

## Cercospora Stem Blotch Disease of Rabbiteye Blueberry

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### ABSTRACT

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A *Cercospora* sp. was isolated and identified as the causal organism of stem blotch of rabbiteye blueberry. Symptoms of the disease appear as red blotches on previous season's growth. Conidia and conidiophores are produced on a fasciculate stroma that emerges from stomates. Hyphal

growth is intercellular, causing hyperplasia and hypertrophy of the cortical parenchyma. Periderm was produced by the phloem parenchyma in response to the pathogen. Typical lesions exhibit collapse and necrosis of cells in the cortex, pericycle, and phloem within 1 yr after infection.

*Additional key words:* *Vaccinium ashei*, histopathology.

A previously unreported stem disease of rabbiteye blueberry (*Vaccinium ashei*, Reade) was observed in a Georgia blueberry planting in 1974. Symptoms of the disease appear as light to dark red discolorations on previous season's growth (Fig. 1). Lesions do not conform to any particular shape or size but occur as a blotching of the stem. Isolation of the pathogen was attempted in May, 1975, from diseased stems of rabbiteye cultivars Delite, Southland, Woodard, and Tifblue obtained from the blueberry planting in Georgia. A *Cercospora* sp. was isolated from 68% of the 96 lesions sampled. The disease was found later on the rabbiteye selection NC911 growing at the Horticultural Crops Research Station, Castle Hayne, North Carolina, and isolation attempts from all 20 lesions that were cultured yielded a *Cercospora* sp. The fungus was observed sporulating on 1-yr-old infected stems of NC911 in May and June 1975.

These studies were made to determine the causal agent of the blueberry stem disease and to study its relationship to disease development.

### MATERIALS AND METHODS

The *Cercospora* sp. used in these studies was isolated from an infected stem of the rabbiteye selection NC911. Conidia obtained from naturally infected stems and from a 14-day-old culture grown on potato-dextrose agar (PDA) were used to determine morphology of the spores. Conidia used to study effects of temperature on germination were harvested from naturally infected stems of NC911 with a camel's-hair brush and placed on water agar at 10, 15, 20, 25, and 30 C. Each treatment was replicated twice, and percentage of germinated spores was recorded after 4 and 24 hr.

Conidia used to determine pathogenicity were obtained from a 1-mo-old culture on PDA by scraping the surface

with a razor blade, flooding the plate with 20 ml of sterile distilled water and then streaking the mycelial suspension over the surface of fresh PDA plates. Cultures were grown at 25 C, and conidia were harvested after 14 days.

To determine pathogenicity and cultivar susceptibility,  $5 \times 10^4$  conidia/ml were sprayed onto the stems of two rabbiteye cultivars (NC911 and Tifblue) and two highbush cultivars (Croatan and Wolcott). Three plants of each cultivar were inoculated and one plant was used as a noninoculated control. The plants were placed in a moist chamber at 25 C for 72 hr and then placed on a greenhouse bench. Plants were grown in a peat:sand (1:1, v/v) mixture and forced from well-rooted cuttings in the greenhouse prior to inoculation.

Histological examinations of infected stems were made 2, 6, and 12 mo after inoculations. Stems were cut into 15-mm sections, which were cleared and fixed in Formalin-acetic-alcohol (FAA) for 2 wk (3). Stems were sectioned on a sliding microtome to a thickness of 20  $\mu$ m, mounted on slides with Haupt's adhesive, and stained with Triarch's quadruple stain (Triarch Incorporated, Ripon, WI 54971).

### RESULTS

Conidia of the *Cercospora* sp. isolated from blueberry stems were hyaline, obclavate-cylindrical, straight to slightly curved, with obconic-truncate base and subacute tip; septations were indistinct in many spores. Conidia, with 3 to 8 septa, obtained from naturally infected stems measured  $35-90 \times 1.5-3.0 \mu$ m. Conidia, with 3 to 10 septa, obtained from PDA cultures measured  $30-110 \times 1.5-3.0 \mu$ m.

Conidia germination after 4 hr at 10, 15, 20, 25, and 30 C, was 1, 6, 15, 8, and 8%, respectively. After 24 hr, conidia germination was 1, 64, 97, 98, and 97%, respectively. Germ tubes averaged 5, 50, 64, 119, and 95  $\mu$ m in length, respectively.

Small red lesions had developed on succulent stems of

NC911 and Tifblue 2 mo after inoculation, but none had developed on the cultivars Croatan and Wolcott. Lesions on succulent stems were slightly raised and measured 2-5 mm in length. Lesions that developed on woody stems were not raised but were typical of the dark red blotches that occurred naturally on infected stems. Within 6 mo, some of the lesions had almost encircled the stems and in several instances had coalesced to form elongated red lesions about 10 mm in length. The color of the lesions turned from red to almost black and centers became slightly sunken within 1 yr after infection. Isolations from

