

A Histological Study of Anthracnose on *Carica papaya*

K. F. Chau and A. M. Alvarez

Graduate student and associate professor, respectively, Department of Plant Pathology, University of Hawaii, Honolulu 96822. Journal Series Paper 2681 of the Hawaii Agricultural Experiment Station.

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ABSTRACT

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The infection process of *Colletotrichum gloeosporioides* on papaya (*Carica papaya*) fruit was examined via light microscopy and transmission and scanning electron microscopy. Penetration of the cuticle by an infection peg, which was produced by an appressorium, was observed 3-4 days after inoculation. A hypha then formed between the cuticle and the epidermal

wall. Mycelium grew intra- and intercellularly, and infected cells eventually separated, collapsed, and formed a sunken lesion that became visible about 5 days after inoculation. The cuticle of the fruit ruptured during the sporulation process.

Additional key words: anthracnose.

Colletotrichum gloeosporioides Penz. causes anthracnose of several fruit crops both in orchards and during storage (1). In histological studies of the pathogenesis of this fungus on avocado and orange, two penetration patterns have been observed: occasionally, hyphae growing from germinated spores penetrate host epidermis directly (6); and more frequently, the germ tubes from spores form appressoria with infection pegs that penetrate the host's epidermal wall (3,4-6,13). However, in studies (14) of anthracnose on papaya, these penetration patterns were not observed.

The objective of this study was to provide information on the penetration and establishment of infection of *C. gloeosporioides* in detached papaya fruit using both light and electron microscopy.

MATERIALS AND METHODS

Mature green papaya fruit of cultivar Kapoho Solo were surface disinfested with hot water (48 C, 20 min) (2), cooled, and inoculated with spore suspensions from 7- to 10-day-old cultures of *C. gloeosporioides* (strain C-747). Five microliters of a suspension containing $2-3 \times 10^3$ spores was placed within a circled area (1.0 cm in diameter) on the fruit surface. Inoculated fruits were incubated at room temperature (~24 C) in moist chambers. Samples were excised from the inoculated areas at 18, 24, 30, 48, 96, and 120 hr

after incubation.

Two methods were used for examining infection: either a thin (<2-3 mm) tangential layer was cut (freehand with a razor blade) from the fruit surface, stained with a drop of 2% gentian violet in 50% EtOH for 30 sec, rinsed with water, and examined via transmission light microscopy (LM) at $\times 400$; or samples were fixed with Navashin's fixative (9), dehydrated with an ethanol and *t*-butyl alcohol series, embedded in paraffin, sectioned at 8-10 μ m, and stained with Flemming's triple stain (9).

For transmission electron microscopy (TEM), samples about $1 \times 3 \times 1$ mm were excised 3-4 days after inoculation when (as determined by LM) infection pegs had formed. Samples were fixed for 3 hr with 3% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 6.8-7.0, and postfixed with 2% OsO₄ in phosphate buffer for 3 hr. Samples were stained for 30 min with 2% uranyl acetate in distilled water, dehydrated with acetone, and embedded in Spurr's plastic (formula for medium hardness). Samples were sectioned with a glass knife mounted in a Reichert OM-U2 or a Sorvall Porter-Blum ultramicrotome. The sections were mounted on copper grids, stained for 8-10 min with a modified Sato's staining solution (2.0 g of lead citrate and no lead acetate) (8) and examined via a Hitachi EM HS-8 electron microscope operating at 50 kV.

For scanning electron microscopy (SEM) studies, samples ~5 \times 5 \times 2 mm were taken from 1 to 10 days after inoculation, when sunken lesions typical of anthracnose had formed. Samples were fixed and rinsed with buffer and water as described for TEM studies; samples were then critical-point dried for surface studies or frozen in a dry ice (CO₂)-acetone mixture, fractured with a cold

