The Effect of Leaf Exudates on Blueberry Leaf Spot Caused by Gloeosporium minus

R. D. Milholland

Associate Professor of Plant Pathology, North Carolina State University, Raleigh, 27607.

Journal Series Paper Number 2970, North Carolina State University Agricultural Experiment Station.

The author acknowledges the assistance of Larry Hammett in determining the amino acid content of the leaf exudate.

Accepted for publication 31 October 1969.

ABSTRACT

Stem and leaf flecks on the highbush blueberry (*Vaccinium corymbosum*) caused by *Gloeosporium minus* have previously been reported. In addition to these symptoms, *G. minus* produces large, brown, circular to irregular shaped lesions 5-10 mm in diam on blueberry leaves in the field, and is capable of causing a serious fruit rot of blueberries. Infection occurs through wounds or hydathodes. All blueberry varieties were susceptible to *G. minus*, but some are more resistant than others. Minimum, optimum, and maximum temperatures for growth in culture are 12, 30, and 36 C. The importance of leaf exudates in increasing the inoculum potential of *G. minus* on blueberry leaves is suggested. Lesions were larger near the hydathodes where high concentrations of carbohydrates were exuded, and in wound inoculations. High concentrations of glucose added to the inoculum increased the pathogenicity of *G. minus*. Inoculations with conidia suspended in distilled water resulted in small flecks. Phytopathology 60:635-640.

Gloeosporium minus Shear was first described as the cause of a storage rot of cranberries (8), and Taylor (9) reported it to cause a stem and leaf fleck disease of blueberry (*Vaccinium corymbosum* L.). According to Taylor (9) and Taylor & Clayton (10), the disease first appears as small reddish flecks on young leaves and stems of succulent shoots. Leaf flecks did not develop further, but as the leaves developed they became puckered and malformed. Stem lesions developed slowly and remained as small brownish pimples which reached 1-2 mm diam.

In 1961, a leaf spot disease of highbush blueberry caused by an unidentified *Gloeosporium* sp. was observed by Fox (2) in a commercial planting in southeastern North Carolina. According to Fox, the leaf spot was more severe on the varieties Croatan and Wolcott than on Murphy. Four years later, the same blueberry planting was severely infected with this leaf spot, resulting in heavy defoliation.

The results reported in this investigation describe additional leaf spot and fruit rot symptoms caused by *G. minus* and the importance of leaf exudates in increasing disease severity on highbush blueberry.

MATERIALS AND METHODS.—*Cultural studies.*—Cultures of *Gloeosporium minus* were obtained from large lesions on blueberry leaves collected from 12 different locations in southeastern North Carolina. Monoconidial isolates were obtained from these cultures and used in all inoculation and spore germination tests. To determine the range of spore size, measurements were made of conidia from 12 different isolates.

Growth rate, habit, and conidial production were studied in culture. Ten petri dishes of each of the following solid media were used: (i) potato dextrose (PDA); (ii) V-8 juice (V-8A); (iii) cornmeal (CMA); and (iv) oatmeal (OMA) agars. Half of the dishes were placed under light (200-400 ft-c) at 25 C; the remaining petri dishes were placed in the dark at 25 C. The relationship of temperature to growth of the fungus was also studied. Five-mm discs of the fungus were placed upside down on PDA plates. Five plates were placed at each of the following temperatures: 6, 12, 18, 24, 30, 36, and 42 C. Diameter of colonies were recorded after 24, 48, 72, and 96 hr.

Pathogenicity.—Plants were grown in a 1 to 1 peat: sand mixture and forced from well-rooted cuttings in the greenhouse prior to inoculation.

Inoculations to determine pathogenicity and varietal susceptibility were made by spraying a standardized inoculum suspension (10^6 conidia/ml) onto blueberry leaves. The plants were placed in a moist chamber at 25-30 C for 72 hr, then removed to a greenhouse bench under natural light at 25-30 C. Four plants each of the varieties Weymouth, Wolcott, and Bluecrop were used in the pathogenicity tests. In a study of the relative susceptibility of eight blueberry varieties, three plants each of Wolcott, Murphy, Morrow, Croatan, Weymouth, Bluecrop, Berkeley, and Jersey were sprayed with a conidial suspension and placed with one noninoculated plant of each variety in a moist chamber. The plants were removed after 72 hr to a greenhouse bench, and the number of large lesions was recorded 8 weeks later.

