Histopathology and Pathogenicity of Botryosphaeria dothidea on Blueberry Stems

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ABSTRACT

Infection of blueberry stems by *Botryosphaeria dothidea* resulted from both wounded and nonwounded inoculation. Mode of penetration and stage of plant development influenced both the type and extent of disease development. Penetration of stems by the fungus through open stomata did not result in necrosis or dieback; instead, small raised lesions developed but failed to enlarge. The fungus was restricted to the outer portion of the lesion. Infection of succulent stems by *B. dothidea* through wounds resulted in a rapid breakdown of all tissues. As the fungus progressed down the stem, tyloses were formed causing partial or complete occlusion of the xylem vessels. In addition to tyloses, dark-stained protrusions of an undetermined nature were formed in infected woody stems. These protrusions originated from parenchyma cells adjacent to the xylem vessels in a manner similar to formation of tyloses.

In a susceptibility trial, 10 highbush blueberry cultivars were highly susceptible to six isolates of *B. dothidae*, whereas eight rabbiteye blueberry cultivars were susceptible to only five.

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Additional key words: Vaccinium corymbosum, Botryospaheria ribis, stem blight.

Stem blight of highbush blueberry (*Vaccinium corymbosum* L.) is caused by the fungus *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not (*B. ribis* Gross. & Dugg.) (8). Witcher & Clayton (8) compared the stem blight fungus, *B. dothidea,* to the fungus formerly known as *B. ribis,* and found the two to be identical. Symptoms of stem blight include yellowing, wilting, and browning of leaves on individual branches (6, 8). The woody tissue of infected branches is pecan-brown colored, frequently occurring only on one side of an affected stem (6). Infections near the tip of succulent twigs may be confused with winter injury and certain twig blight diseases (8).

Botryosphaeria dothidea has been found by several workers (1, 5, 7, 8) to be a wound parasite. However, Luttrell (3) found that *B. dothidea* was able to establish itself in elm stems that showed no apparent wounds, and that infections resulting from conidia inoculations were more severe than mycelial inoculations. Witcher & Clayton (8) considered that infection of blueberry by the stem blight organism

resulted only from wound inoculations.

Stem blight is a limiting factor in the production of highbush blueberry in North Carolina. Stem blight was found in 37 of 39 blueberry fields surveyed in 1959, and 9% of the plants were affected (8). With the trend toward the use of mechanical harvesters and the subsequent wounding of blueberry plants by these machines, the probability of stem blight incidence increasing is great.

The research reported herein was undertaken to determine the mode of infection and the histological effects of the pathogen on stem tissues, and the relative susceptibility of blueberry cultivars to isolates of *B. dothidea.*

MATERIALS AND METHODS.—*Botryosphaeria dothidea* was isolated from infected highbush and rabbiteye (*V. ashei* Reade) blueberry stems collected from five different locations in southeastern North Carolina. Monoconidial cultures were made of all isolates.

Inoculations to determine pathogenicity and cultivar susceptibility were made by introducing aerial mycelia removed from oatmeal agar (OMA) cultures into stem wounds. Ten commercial highbush cultivars (Angola, Croatan, Wolcott, Morrow, Murphy, Berkeley, Jersey, Bluecrop, Stanley, and Earliblue), and eight rabbiteye cultivars (Garden Blue, Tifblue, Woodard, Homebell, Delite, Southland, Briteblue, and Menditoo) were used. Three single-stem, greenhouse-grown plants of each cultivar were inoculated with each isolate. An equal number of plants was wounded but not inoculated. The plants were placed in a moist chamber at 25-30 C for 48 hr, after which they were transferred to a greenhouse bench. Length of the lesion from point of inoculation down the stem was recorded after 7 and 14 days. Tests were repeated two times. Analysis of variance was utilized to determine significant differences (5% level) between means.

