Histology of Botryosphaeria Canker of Susceptible and Resistant Highbush Blueberries

R. D. Milholland

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

Botryosphaeria corticis penetrated highbush blueberry stems through open stomates 6 hr after inoculation. No evidence of direct penetration was obtained. Enlargement of the stem canker is due to increased cell numbers, and not to increased cell size. Hyphae were both intra- and intercellular, and ranged from 2-12 μ in diam. Mature perithecia were observed 1 year after infection. The cortex and phloem were completely disorganized in susceptible blueberry stems, but no disorganization of these tissues was observed in resistant stems. Xylem invasion was associated with the proliferation of the cortical parenchyma into xylem rays. Rays were larger in infected than in normal wood. Lesions in highly resistant cultivars result from cell division in the epidermis. No fruiting structures, pycnidia, or perithecia were observed in cankers of resistant stems 18 months after inoculation. Phytopathology 60:70-74.

The most important disease of highbush blueberries (*Vaccinium corymbosum* L.) in North Carolina is stem canker caused by *Botryosphaeria corticis* (Demaree & Wilcox) Arx & Muller. The causal fungus was identified and the disease symptoms described by Demaree & Wilcox (2) in 1942. Demaree & Morrow (1) found that symptoms of stem canker varied with the susceptibility of the cultivar. On susceptible cultivars, cankers enlarge rapidly during the second season and become rough, swollen, and deeply fissured. Cankers enlarge slowly, but do not swell appreciably on the more resistant cultivars.

Demaree & Wilcox (2) reported that the pathogen enters the stem through unbroken bark, probably through lenticels of current year shoots, and then penetrates the cortex and cambium into the xylem, causing some discoloration of the wood. The research reported herein was undertaken to determine the effects of the pathogen on stem tissues of resistant and susceptible cultivars.

MATERIALS AND METHODS.—Canker-free plants of the cultivars Stanley, Weymouth, Wolcott, Murphy, and Angola were inoculated with isolate 52 of *B. corticis* (6). The inoculum was standardized to a concentration of 3×10^4 conidia/ml of water, and applied to stems of rapidly growing blueberry plants with an air compressor sprayer. Three single-stem, greenhouse-grown plants of each cultivar were inoculated. One plant of each cultivar was sprayed with distilled water as a control. The plants were placed in a moist chamber at 25-30 C for 72 hr, after which they were removed to a greenhouse bench. The stems were examined for canker development 6, 12, 18, 24, and 48 months after inoculation.

The stems were cut into 10-mm sections and cleared in Formalin-acetic-alcohol (FAA) for 2 weeks. They were then placed in boiling water for 5 min before being softened in nine parts of 60% ethyl alcohol to one part of glycerin for 30 days. Stems were sectioned on a sliding microtome to a thickness of 20 μ. Sections were mounted on slides with Haupt's adhesive, and stained with safranin and fast green (4).

Penetration by the fungus was studied, using stems removed from Weymouth plants 2, 4, 6, 12, 24, 48, and 72 hr after inoculation. The stems were cut into small sections, cleared, and stained with cotton blue in lactophenol (7). The epidermis was removed and examined microscopically for penetration by the fungus.

RESULTS.—Conidia of *B. corticis* germinated on blueberry stems within 2 hr. Germ tubes emerged from both ends of the conidia. Length of germ tubes 4 hr after inoculation averaged 72 μ. Germ-tube length after 48 hr was approximately 400 μ, with extensive branching. The fungus penetrated only through open stomata. In many instances, the germ tube would encircle the guard cells or grow over the stoma without penetration. Penetration was observed as early as 6 hr after inoculation, but most penetrations occurred after 24 hr. Upon entering a stoma, an appressoriumlike structure was produced at the tip of the germ tube. Ramification of hyphae through the host tissue was not observed.

