A Leaf Spot Disease of Highbush Blueberry Caused by Alternaria tenuissima

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Journal Series Paper No. 4002 of the North Carolina State University Agricultural Experiment Station, Raleigh.

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The assistance of E. G. Simmons in identifying the species involved in these studies is gratefully acknowledged.

Accepted for publication 30 April 1973.

ABSTRACT

Cultures of *Alternaria tenuissima* isolated from blueberry leaf lesions and decayed fruit were pathogenic on highbush blueberry leaves. Symptoms appear as circular to irregular-shaped brown lesions, 1- to 5-mm in diam, surrounded by a red border. Lesions continued to enlarge under high humidity; whereas, those developing after removing the plant from a saturated atmosphere remained as small red flecks. Optimum temperature for fungal growth in vitro was 28 C; whereas, the optimum temperature for disease development was 20 C. More spores were produced by the cultures grown at 25 C under 2,583 lux than in the dark. All blueberry cultivars were susceptible to *A. tenuissima*; 'Wolcott' was the most susceptible and 'Angola' the least susceptible. Spore concentration was also a major factor in disease development. A three-fold increase in leaf lesions was obtained with a concentration of 10^6 spores/ml as compared with 10^5 spores/ml.

Phytopathology 63:1395-1397

Additional key words: Alternaria alternata, Alternaria tenuis, Vaccinium corymbosum, pathogenicity, leaf spot.

A leaf-spotting disease of highbush blueberry (*Vaccinium corymbosum* L.) not previously described was observed for the first time in North Carolina. Symptoms of the disease on the 'Weymouth' cultivar first appeared as tan to light brown circular lesions surrounded by a dark red border (Fig. 1A). Lesions 3- to 5-mm in diam were located on the lower leaves of the plant. Isolations from these lesions yielded an *Alternaria* sp. In late June, *A. tenuissima* (Fries) Wilts was isolated from several 'Wolcott' plants heavily damaged by a leaf spot disease in Pender Co. Later in the season it caused severe defoliation. The lesions were light to dark brown, circular to irregular, surrounded by a dark red border, and varied in size from 2- to 10-mm in diam. *Alternaria tenuissima* was isolated from brown, irregular-shaped lesions on highbush blueberry seedlings growing in the greenhouse at the Horticultural Crops Research Station, Castle Hayne, North Carolina (Fig. 1B).

Alternaria sp. has been isolated from blueberry stems and leaves by several workers (5, 6), but was primarily considered as a secondary invader. In 1971, *A. tenuissima* was identified as the principal cause of fruit decay of blueberries in North Carolina (3). Although not considered to be an economic problem in New Jersey, *A. tenuis* Auct. was isolated from molded blueberry fruit with a relatively high frequency (1). Emory G. Simmons identified a culture from a leaf lesion and one from decayed fruit as belonging to the "*A. tenuissima* group" (*Personal communication*, letter dated 6 Dec 1971 from Emory G. Simmons, Dept. of the Army, U.S. Army Natick Lab, Natick, Massachusetts).

These studies were made to determine the causal agent associated with the blueberry leaf spot and to study its relationship to disease development.

MATERIALS AND METHODS.—*Alternaria tenuissima* used in these studies was isolated from Wolcott leaf lesions and decayed fruit.

To determine pathogenicity and cultivar susceptibility, 10^6 spores/ml were sprayed onto blueberry leaves. The plants were placed in a moist chamber at 25 C for 24 hr, removed to a greenhouse bench in natural light at 20 to 30 C, or maintained at constant temperature of 15, 20, 25, or 30 C in Sherer-Gillette CEL 25-7HL chambers. The chambers were maintained at 1.076×10^4 lux for 16 hr daily, using cool-white 40 w fluorescent lamps and 25 w incandescent bulbs. Plants were grown in 1:1, peat: sand (v/v) mixture and forced from well-rooted greenhouse cuttings.