In additional pathogenicity tests, young succulent stems of the cultivar Berkeley were inoculated with conidia of *B. dothidea.* The test consisted of five treatments, with six stems inoculated/treatment. Two noninoculated stems served as controls for each treatment. The treatments included: (i) cut made at leaf axil with razor blade; (ii) shoot tip excised; (iii) succulent stem wounded with a sterile needle; (iv) cut made with a razor blade in 1-year-old stem; and (v) nonwounded succulent stem. Inoculations were made by spraying an inoculum suspension (10^5 conidia/ml) onto blueberry stems. Plants were placed in a moist chamber at 25 to 30 C for 48 hr, then removed to a greenhouse bench under natural light at 25 to 30 C. Number and length of lesions were recorded after 14 days.

Penetration by the fungus was studied histologically, using nonwounded stems removed from Berkeley plants 2, 6, 24, and 48 hr after inoculation. The stems were cut into small sections, cleared, and stained with cotton blue in lactophenol (4). 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 in the greenhouse and from naturally infected plants in the field. Stems were examined 1, 2, 4, and 8 weeks after inoculation. Stems were cut into 10- to 20-mm sections and cleared and fixed in Formalin-acetic-alcohol (FAA) for 2 weeks. Fixed woody stems were placed in boiling water for 5 min before being softened in a 9:1 mixture of 60% ethyl alcohol and glycerine 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 (2).

RESULTS.—In susceptibility studies with 18 different blueberry cultivars, all plants inoculated with each of 6 isolates of *B. dothidea* became infected. The length of the lesions for cultivars inoculated with isolates 2 to 6 ranged from 20 to 60 mm after 14 days. Irrespective of isolate, disease severity was greatest on the highbush cultivar Bluecrop and the rabbiteye cultivar Woodard. The average rate of disease development in inoculated

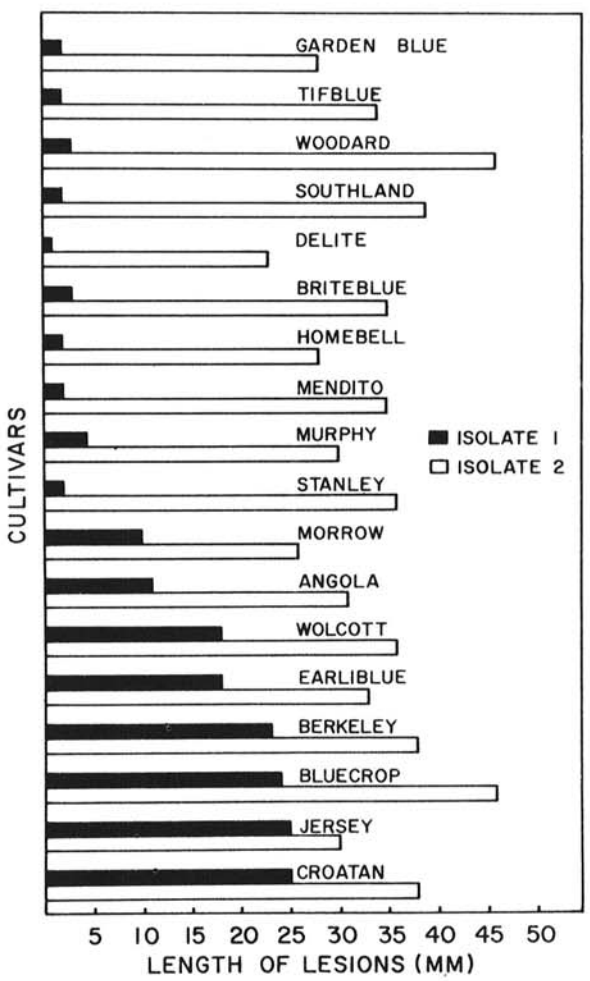

Fig. 1. Relative susceptibility of blueberry cultivars to stem blight caused by *Botryosphaeria dothidea.* Stems inoculated in the greenhouse and length of lesions recorded after 14 days.