Small red lesions were formed on the succulent stems 4-5 days after inoculation. The rate of canker development varied on the cultivars tested. Eighteen months after inoculation, cankers on Angola and Murphy (resistant) were small, slightly raised lesions; on Stanley, Weymouth, and Wolcott (susceptible) they were large and swollen (Fig. 1-A, B).

Cankers enlarged through increased cell numbers (hyperplasia), and not through increased cell size (hypertrophy). Hyphae in the cortex were intercellular, and measured 2-4 μ in diam. In addition, large, thick-walled hyphae measuring 7-12 μ in diam were observed (Fig. 1-C). Both types of hyphae are produced on oatmeal agar. The large hyphae appear to grow from a stromalike structure at the outer edge of the cortex (Fig. 1-D). Growth of hyphae and proliferation of parenchyma cells caused the cortex of susceptible blueberry stems to become completely disorganized within 6 months of inoculation (Fig. 1-E, F).

Immature pycnidia were present in cankers on susceptible stems 6 months after inoculation. Mature perithecia were observed in cankers of susceptible blueberry stems 1 year after infection (Fig. 2-A). Perithecia were black, globose to conical, and 225 μ high and 200 μ wide. Asci and ascospores were similar to those described by Demaree and Wilcox (2).

The cortex and phloem were disorganized in 2- and 3-year-old cankers. Rapid division of the cells in the cortex caused many of the parenchyma cells to become crushed, leaving large cavities filled with mycelium.

