Stem and Root Rot of Blueberry Caused by Calonectria crotalariae

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ABSTRACT

Calonectria crotalariae was isolated from blueberry seedlings and identified as the causal organism of a stem- and root-rot disease. Symptoms first appear as a browning of the stem near the soil line, followed by a wilting of the plant. Within 1 wk after infection, the entire stem turns brown and dies. The pathogen also causes root necrosis and a leaf spot consisting of brown, circular lesions surrounded by a red border measuring 1-3 mm in diam. Both highbush and rabbiteye blueberry cultivars are susceptible. A morphological and pathological comparison was made between the blueberry isolate, and a peanut isolate of *C. crotalariae*.

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Additional key words: Cylindrocladium crotalariae, Vaccinium corymbosum, Vaccinium ashei.

A severe blighting and death of highbush blueberry seedlings (*Vaccinium corymbosum* L.) was observed in July 1973 at the Horticultural Crops Research Station, Castle Hayne, N.C. The seedlings were growing in ground beds in a peat/sand mixture (1:1, v/v) that had previously been sterilized with methyl bromide. Disease symptoms first appeared as a browning or blackening of the stem near the soil line followed by wilting and death of the plant (Fig. 1-A). Several orange-colored perithecia of *Calonectria* sp. developed on the affected tissue. Some root necrosis was also observed when the infected plants were removed from the ground beds.

Studies were initiated to determine the etiology, and pathogenicity of the causal agent to blueberry.

MATERIALS AND METHODS.—*Causal organism.*—Morphological and pathological comparisons were made between the *Calonectria* sp. isolated from blueberry and *Calonectria crotalariae* (Loos) Bell and Sob. obtained from R. C. Rowe, North Carolina State University, Raleigh. The two cultures were grown on potato-dextrose agar (PDA) at 25 C and measurements of the imperfect stage were recorded after 7 days.

The influence of temp on growth rate, habit, and conidial production were studied in culture. Square (5-mm) blocks were removed from 7-day-old cultures of the blueberry and peanut isolates and placed on PDA. Four petri plates of each isolate were placed at 5, 10, 15, 20, 25, 30, and 35 C.

Conidia used in the germination tests were harvested from 7-day-old PDA cultures by washing the surface of a culture with sterile distilled water. The conidial suspension was sprayed onto young succulent stems of the blueberry cultivar Jersey, and placed in a moist chamber at 25 C. The stems were excised into 5-cm sections after 4 and 6 h, and stained with cotton blue and lactophenol (3). The epidermis was removed and examined microscopically for germination and penetration.

Pathogenicity tests.—Rooted cuttings of the highbush blueberry cultivars Bluecrop, Croatan, Jersey, Morrow, and the rabbiteye (*V. ashei*) cultivars Garden Blue and Tifblue were inoculated by spraying a spore suspension (10^5 spores/ml) onto the succulent stems and leaves. Twelve plants of each cultivar were inoculated with the blueberry and peanut isolates. Four noninoculated plants of each cultivar served as controls. Plants were placed in a moist chamber at 25 C for 24 h and then transferred to Sherer-Gillette CEL 25-7HL constant temp chambers at 25 C.

Root inoculations were made by scraping the surface of a 7-day-old culture with a razor blade, flooding the plate with 50 ml of distilled water, straining the suspension through cheesecloth into a beaker, and pouring 5 ml of the conidial suspension over the roots of each plant. Six plants of the cultivars Bluecrop, Croatan, Jersey, and Morrow were inoculated. Two plants of each cultivar served as noninoculated controls. Only the blueberry isolate was used in these tests. The plants were grown in a peat/sand mixture (1:1, v/v) in 10.2-cm (4-in) diam clay pots.

The leaves and stems of three plants of each of the following species and cultivars were tested for susceptibility to the blueberry and peanut isolates: *Arachis hypogaea* L. 'Florigiant', *Crotalariae mucronata* Desv., *C. spectabilis* Roth, and *Rhododendron obtusum* (Lindl.) Planch. 'Hino Crimson'. A spore suspension of 10^5 spores/ml was sprayed onto the stems and leaves of plants which were placed in a moist chamber for 24 h, and then removed to a greenhouse bench.