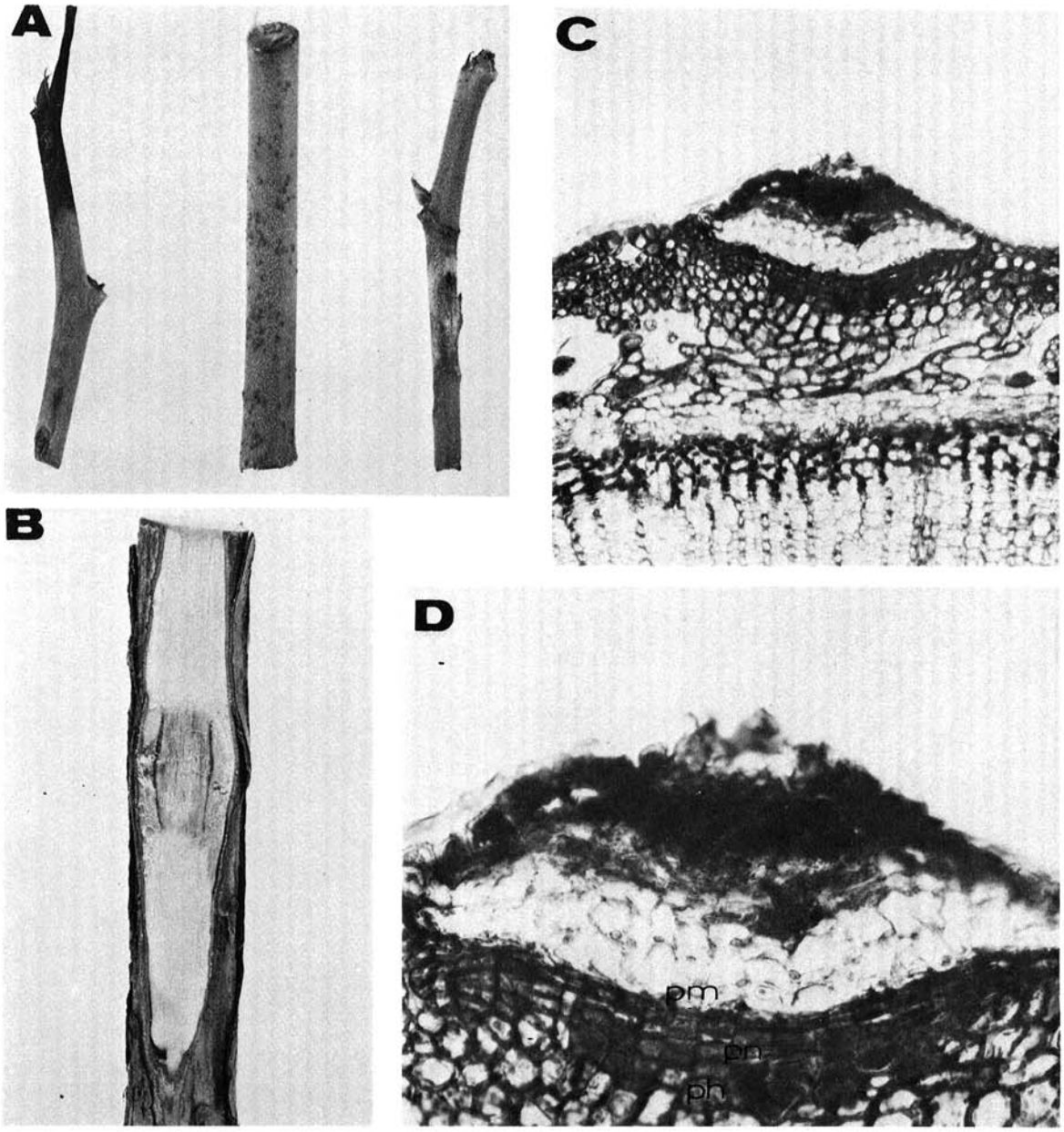

Fig. 2. A) (Left) Stem blight development on blueberry 14 days after infection with *Botryosphaeria dothidea* through shoot tip. (Center) Lesion development after infection through stomata. (Right) Necrosis of stem after infection through a wound. **B)** Stem blight development on wounded 1-year-old stem showing pecan-brown discoloration of the wood. **C)** Transverse section of stem lesion 1 month after infection through a stomate by *B. dothidea* (× 120). **D)** Periderm formation beneath the lesion, acting as a barrier to hyphal invasion: ph = phelloderm; pn = phellogen; pm = phellem (× 300).

