Histopathology of Ripe Rot Caused by *Colletotrichum gloeosporioides* on Muscadine Grape

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ABSTRACT

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In a histological study of ripe rot on attached fruit of muscadine grape (*Vitis rotundifolia*), the susceptible cultivar, Carlos, and the resistant cultivar, Pride, responded similarly to the early stages of infection by *Colletotrichum gloeosporioides*. Conidia germinated and produced appressoria, and hyphae penetrated the cuticle within 1 wk after inoculation on green or ripening fruit. Further fungal growth ceased until the fruit ripened. Plant responses to the penetration hyphae varied from a slightly darker staining reaction in protoplasm adjacent to the hyphae, to a necrosis of the epidermal cells below the hyphae. The necrotic reaction in Carlos often was accompanied by hyperplasia in the subepidermal cells. When Carlos fruit ripened, *C. gloeosporioides* colonized the pericarp inter- and intracellularly and produced acervuli, but fungal growth was not resumed in ripe fruit of the resistant cultivar, Pride.

Additional key words: latent infection.

Ripe rot, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., is a serious disease of muscadine grape (*Vitis rotundifolia* Michx.) in North Carolina (5). Although fruit rot symptoms do not appear until harvest, field studies indicate that infections occur much earlier in the season and remain latent until ripening (7).

Histological studies of infection by *C. gloeosporioides* on other fruit crops reveal that conidia germinate and produce dark appressoria within 24 hr of inoculation on green fruit (3,4,14). Appressoria either remain dormant, giving rise to penetration hyphae and infection hyphae only at ripening (2,6), or germinate immediately, penetrate, and produce a few latent subcuticular hyphae (1,3,12). When fruit are ripe, hyphae colonize the pericarp inter- and intracellularly, and acervuli are formed on the fruit surface.

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A survey of muscadine grapes at the Horticultural Crops Research Station, Castle Hayne, NC, in 1981 showed that the incidence of ripe rot on the bronze-fruited cultivars (Carlos, Fry, Magnolia, and Scuppernong) ranged from 6.7 to 33.5%, while symptoms of ripe rot were never observed on the black-fruited cultivars, which included Noble, Tarheel, and Pride (M. E. Daykin, *unpublished*).

The purpose of this study was to examine the infection process of *C. gloeosporioides* on muscadine grape and to compare histologically the reactions of a susceptible and a resistant cultivar.

MATERIALS AND METHODS

Field inoculations were conducted at the Horticultural Crops Research Station, Castle Hayne, NC, on 11- to 12-yr-old vines of muscadine grape. Fruit clusters of the susceptible cultivar, Carlos, were inoculated with a suspension of 10^6 conidia of *C. gloeosporioides* per milliliter on 29 June, 27 July, 24 August, and 22 September 1982 at the small green fruit, large green fruit, fruit turning color, and ripe fruit stages of development, respectively. The isolate of *C. gloeosporioides* used was obtained from an

infected grape mummy. Inoculum was grown on potato-dextrose agar for 6 days at 25 C and a conidial suspension was prepared by flooding the plates with sterile distilled water, scraping the surface of the culture, and filtering the suspension through two layers of cheesecloth. The suspension was atomized onto the fruit surfaces and the clusters were immediately covered with two layers of wet cheesecloth and a plastic bag. The cheesecloth and plastic bags were removed after 3 days and replaced with paper bags to prevent the occurrence of natural infections. Additional fruit clusters sprayed with sterile distilled water and bagged on the same inoculation dates served as controls. In 1983, inoculations were made again on 26 July, 9 and 30 August, and 20 September on Carlos and also on a highly resistant cultivar, Pride, with an inoculum suspension containing 6×10^6 conidia of *C. gloeosporioides* per milliliter.

