Factors Affecting Sporulation and Infection by the Blueberry Stem Canker Fungus, Botryosphaeria corticis

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Journal Series Paper No. 3508 of the North Carolina State University Agricultural Experiment Station, Raleigh.

Accepted for publication 10 August 1971.

ABSTRACT

Temperature affects both the number and type of stem canker lesions produced by *Botryosphaeria corticis* on blueberry. The cultivar Wolcott was susceptible to *B. corticis* at 27 C, but showed a resistant reaction at 16 and 21 C. The resistance of the cultivar Bluecrop was not affected by temperature. The optimum temperature for fungal growth, sporulation, and spore germination was 27 C. No growth occurred at 10 C. Cultures of four races of *B. corticis* failed to sporulate when maintained in the dark. Light intensities ranging from 60 to 960 ft-c induced fertile pycnidia. Sporulation was optimum for all races when cultures were grown under 480 ft-c of light. A minimum of 6 days of continuous light at 480 ft-c was necessary to induce sporulation. Cultures grown for 8 days under light and 6 days in the dark produced more spores than those grown for 14 days under continuous light. A single 24-hr exposure to light during different phases of growth was not sufficient to produce fertile pycnidia.

Phytopathology 62:137-139.

Additional key words: canker resistant, pycnidia production.

Botryosphaeria corticis (Demaree & Wilcox) Arx & Muller causes a serious stem canker disease of highbush blueberry (*Vaccinium corymbosum* L.) in North Carolina (2, 3, 6). Demaree & Morrow (1) found that symptoms of stem canker varied with the susceptibility of the cultivar. Large swollen cankers with deep fissures and cracks develop on very susceptible cultivars. Cankers are restricted in size or absent on the more resistant cultivars. Resistance of the blueberry plant to the stem canker fungus is related to fungal development after infection rather than to the establishment of infection (4). Taylor (6) studied the cultural requirements of the fungus and the development of the disease under field and greenhouse conditions.

With the recent demonstration that there are at least six pathogenic races of *B. corticis* infecting highbush blueberry in North Carolina (5), tests were initiated to evaluate blueberry seedlings for resistance. The inoculation of blueberry seedlings in the greenhouse requires the production of large amounts of inoculum. Because most of the races produce very few spores in culture, the present studies were made (i) to determine the influence of temperature and light on growth and sporulation of *B. corticis* in culture and to manipulate these factors for maximum spore production; and (ii) to determine the effects of temperature on canker development on highbush blueberry.

MATERIALS AND METHODS.—Canker-free plants of the cultivars Wolcott and Bluecrop were inoculated with races SC-2, SC-3, and SC-4 of *B. corticis* (5). Inoculum concentration was standardized to 3×10^4 conidia/ml of water, and 2 ml was applied to each stem of rapidly growing blueberry plants with an air compressor sprayer. Twelve single-stem, greenhouse-grown plants of each cultivar were inoculated with each race. Four plants of each cultivar were sprayed with distilled water as controls. Plants were placed in a moist chamber at 25 to 30 C for 72 hr, then maintained in constant-temperature chambers at 16, 21, and 27 C plus one chamber which was maintained at 16 and 27 C, respectively, on a 12-hr cycle. Each race was tested on a three-plant replicate plus the controls at each of the four temperatures. Light intensity was maintained at 3,800 ft-c for 16 hr daily, using cool-white 40-w fluorescent lamps and 25-w incandescent bulbs. Plants were observed for stem canker after 4 months, and disease development was rated on a scale of 0 to 5 (5). The five disease reaction types ranging from highly resistant to highly susceptible were characterized as follows: type 1 = small red flecks; type 2 = small, slightly raised lesions with little or no discoloration (0.5 to 1 mm in diam); type 3 = mixture of red flecks and small slightly raised lesions as well as large swollen lesions (2 to 4 mm in diam); type 4 = large broadly conical swellings often surrounded by a green border; type 5 = large, swollen, broadly conical lesions surrounded by abundant red discoloration.

Growth rate, pycnidium and spore production, and spore germination for each of 4 races of *B. corticis* were compared at 10, 16, 21, 27, and 32 C. Three cultures of each race were grown on oatmeal agar (OMA) under 480 ft-c of light. Pycnidium and spore counts were made after 14 days. Spores were harvested by scraping the surface of the culture with a razor blade, then flooding the plate with 20 ml of water. The suspension was screened through cheesecloth, and spores were counted with a hemacytometer. Conidia used in the germination test were collected by flooding the surface of 2-week-old cultures grown on OMA with sterile distilled water. Conidia were washed 3 times in sterile glass-distilled water by centrifugation. A drop of the conidial suspension was placed on acid-washed slides, placed in moist petri dishes and incubated at 10, 16, 21, 27,

and 32 C. Per cent germination and germ tube length was recorded after 4 hr, counting 100 conidia/treatment. Each treatment was replicated 3 times.