stems in the greenhouse was 25 mm/month, but ranged from 1 to 75 mm, depending on the isolate, cultivar, and stage of plant growth. No significant differences in susceptibility of highbush or rabbiteye cultivars to stem blight were observed using isolates 2 to 6. Isolate 1 was less pathogenic on both highbush and rabbiteye plants. The length of the lesions for the highbush plants ranged from 2 to 25 mm (avg 16 mm), whereas the range for the 8 rabbiteye cultivars was only 1 to 3 mm (avg 1.7 mm) after 14 days. Data for 2 of the 6 isolates are given in Fig. 1. Dieback symptoms were similar to those described by Witcher & Clayton (8). Pycnidia formed on the necrotic stems within 14 days after inoculation. The diameter of pycnidia for all isolates was 120 to 240 μ, with conidia measuring 15 to 27 μ long and 6 to 9 μ

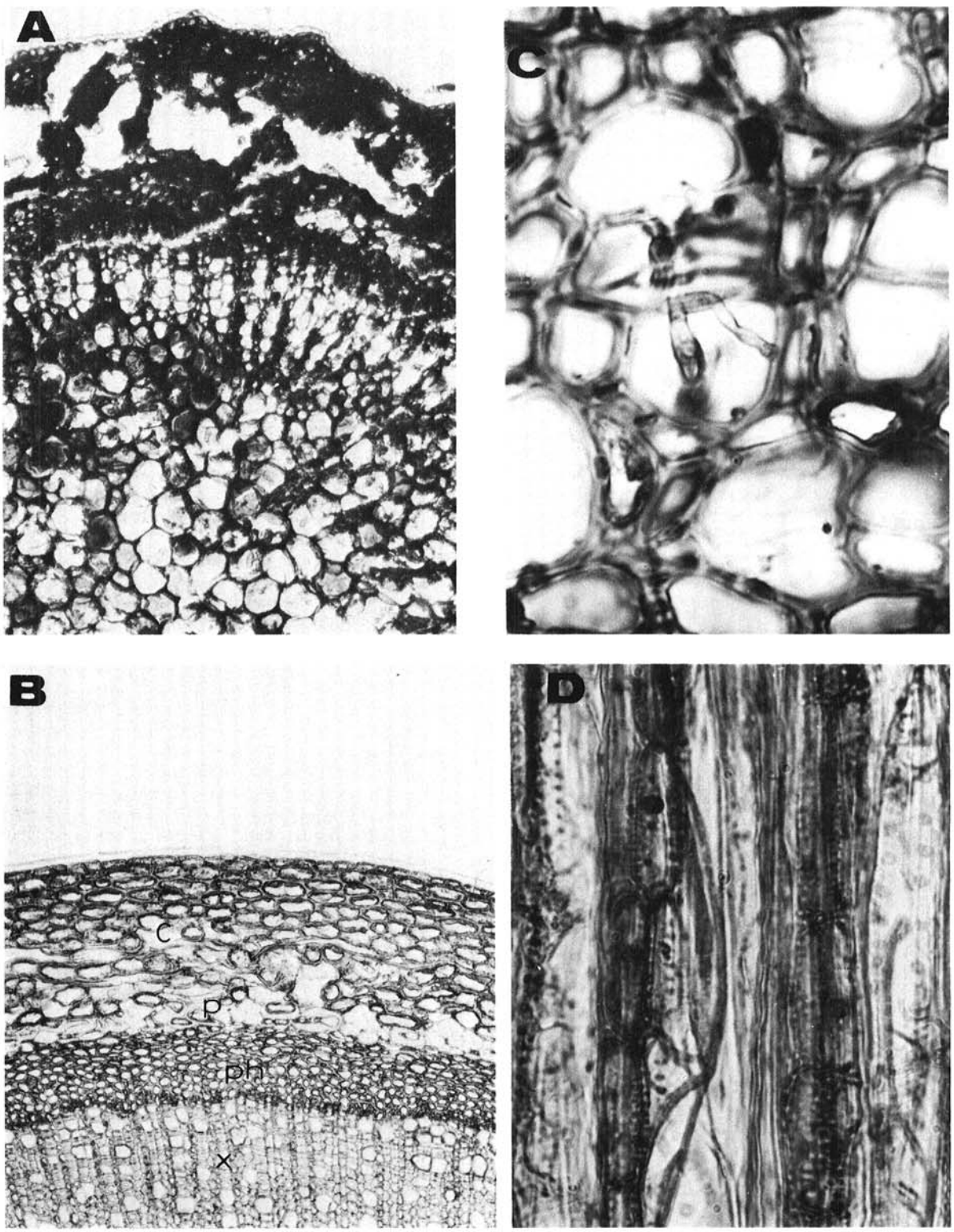

Fig. 3. A) Transverse section of a 14-day-old lesion on blueberry caused by *Botryosphaeria dothidea* showing disorganization of cortex (× 120). **B)** Portion of a transverse section of a healthy blueberry stem: c = cortex; p = pericycle; ph = phloem; x = xylem (× 120). **C)** Transverse section of infected blueberry stem, showing hyphae of *B. dothidea* in the vessels (× 480). **D)** Tangential section of infected blueberry stem, showing hyphae inside the vessels (× 480).

wide (average 19.2 X 7.7 μ). No necrosis or dieback occurred on noninoculated controls.

Stem blight developed on all wounded Berkeley stems inoculated with a conidial suspension (10^5 conidia/ml). Lesions on succulent stems wounded at the leaf axil averaged 10 mm in length after 14 days, whereas lesions on stems excised at the shoot tip averaged 30 mm (Fig. 2-A). Stem blight lesions on succulent stems wounded with a sterile needle at time of inoculation varied from a small (1 to 2 mm) raised lesion to an elongated, slightly sunken lesion 20 mm in length. While