Wound inoculations.—Six plants each of Bluecrop and Weymouth were used in these studies. Leaves of two plants of each variety were wounded with a small sterile needle, then sprayed with a conidial suspension of *G. minus*. The same number of plants of each variety was inoculated without wounding the leaves. Noninoculated, wounded, and nonwounded plants served as controls.

Inoculations with leaf exudates.—Bluecrop and Weymouth plants were inoculated with a 1:1 mixture of (i) leaf exudate and conidial suspension and (ii) distilled water and conidial suspension. Inoculations with the exudate, glucose, and amino acids were all made by placing 10 drops of a standardized inoculum suspension (10^6 conidia/ml) from a 50-µliter pipette on the axial side of each leaf. Ten leaves of each plant were inoculated. Disease severity was evaluated 4

Fig. 1. **A)** *Gloeosporium* leaf spot caused by *Gloeosporium minus*. **B)** Leaf flecks and puckering 14 days after inoculation with *G. minus*. **C)** Lesion development by *G. minus* after infection through hydathodes and along main veins. **D)** Leaf spot development on Weymouth leaves 2 weeks after inoculation with *G. minus*. Upper leaves were wounded prior to inoculation and the lower leaves were not wounded. **E)** Effect of amino acids and glucose on leaf spot development by *G. minus* 30 days after inoculation. (A) Aspartic acid; (B) aspartic acid plus 50% glucose; (C) distilled water; and (D) 50% glucose. **F)** Mature blueberry fruit (upper) infected with *G. minus* 3 weeks after inoculation. Lower, noninoculated controls.

weeks after inoculation on the basis of number and size of leaf lesions.

Guttation fluids that were exuded from the hydathodes of young succulent blueberry leaves were collected with a 50-µl pipette and stored in a freezer. Total carbohydrates of the leaf exudate were determined by the anthrone method (5), using glucose as a standard. The procedure described by Gehrke & Stalling (4) was used for amino-acid analysis.

Spore germination studies.—Conidia used in the germination tests were harvested when 2 weeks old by washing the surface of a culture grown on OMA with sterile distilled water. The conidia were washed three times in sterile glass-distilled water by centrifugation. Spores were added to 2 ml of the germinating medium, and a drop of the conidial suspension was placed on acid-washed glass slides. The slides were placed in moist petri dishes and incubated at 25 C. Each treatment was replicated three times. One hundred conidia were counted for each treatment. Per cent germination and germ tube length and width were recorded after 12, 24, 48, and 72 hr.

Fruit inoculations.—Inoculations of blueberry fruit were made by wound inoculations using mycelia or by inoculations using conidia on nonwounded fruit. Green, unripened blueberries were inoculated by making a small wound in the fruit with a sterile needle and placing a small amount of mycelium in the wound. Wounded, noninoculated berries served as controls. Inoculations using conidia were made by dipping ripened blueberries into a spore suspension and placing them into sterile, moist petri dishes. The same procedure with noninoculated berries was followed for the controls. The berries were incubated at 25 C for 3 weeks.

RESULTS.—Large brown, circular to irregular-shaped lesions surrounded by a dark border were observed on naturally infected leaves (Fig. 1-A). Numerous acervuli were produced on both the upper and lower portion of the older lesions. Conidia were hyaline, oblong, elliptical, often slightly curved, measuring 6-10 µ × 3-4 µ, fitting the description of *G. minus* by Shear on cranberry (8) and by Taylor on blueberry (9).

Cultural studies.—Growth rate, habit, and conidial production were identical to those described by Taylor & Clayton (10) when the fungus was grown on PDA. The fungus sporulated in culture on all media tested when grown under light; little or no sporulation occurred when grown in the dark. The fungus sporulated best on OMA and PDA with little sporulation on V-8A and CMA. The cardinal temperatures for growth in culture were 12, 30, and 36 C (Fig. 2). Growth was very good at 24 C, and none occurred at 6 C or 42 C after 7 days.