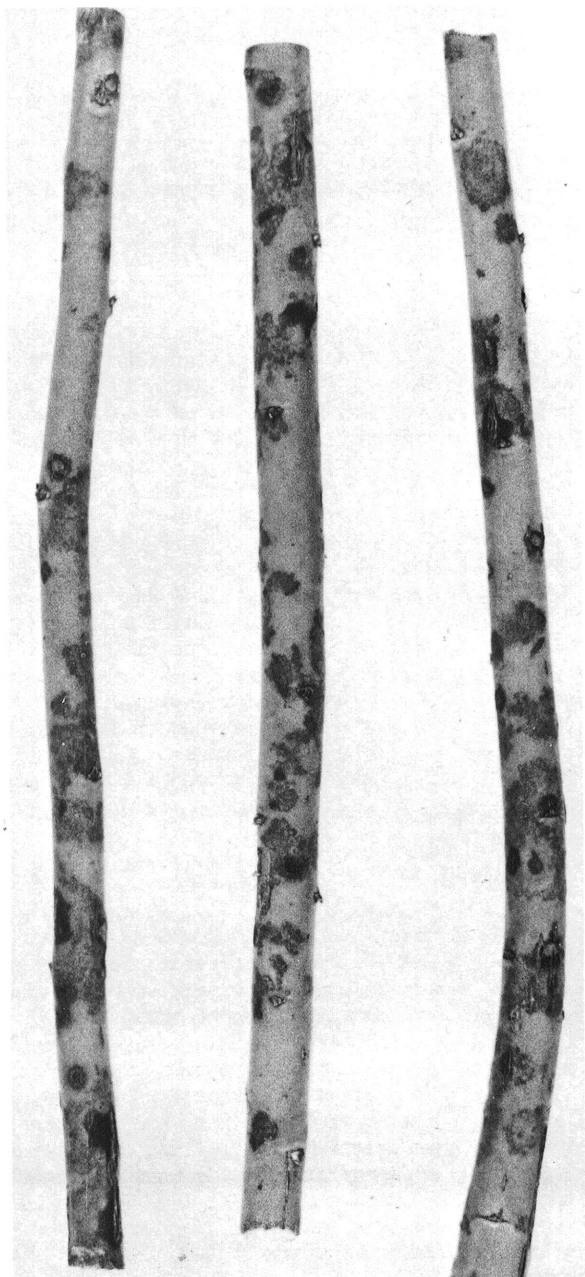


Fig. 1. Stem lesions caused by *Cercospora* sp. on rabbiteye blueberry.

lesions on succulent and woody stems yielded cultures of *Cercospora* sp. No lesions developed on inoculated highbush plants or noninoculated controls.

Penetration through open stomata occurred within 48 hr after inoculation. The fungus grew intercellularly within the stem cortex, and the hyphae measured 3-4  $\mu\text{m}$  in width. Sporulation occurred when the hyphae accumulated in the substomatal cavity and formed a light- to olive-brown fasciculate stroma that measured 20-25  $\times$  10-15  $\mu\text{m}$  (Fig. 2-A). The stroma emerged from the stoma and produced conidiophores and conidia (Fig. 2-B). Conidiophores were hyaline to light olive-brown in mass, rounded at the ends, and measured 15-35  $\times$  2-3  $\mu\text{m}$ .

The small raised lesions developed as a result of hyperplasia and hypertrophy in the cortex (Fig. 2-C, D). Intercellular growth of the fungus caused some of the cells in the cortex to collapse, often leaving large holes in the tissue. The fungus was confined primarily to the outer portion of the cortex after 2 mo, and about two to three layers of periderm were initiated by the phloem parenchyma. Most of the cells in the cortex had become infected after 6 mo, and were necrotic (Fig. 2-E). The fungus also had penetrated into the pericyclic fibers and periderm causing some disruption of these tissues. Within 1 yr after infection, the fungus had completely encircled the stem, and the cortical cells at the site of infection were completely collapsed and necrotic. Ingress into the phloem occurred and cells in the periderm were almost completely destroyed. Only the first few layers of cells in the cortex at the farthest point from the site of infection were necrotic. Several layers of periderm were formed beneath these necrotic cells.