wounded 1-year-old stems were infected by conidia, discoloration of the stem extended only 2 to 3 mm below the infection site after 14 days (Fig. 2-B). No necrosis or dieback occurred as the result of conidial inoculations on nonwounded stems. Instead, small slightly raised lesions developed but failed to enlarge. No lesions developed on noninoculated stems.

Conidia of *B. dothidea* germinated on blueberry stems within 2 hr. Per cent germination after 2 and 6 hr was 60 and 98%, respectively. Average length of germ tubes after 2 and 24 hr was 11 and 166 μ, respectively. The fungus penetrated only through open stomata on nonwounded stems. Penetration was observed as early as 2 hr after inoculation, but most penetration occurred after 48 hr,

Small, slightly raised lesions developed as the result of penetration by the fungus through open stomata (Fig. 2-C, D). The layer of cells beneath the epidermis gave rise to the lesion. Lesions developed as the result of hyperplasia and not hypertrophy. The four or five layers of cells beneath the lesions were closely packed and rectangular in shape. These dark-stained cells are the periderm consisting of the phellogen, the phellem or cork (produced by the phellogen toward the outside), and the phelloderm. The layer of cells which separates the necrotic tissue from the healthy parenchyma extended across the entire lesion. The fungus was restricted to the outer portion of the lesion, with no hyphae being observed in the cortex. No fruiting structures developed in lesions of nonwounded inoculated stems.

Establishment of mycelium in wounded, succulent stems resulted in a rapid breakdown of individual cells within 2 to 3 days (Fig. 3-A, B). Hyphae measured 1 to 4 μ in diam, and grew intracellularly in the cortex, phloem, xylem vessels, and pith parenchyma (Fig. 3-C, D). Hyphae were also observed growing intercellularly in the air spaces of the cortex.