The relationship of temperature to fungal growth was determined by placing 5-mm disks of the fungus on potato-dextrose agar (PDA) plates, incubating them at 7, 14, 21, 28, and 35 C for 7 days and measuring the colony diam. Conidia used to study temperature effects on germination were harvested from 2-week-old cultures by scraping the surface with a razor blade, flooding the plate with 30 ml of sterile distilled water, and screening the suspension through cheesecloth. A drop of the spore suspension was placed on sterile glass slides in moist petri dishes and incubated at 7, 14, 21, 28, and 35 C. One hundred conidia were counted for each treatment. Percent germination was recorded after 4 hr.

The effect of light on sporulation of the fungus incubated at 25 C was studied by growing *A. tenuissima* on PDA at 2,583 lux, and in light-proof containers held at the same conditions. Spores were harvested by scraping the surface of the culture with

a razor blade, flooding the plate with 30 ml of water, screening the suspension through cheesecloth, and counting the spores in a hemacytometer.

RESULTS.—*Fungus description.*—Conidiophores were yellow-brown, simple smooth, 3- to 5-septate, tips slightly swollen, and measured 30-50 μ X 4-5 μ. Conidia of the leaf isolate were ovoid, obclavate, pyriform, muriformly divided with 1-7 cross septa, and 1-3 vertical septa. Conidia measured 15-55 μ X 7-15 μ (avg 35.8 μ X 10.8 μ). Beaks were cylindrical in shape, 2-3 μ in diam, and measured up to 15 μ in length. These measurements were similar to those reported earlier for the fruit isolate (3).

Cultural studies.—Colonies of both isolates were olive to dark green when grown at 2,583 lux at 25 C. When grown in the dark, colonies were light gray in color, with abundant aerial mycelium. The average number of spores/ml produced by the leaf and fruit isolates when grown under light were 2×10^6 and 6×10^5, respectively. When grown in the dark, the average number of spores/ml produced by the leaf and fruit isolates were 3×10^4 and 5×10^3, respectively.

Growth in diam of the fungus on PDA for 7 days at 7, 14, 21, 28, and 35 C was 10, 28, 40, 51, and 28 mm, respectively. Percent spore germination at the five different temperatures was 11, 30, 45, 55, and 37%, respectively.

Pathogenicity studies.—Pathogenicity studies were conducted in the greenhouse on the cultivars Weymouth and Wolcott. Three plants of each cultivar were inoculated with the isolate from the leaf lesion and decayed fruit. One noninoculated plant of each cultivar served as controls.

Numerous small red flecks were observed on the young succulent leaves 3 days after inoculation. Circular- to irregular-shaped brown lesions with a red border were observed on both cultivars after 7 days (Fig. 1C). Both isolates of the fungus were pathogenic. Lesions varied in size from small red flecks to large necrotic lesions 5-mm in diam. Lesions failed to develop on leaves inoculated on the upper surface; however, symptoms were evident on leaves inoculated on the lower surfaces. Lesions failed to develop on mature leaves. Small red lesions developed on young succulent shoots, but failed to develop further. Reisolations from the small red flecks and the larger necrotic lesions produced cultures typical of the original isolate.

Inoculations to determine cultivar susceptibility were made by spraying a standardized spore suspension on leaves of five commercial highbush cultivars: 'Angola', 'Bluecrop', 'Croatan', 'Morrow', and Wolcott. Three plants of each cultivar were inoculated with each of the two isolates, and one plant of each cultivar served as noninoculated controls. Plants were removed from the moist chamber after 24 hr, and placed at a constant temperature of 21 C.

Small dark necrotic lesions were observed on succulent leaves of all inoculated plants 24 hr after inoculation. Numerous small red flecks were observed on succulent leaves after 3 days. Small (1- to 2-mm diam) brown, irregular-shaped lesions surrounded by a red border developed on inoculated leaves of all cultivars. The young, succulent, immature leaves at the growing point were so heavily infected that the leaves became distorted. The average number of lesions/leaf on Wolcott, Bluecrop, Croatan, Morrow, and Angola inoculated with the leaf isolate after 7 days were 15, 13, 10, 7, and 2, respectively. The average number of lesions/leaf on Morrow, Croatan, Wolcott, Bluecrop, and Angola inoculated with the fruit isolate were 7, 6, 5, 5, and 1, respectively. No lesions were observed on noninoculated controls.