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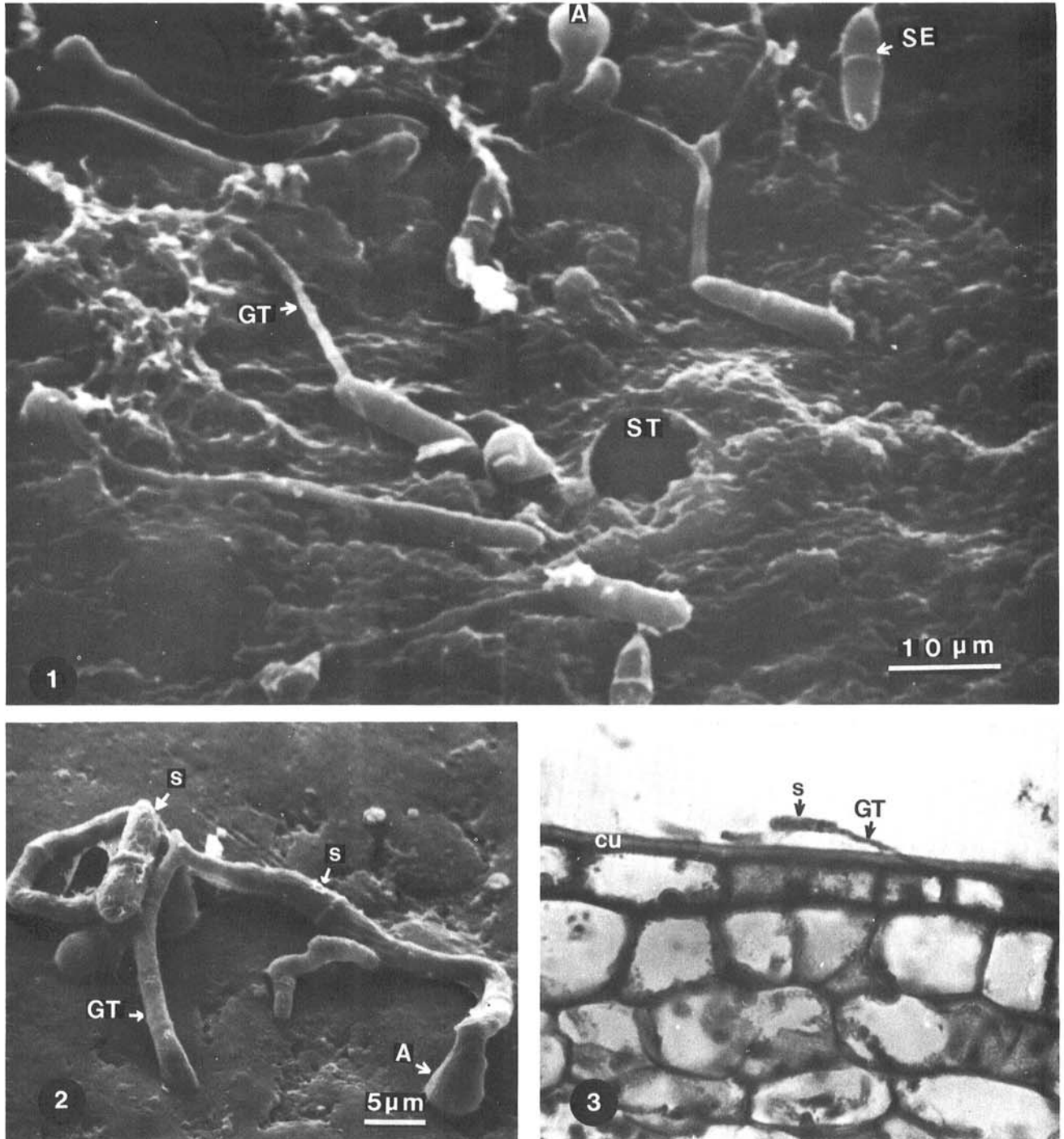
razor blade, and lyophilized to permit the viewing of internal structures. Scanning electron microscopy samples were sputter coated with gold and platinum, and examined with either a Cambridge S4-10 or 150 Stereoscan scanning electron microscope operated at 5–20 kV.

RESULTS

Appressoria at the tips of germ tubes were observed within 24 hr postinoculation (Fig. 1). Usually one or two appressoria were formed on the fruit surface by each germinated spore (Fig. 2). Germ

tube growth was not directed toward the stomates, but appressoria occasionally were observed in stomatal cavities. Direct penetration of the cuticle by germ tubes was observed (Fig. 3); more frequently, the fungus penetrated the fruit cuticle via infection pegs within 3–4 days after inoculation (Fig. 4).

