

Blueberry Stem Canker and Dieback Caused by *Gloeosporium minus*

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ABSTRACT

Stem canker and dieback symptoms caused by *Gloeosporium minus* were observed on highbush blueberry (*Vaccinium corymbosum*) for the first time in North Carolina. Symptoms first appear as dark-red, circular-to-elliptical lesions surrounding a leaf scar on young succulent shoots. As the cankers enlarge, affected stems turn brown, become gray, and die. By the second year, cankers range in size from 10 to 30 mm in length, and often result in severe dieback. Numerous black acervuli are produced over the entire lesion. Six blueberry cultivars tested were susceptible to *G. minus*, although resistance varied. The severity of the disease depended upon mode of infection and stage of plant

growth. Invasion of leaf scars via attached petioles resulted in stem canker development. Penetration of nonwounded stems through the epidermis resulted in small raised lesions that failed to enlarge. A temp of 25-30 C was optimum for canker development and spore production. Histological observations indicated that the fungus apparently invades the xylem vessels of the stem only through vascular tissue of the leaf scar or flower buds. Death of the stem is apparently due to vascular occlusion by hyphae and tyloses. Penetration and infection of nonwounded stems through the epidermis results only in a fleck reaction.

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Additional key words: pathogenicity, canker, histology, infection.

Numerous leaf and stem diseases of highbush blueberry (*Vaccinium corymbosum* L.) cause considerable damage each year in North Carolina. The fungus *Gloeosporium minus* Shear, causal agent of *Gloeosporium* leaf spot on blueberry, is present in most commercial plantings in southeastern North Carolina (3, 4). Shear (6) first described this fungus as the cause of a storage rot of cranberries, and Taylor (7) reported the fungus caused a stem and leaf fleck disease of blueberry. On blueberry stems, the symptoms first appear as small reddish flecks on young succulent shoots, then develop into small (1-2 mm in diam) brown pimples during the second growing season.

In 1972, a stem canker disease of highbush blueberry caused by *G. minus* was observed for the first time in a commercial planting in North Carolina. A 4.9-hectare (ha) [2-acre] planting of the highbush blueberry cultivar

Jersey was severely affected, with ca. 95% of the plants showing symptoms. Symptoms first appeared as dark-red, circular-to-elliptical lesions surrounding a leaf scar and later around flower buds of the current season's growth. As the cankers enlarged, the affected stems turned brown, and eventually become gray and died (Fig. 1). The disease often resulted in severe cane dieback measuring as much as 45 cm in length. Numerous acervuli were produced over the entire lesion.

In addition to stem symptoms, numerous large, brown, circular to irregular-shaped lesions caused by *G. minus* were observed on the foliage. By mid-July, leaf lesions developed along the lower leaf margin and continued to develop along the petiole, with the fungus invading the stem tissue. Numerous red lesions surrounding the leaf scars were observed on young succulent stems. Isolations were made from 21 stem lesions and *G. minus* was

recovered from all lesions. Isolations were also made from stem cankers on previous years growth, and *G. minus* was recovered from 35 of 36 cankers.

The results reported in this investigation describe additional stem symptoms caused by *G. minus*, the mode of infection and the histological effects of the pathogen on stem tissues.

MATERIALS AND METHODS.—Cultures of *G. minus* used in these studies were isolated from *V. corymbosum* 'Jersey' leaf lesions (GL-1) and stem cankers (GS-1). Monoconidial isolates obtained from these cultures were maintained on potato-dextrose agar (PDA) at 25 C.

Growth rate, habit, and conidial production were studied in culture. Five petri plates of each of the

following solid media were used: (i) potato-dextrose agar (PDA); (ii) V-8 juice agar (V-8A); (iii) yeast-extract agar (YEA); and (iv) malt agar (MA). The cultures were grown at 25 C under continuous light at 2,000 lumens/square meter (lm/m^2). The influence of temp on spore germination was also studied. Conidia were harvested when cultures were 2 wk old by scraping the surface of the culture with a razor blade, then flooding the plate with 30 ml of sterile distilled water. The spore suspension was passed through cheesecloth into a beaker, and the conidia were washed in 30 ml of sterile distilled water by centrifugation. The water was decanted and the conidia were resuspended in 30 ml of sterile distilled water. The spore suspension (10^6 conidia/ml) was sprayed onto young succulent stems of the cultivar 'Croatan.' Stems