Tyloses formed in the xylem vessels of infected stems within 6 days after inoculation. These protrusions from adjacent parenchymatous cells caused partial or, frequently, complete occlusion of the xylem vessel elements (Fig. 4-A, B). Tyloses and hyphae were observed to occupy the same vessel. Tyloses formation was abundant in stems showing discoloration, but sparse in stem tissue which was just below or above the discolored area. Tyloses were observed as far as 20 mm in advance of the fungus. The hyphae in the discolored portion of the stem vessels spread longitudinally, with several strands occupying a single vessel. Some branching of the hyphae inside the xylem vessels occurred, resulting in invasion of adjacent tracheids. No hyphae were found in stem tissue beyond the discolored area.

Although tyloses were observed in xylem vessels of wounded controls, tyloses formation was sparse and occurred near the wound. No tyloses were observed in nonwounded controls.

In addition to tyloses formation, dark-staining protrusions in various stages of development were observed in the xylem vessel elements of infected woody stems (Fig. 4-C, D). These self-contained protrusions were somewhat different from tyloses formation, in that no definite cell membrane could be observed. Like tyloses, these protrusions originated from adjacent parenchymatous cells and caused complete occlusion of the vessels. Neither tyloses nor the dark-stained protrusions were observed in the healthy woody stems.

DISCUSSION.—In susceptibility studies with blueberry plants of 18 different cultivars, all isolates of *B. dothidea* tested were pathogenic to some blueberry cultivars. Significant differences in disease development were noted between cultivars and between isolates. In general, the highbush cultivars were highly susceptible to all six isolates tested, whereas the rabbiteye cultivars were susceptible to only five of the six isolates of *B. dothidea.*

Conidial inoculations were successful in both wounded and nonwounded stems. According to Witcher & Clayton (8), conidial inoculations with the stem blight organism were unsuccessful on nonwounded stems. This difference, although not tested, might be due in part to the concentration of inoculum used in the different studies. The severity of disease depended upon the type of wound and stage of plant growth. Succulent stems in which the vascular tissue was completely exposed were the most susceptible to stem blight development. The fungus was unable to reach the vascular tissue of nonwounded stems, and therefore did not cause necrosis or dieback of the stem. Instead, small raised lesions developed but failed to enlarge.

Spore germination, penetration, and establishment of *B. dothidea* in blueberry stems occurs rapidly. Penetration of nonwounded stems takes place through open stomata. No evidence of direct penetration was observed. Resistance of the blueberry plant to the stem blight fungus is related to fungal development after infection rather than to the establishment of infection. When infection takes place through an open stomate, the layer of cells beneath the epidermis undergoes cell division. Apparently this thickened layer of periderm restricts the fungus to the outer portion of the lesion.

Invasion of wounded, succulent stems causes a rapid breakdown of the cortex and phloem tissue in a few days. Once the fungus invades the vascular tissue, the mycelium moves down the stem rapidly, as much as 75 mm/month. In many instances, only one side of an affected branch may show the brown discoloration. Lateral movement of the fungus occurs very slowly.

