Scanning Electron Microscopy of Cotton Boll Invasion by Diplodia gossypina

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ABSTRACT

SNOW, J. P., and M. G. SACHDEV. 1977. Scanning electron microscopy of cotton boll invasion by Diplodia gossypina. Phytopathology 67: 589-591.

Diplodia gossypina readily entered cotton bolls (*Gossypium hirsutum*) of various ages. Penetration was achieved in three ways: directly through wax and epidermal layers on the surface of the boll, directly through unwaxed multicellular epidermal hairs, and through open stomata. Invasion occurred through open stomata of bolls 20 days or older. Direct penetration of wax, epidermal cells, and epidermal hairs was observed in surface views and cross sections of bolls of all ages studied.

In the southeastern United States, cotton (*Gossypium hirsutum* L.) is plagued by numerous boll rotting microorganisms (9). In Louisiana, boll rot losses over the past 10 yr have averaged about 10% (10). *Diplodia gossypina* Cke. is one of the major pathogens causing boll rot of cotton in Louisiana.

The mode of infection of cotton bolls by *D. gossypina* has received limited attention. Permanent opening of cotton boll stomata occurs about 30 days after anthesis (1, 8) exposing the boll interior to fungal invasion through these natural openings. The method of infection prior to 30 days is not well understood.

The bract (6) and dehiscing sutures (1) have been suggested as possible avenues of entry for boll rotting organisms. However, no evidence was found that *D. gossypina* infected the boll internally via the bract (3). Direct penetration of the boll wall (9) and multicellular epidermal hairs (2, 4) also has been proposed.

This investigation was initiated to gain more information about the infection of cotton bolls by *D. gossypina*.

MATERIALS AND METHODS

Cotton (*G. hirsutum* 'Deltapine 16') was grown in the greenhouse from seed supplied by Delta and Pineland Company, Scott, Mississippi 38772. Greenhouse temperatures ranged from 25 C at night to 40 C during the day. White blooms were tagged daily to provide bolls of known ages (7). Five-, 10-, 20-, 30-, and 40-day-old bolls were collected and the bracts removed. Bolls were surface-sterilized for 3 min in 0.5% sodium hypochlorite and inoculated by transferring a 3-mm disk of agar containing *D. gossypina* onto each boll. Inoculated and control bolls were incubated on sterile moist paper towels at 30 C for 6-9 days until rotting was observed.

Tissue specimens (1 mm^2) from control and infected bolls were fixed with Formalin-acetic acid-alcohol; 3.5% glutaraldehyde in 0.2 M phosphate buffer, pH 6.8; or 3% glutaraldehyde- 3% acrolein in a 0.1 M K_2HPO_4 buffer, pH 6.8. Several specimens then were postfixed in 2% OsO_4 and subjected to cytoplasm hydrolysis as described by Kinden and Brown (5) for locating fungal parts within the host cells. Samples were dehydrated through a graded ethanol series, followed by critical-point drying with a Model DCP-1 Denton Critical Point Dryer (Denton Vacuum, Inc., Cherry Hill Industrial Center, Cherry Hill, NJ 08003) using liquid CO_2.

Dried specimens were mounted on aluminum stubs using aluminum paint as an adhesive and conductor. Tissues treated by the Kinden and Brown (5) procedure were oriented on the stubs to provide cross sections of boll tissue. Other tissues were oriented to provide a view of the boll surface. Specimens were coated with gold in a vacuum evaporator (Denton 500), examined with a Joelco JSM-2 scanning electron microscope (JEOL Application Laboratory, 477 Riverside Ave., Medford, MA 02155) operating at 25 Kv and photographed using Polaroid P/N 55 or Tri X Ortho 4/5 film.

RESULTS

Scanning electron microscopic observations of the noninoculated cotton boll surface revealed a coating of wax deposited on the boll in an intricate pattern except on stomata and epidermal hairs. Young boll tissue (5 days old) had less wax deposited on the surface as evidenced by the distinctly visible underlying epidermal cells (Fig. 1) compared to older tissue (Fig. 2, 3).

The surface of inoculated boll tissue of all ages showed extensive and well-branched mycelial growth. Fungal hyphae penetrated multicellular epidermal hairs on bolls of all ages (Fig. 4). Hyphae often entered and exited through open stomata of 30- and 40-day-old bolls,