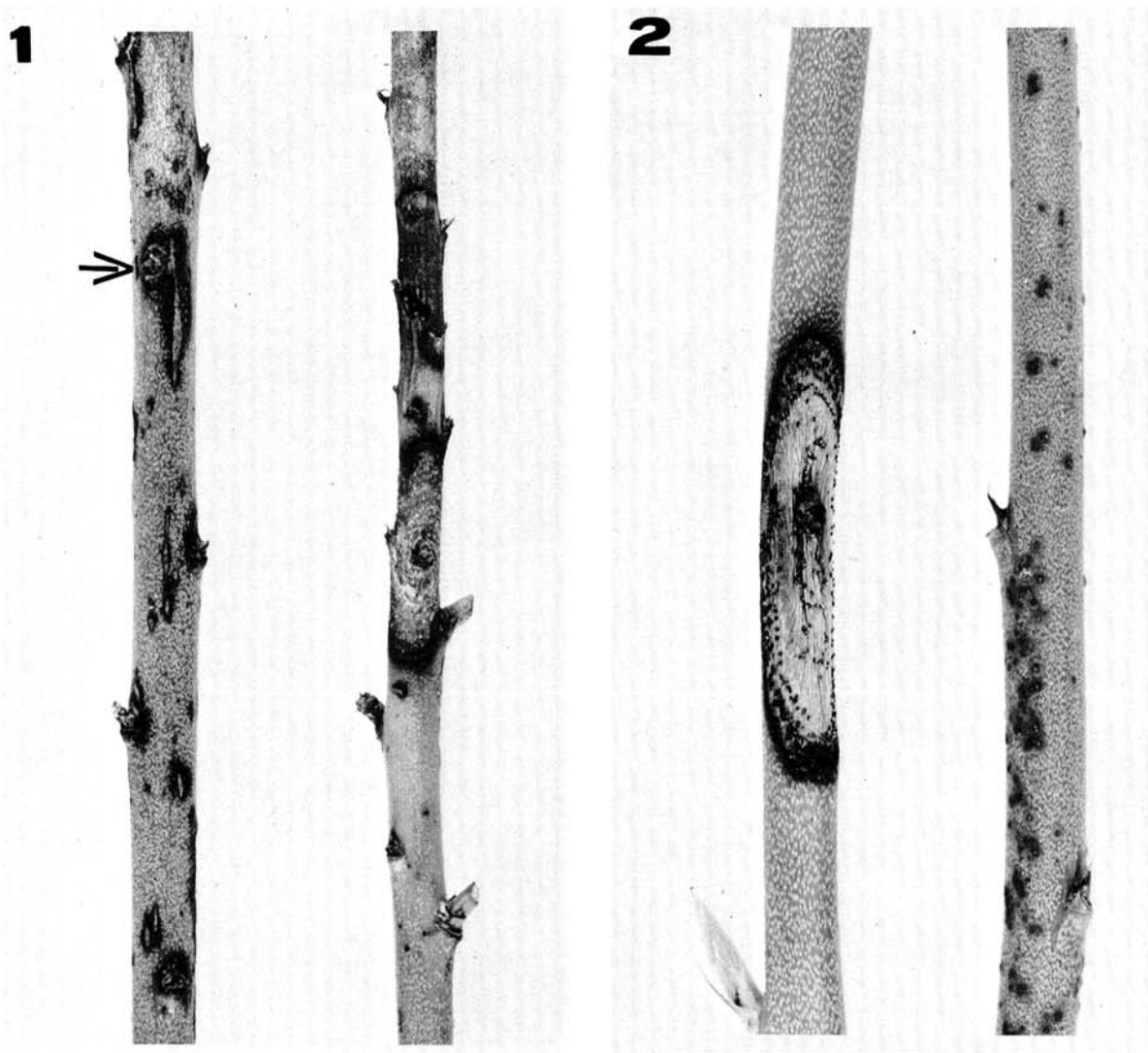


Fig. 1-2. 1) Naturally infected 'Jersey' blueberry stems showing different stages of canker and dieback symptoms caused by *Gloeosporium minus*. Note lesion development around leaf scar (arrow). 2) Stem canker and fleck development on blueberry stems inoculated with *Gloeosporium minus*. Stem canker (left) developed from infection of leaf scar via petiole, and flecks (right) developed from infection through epidermis.

were excised into 5-cm sections, placed on moist filter paper in petri plates, and incubated at 10, 15, 20, 25, 30, and 35 C. Tests were repeated twice. One hundred conidia were counted for each treatment. Percent germination and germ tube length were recorded after 24, 48, and 72 h.

Plants were grown in a peat:sand (1:1, v/v) mixture and forced from well-rooted cuttings in the greenhouse prior to inoculation.

Inoculations to determine pathogenicity and cultivar susceptibility were made by removing the leaf blade from the petiole and inserting aerial mycelium removed with a sterile needle from 2-wk-old cultures grown on PDA into the wounded petioles. Two inoculations, 5 and 20 cm from the growing point, were made on each stem. Six commercial highbush cultivars ('Angola', 'Bluecrop', 'Croatan', 'Jersey', 'Morrow', and 'Wolcott') were used. Three single-stem, greenhouse-grown plants of each cultivar were inoculated with isolates GS-1. One noninoculated plant of each cultivar served as controls. The plants were placed in a moist chamber at 25 C for 48 h, and then transferred to a greenhouse bench. The test was repeated twice.