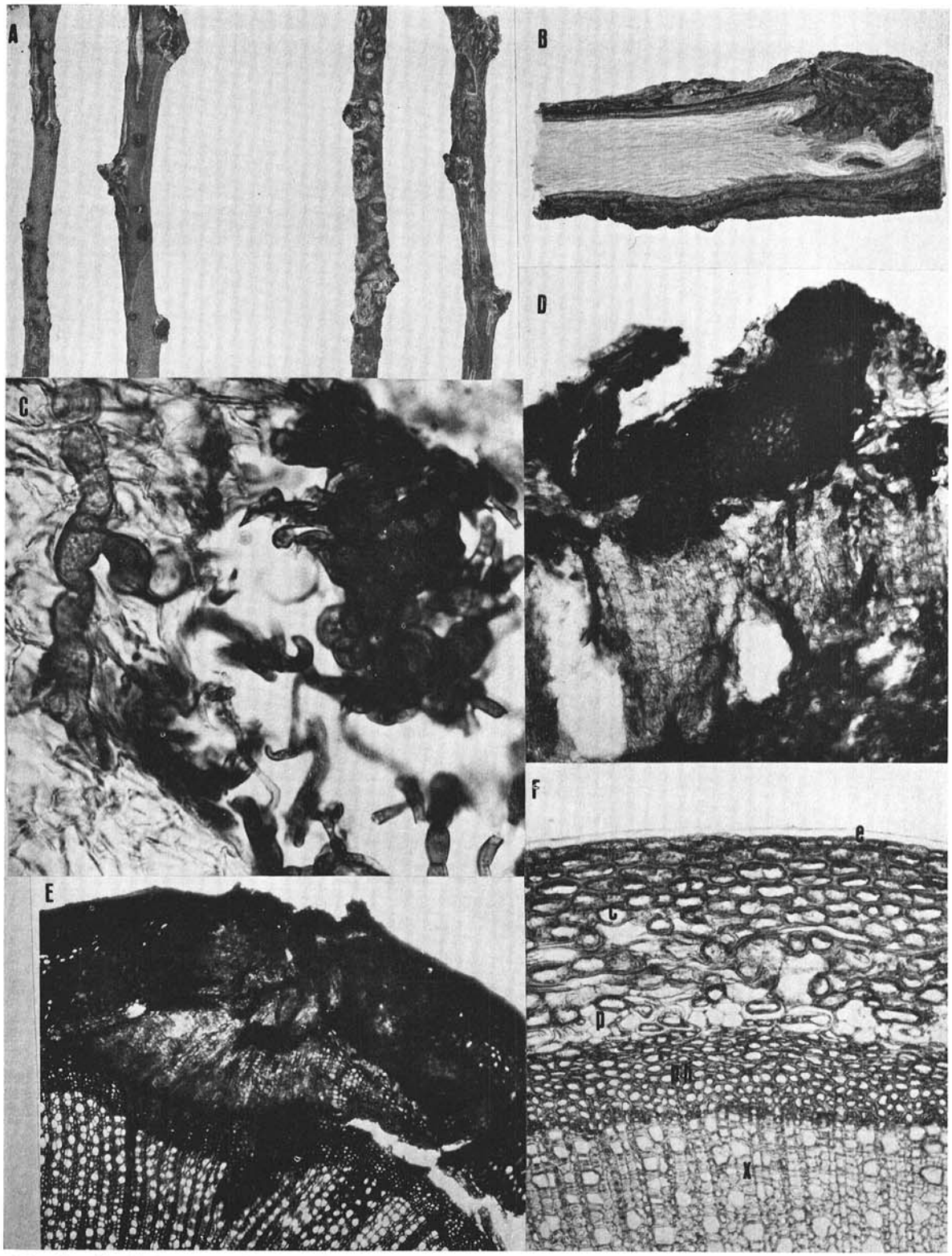

Fig. 1. **A)** Cankers on resistant (left) and susceptible (right) blueberry stems 18 months after infection. **B)** Transverse view of stem canker on the susceptible cultivar Wolcott, showing infected portion of the wood. **C)** Hyphae of *Botryosphaeria corticis* in the stem cortex (× 480). **D)** Transverse section of a 1-yr-old infected stem, showing stroma and enlarged hyphae (× 120). **E)** Transverse section of a 6-month-old canker, showing disorganization of cortex (× 120). **F)** Section of normal blueberry stem: e = epidermis; c = cortex; p = pericycle; ph = phloem; and x = xylem (× 120).