Electron microscopy revealed that septa had formed between the appressoria and the germ tubes (Fig. 5). The cell wall of the appressorium sleeved the infection pegs that had penetrated the cuticle (Fig. 6). An electron-dense region was observed in the cell wall below the infection peg. An extension of the infection peg was observed between the cuticle and the epidermal cell. Hyphae grew



Figs. 1–3. 1, Microscopy of spore germination and initial cuticle penetration by *Colletotrichum gloeosporioides* on a detached papaya fruit. 1, Scanning electron micrograph of germinated spores of *C. gloeosporioides* on a fruit surface showing septa (SE) at the middle of spores, and appressoria (A) at tips of the germ tubes (GT). Note that germ tube growth does not appear to be directed toward the stoma (ST). 2, Scanning electron micrograph of a germinated spore (S) with two germ tubes (GT). Note the appressorium (A) to right. 3, Light micrograph of direct penetration of cuticle (CU) by a germ tube (GT) of spores of *C. gloeosporioides*.

between the cuticle and the epidermal cell wall (Fig. 7).

A sunken lesion was formed as the fungus continued to develop in the host tissue. In this advanced stage of infection, some parenchyma cells with intercellular hyphae were observed in the central areas of sunken lesions. At the edge of the lesions, granular materials and intracellular hyphae were observed.

A compact mycelial stroma separated the cuticle from the collapsed fruit tissue and the cuticle was distended upward (Fig. 8). During sporulation the cuticle ruptured and spores were released (Fig. 9). Ridges were observed on the under side of the ruptured cuticle (Fig. 10).

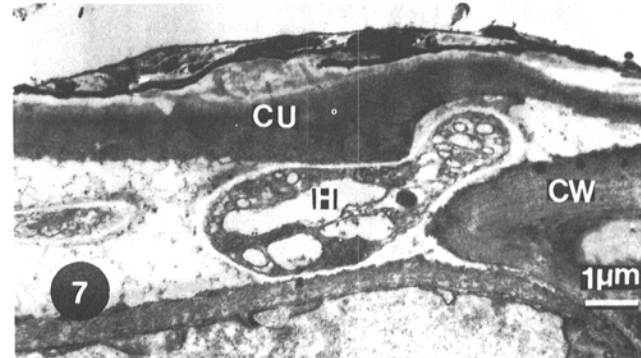
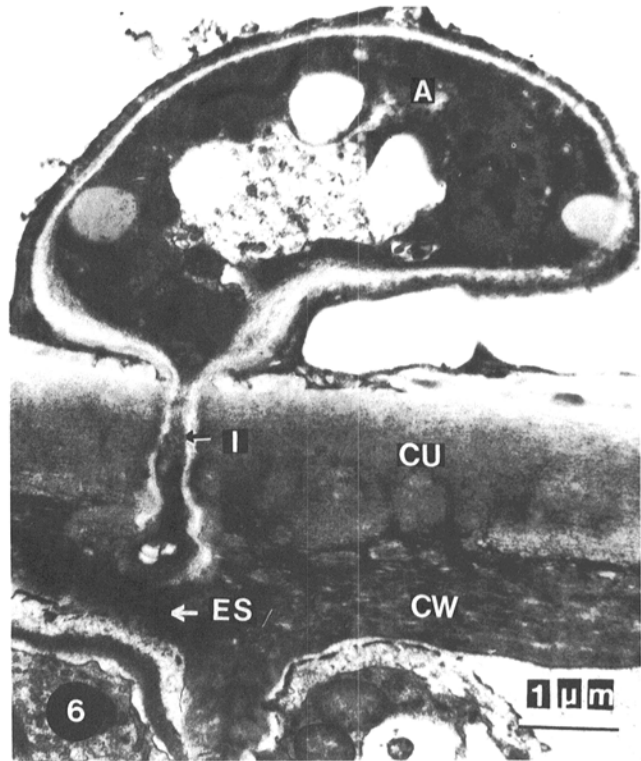
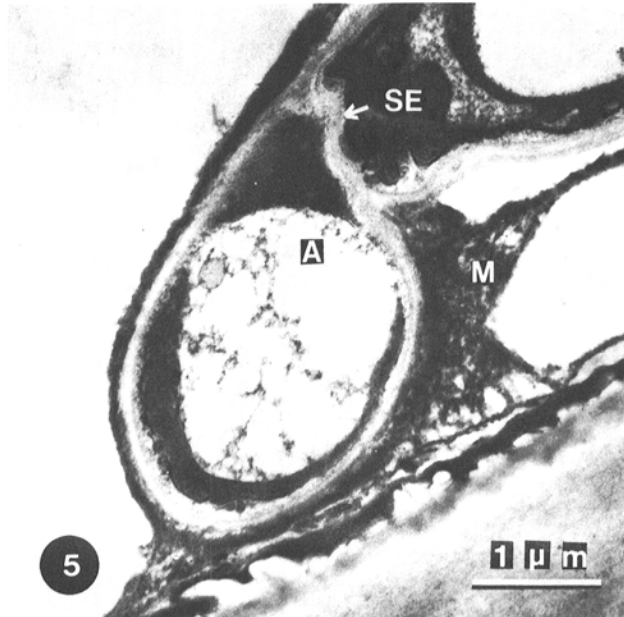
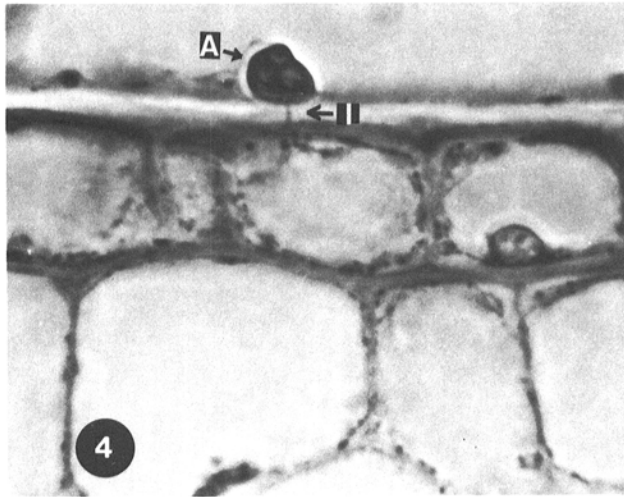
DISCUSSION

