Infection of Blueberry Fruit by *Colletotrichum gloeosporioides*

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ABSTRACT

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Blueberry fruit were susceptible to infection by *Colletotrichum gloeosporioides* at all stages of development but did not show symptoms until ripening. The infection process, spore germination, appressorium formation, and penetration occurred within 1 wk of inoculation of green fruit, but the fungus did not develop further until the fruit ripened. In a survey of anthracnose fruit rot on ripe fruit of eight blueberry cultivars, Powderblue and Morrow were the most resistant, whereas Jersey, Harrison, and Blueray were the most susceptible.

Anthracnose fruit rot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is an important disease of highbush (*Vaccinium corymbosum* L.) and rabbiteye (*V. ashei* Reade) blueberry (5,7,10). The primary symptom of this disease is rotting of ripe fruit both in the field at harvest and later during storage. Distinctive masses of salmon-colored conidia are usually present on surfaces of rotted fruit. In North Carolina, losses from preharvest rots are estimated at 3% annually; however, losses during storage can be much higher.

A limited amount of work has been done on the epidemiology of anthracnose fruit rot. In Michigan, conidia of *C. gloeosporioides* were released from diseased bushes from May to August while flowers and developing fruit were present (5). Field inoculations of green fruit with conidial suspensions of *C. gloeosporioides* resulted in postharvest fruit rots. *C. gloeosporioides* has been isolated readily from dead blueberry twigs during the winter (4,5,10). Dead twigs, therefore, are considered important sources of primary inoculum.

The purpose of this study was to determine the periods of infection by *C. gloeosporioides* on blueberry in North Carolina and to examine the infection process on fruit at different developmental stages. Cultivar susceptibility to *C. gloeosporioides* was also evaluated.

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MATERIALS AND METHODS

Infection periods. The periods when fruit became infected in the field were determined by isolations from naturally inoculated green fruit. In 1980, collections of 500 fruits from cultivar Harrison were made weekly at the Horticultural Crops Research Station, Castle Hayne, NC, from 22 April (when small green fruit were present) until 3 June (when the fruit were ripe). After each collection, fruit were surface-disinfested in 0.53% sodium hypochlorite for 2.5 min, rinsed in sterile distilled water, and incubated in sterile moist chambers at 25 C. Observations on the numbers of fruit sporulating with *C. gloeosporioides* were made at 3-day intervals for 3 wk.

In 1982, 250 fruits from cultivar Harrison were collected weekly from 13 April (green fruit) until 8 June (ripe fruit). The berries were surface-disinfested, halved, and plated on water agar plus 100 μg/ml of vancomycin hydrochloride. The berry halves were observed periodically during the next 5 wk for sporulation by *C. gloeosporioides*.

Infection process. Field inoculations were conducted at Castle Hayne in 1983 on 5-yr-old plants of the highbush cultivars Wolcott and Croatan. Inoculations were made on 27 April, 17 May, and 31 May 1983 at the green fruit, pink fruit, and blue fruit stages, respectively. Fruit clusters were inoculated by atomizing with a suspension of 6×10^6 conidia per milliliter of *C. gloeosporioides*. Sprays of sterile distilled water served as controls. Treated clusters were wrapped in two layers of wet cheesecloth and covered with plastic bags. Paper bags were tied over the cheesecloth and plastic bags to reduce heat accumulation. The cheesecloth and plastic bags were removed from the clusters after 3 days and replaced with paper bags alone until the fruit were collected. Fruit from one cluster per treatment (about 10 berries per cluster) were collected and 3-mm-thick tangential sections fixed in formalin-propionopropanol (FPP) at 2 days and 1 wk, then once every 2 wk after inoculation until the fruit were ripe. The tissue was dehydrated in an isopropyl alcohol series and embedded in Paraplast+ (Sherwood Medical Industries, St. Louis, MO). Sections 8–12 μm thick were cut on a rotary microtome and stained with Triarch's quadruple stain (Triarch, Inc., Ripon, WI). Epidermal peels of fresh tissue also were cleared and stained in chloral hydrate and aniline blue and mounted in lactophenol (6) to observe spore germination and appressorium formation.