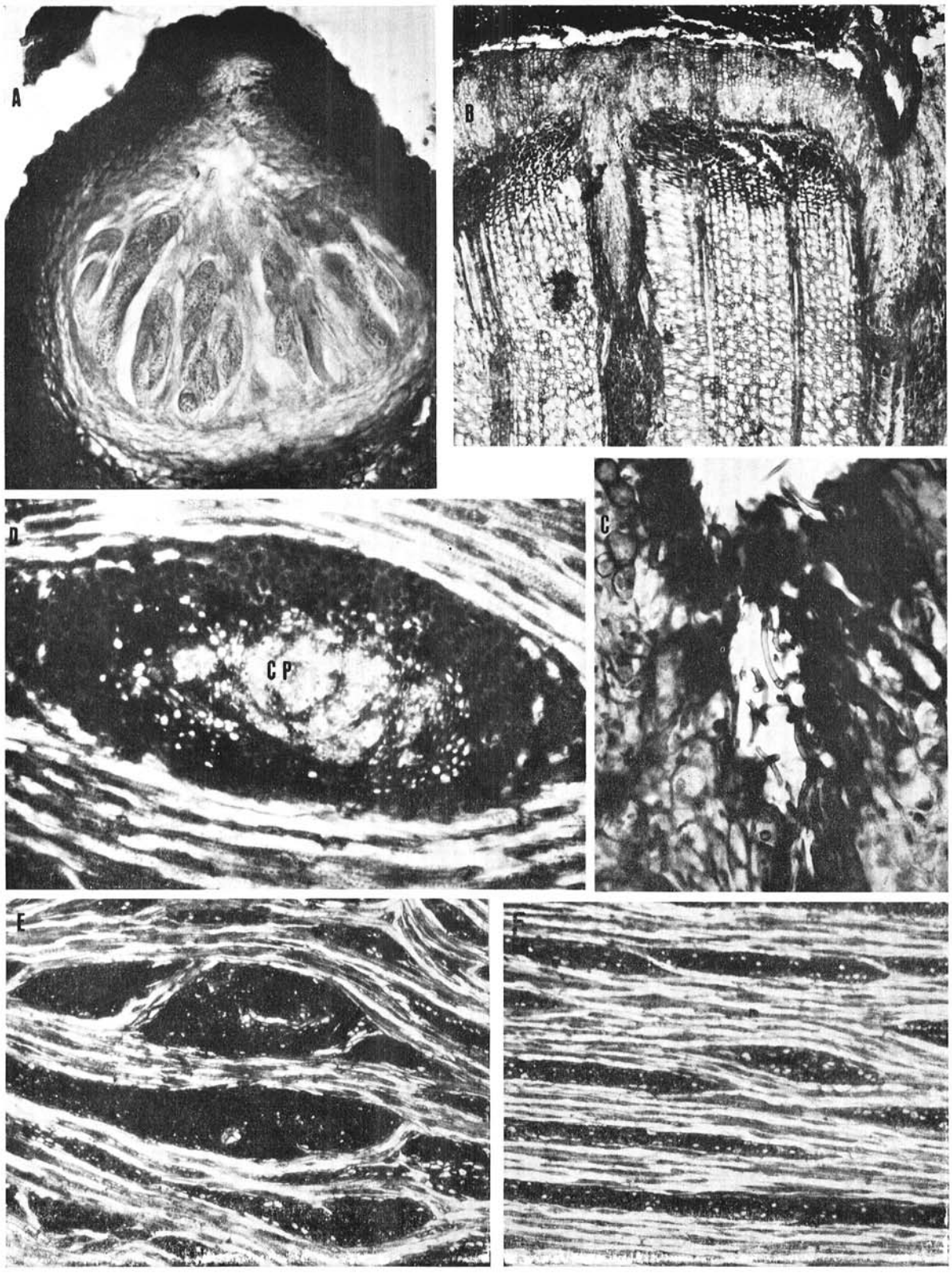

Fig. 2. **A)** Mature perithecium of *Botryosphaeria corticis* (× 300). **B)** Transverse section of a 1-yr-old infected stem, showing proliferated cortical parenchyma in the xylem (× 48). **C)** Hyphae of *B. corticis* in the proliferated parenchyma (× 300). **D)** Tangential section of infected xylem ray with cortical parenchyma and hyphae in the center: cp = cortical parenchyma (× 120). **E)** Tangential view of infected xylem rays (× 48). **F)** Tangential view of normal xylem rays (× 48).

Invasion of the xylem was associated with the xylem rays (Fig. 2-B). The fungus did not penetrate through the phloem and cambium into the xylem without being associated with the proliferated parenchyma cells (Fig. 2-C). Figure 2-D shows a tangential view of an enlarged ray with the cortical parenchyma cells in the center.

The anatomy of highbush blueberry stem is characterized by small uniseriate rays and large multiseriate rays that are generally fusiform in shape (3). The large ray is composed of two kinds of cells, the light ones which represent modified fibers and the dark, smaller parenchyma cells. The multiseriate rays were significantly wider in infected wood than normal wood (Fig. 2-E, F). Because the size of the ray parenchyma in infected and normal rays did not differ significantly, the increase in the width of the infected rays was from an increase in cell numbers rather than cell size. Xylem rays were 50-400 μ wide in infected tissue and 40-70 μ in normal tissue. Infected rays averaged 16 cells in width, with some rays having as many as 30-40 cells. Normal rays averaged 5 cells in width. Whorls of tracheids and rays were occasionally seen in the infected tissue. Hyphae were observed within some tracheids adjacent to the proliferated parenchyma.