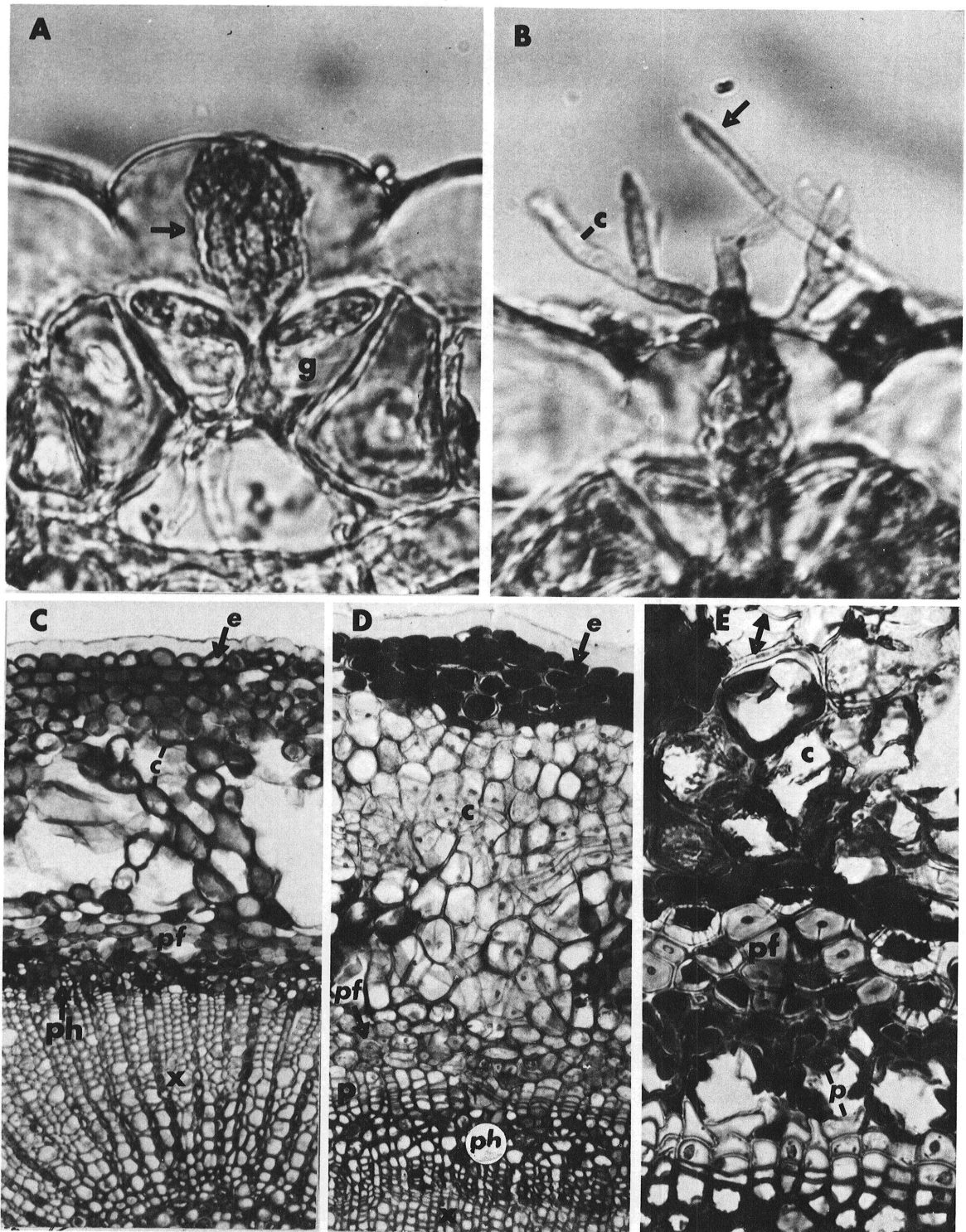
#### DISCUSSION

This is the first report of a *Cercospora* sp. causing a disease of rabbiteye blueberry. Almost 2,000 *Cercospora* spp. have been distinguished by differences in host specificity and morphology of conidia (1). However, no attempt was made to identify the species of *Cercospora* involved in these studies.

In pathogenicity tests with the two highbush and two rabbiteye cultivars, only the rabbiteye cultivars became infected. Furthermore, the disease has not been observed on highbush blueberry plants in the field. Observations from greenhouse inoculations indicated that conidial germination and penetration occurs within 48 to 72 hr at 25 C; however, growth of the fungus and disease development are relatively slow.

The name given to this disease, "stem blotch", characterizes the symptoms produced by the host-pathogen interaction. Symptoms, in an early stage of disease development, could easily be confused with the red ringspot virus disease of blueberry (2). Under favorable conditions, sporulation occurred about 6 mo to 1 yr after infection. Conidia developed from a stroma that emerges through stomata on the stem. Conidia were present on naturally infected stems from mid-May to mid-July in 1975. However, because of the variability in weather conditions in southeastern North Carolina, this may not be true every year.

The host response to invasion by the *Cercospora* fungus is different from that induced by infection with *Botryosphaeria dothidea* wherein a periderm layer is



**Fig. 2-(A to E).** Histopathology and sporulation of *Cercospora* sp. on rabbiteye blueberry stems. **A)** Fasciculate stroma (arrow) emerging from stoma. g = guard cell ( $\times 1,000$ ). **B)** Conidium (arrow) and conidiophores (c) ( $\times 1,000$ ). **C)** Healthy stem. e = epidermis; c = cortex; pf = pericyclic fibers; ph = phloem; x = xylem ( $\times 165$ ). **D)** Two-month-old lesion showing necrosis of epidermis and parenchyma cells (e), hyperplasia and hypertrophy of cortex (c), and periderm (p) initiated by the phloem (ph). pf = pericyclic fibers; x = xylem ( $\times 175$ ). **E)** Six-month-old lesion showing hyphae (arrow) and necrotic cortex (c), pericycle (pf), and periderm (p) ( $\times 400$ ).

formed in the cortex beneath the site of infection (4). In contrast, periderm formation was initiated by the phloem parenchyma following invasion of rabbiteye stem tissue by *Cercospora* sp.

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