. There is a collapsing of epidermal cells and accumulation of dark-staining material around infection site. Note hyperplastic reaction in mesophyll tissue (× 200). **H)** Transverse section of healthy blueberry leaf (× 200).

the finding for inoculum amended with glucose which resulted in 88% spore germination with 72% of these germinated conidia producing appressoria, which yielded 63% potentially infective propagules. This is a 2.5-fold increase in inoculum potential.

On several occasions it was noted that the hyphae without forming appressoria were able to penetrate through open stomates. The high concentrations of sugars and amino acids that are exuded through the specialized hydathodes or glands provide the fungus with additional nutrients, presumably increasing the infective potential of the spores.

The defense mechanism responsible for the fleck reaction appears to involve a hypersensitive reaction, indicating incompatibility between the host and pathogen. Upon penetration of the epidermis by the fungus, the host tissue reacts immediately as evidenced by the accumulation of a dark-staining material at the infection site and restriction of the fungus to a very few cells in the vicinity of the infection court. Within 72 hr after inoculation, the infected cells and those adjacent to them collapsed, necrosis occurred, and further growth of the fungus was prevented.

In this host-pathogen interaction it appears that compatibility is primarily dependent upon the nutritional environment at the host surface, which presumably affects the fungus prior to penetration of the host. The additional source of energy in the form of nutrients available for growth of the fungus at the leaf surface apparently alters its pathogenic potential, thereby allowing the fungus to overcome the host's natural defense mechanisms.

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