Pathogenicity.—Numerous, small red flecks were observed on the young succulent leaves and shoots 7 days after inoculation. Heavily infected leaves became puckered and malformed (Fig. 1-B). On several of the inoculated leaves, small dark lesions were evident at the lower edges near the hydathodes. Leaf flecks did not develop further, but large (5-10 mm in diam), irregular-shaped brown lesions surrounded by a purple border developed where infection had taken place at the hydathodes or along the main vein (Fig. 1-C). A few scattered acervuli were produced 30 days after inoculation. Small, raised, reddish brown lesions were produced on succulent shoots of all inoculated plants. Stem lesions developed slowly and reached diameters of 1-2 mm after 6 months. No lesions developed on older stems. Reisolations from the small red flecks and the large brown lesions produced cultures typical of the inocula.

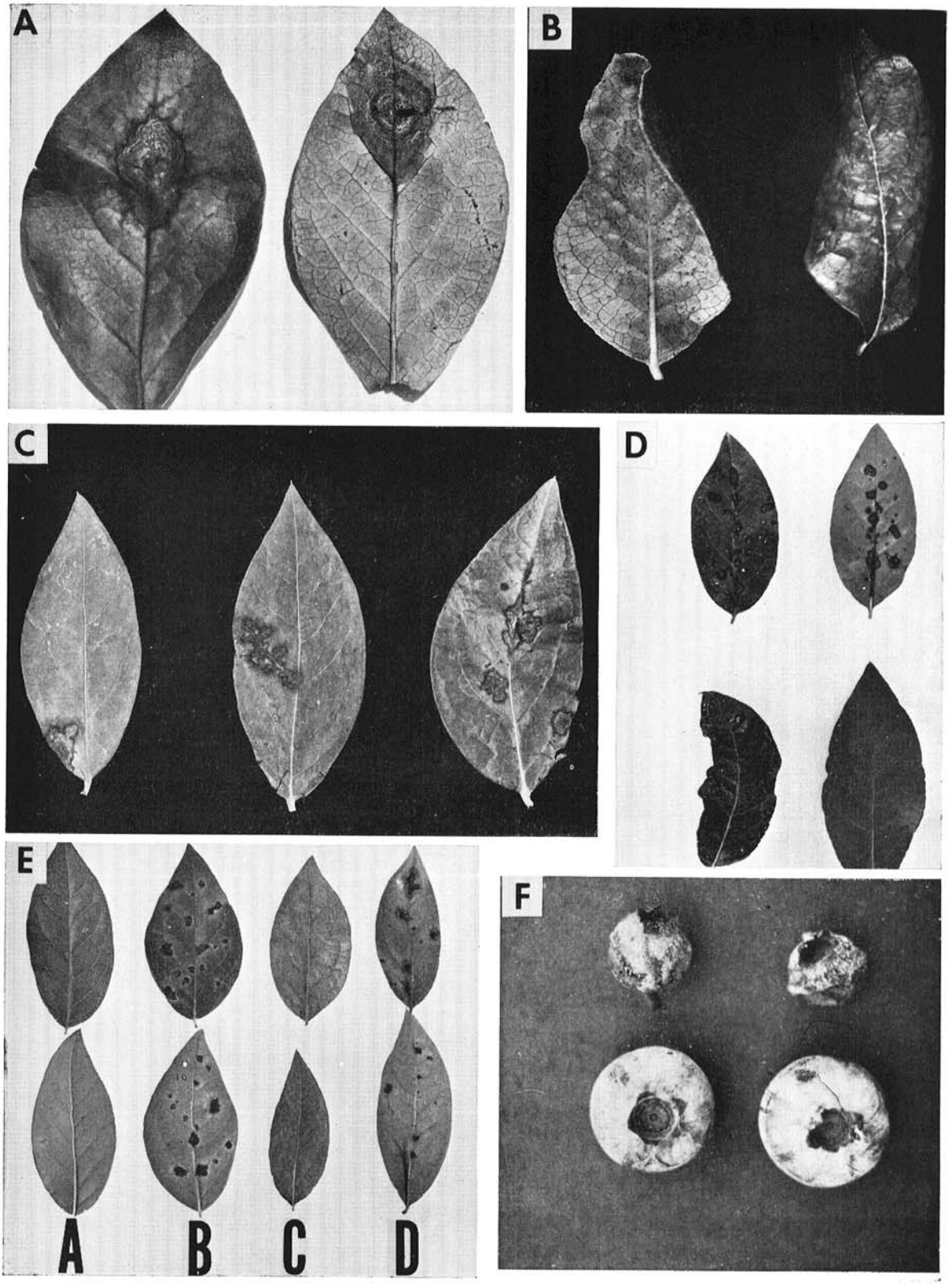
Although young succulent leaves of inoculated plants of all eight varieties tested were highly susceptible to the leaf fleck stage, the varieties differed markedly in susceptibility to the development of large anthracnose lesions (Fig. 3). The variety Bluecrop was most susceptible to the development of the large lesions; Morrow was least susceptible.