In an additional pathogenicity test, young succulent stems of the cultivar Jersey were inoculated with conidia of *G. minus*. The test consisted of four treatments with six stems inoculated/treatment. One noninoculated stem served as a control for each treatment. Inoculations were made by placing a drop of the spore suspension (10^6 conidia/ml) on (i) the petiole, (ii) leaf scar, (iii) leaf axil, and (iv) by spraying the spore suspension onto nonwounded blueberry stems. With the exception of the latter treatment three inoculations were made per stem. Plants were placed in a moist chamber at 25 C for 72 h, then removed to a greenhouse bench under natural light at 25 to 30 C. Number of lesions were recorded after 14 days.

The effect of temp on disease development was studied by removing the leaf blade and inserting mycelium into the wounded petiole of the cultivar Bluecrop. The isolates GL-1 and GS-1 were used to inoculate 12 stems with each isolate. Two inoculations were made on each stem. The plants were placed in a moist chamber with 100% relative humidity (RH) at 25 C for 48 h, then transferred to Sherer-Gillett CEL 25-7HL constant-temp chambers at 15, 20, 25, and 30 C. The chambers were maintained at ca. 2,500 lm/m² for 16 h daily, using cool-white 40 W fluorescent lamps and 25 W incandescent bulbs. Six inoculated and one noninoculated plants were placed at each temperature.

Penetration by the fungus was studied using stems removed from Jersey and Croatan plants 24, 48, and 72 h after inoculation. The stems were cut into small sections, and stained with cotton blue in lactophenol (5). The epidermis was removed and examined microscopically for penetration by the fungus. Infected stems used for histological studies were obtained from plants inoculated with conidia and mycelium in the greenhouse and from naturally infected plants in the field. Stems were examined 2 and 8 wk after inoculation. Stems were cut into 20-mm sections, cleared and fixed in formalin-acetic-alcohol (FAA), dehydrated in tertiary butyl alcohol, and embedded in paraffin. Sections 10- μ m thick were mounted on slides with Haupt's adhesive, and stained with safranin and fast green (2).

RESULTS.—Growth rate, habit, and conidial production on PDA of *G. minus* isolated from stem cankers were identical to those described by Taylor and Clayton (8), and for *Gloeosporium* leaf spot (3). Conidia were hyaline, elliptical to fusoid, measuring 6-10 μ m \times 3-4 μ m, fitting the description of *G. minus* from cranberry (6), and blueberry (7). The fungus sporulated after 14 days on all media tested, with the exception of YEA.

Percent germination after 72 h at 10, 15, 20, 25, 30, and 35 C was 4, 36, 41, 84, 41, and 11%, respectively. The average germ tube length at the above temp was 6, 22, 30, 67, 30, and 13 μ m, respectively.

In susceptibility studies with six different blueberry cultivars, all plants inoculated with GS-1 isolate of *G. minus* became infected. Dark-brown to black lesions with gray centers surrounded by red discoloration were observed on infected stems four days after inoculation. A few scattered acervuli were produced on the lesions after 7 days. Stem cankers were light- to dark-brown, elliptical, and varied in length from 6 to 20 mm after 14 days (Fig. 2). The average length of cankers for the cultivars Jersey, Bluecrop, Morrow, Croatan, Angola, and Wolcott were 20, 15, 15, 12, 9, and 6 mm, respectively. Cankers developing near the growing point tended to be larger than those lower on the stem. For all cultivars, the average length of stem cankers which developed 5 and 20 cm from the growing point was 16 and 10 mm, respectively. The number of acervuli per canker for Bluecrop, Croatan, Jersey and Morrow ranged from 25 to 50, while Angola and Wolcott had less than five acervuli after 14 days.

Dark-brown to black lesions developed on all Jersey stems inoculated with a conidial suspension at the wounded petiole or leaf scar. Nine lesions developed from the 18 inoculations at the petioles, and eight lesions developed at the leaf scars. No lesions developed when spores were placed at the leaf axil. Small red flecks that failed to enlarge were observed when spores were sprayed onto nonwounded stems.

Stem canker development was favored by temp of 25 to 30 C. No significant difference in canker development was observed between isolates GL-1 and GS-1 at any temp tested. The average length of cankers after 14 days for the two isolates at 15, 20, 25, and 30 C was 3, 5, 8, and 11 mm, respectively. Cankers were light- to dark-brown, surrounded by a red border, sunken and elliptical in shape. No acervuli were produced on any cankers at 15 C, and only an occasional acervulus developed at 20 C. Numerous acervuli were produced at 25 and 30 C after 14 days. No lesions developed on stems of noninoculated controls.