The small, slightly raised lesions on highly resistant varieties appear to have developed as the result of cell division in the epidermis (Fig. 3-A, B). The layer of cells (epidermis) which separated the necrotic tissue from the healthy parenchyma stained a dark color and extended across the entire lesion. Approximately the same number of cells were counted between the epidermis and phloem in healthy and diseased tissue of Angola stems 18 months after infection. According to Mahlstede & Watson (5), the outer cortical region will vary from 5-12 cells in depth. Similar findings are reported in these studies. Very few hyphae were observed in the resistant tissue, with all of the hyphae confined to the outer portion of the canker. No fruiting structures, pycnidia, or perithecia were observed in cankers of resistant stems 18 months after inoculation.

DISCUSSION.—The infection process, spore germination, penetration, and establishment of *B. corticis* in blueberry stems occurs quite rapidly; however, the development of the disease is a relatively slow process. The fungus is a relatively slow-growing organism, and is confined to the cortex and phloem during the first year. In highly susceptible cultivars the fungus invades the cortex, causing severe hyperplasia of the cortical parenchyma. As the cells continue to divide and proliferate, the fungus invades the phloem, resulting in further disorganization of developing tissues. Viability and continued growth of the fungus appear to depend on close relationship with dividing parenchyma cells. Hyphae were not found in advance of these cells. As the fungus continues to grow and invade the wood, cankers develop and the stem finally becomes girdled, weakened, and eventually dies.

Resistance of the blueberry plant to the stem canker fungus is related to fungal development after infection rather than to the establishment of infection. All inoculated plants were infected. The type of lesion which develops after infection rather than the number of infection sites is significant in determining resistance or susceptibility. In the resistant cultivar Angola, the epidermal cells apparently undergo cell division after penetration by the fungus, giving rise to small, slightly raised lesions. However, the fungus is restricted to the outer portion of the canker; i.e., hyphae neither penetrated into the cortex nor caused any proliferation or disorganization of the cortical parenchyma. The cells in the raised lesion become necrotic and may eventually be sloughed.

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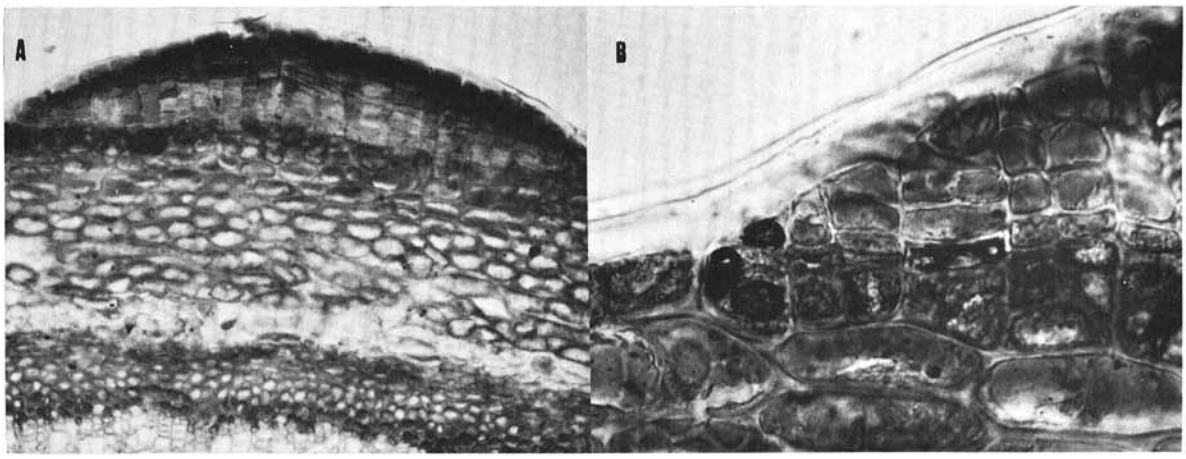

Fig. 3. A) Transverse section of stem lesion on the resistant cultivar Angola 18 months after infection by *Botryosphaeria corticis* (× 120). **B)** Development of stem lesion on Angola as the result of cell division in the epidermis (× 480).

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