Wound inoculations.—Tests were conducted to determine if infection through wounds would result in development of the large lesions similar to those observed when infection occurred through the hydathodes.

Dark brown, circular lesions with a purple border were observed on all wounded inoculated leaves (Fig. 1-D). Lesions varied in size from 3 to 10 mm in diam 30 days after inoculation. Acervuli were produced on both sides of the lesion. Numerous, small red flecks were observed on nonwounded inoculated leaves. The only large lesions that developed on nonwounded leaves were from infections at the hydathodes. The average number of lesions per leaf was wounded, 6.6; nonwounded, 0.1. No lesions developed on the noninoculated controls.

Leaf exudate.—Inoculations were made to determine whether the guttation fluids that exuded from the hydathodes influenced development of the large lesions.

Large, brown, irregular-shaped lesions 4-5 mm in diam, surrounded by a dark border, were produced on leaves of both varieties inoculated with conidia suspended in the leaf exudate 30 days previously. The numbers of lesions developing from 10 inoculations/leaf were 5.0 and 4.2 for Weymouth and Bluecrop, respectively. A few small red flecks were produced on leaves inoculated with conidia suspended in water. No lesions developed on leaves of controls. Reisolations were typical of the inocula.


A
B
C
D
E
A
B
C
D
F

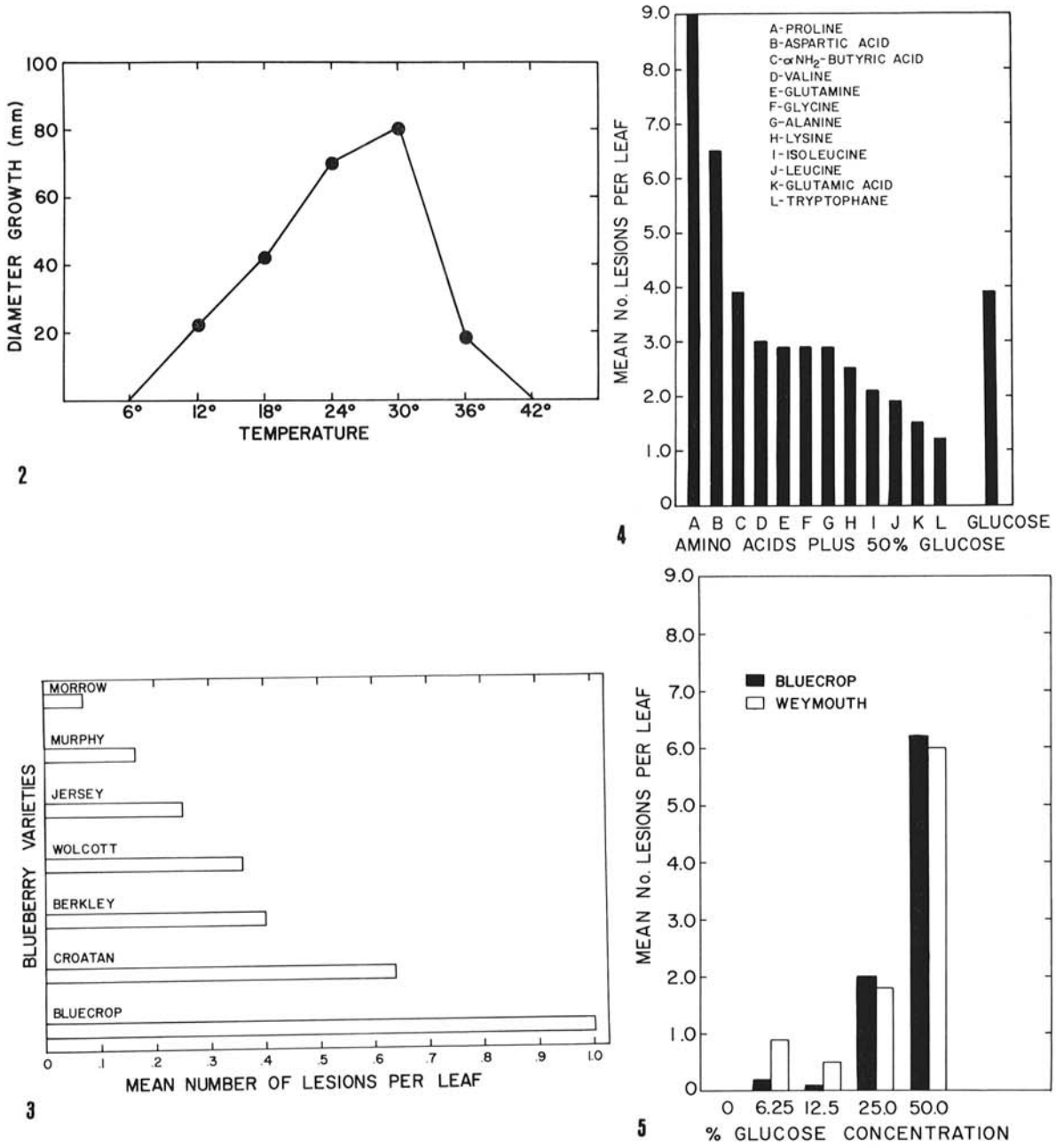


Fig. 2-5. **2)** Effect of temperature on growth of *Gloeosporium minus* on PDA. **3)** Relative susceptibility of blueberry varieties to leaf spot caused by *G. minus*. **4)** Effect of glucose alone and in combination with amino acids on leaf spot development by *G. minus* on highbush blueberry. **5)** Effect of glucose concentration on leaf spot development by *G. minus* on highbush blueberry.

