Effect of Temperature and Boll Injuries on Development of Diplodia Boll Rot of Cotton

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ABSTRACT

Diplodia gossypina grew best on potato-dextrose agar and rotted cotton bolls most rapidly at 30 C. At 30 C, bolls were often completely decayed in 4 days when mycelial inoculum was placed over a deep wound made through the pericarp with a dissecting needle, whereas 9 to 11 days were usually required for complete rot of noninjured bolls. Decay of the entire boll occurred in 5 to 7 days when inoculum was placed over a shallow wound (surface scratched) or between artificially cracked sutures. Sixteen isolates of *D. gossypina* from cotton bolls differed more in aggressiveness on noninjured bolls than on bolls punctured with a dissecting needle. These results indicate that both high temperatures and boll injuries are important factors in the rapid development of Diplodia boll rot.

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Diplodia gossypina Cke. is an important cause of cotton (*Gossypium hirsutum* L.) boll rot in the southeastern United States (1, 2, 4, 8, 10, 11, 13). Early workers (8, 13) concluded that the fungus entered bolls only through wounds. However, Bagga (5) classified *D. gossypina* as a primary pathogen since he found that it penetrated bolls directly after contact inoculation of the pericarp. Recent work (3, 7) indicated that the organism enters bolls through open stomates or through parenchyma of carpel sutures as bolls reach maturity. The relative importance of these and other portals of entry under field conditions is not known. Also, little information is available on the precise environmental conditions conducive to rot development, although Diplodia rot is reported to develop rapidly in the "warm and humid climate" of the cotton belt (14). This paper reports an investigation of the influence of temperature, portal of entry, and various geographical isolates on development of Diplodia boll rot. An abstract of a portion of this work has been published (9).

MATERIALS AND METHODS.—Mature (35 days old), apparently healthy, green Coker 201 bolls were collected from field plants during the growing season and from greenhouse-grown plants during the remainder of the year. Bolls, with bracts removed, were washed several times with tap water, surface-sterilized for 5 min in a 0.5% sodium hypochlorite solution containing 1.5 g of Alconox detergent/liter, and rinsed three times in tap water. Cultures of *D. gossypina* were grown on potato-dextrose agar (PDA, Difco) for 3 to 4 days at 30 C. Mycelial plugs, 3 mm in diam, cut with a sterile cork borer from the periphery of the colonies, were placed on the

sterilized bolls with the mycelium side in contact with the boll surface, and held in place with a 22-mm circular adhesive bandage (6). Control bolls were treated similarly with sterile PDA plugs. Inoculated and control bolls were placed in sterilized humidity chambers (9 X 25 cm) equipped with a wire rack that held the bolls upright with their pedicels immersed in water. Disease readings (percent of boll surface decayed) were taken daily for 10 to 14 days after initial symptoms appeared. Bolls decayed by contaminants were discarded during the course of the study. All trials were repeated at least once.

Temperature studies.—Two methods of inoculation were used. Inoculum was placed either on noninjured pericarp walls or over deep puncture wounds made with a blunt dissecting needle. Fifteen bolls were inoculated by each method, and five were maintained as controls. All bolls were placed in incubators maintained at 10 through 35 C at 5-C intervals. Disease readings were taken daily for 15 days after inoculation. The fungus isolate used in the temperature studies and 12 other isolates from cotton bolls were also grown on PDA plates at 5 through 40 C at 5-C intervals to determine the influence of temperature on in vitro growth.

Portal of entry studies.—I inoculated bolls by placing inoculum (i) on the pericarp surface of noninjured bolls; (ii) over deep wounds made by inserting a blunt dissecting needle through the boll walls; (iii) over a shallow wound ($\leq$ 0.5 mm deep) made by scratching the surface with a dissecting needle; and (iv) on the pericarp surface of bolls midway between sutures cracked with a scalpel. Inoculated and appropriate control bolls were placed at 30 C. Disease readings were taken daily for 10 days.