Light intensity was varied by increasing the distance between culture and light source. Five-mm discs of races 1, 2, 3, and 4 of *B. corticis* were transferred to OMA and irradiated with fluorescent light for 14 days at intensities of 60, 120, 240, 480, and 960 ft-c at 27 C. Dark-grown cultures were placed in lightproof containers and held at the same temperature. The number of mature pycnidia per cm^2 of colony surface was counted in three random areas of each culture. Four replicated plates were used per treatment.

Tests were also made to measure the effect of varying periods of light on spore production. Immediately upon transfer of the fungus, cultures were placed at 27 C under 480 ft-c of light, then placed in the dark after 2, 4, 6, 8, 10, and 12 days' exposure to light. Controls consisted of cultures grown continuously in the dark and at 480 ft-c of light for 14 days. Each treatment was replicated 3 times. Spores were collected after 14 days and counted with a hemacytometer. Analysis of variance and multiple range tests were utilized to determine significant differences (5% level) between means.

RESULTS.—*Effect of temperature on canker development.*—Small, dark lesions were observed on inoculated stems of the susceptible (Wolcott) and resistant (Bluecrop) cultivars 7 days after inoculation at 21 and 27 C and after 14 days at 16 C. None of the plants inoculated was immune to infection. The number of lesions formed on the two cultivars 4 weeks after inoculation was consistently lower for all races at 16 C than at the other temperatures tested (Table 1). Canker development on the resistant cultivar Bluecrop was not affected by temperature; i.e., numerous small red lesions developed but failed to enlarge. However, the susceptible cultivar Wolcott showed a resistant reaction at 16 and 21 C, but a susceptible reaction at both 27 C and when temperature fluctuated between 16 and 27 C (Table 1). No canker infection occurred on the control plants.

Effect of temperature on growth and sporulation.—According to Taylor (6), the optimum temperature for growth of *B. corticis* on potato-dextrose agar was near 28 C, the minimum around 12 C, and the maximum was between 32 and 36 C. In the present studies, the relative growth rate of the four races on OMA after 96 hr was greatest at 27 C and the least at 16 C. No growth occurred at 10 C. While cultures of all four races covered the petri dish (85 mm) after 7 days at 21 and 27 C, the average colony diameter at 32 C was 40 mm.

The optimum temperature for sporulation and pycnidial formation was 27 C; however, considerable variation in sporulation existed between races at the same temperature. Race 1 produced the greatest number of spores at 27 C, ca. 8 times the amount produced by race 3. However, the reverse occurred at 32 C.

Spore germination tests.—Because conidia of *B. corticis* usually germinate within 2 hr in distilled water with rapid germ tube growth (4, 6), per cent germination and length of germ tubes were recorded after 4 hr. Conidia germinated within 4 hr at all temperatures tested except 10 C. Spore germination was ca. 90% for all races at 27 C after 4 hr. While very little difference in per cent spore germination was observed between races at 21, 27, and 32 C, germ tube length was greatest at 27 C. Conidia maintained at 27 C for 4 hr had an average germ tube length of 154 μ in contrast to 48 μ and 92 μ for 21 and 32 C, respectively. Germ tubes from conidia had grown and intermingled so much after 24 hr that accurate germination and germ tube measurements could not be made.

Effect of light intensities on formation of pycnidia and spores.—Continuous fluorescent light for 14 days resulted in fertile pycnidia at all intensities tested. There was a gradual increase in pycnidial production from 60 to 240 ft-c, with the greatest number produced at 480 ft-c. Sporulation was significantly greater for most races tested when cultures were grown under 480 ft-c of light. A significant decrease in sporulation was observed when the light intensity was increased to 960 ft-c. No pycnidia or spore production occurred when cultures were maintained in the dark. Cultures exposed to light were pale greenish yellow, and those grown in the dark were pale to dark gray.

Light duration tests.—A minimum of 6 days of

TABLE 1. Effect of temperature upon canker development on Wolcott and Bluecrop cultivars of blueberry inoculated with three races of *Botryosphaeria corticis*

		Mean no. canker lesions per plant after 28 days				Mean canker susceptibility rating[a]			
Cultivar	Race	16 C	21 C	27 C	16-27 C	16 C	21 C	27 C	16-27 C
Wolcott	SC-2	5	80	179	32	1	3	4	4
	SC-3	2	16	28	8	1	3	4	4
	SC-4	12	15	20	15	1	2	4	4
Bluecrop	SC-2	28	53	36	52	1	1	1	1
	SC-3	6	25	20	14	1	1	1	1
	SC-4	8	30	20	16	1	1	1	1

[a] Plants rated 1 and 2 were classed as canker-resistant; 3, as intermediate; and those rated 4 and 5, as canker-susceptible. Disease ratings were made after 4 months.

continuous light was necessary to induce sporulation in all cultures. Sporulation was greatest for all races when cultures were exposed to 8 days of light followed by 6 days of darkness. None of the races produced spores in the nonirradiated cultures. The pycnidia produced in cultures grown for 8 days under light and 6 days in the dark were more numerous, but smaller than those in cultures grown under continuous light for 14 days. There was considerable variation in sporulation among the four races for a given exposure period.