Analysis of carbohydrate content showed the exudate to contain 500,000 ppm of glucose equivalent. Twelve amino acids were identified from the leaf exudate, which included proline (4 ppm), isoleucine (6 ppm), valine (7 ppm), leucine (10 ppm), NH_2—butyric acid (10 ppm), glycine (22 ppm), alanine (24 ppm), aspartic acid (30 ppm), glutamine (30 ppm), glutamic acid (140 ppm), lysine (290 ppm), and tryptophane (390 ppm).

Effect of glucose and amino acids on lesion development.—Conidia of *G. minus* were mixed in a solution containing the major compounds occurring in the leaf exudate, and applied to blueberry leaves. The twelve amino acids were used alone and in combination with a 50% solution of glucose to inoculate leaves of Bluecrop and Weymouth plants (Fig. 4).

Only small necrotic flecks developed when blueberry leaves were inoculated with conidia suspended in the

12 different amino acids. No necrosis or lesion development resulted when amino acids or glucose controls were applied to blueberry leaves. Small (1-4 mm in diam), brown lesions with a dark border were observed on leaves inoculated with a 50% glucose solution singly or in combination with one of the 12 amino acids 30 days after inoculation (Fig. 1-E). The lesions were circular to irregular in shape, and continued to enlarge; they measured 5-10 mm in diam after 60 days. The larger lesions usually developed along one of the main veins. Acervuli were produced on both sides of the lesions. A red discoloration was associated with many of the larger lesions.

Effect of glucose concentration on lesion development.—To determine what effect the concentration of glucose would have on leaf spot development, inoculations were made with 50%, 25%, 12.5%, and 6.25% concentrations of glucose. The various concentrations of glucose without conidia of *G. minus* served as controls.