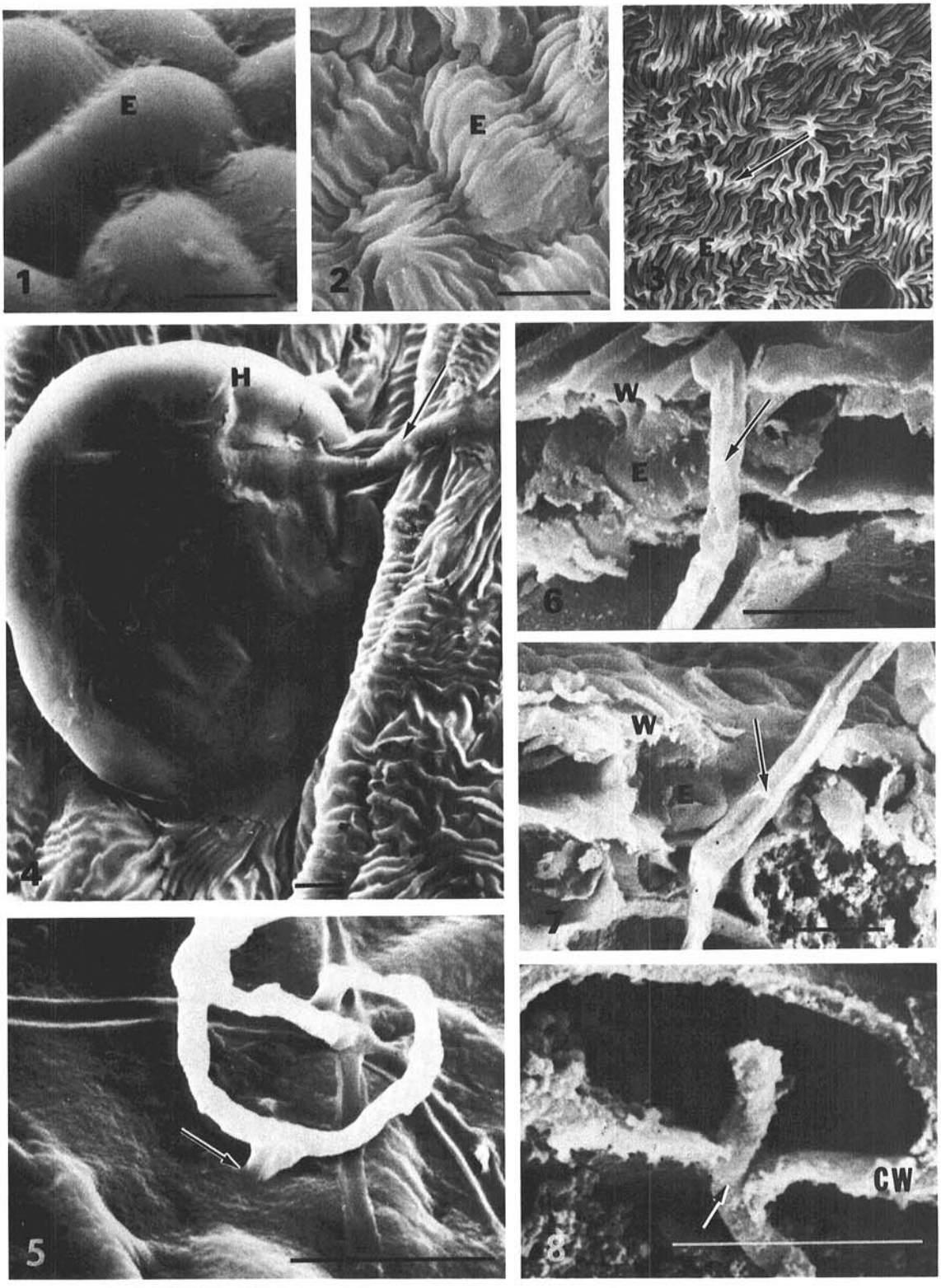
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Fig. 1-8. Scanning electron micrographs of the infection of cotton bolls by *Diplodia gossypina* (1-5). The surface of Deltapine 16 cotton bolls. Scale bars = 10 μm. **1)** Five-day-old boll. Note epidermal cell (E) with no surface wax. **2)** Ten-day-old boll. Epidermal cells are covered with wax layer. **3)** Fifteen-day-old boll. Epidermal cells are covered with intricate lacework of wax (arrow). **4)** Hypha (arrow) of *Diplodia gossypina* entering unwaxed multicellular epidermal hair (H) of a 10-day-old boll. **5)** Hypha of *D. gossypina* (arrow) entering wax coating of a 20-day-old boll. (6-8) Scanning electron micrographs of cross-sectional views of Deltapine 16 cotton bolls infected with *Diplodia gossypina*. Scale bars = 10 μm. **6)** Hypha (arrow) penetrating wax (W) and epidermal cell (E) of a 20-day-old boll. **7)** Hypha (arrow) penetrating wax (W) and epidermal cell (E) of a 20-day-old boll. **8)** Parenchyma cell of a 20-day-old boll showing tearing of cell wall (CW) caused by hypha (arrow).

occasionally entered stomata of 20-day-old bolls, but were not seen entering the open stomata of 5- and 10-day-old bolls. Direct penetration of the wax layer was observed in the boll tissue of all ages (Fig. 5). Penetration of wax and epidermal cells was distinctly evident in cross sectional view of cotton boll tissue (Fig. 6, 7). Fungal hyphae appeared to penetrate cuticle and wall material with ease. No appresorium or infection peg was seen but the host cell wall was torn by the advancing hypha (Fig. 8). In cross section the interior cell surface was covered by a dense mycelial mat. Some of the cells near the boll surface were packed with hyphae. Hyphal growth was both intercellular and intracellular in the host tissue.

DISCUSSION

Diplodia gossypina penetrated multicellular epidermal hairs, the waxy covering, and the epidermal cells of bolls prior to the period of permanent stomatal opening. The frequency of penetration by these routes appeared to remain constant with increasing age of the boll. Penetration through stomata, however, was not seen in younger bolls. Instead, this method of entry was observed most often in bolls 30 days old or older.

Some of the damage done by boll rotting fungi such as *D. gossypina* occurs prior to the time of permanent stomatal opening (9). Direct penetration through multicellular epidermal hairs and the wax and epidermal cells covering the bolls could account for some of this early damage. Entry of *D. gossypina* and subsequent damage after permanent stomatal opening is probably the result of a combination of direct penetration through epidermal hairs, direct penetration through wax and epidermal cells, and entry through open stomata.

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