This report clarifies the mode of penetration of papaya cuticle by *C. gloeosporioides*. Since fungal germ tube growth is not directed toward the stomates on papaya fruit surfaces, and appressoria were observed only occasionally in stomatal cavities, stomates do not appear to be the usual site of entry. Brown (4) reported that appressoria of *C. gloeosporioides* were rarely observed in stomatal cavities of citrus fruits. Penetration of papaya cuticle via

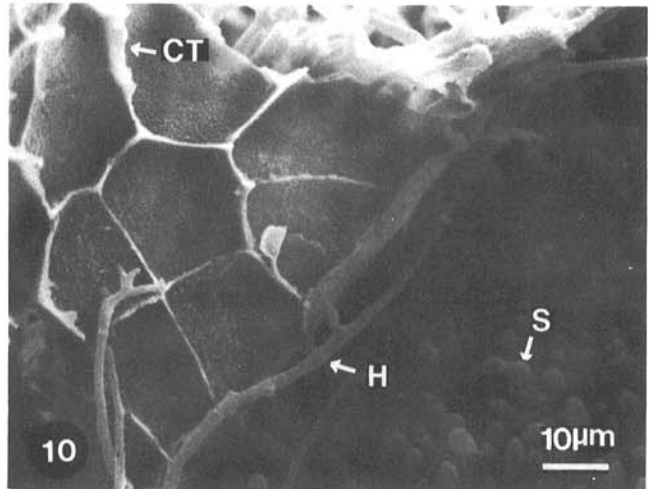
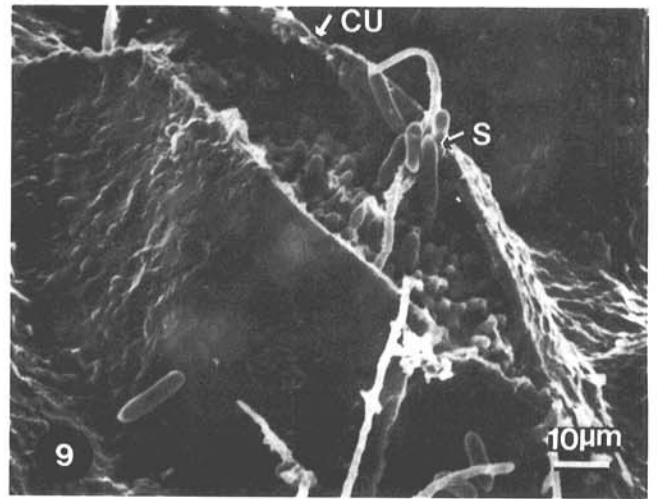
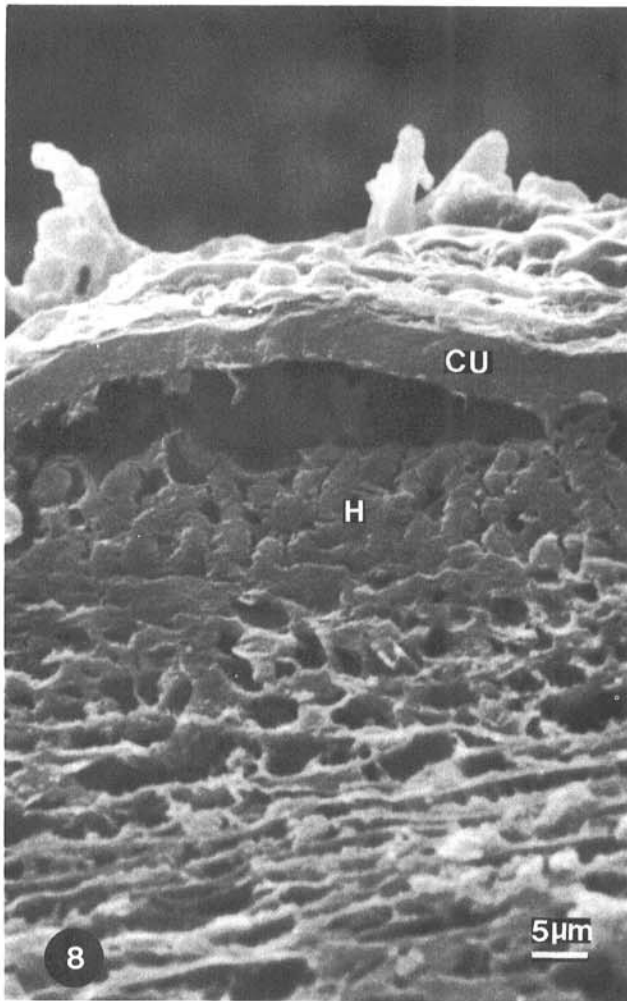
appressoria and infection pegs appears to be the most common mode of entry, although direct penetration of cuticle by germ tubes also occurs.