Small, circular- to irregular-shaped lesions were observed on all inoculated plants after 7 days. The average numbers of lesions per leaf for the variety Weymouth were 6.0, 1.8, 0.5, and 0.9 when conidia were suspended in 50%, 25%, 12.5%, and 6.25% glucose solutions, respectively (Fig. 5). Very little difference in lesion development was observed between the varieties Bluecrop and Weymouth. Lesions produced from inoculations with the two higher concentrations of glucose continued to enlarge, and measured 3-5 mm in diam after 30 days. Numerous small necrotic lesions which failed to enlarge were produced on leaves inoculated with 12.5% and 6.25% concentrations of glucose.

Effect of spore concentration on lesion development.—Leaves of the variety Bluecrop were inoculated with different concentrations of conidia suspended in (i) 50% glucose solution; (ii) 30 ppm aspartic acid; and (iii) distilled water. Two plants, a total of 20 leaves, were inoculated with each treatment; three plants served as controls. No lesions developed on leaves inoculated with the conidia suspended in aspartic acid or distilled water. However, a few necrotic flecks were observed on leaves inoculated with aspartic acid, and several small red flecks were observed on leaves inoculated with conidia suspended in water. No necrosis or lesion development occurred on the noninoculated controls.

Dark brown, irregular-shaped lesions with a purple border were produced on leaves inoculated with the 50% glucose spore suspension. The lesions ranged in size from 2 to 5 mm in diam, with the larger lesions being in close proximity to the main veins. The lesions continued to enlarge, with those developing along the main vein measuring 10-12 mm in diam after 60 days. Plants inoculated with a concentration of 10^7 conidia/ml showed a 5-fold increase in leaf spot disease as compared with those inoculated with a concentration of 10^4 conidia/ml. The average numbers of lesions per leaf were 0.8, 0.8, 2.3, and 4.3 when inoculated with concentrations of 10^4, 10^5, 10^6, and 10^7 conidia/ml, respectively. A considerable amount of red discoloration was also associated with the larger lesions. Several small, irregular-shaped red flecks were produced on leaves inoculated with concentrations of 10^4 and 10^5 conidia/ml.

Spore germination tests.—Per cent germination, length, and width of germ tubes were recorded after 48 hr (Table 1). Conidia of *G. minus* germinated in all media tested except 50% glucose within 12-24 hr. Spore germination was 50% higher in the 50% glucose plus aspartic acid solution than in the 50% glucose alone. Spore germination was approximately 95% for all treatments after 72 hr.

Aspartic acid stimulated germ tube growth (length). The high concentration of glucose (50%) retarded growth. Very little difference in length of germ tubes was observed when conidia were germinated in distilled water or the two highest glucose concentrations after 72 hr. The average length of germ tubes after 72 hr was 51 μ in distilled water, 48 and 49 μ in 25% and 50% glucose, respectively. Germ tube length was greatly increased when conidia were germinated in the aspartic acid solutions.

A significant difference in width of germ tubes was noted when conidia were germinated in the 50% glucose with or without the aspartic acid as compared with the other treatments. The average width of germ tubes was 2.2 μ for the 50% glucose and 1.2 μ for distilled water and aspartic acid.

Fruit inoculations.—Mycelia.—Twenty blueberries each of the varieties Wolcott and Weymouth plus twenty cranberry fruits were inoculated with three isolates of *G. minus*. The berries were submerged for 30 sec in a 1%-sodium hypochlorite solution, rinsed in sterile water, and inoculated. Large (15 mm), brown, sunken lesions were produced 4 days after inoculation. After 7 days, 90% of the inoculated blueberries were completely rotted and had turned brown. Numerous black fruiting structures (acervuli) were produced on the rotted fruit. No rot occurred on the noninoculated checks. A soft rot occurred at point of inoculation on all 20 cranberry fruits.

Conidia.—Small (1 mm), raised lesions were produced on inoculated berries 72 hr after inoculation.

TABLE 1. Effect of glucose concentration on spore germination and growth of *Gloeosporium minus*

Treatments	Germination and growth after 48 hr[a]		
	Spore germination	Length of germ tubes	Width of germ tubes
	%	μ	μ
50% Glucose	59	16	2.2
25% Glucose	54	30	1.5
12.5% Glucose	65	24	1.2
6.25% Glucose	90	58	1.2
30 ppm Aspartic acid	60	86	1.2
30 ppm Aspartic acid plus 50% Glucose	93	68	2.0
H_2O	77	52	1.2

[a] Means based on 100 spores counted/treatment. Each treatment replicated three times.