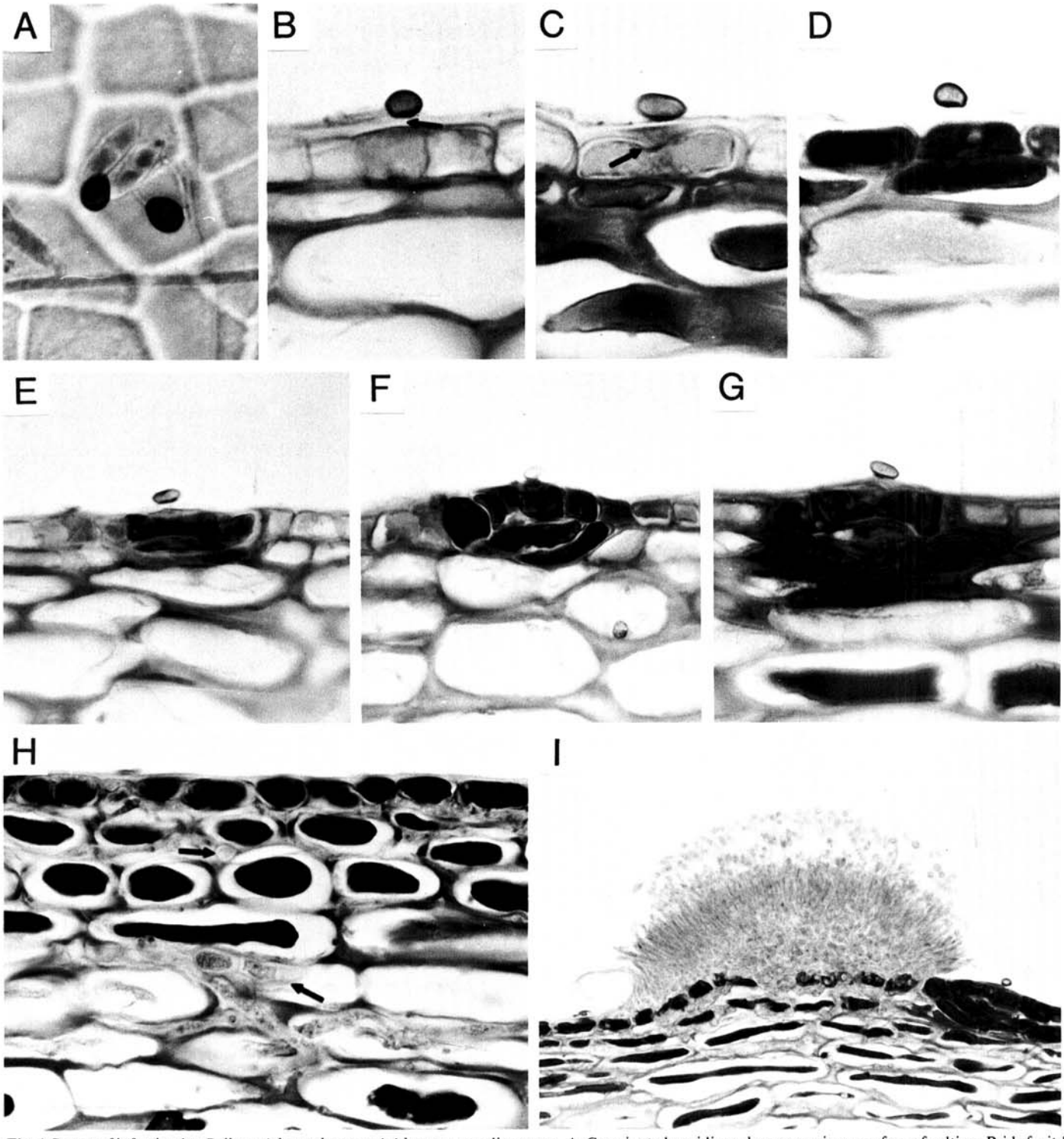

Fig. 1. Stages of infection by *Colletotrichum gloeosporioides* on muscadine grape. **A,** Germinated conidia and appressoria on surface of cultivar Pride fruit 1 wk after inoculation (×1,025). **B,** Appressorium on fruit of cultivar Carlos 5 wk after inoculation. Although a penetration hypha is present (arrow), there is no necrosis in the tissue (×1,025). **C,** Papilla (arrow) formed beneath an appressorium on Carlos 1 wk after inoculation (×1,025). **D,** Necrosis in cells beneath penetration hypha on Pride 3 wk after inoculation of green fruit. The necrotic cells are stained darkly with safranin (×1,025). **E, F, and G,** Proliferation and necrosis of epidermal and subepidermal cells of Carlos at 5, 7, and 11 wk after inoculation of green fruit. The cellular contents are collapsed and stained darkly with safranin. (×715). **H,** Intercellular hyphae (arrows) in pericarp of ripe Carlos fruit (×500). **I,** Mature acervulus on ripe Carlos fruit. Appressorium and necrotic cells are also present (×310).