Cultivar susceptibility. In 1981 and 1983, surveys were made of anthracnose fruit rot on ripe berries of the highbush cultivars Blueray, Bluechip, Croatan, Harrison, Jersey, Morrow, and Murphy and the rabbiteye cultivar Powderblue. About 700 ripe berries were harvested three times during a 2-wk period in 1981 from four bushes of each cultivar. Green fruit on these bushes had been sprayed with a suspension of *C. gloeosporioides* conidia ($>1 \times 10^6$ conidia per milliliter) on 23 and 28 April and 5, 12, and 19 May to increase the probability of infection. In 1983, about 400 ripe berries were harvested three times during a 2-wk period from four bushes of each cultivar. The bushes used in the 1983 survey were in a different section of the research station from those used in the 1981 survey. After picking, the berries were stored at room temperature in plastic

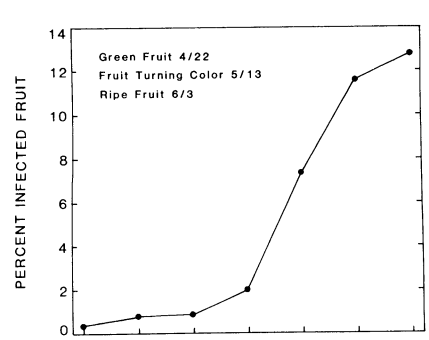

Fig. 1. Percentage of cultivar Harrison blueberries naturally infected with *Colletotrichum gloeosporioides* in a field at the Horticultural Crops Research Station, Castle Hayne, NC, in 1980. Percentages are based on collections of 500 fruits.

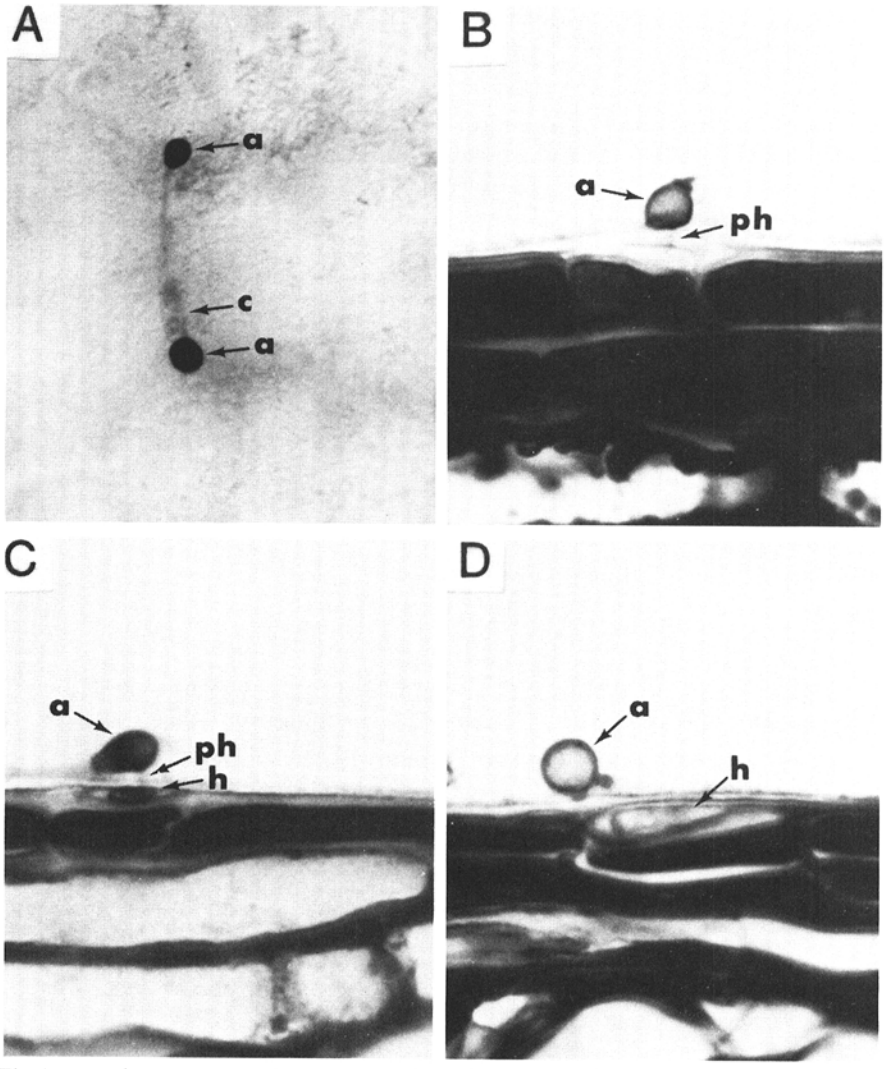

Fig. 2. Stages in penetration of blueberry fruit by *Colletotrichum gloeosporioides*. **(A)** Germinated conidium (c) and appressoria (a) on surface of fruit 1 wk after inoculation (×670). **(B)** Penetration hyphae (ph) in cuticle below appressorium (a) (×960). **(C)** Appressorium (a) and large infection hypha (h) developing from penetration hypha (ph) in ripe fruit (×960). **(D)** Appressorium (a) with large infection hypha (h) between cuticle and epidermal cell wall of ripe fruit (×960).

buckets enclosed in plastic bags for 6–7 days, sorted, and the berries with anthracnose fruit rot counted.