Effect of temperature on disease development.—The lower leaf surface of 12 plants of

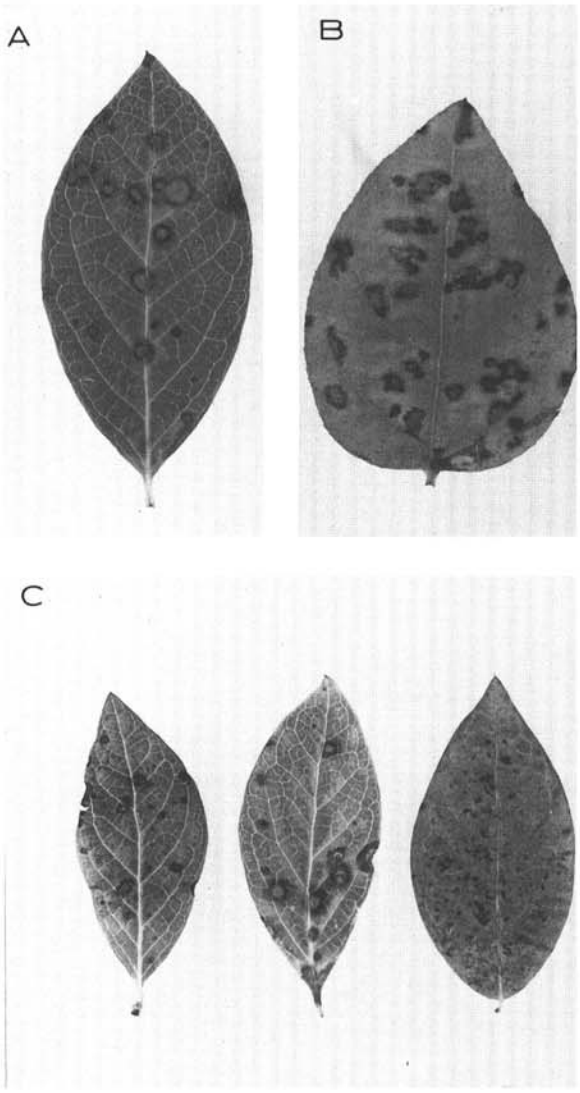

Fig. 1. A) Leaf spot symptoms on 'Weymouth' blueberry leaves caused by *Alternaria tenuissima.* **B)** Irregular-shaped lesions on highbush blueberry seedlings caused by *Alternaria tenuissima.* **C)** Leaf spot symptoms on blueberry leaves showing the circular- to irregular-shaped necrotic lesion and the small red flecks, 7 days after inoculation.

the cultivar Wolcott were sprayed with an aqueous suspension containing 10^6 spores/ml. Three inoculated plants and one noninoculated plant were placed at each of the following temperatures: 15, 20, 25, and 30 C. The test was repeated twice.

Numerous small red flecks or lesions 0.5 mm or less in diam developed on all inoculated succulent leaves placed at 15, 20, and 25 C. No red flecks developed on leaves at 30 C. Circular- to irregular-shaped brown lesions surrounded by a red border, were also observed on succulent leaves at 15, 20, or 25 C. Lesion size varied from 1- to 5-mm in diam after 7 days. Lesions which developed on inoculated leaves at 30 C remained as small necrotic lesions without a red border. Lesions were often surrounded by chlorosis. No lesions or flecks were observed on noninoculated controls. Three times as many lesions developed on inoculated leaves at 20 C, than those placed at 15 and 25 C, and almost ten times as many as those at 30 C.