Light effect was also tested by placing cultures at 27 C in the dark immediately upon transfer of the fungus, followed by exposure to 480 ft-c of light after 2, 4, 6, 8, 10, and 12 days in the dark. No pycnidia or spores were produced in cultures maintained in the dark for a period longer than 8 days. Cultures grown in the dark for 8 days, then placed under light for 6 days, yielded ca. 1×10^5 spores/culture, whereas cultures grown for 6 days in the dark, then transferred to 480 ft-c of light, yielded 3 times as many spores. There was a decrease in sporulation for all cultures as the number of days in the dark increased.

Cultures of *B. corticis* were also grown continuously in the dark at 27 C except for a single exposure to 480 ft-c of light for one 24-hr period at the 2nd, 4th, 6th, 8th, 10th, and 12th day after transfer. These studies were conducted to determine if a single 24-hr exposure to light during different periods of growth in culture would stimulate pycnidial production. No pycnidia or spores were produced in any of the cultures.

DISCUSSION.–Although temperature affects both the number and type of lesions produced by *B. corticis* on blueberry stems, the effect of temperature appears to be primarily on the pathogen rather than on the host. The temperature curve for spore germination and for growth of *B. corticis* in culture corresponds closely with that for stem canker development on blueberry stems. The optimum temperature for both growth in culture and canker development on the susceptible host was 27 C. Progressively lower temperatures restrict both fungal growth and canker development. Host reaction is expressed in increased resistance at progressively lower temperatures; e.g., at 21 C, both large and small lesions are produced; at 16 C, the disease reaction is limited to small red flecks. Alternating moderately low (16 C) and relatively high (27 C) temperatures did not appear to be a critical factor in stem canker development, whereas a constant low temperature appeared to be a significant factor in restricting development of the disease in susceptible plants. The resistance of Bluecrop was unaffected by temperature.

In general, these data substantiate the observations of Taylor (6) that the fungus would grow readily on a wide variety of media, with pycnidia production being more abundant on oatmeal agar. In these studies, the optimum temperature for growth of all races of *B. corticis* was 27 C, with some growth occurring at 16 and 32 C. Conidia were found in irradiated cultures after 14 days at 21, 27, and 32 C, but none was present at 16 C.

Although Taylor (6) reported that light is an essential factor in the sporulation of *B. corticis*, effects of intensity or duration of exposure were unknown. It is now apparent that the quantity of spores produced in culture is dependent not only on light intensity but also on duration of exposure. Sporulation increased progressively as light intensity was increased up to 480 ft-c, but decreased at 960 ft-c.

Six days of continuous light at 480 ft-c was necessary to induce sporulation for all races. Whether or not a longer period of time would be required under lower light intensities is not known. Cultures grown for 8 days under light and 6 days in the dark consistently produced the greatest number of spores. Exposure to light during the early phase of growth does not appear to be a critical factor in spore production, as cultures grown in the dark immediately after transfer for 8 days and then placed under light sporulated in culture. However, spore production was not so great as those grown for 8 days under light and then placed in the dark. A single 24-hr exposure to light did not initiate pycnidial production and sporulation.

Knowledge of the effects of different environmental conditions on disease development is essential in the evaluation of breeding material for canker resistance. Evaluation of blueberry plants for canker resistance in the greenhouse shows promise for screening blueberry seedling progenies for resistance prior to transplanting them to the field. This technique eliminates handling large numbers of seedlings in the field, reduces the time necessary for evaluating canker resistance, and facilitates testing for resistance against all known races of *B. corticis*.

LITERATURE CITED

1. DEMAREE, J. B., & E. B. MORROW. 1951. Relative resistance of some blueberry varieties and selections to stem canker in North Carolina. Plant Dis. Reptr. 35:136-141.
2. DEMAREE, J. B., & MARGUERITE S. WILCOX. 1942. Blueberry cane canker. Phytopathology 32:1068-1075.
3. DEMAREE, J. B., & MARGUERITE S. WILCOX. 1947. Fungi pathogenic to blueberries in the eastern United States. Phytopathology 37:487-506.
4. MILHOLLAND, R. D. 1970. Histology of Botryosphaeria canker of susceptible and resistant highbush blueberries. Phytopathology 60:70-74.
5. MILHOLLAND, R. D., & G. J. GALLETTA. 1969. Pathogenic variation among isolates of Botryosphaeria corticis on blueberry. Phytopathology 59:1540-1543.
6. TAYLOR, JACK. 1958. Stem canker and related blueberry diseases. North Carolina Agr. Exp. Sta. Tech. Bull. 132. 24 p.