Results of these studies indicate that death of

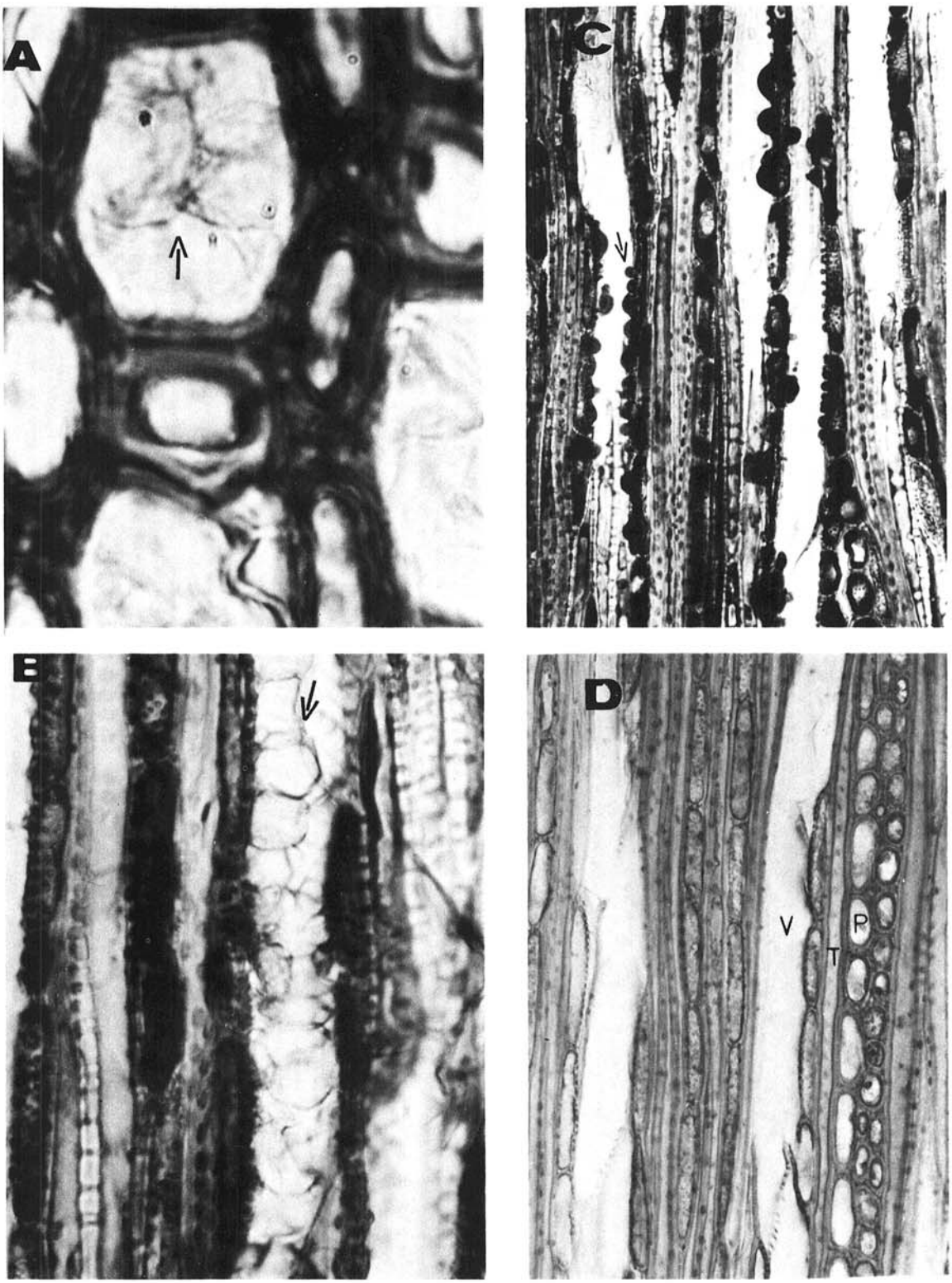

Fig. 4. A) Transverse section of blueberry stem infected with *Botryosphaeria dothidea* showing tyloses formation (arrow) (× 1,600). **B)** Tangential section of a 14-day-old infected blueberry stem, showing complete occlusion of vessel by tyloses (arrow) (× 640). **C)** Tangential section of a 2-year-old infected stem, showing dark-stained protrusions inside the xylem vessels (× 300). **D)** Tangential section of a 2-year-old healthy stem: v = vessel; p = parenchyma; t = tracheids (× 480).

blueberry stems infected with *B. dothidea* is primarily due to the partial or complete occlusion of the xylem vessels by tyloses and the presence of mycelium which will impede or restrict the flow of water. Dark-stained protrusions, the nature of which was undetermined, were also present in the xylem vessels of infected woody stems. The formation of these protrusions appears to be initiated by the fungus since they were not observed in healthy stems.

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