Ninety per cent of the berries had rotted after 3 weeks (Fig. 1-F). Rotting started at point of attachment of the pedicel. No rot occurred on noninoculated checks.

DISCUSSION.—Most commercial blueberry plantings in eastern North Carolina are affected with *Gloeosporium* leaf spot, and severe defoliation of several blueberry plantings occurred in 1965. Large brown lesions on infected blueberry leaves are the characteristic symptom in the field. Development of the large anthracnose lesions results primarily from infection at the hydathodes, and to a lesser extent through wounds.

Literature on plant root exudates and their influence on soil microorganisms is extensive (6, 7). Nutrients released from plant tissues influence the inoculum potential of associated pathogens (1, 11). The influence of leaf exudates on foliar pathogens is not so well known.

Inoculum potential, as defined by Garrett (3), is the growth energy available to the fungus pathogen at the surface of the host organ to be infected. The observation that the development of large lesions is restricted to areas where leaf exudate is available to the fungus suggests that leaf exudates may be important in increasing the inoculum potential of *G. minus*. Lesions were larger near the hydathodes where carbohydrates and amino acids were exuded, and in wound inoculations. Inoculations with the leaf exudate or a synthetic exudate containing the major components of leaf sap increased leaf spot severity: inoculations with conidia suspended in distilled water resulted in small flecks.

Although amino acids were not directly responsible for lesion development, the presence of some amino acids appeared to increase disease severity. When combined with glucose, proline and aspartic acid had a stimulatory effect, whereas some amino acids such as glutamic acid and tryptophane appeared to inhibit lesion formation. Concentration of glucose was also important in determining both the size and number of lesions produced. High concentrations of glucose inhibited germination during the first 24 hr. Low concentrations of glucose stimulated germination. Length of germ tubes appeared to be stimulated by the addition of aspartic acid, but the width of germ tubes was increased by the 50% glucose solution. Many of the germ tubes growing in the 50% glucose solution were 2-3 times larger than those growing in distilled water. This increase in size of germ tubes could possibly be an indication that the growth energy available to the fungus had been augmented, increasing its pathogenic potential.

LITERATURE CITED

1. BEUTE, M. K., & J. L. LOCKWOOD. 1968. Mechanism of increased root rot in virus-infected peas. Phytopathology 58:1643-1651.
2. FOX, J. A. 1962. *Septoria* leaf spot and *Septoria* canker on highbush blueberry caused by *Septoria albopunctata*. M.S. Thesis. N.C. State Univ. 42 p.
3. GARRETT, S. D. 1956. Biology of root-infecting fungi. Cambridge Univ. Press. 292 p.
4. GEHRKE, C., & D. STALLING. 1967. Quantitative analysis of the twenty natural protein amino acids by gas-liquid chromatography. Separation Sci. 2(1): 101-138.
5. MORRIS, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254-255.
6. ROVIRA, A. D. 1965. Plant root exudates and their influence upon soil microorganisms, p. 170-184. *In* K. F. Baker & W. C. Snyder [ed.] Ecology of soil-borne plant pathogens. Univ. Calif. Press.
7. SCHROTH, M. N., & D. C. HILDEBRAND. 1964. Influence of plant exudates on root-infecting fungi. Annu. Rev. Phytopathol. 2:101-132.
8. SHEAR, C. L. 1907. New species of fungi. Bull. Torrey Bot. Club 34:305-317.
9. TAYLOR, J. 1958. Stem canker and related blueberry diseases. N.C. Agr. Exp. Sta. Tech. Bull. 132.
10. TAYLOR, J., & C. N. CLAYTON. 1959. Comparative studies on *Gloeosporium* stem and leaf fleck and *Dothichiza* leaf spot of high bush blueberry. Phytopathology 49:65-67.
11. TOUSSOUN, T. A., S. M. NASH, & W. C. SNYDER. 1960. The effects of nitrogen sources and glucose on the pathogenesis of *Fusarium solani* f. *phaseoli*. Phytopathology 50:137-140.