Samples of fruit from one cluster (five to 10 grapes per cluster) were fixed in formalin-propiono-propanol at 3 days, 1 wk, and then every 2 wk after inoculation until the fruit were ripe. The tissue was dehydrated in an isopropyl alcohol series, embedded in Paraplast+ (Sherwood Medical Industries, St. Louis, MO 63100), and sectioned on a rotary microtome at 8–12 μm. Sections were stained with Triarch's Quadruple Stain (Triarch Inc., Ripon, WI 54971) and thionin and orange G (11). Phloroglucinol and Sudan IV were used to test for lignin and suberin, respectively, and resorcin blue and aniline blue fluorescence were used to test for callose (8,10). Epidermal peels of fresh fruit were cleared in chloral hydrate, stained with aniline blue, and mounted in lactophenol to observe spore germination and appressorium formation (9).

RESULTS

Results of the 1982 and 1983 inoculations on Carlos were combined as the cultivar responded the same in both years. Conidia germinated on Carlos and Pride, each conidium producing one or two dark appressoria 5–8 μm in diameter, within 3 days after inoculation. The appressoria were borne either sessile or on the ends of germ tubes (Fig. 1A). In Carlos, fine penetration hyphae that pierced the cuticle were produced from appressoria during this time. Similar penetration hyphae also were observed in the cuticle of Pride in tissue collected 1 wk after inoculation. In 1983, penetration hyphae developed from 36 and 55% of 200 appressoria observed on green fruit of Carlos and Pride, respectively. Further growth of the fungus did not occur until the fruit was ripe. However, in both cultivars, varying amounts of discoloration were usually visible in the epidermal cells beneath the penetration hyphae. In some responses, the protoplasm near the epidermal wall under the penetration hypha stained slightly darker with safranin (Fig. 1B). Sometimes the epidermal cell wall adjacent to the penetration hypha was thickened and formed a papilla (Fig. 1C). In other responses, one or several cells adjacent to the penetration hyphae were necrotic (Fig. 1D to G). When epidermal cells of Pride showed a necrotic reaction, there were no accompanying cell divisions (Fig. 1D). However, on Carlos, the necrotic cells often were hypertrophied and several periclinal divisions occurred in the layers below the epidermis (Fig. 1E to G). Appressoria associated with this reaction tended to be lighter in color and often were collapsed. Grapes inoculated both as green and ripening fruit showed these responses which ranged from a slightly darker staining reaction in protoplasm near the penetration hyphae to a cellular necrosis; it was more difficult to observe in fruit that were beginning to color, as the entire epidermal layer stained darker. Histochemical tests on the papillae and necrotic epidermal cells were negative for callose and lignin. However, some suberization was present in the proliferated epidermal cells of Carlos.

Regardless of fruit development at the time of inoculation, large hyphae colonized the pericarp inter- and intracellularly only on ripe fruit of the susceptible cultivar, Carlos (Fig. 1H). This fungal growth resulted in a collapse and necrosis of the cells. Mature acervuli were then produced from stromatic tissue that had formed between the cuticle and epidermis (Fig. 1I) and symptoms of ripe rot developed. In contrast, fungal growth beyond the penetration hyphae was never observed in ripe fruit of the resistant cultivar Pride and ripe rot was never observed on any of the inoculated fruit.

DISCUSSION

C. gloeosporioides survives the latent period in unripe muscadine grapes as penetration hyphae in the cuticle. In many cases, the appearance of these fine hyphae was probably obscured by the thickness of the sections. However, their presence could be inferred by the similar types of reactions occurring in the epidermal cells beneath appressoria with and without visible penetration hyphae.

This form of latency for *C. gloeosporioides* is unlike that occurring on avocados (2) and mangoes (6) in which the dormant structure reportedly consists of an ungerminated appressorium, but is similar to that observed in infections on blueberries (M. E. Daykin, *unpublished*). Several workers have reported that *C. gloeosporioides* survives as subcuticular hyphae in oranges (1) and papaya (12). However, their conclusions were based on inoculations of detached fruit, which respond differently to infection than attached fruit (13). The cellular proliferation that occasionally occurred beneath appressoria on Carlos grapes also was reported by Stanghellini and Aragaki (13) in inoculations of attached unwounded papaya, except that callose deposits were not observed in our studies.

Carlos and Pride differ greatly in susceptibility to ripe rot, but their initial responses to infection by *C. gloeosporioides* were similar. The only difference in their initial responses was that the necrotic reaction in Carlos was sometimes accompanied by additional cell divisions. Although fruit of Pride never showed symptoms, reisolations of *C. gloeosporioides* from surface-sterilized ripe fruit that had been inoculated while green showed that the fungus was still viable. The resistance mechanism in Pride, therefore, must operate to prevent inter- and intracellular colonization from occurring. Additional physiological studies are needed to determine the factors inducing hyphal growth and colonization in ripe Carlos fruit and preventing its development in Pride.

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