Effect of spore concentration.—Four plants of the cultivar Wolcott were inoculated with each of three different spore concentrations (10^6, 10^5, and 5×10^4 spores/ml) of the leaf isolate. Plants were removed from the moist chamber after 24 hr and placed at a constant temperature of 21 C.

Small, brown, irregular-shaped lesions surrounded by a dark red border developed on inoculated succulent leaves 3 days after inoculation. Lesions varied in size from 1- to 5-mm in diam. Several large gray lesions (5- to 10-mm in diam) developed along the margins of succulent leaves inoculated with 10^6 spores/ml, causing the leaf to become malformed. In addition, numerous pinpoint lesions or flecks 0.5 mm or less in diam occurred on the succulent leaves inoculated with the high spore concentration. The average number of lesions/leaf that developed from inoculations with 10^6, 10^5, and 5×10^4 spores/ml were 18, 6, and 3, respectively.

DISCUSSION.—*Alternaria* is an omnivorous fungal organism that not only attacks many host plants, but it also causes problems for the pathologist in his attempt to properly identify certain type species. There is some disagreement as to the correct name for the *Alternaria* causing "brown-spot" of tobacco, and the type of characters suitable for species identification. According to Lucas (2) there is no consistently reliable way to distinguish between the fungi *A. tenuis* and *A. tenuissima*. Simmons (4) redescribed *A. tenuis* and indicated that the valid name should be *A. alternata* (Fries) Keissler. Until further data are available, and on the basis of Simmons' identification of the isolates used in this study, the name *Alternaria tenuissima* is accepted.

The optimum temperature for growth of the organism in culture and spore germination was about 28 C. The quantity of spores produced in culture is dependent not only on temperature but also on light intensity. There were 50 to 100 times more spores produced when cultures were grown at 2,583 lux than when grown in the dark at the same temperature. Of the temperatures tested, 20 C is optimum for infection and disease development.

Results of pathogenicity tests indicate that small pinpoint necrotic lesions on succulent tissue, in a saturated atmosphere, continue to develop into irregular shaped brown lesions measuring 1- to 5-mm in diam. Inoculated plants removed from the moist chamber prior to lesion development developed fleck formation only. Lesions on succulent stems are restricted to small red flecks. Differences in cultivar susceptibility were also noted. Wolcott appears to be the most susceptible with Angola showing the least amount of infection.

In addition to temperature and high humidity, spore concentration is also a major factor in disease development. Plants inoculated with a concentration of 10^6 spores/ml showed a three-fold increase in leaf spot disease as compared with those inoculated with a concentration of 10^5 spores/ml.

Although *A. tenuissima* is capable of causing a leaf spot disease under certain conditions, the fungus does not appear to be a serious or widespread leaf spot problem on highbush blueberry. Results of the present studies indicate that only during prolonged periods of cool (20 C) wet weather, when high concentrations of the inoculum are available, would the disease be of any importance on blueberries in North Carolina. Under these conditions, and because of its importance as a post-harvest fruit decay organism (3), any increase of *A. tenuissima* on blueberry leaves in May could cause considerable damage to the quality of the fruit when harvested in June.

LITERATURE CITED

1. CAPPELINI, R. A., A. W. STRETCH, & J. M. MAIELLO. 1972. Fungi associated with blueberries held at various storage times and temperatures. Phytopathology 62:68-69.
2. LUCAS, G. B. 1971. Alternaria alternata (Fries) Keissler, the correct name for A. tenuis and A. longipes. Tob. Sci. 15:37-42.
3. MILHOLLAND, R. D., & R. K. JONES. 1972. Post-harvest decay of highbush blueberry fruit in North Carolina. Plant Dis. Reptr. 56:118-122.
4. SIMMONS, E. G. 1967. Typification of Alternaria, Stemphylium and Ulocladium. Mycologia 59:67-92.
5. WILCOX, M. S. 1936. Notes on blueberry fungi. Plant Dis. Reptr. 20:106-107.
6. ZUCKERMAN, B. M. 1960. Fungi collected from blueberry stems in Massachusetts. Plant Dis. Reptr. 44:416.