RESULTS AND DISCUSSION

Infection periods. In 1980, 0.4% of the fruit were infected with *C. gloeosporioides* by 22 April, 1 wk after full bloom (Fig. 1). The percentage of infected fruit increased to 12.8% on 3 June. The greatest increase in incidence occurred between 13 May (2.0%) and 27 May (11.6%).

In 1982, sporulation on detached fruit was not detected until 28 April (green fruit), when 1.2% of the fruit were infected. Incidence fluctuated between 0.8 and 2.8% until 1 June, then rose to 8% on 8 June.

Fruit were susceptible to infection at all stages of development. This agrees with previous results (5) that showed that *C. gloeosporioides* causes latent infections on green blueberry fruit and that postharvest decay is often the result of early-season infection. Losses from blueberry fruit rots can be reduced by fungicidal sprays (R. D. Milholland, *unpublished*). On the basis of our studies, control of anthracnose fruit rot is best achieved with applications of an effective fungicide, such as captafol, beginning at petal fall and repeated throughout the period of fruit development.

Infection process. Conidia of *C. gloeosporioides* germinated and produced one or two dark appressoria that were either sessile or borne at the ends of short germ tubes 2 days after inoculation (Fig. 2A). Penetration hyphae were observed in the cuticle 1 wk after inoculation (Fig. 2B). Penetration hyphae developed from 3 and 11% of the 200 appressoria observed on green fruit of cultivars Wolcott and Croatan, respectively. No further growth by the fungus was seen in green and pink fruit of either cultivar. There also was no reaction in the epidermal layer to the penetration hyphae. When fruit ripened, fungal growth resumed and large infection hyphae were observed between the cuticle and outer epidermal cell wall (Fig. 2C). These hyphae usually collapsed the cell wall as they enlarged (Fig. 2D). Additional hyphae subsequently invaded the remainder of the fruit, resulting in the fruit rot symptom.

Histological studies of field-inoculated fruit support the existence of extended latency. Conidia of *C. gloeosporioides* germinated, produced appressoria, and in some cases penetrated green fruit by 1 wk after inoculation. A greater percentage of penetration hyphae may have been present but were not observed because of the thinness of the cuticle and the fineness of the penetration hyphae. Fungal activity ceased until the fruit were ripe.

Many of the histological studies with *C. gloeosporioides* on other fruit have used detached fruit and workers have reported that the fungus survives latency as subcuticular hyphae (1,8). These conclusions may be inaccurate, however, because *C. gloeosporioides* is able to colonize and sporulate on green fruit once it is removed from the plant (3,9). In histological studies with field-inoculated attached fruit of mango and avocado, *C. gloeosporioides* survived the latent period as dormant appressoria (2,3). Our results, however, are in contrast because the fungus also persisted as germinated appressoria and penetration hyphae.

Cultivar susceptibility. The eight cultivars surveyed differed somewhat in susceptibility to anthracnose fruit rot (Table 1). The rabbiteye cultivar Powder Blue and the highbush cultivar Morrow consistently had low incidence of anthracnose fruit rot (5.2% or less), whereas cultivars Jersey and Harrison consistently had higher incidence of anthracnose fruit rot (12.1% or more).

Table 1. Incidence of anthracnose fruit rot on eight blueberry cultivars

Cultivar	Berries with anthracnose fruit rot (%)[a]	
	1981[b,c,d]	1983[d,e]
Powderblue	0.8	1.1
Morrow	5.2	1.3
Croatan	8.1	1.8
Bluechip	3.9	10.7
Murphy	10.7	5.6
Blueray	13.0	8.6
Harrison	12.8	12.1
Jersey	17.7	34.1

[a] Berries were evaluated for anthracnose fruit rot 6–7 days after harvest.
[b] Green fruit were inoculated with a conidial suspension of *Colletotrichum gloeosporioides* ($>1 \times 10^6$/ml) on 23 and 28 April and 5, 12, and 19 May.
[c] About 700 ripe fruits were harvested three times during a 2-wk period from four bushes of each cultivar.
[d] Berries were stored in plastic buckets enclosed in plastic bags at room temperature for 6 or 7 days before rot evaluation.
[e] About 400 ripe fruits were harvested three times during a 2-wk period from four bushes of each cultivar.

Cultivars Croatan, Bluechip, Murphy, and Blueray were intermediate in susceptibility. The resistance found in cultivars such as Powderblue and Morrow should be used in future breeding programs. More refined techniques should first be developed, however, to distinguish between different levels of susceptibility.

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