RESULTS.—*Causal organism.*—Perithecia of the *Calonectria* sp. isolated from blueberry stems are orange, oval-to-globose, 260-400 μm wide, and are scattered to gregarious. Asci are hyaline, clavate, long-stalked, 75-120 μm × 12-20 μm, and contain eight ascospores. Ascospores are hyaline, fusoid to

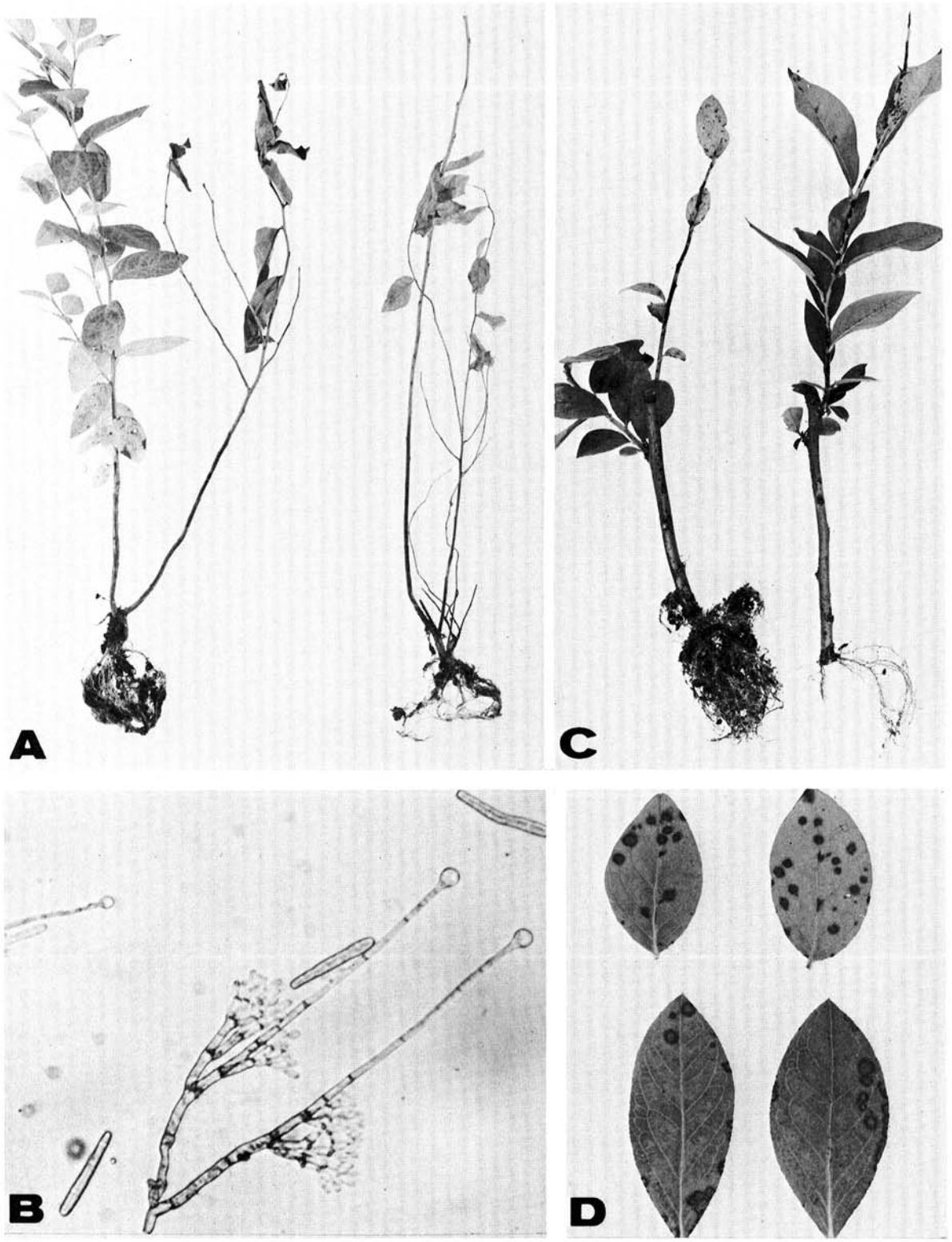

Fig. 1-(A to D). A) Blueberry seedlings infected with *Calonectria crotalariae.* Infected stems (right) turn brown, wilt, and eventually die; **B)** Conidiophores, stipes, vesicles, and conidia of *Calonectria crotalariae.* **C)** Highbush blueberry plants of the cultivar Jersey 24 h after inoculation with *Calonectria crotalariae.* Note large black irregular-shaped lesions on stems. **D)** Leaf spots of highbush (top), and rabbiteye (bottom) blueberry 4 days after inoculation with *Calonectria crotalariae.*

falcate, granular, one-to-three septate, constricted at the septa, 22-52 μm × 5-8 μm and avgd 36.6 × 6.0 μm.

Cylindrocladium sp. was consistently isolated from infected blueberry seedlings. Mycelium of the fungus is white, later turning a brown to reddish-brown color when grown on PDA at 25 C. Conidiophores are borne laterally on a stipe that terminates in a hyaline globose vesicle measuring 6-16 μm in diam (Fig. 1-B). Conidia are hyaline, cylindrical, slightly tapered towards the basal end, mostly three-septate, 55-95 μm × 4-8 μm, and avg 70.2 × 6.0 μm.