Spore germination and appressoria development were observed 48 h after inoculation. Small, slightly raised lesions on nonwounded stems developed as a result of direct penetration by the fungus through the cuticle and the epidermal cell wall. Enlargement of the lesion resulted from hyperplasia and not hypertrophy. The lesion consisted of 10 to 20 layers of closely packed cells which were rectangular in shape. The layer of cells beneath the epidermis gives rise to the lesion. The necrotic cells of the raised lesion were stained dark and hyphae were not observed in the cells. Hyphae was not observed in living cells below the lesion. No fruiting structures were observed after 8 wk.

Observations of transverse and tangential sections of succulent stems inoculated with mycelium showed that a rapid breakdown of individual cells occurred within 14 days. Hyphae measured 1-2 μm in diam, and grew intracellularly in the cortex, phloem, and xylem vessels of inoculated and naturally infected stems. Hyphae were most abundant within dead cells and in the air spaces of the cortex. Hyphae grew longitudinally in the discolored portion of the vessels, with several strands occupying a single vessel.

Abundant tyloses formed in the xylem vessels of infected stems within 14 days after inoculation, causing partial or complete occlusion of the vessels. Very little tylose formation was observed in the naturally infected Jersey stems.

Acervuli that formed in the outer layers of necrotic cortex tissue 14 days after inoculation were large (200-400 μm in diam), black, subepidermal, and erumpent when mature.

DISCUSSION.—This is the first report of *Gloeosporium* stem canker and dieback caused by *Gloeosporium minus* on highbush blueberry. A leaf spot disease of highbush blueberry caused by an unidentified *Gloeosporium* sp. was first observed in 1961 (1). The causal agent was later identified and its pathogenicity demonstrated by Milholland (3) in 1970. During the past 10 yr, the disease has increased in its occurrence and severity, and has almost reached epidemic proportions in North Carolina.

Gloeosporium minus was obtained from 98% of the 57 stem canker isolations made in 1973. All of the stem cankers were observed to develop around a leaf scar or flower bud. Many of the isolations were made in July from the small red lesions surrounding a leaf scar. Progress of symptom development from petioles into the stem was observed on numerous occasions in the field. Whether or not the fungus invaded all of the stems via attached petioles or penetrated the leaf scars directly is unknown.

All major blueberry cultivars grown in North Carolina are susceptible to *G. minus*, as well as the leading cultivars grown in New Jersey, Bluecrop and Jersey. Differences in disease development were noted between cultivars, with Jersey being the most susceptible. Conidial inoculations were successful in both wounded and nonwounded stems. The severity of disease depended upon the mode of

infection and stage of plant growth. Invasion of wounded petioles and leaf scars by the fungus resulted in stem canker development. However, when nonwounded stems were inoculated, only small, raised lesions developed, and they failed to enlarge. These findings suggest that the pathogen must colonize the vascular tissue for development of stem canker.

Studies of the effect of temp on disease development indicated that 25-30 C was optimum for canker development and spore production. Although some lesions developed at 15 C, no fruiting structures were formed at this temp.

Histological observations indicated that penetration and infection of nonwounded stem tissue by *G. minus* resulted in fleck formation. It is possible that the fungus was unable to penetrate the pericyclic fibers surrounding the vascular tissue, and that its colonization was thus restricted to the few cells beneath the epidermis. The fungus apparently invades the xylem vessels of the stem only through vascular tissue of the petiole and leaf scar. Invasion of the leaf scar by the fungus causes a rapid breakdown of individual cells within a few days. Death of the stem tissue is due in part to vascular occlusion by tyloses and mycelium.

LITERATURE CITED

1. FOX, J. A. 1962. Septoria leaf spot and Septoria canker on highbush blueberry caused by *Septoria albopunctata*. MS. Thesis, N.C. State Univ. 42 p.
2. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
3. MILHOLLAND, R. D. 1970. The effect of leaf exudates on blueberry leaf spot caused by *Gloeosporium minus*. *Phytopathology* 60:635-640.
4. MILHOLLAND, R. D. 1973. Histopathology of fleck and lesion symptoms on blueberry infected with *Gloeosporium minus*. *Phytopathology* 63:320-323.
5. RIKER, A. J., and R. S. RIKER. 1936. Introduction to research on plant diseases. John H. Swift Co., St. Louis. 117 p.
6. SHEAR, C. L. 1907. New species of fungi. *Bull. Torrey Bot. Club* 34:305-317.
7. TAYLOR, J. 1958. Stem canker and related blueberry diseases. N.C. Agric. Exp. Stn. Tech. Bull. 132. 24 p.
8. TAYLOR, J., and C. N. CLAYTON. 1959. Comparative studies on *Gloeosporium* stem and leaf fleck and *Dothichiza* leaf spot of highbush blueberry. *Phytopathology* 49:65-67.