The absence of mechanical damage and the appearance of electron-dense areas in the cell wall near the infection peg at the penetration site suggested that the papaya cell wall might have been enzymatically dissolved by *C. gloeosporioides*. Maeda (10), Stavely et al (15), and McKeen et al (11) considered increased electron density in the cuticle at penetration sites to be evidence of enzymatic degradation of cuticle during the penetration process. Recently, Dickman et al (7) purified cutinase from *C. gloeosporioides* and showed that this enzyme was involved in penetration. Brown (5) suggested that enzymatic activity was involved in penetration of the cuticle of Robinson tangerine by *C. gloeosporioides*.

Previous studies of latent infection caused by *Colletotrichum* sp. on mango (6), orange (4), avocado (3), and banana (12,13) suggested that fungal appressoria, infection pegs, and subcuticular hyphae were essential for fungal penetration and might play a role in its survival during the "latent stage." Stanghellini and Aragaki (14) did not observe infection pegs or subcuticular hyphae of *C.*



Figs. 4-7. 4, Microscopy of *Colletotrichum gloeosporioides* in the cuticle, penetration, and initial colonization stages of infection of a detached papaya fruit. 4, Light micrograph of an appressorium (A) with an infection peg (I) extending through the cuticle 4 days after inoculation. 5, Transmission electron micrograph of a septum (SE) between the germ tube and an appressorium (A). Note that the electron-dense material (M) appears to cement the appressorium to the cuticle. 6, Transmission electron micrograph of infection peg (I) between cuticle (CU) and cell wall (CW). Electron-translucent area around the infection peg appears to be fungal cell wall. Electron-dense areas (ES) are located around and below the infection peg (white arrow). 7, Transmission electron micrograph of fungal hypha (H) between the cuticle (CU) and cell wall (CW) of the epidermal cell.



Figs. 8–10. 8, Scanning electron microscopy of *Colletotrichum gloeosporioides* in the later phases of colonization of a papaya fruit. 8, Scanning electron micrograph of hyphae (H) between the cuticle (CU) and collapsed fruit cells. 9, Scanning electron micrograph of ruptured cuticle (CU) and an acervulus with a few released spores (S). 10, Scanning electron micrograph showing separated cuticle in an acervulus. Note the cutinized layer (CT) of the cell wall on the lower surface of the cuticle and fungal hyphae (H) located between the cuticle and the spore layer.

gloeosporioides on papaya and questioned the occurrence of latent infections in papaya. Although the present study was not intended to examine the question of latency, ultrastructural evidence indicates that *C. gloeosporioides* does form an appressorium, an infection peg, and subcuticular hyphae during penetration of detached mature green papaya fruit.

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