Perithecia of *C. crotalariae* collected from infected peanuts are orange, and ranged 286-407 μm in diam. Ascospores measured 22-52 μm × 6-8 μm and avgd 37.1 × 6.8 μm. The imperfect stage of the peanut isolate was similar to the blueberry isolate when grown on PDA at 25 C. The globose vesicles borne at the terminal portion of the stipe measured 6-18 μm in diam, and the conidia ranged in size from 55-100 μm × 5-8 μm, averaging 75.3 × 7.2 μm.

Perithecia of both isolates were produced in culture after 14 days at 25 C by growing the fungus on water agar or inoculating 15-mm sections of sterilized blueberry stems placed on water agar. Size and shape of perithecia and ascospores in culture were similar to that obtained from naturally infected blueberry and peanut plants.

The relative growth rate of the blueberry and peanut isolates of *C. crotalariae* on PDA after 7 days was greatest at 25 C, and least at 15 C. No growth was observed at 10 C or 35 C after 7 days. Very few spores were produced at 15 C with a breakdown of cell contents occurring in almost every spore. Abundant sporulation was produced for both isolates at 25 C and 30 C after 7 and 14 days. An optimum temp of 25 C for growth and sporulation in culture of the blueberry isolate of *C. crotalariae* is similar to that previously reported by Bell and Sobers (1).

Spore germination was approx. 92% at 25 C after 4 h. The avg germ tube length after 4 and 6 hr was 27 μm and 148 μm, respectively. Some swelling of the terminal portion of the germ tube was observed after 6 h. Actual penetration of the epidermis by the fungus was not observed.

Pathogenicity tests.—Numerous dark-brown-to-black, irregular-shaped lesions were observed on all inoculated stems after 24 h (Fig. 1-C) The succulent portions of several stems were killed after 48 h. Stems killed by the blueberry isolate of *C. crotolariae* after 7 days for Bluecrop, Tifblue, Garden Blue, Jersey, Morrow and Croatan were 100, 75, 66, 66, 66, and 42%, respectively. All of the stems inoculated with the peanut isolate were killed after 7 days.

Leaf spots first appeared as small pinpoint lesions less than 0.5 mm in diam after 24 h. Lesions continued to develop into brown circular lesions surrounded by a red border measuring 1-3 mm in diam after 4 days (Fig. 1-D). Isolations from stem and leaf lesions yielded cultures identical to that of the inocula.

Although all cultivars were susceptible to root infection, the cultivar Jersey was the most susceptible. Eighty percent of the inoculated Jersey plants had wilted and died within 7 days. Croatan was the least susceptible, with only 50% of the plants wilting after 3 wk. Roots of inoculated plants were necrotic 14 days after inoculation. Isolations from necrotic roots yielded cultures typical of the inoculum.

Greenhouse inoculations with the blueberry and peanut isolates of *C. crotalariae* on azalea, crotalaria, and peanuts proved to be pathogenic. Leaf spot symptoms on the azalea cultivar Hino Crimson were dark-brown, circular-to-irregular-shaped lesions, 0.5 to 4 mm in diam. Defoliation occurred after 14 days. Elongated brown lesions 10 to 15 mm in length were also observed on the stems. Both species of crotalaria (*C. mucronata, C. spectabilis*) were susceptible to the blueberry and peanut isolates. Numerous small black lesions, measuring 1 mm or less in diam, developed on leaves and stems 7 days after inoculation. After 14 days, lesions on stems became elongated and caused some vascular discoloration. Numerous small black lesions, 0.5 mm or less in diam, developed on the stems and leaves of peanut plants inoculated with either isolate 7 days after inoculation. Dark-brown-to-black lesions were also observed on the pegs and pods after 14 days.

DISCUSSION.—This is the first report of *Calonectria crotalariae* causing a stem and root of blueberry. The blueberry and peanut isolates of *C. crotalariae* used in these tests were found to be pathologically and morphologically identical. Both the perfect and imperfect stage of the pathogen is very similar to the description of *C. crotalariae* previously reported on peanuts (1) and papaya (2).

The infection process, spore germination, penetration, and establishment of *C. crotalariae* in blueberry stems occurs quite rapidly. Conidia germinate on blueberry stems within 2-4 h, with germ tubes emerging from both ends of the conidia.

Two major highbush cultivars grown in North Carolina (Croatan and Morrow), and two commercial cultivars grown in New Jersey (Bluecrop and Jersey) were highly susceptible to the pathogen. Although the stems, leaves, and roots of blueberry plants were found to be susceptible, the stem tissue appears to be the most vulnerable. Succulent blueberry stems can be killed within 24-48 h after infection. The disease would be of primary importance in rooting beds where a continual mist is applied to the plants creating an ideal environment conducive to infection and disease development. Although the disease has not been found in mature blueberry fields, the possibility of the fungus spreading from nearby infested peanut fields is great. In a recent report by Rowe, et al. (4), *C. crotalariae* has been found in all 12 major peanut-growing counties in eastern North Carolina. Since a large portion of the blueberry acreage in North Carolina is located in several of these counties, the disease could eventually become a serious threat to the blueberry industry.

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