Isolate comparisons.—Sixteen isolates (14 from Georgia, 1 from Mississippi, and 1 from Louisiana) of *D. gossypina* from cotton bolls were compared for their relative capacity to invade injured (deep wound made with a dissecting needle) and noninjured bolls. Ten bolls were inoculated by each method and were placed at 30 C for 10 days during which disease readings were taken.

RESULTS.—*Temperature studies.*—The rate of disease development in both injured and noninjured bolls increased with increases in temperature to 30 C. Disease development was similar at 30 and 35 C. A pink pigment developed on bolls at 35 C similar to that reported (2) in cultures of *D. gossypina* growing in vitro. No significant rot occurred at 10 C after 14 days, although the fungus penetrated the boll in the immediate vicinity of the inoculum. At all temperatures, rot developed faster in the injured than in the noninjured bolls. Growth rate of this isolate on PDA increased to 30 C and then decreased. Twelve other isolates tested responded similarly to temperature except for differences in growth rate at all temperatures and a slightly higher (35 C) optimum for some isolates.

Portal of entry studies.—Disease developed fastest and most uniformly at 30 C when the inoculum was placed over a deep wound (Fig. 1). Most bolls were completely rotted in 4 to 5 days when inoculated by this method. Bolls also decayed rapidly (many completely in 5 to 7 days) when inoculum was placed over a shallow wound or on bolls with artificially cracked sutures. However, disease development was greatly retarded (9 to 11 days before many bolls rotted) when inoculum was placed on the walls of noninjured bolls. Rate of decay varied more on inoculated noninjured bolls than on wounded bolls. For example, all stages of decay occurred on noninjured bolls at any given time between 6 and 9 days after inoculation.

Isolate comparisons.—Most of the isolates were highly and equally virulent when inoculum was placed on wounded bolls. Nine of the 16 isolates rotted more than 90% of the boll surface, five isolates between 70 and 90%, and two isolates between 50 and 60% during the 6 days after inoculation. All isolates were less aggressive, disease developed less uniformly, and isolates varied more in virulence on noninjured than on injured bolls. Percentage of boll surface rotted 6 days after inoculation of noninjured bolls ranged from about 10 to 50%.

DISCUSSION.—These results indicate that high temperatures and suitable portals of entry are important in the rapid development of Diplodia boll rot. In Georgia and certain other states (1, 8) Diplodia boll rot is more severe in coastal than piedmont areas. Differences in temperature during the period of boll maturity may partially explain this distribution although other factors are likely involved. The 30- to 35-C optimum temperature for growth of *D. gossypina* on PDA was similar to the 30- to 34-C optimum reported by Aycock (2).

Edgerton (8) and later, Walker (13) reported that *D. gossypina* was unable to penetrate noninjured boll tissues. Walker (13) observed that lesions caused by other boll-rotting organisms and insect punctures were common points of entry in the field. Pinckard (12) also noted that Diplodia rot commonly followed insect injury and damage caused by mechanical pickers. My results and those of others (3, 5, 7) indicate that under certain conditions *D. gossypina* also is capable of penetrating mature, noninjured bolls. However, a great advantage is afforded the organism when a wound or partially cracked suture is available for entry. Even relatively shallow surface scratches are conducive to rapid disease development. Large numbers of insect injuries, other wounds, and often partially cracked sutures are present on mature bolls in the field. These probably are essential for the rapid buildup of Diplodia boll rot. These findings suggest the importance of an effective insect control program for the control of Diplodia and probably other boll rots.

The various isolates, all from cotton bolls, were remarkably similar in virulence when injured bolls were inoculated. More differences were evident on noninjured bolls, but all isolates developed slowly with this type of inoculation. The various isolates retained their relative virulence levels throughout the 2-year span of this study. Stability in *D. gossypina* also was reported by others (14), but variation in virulence also is common (2).

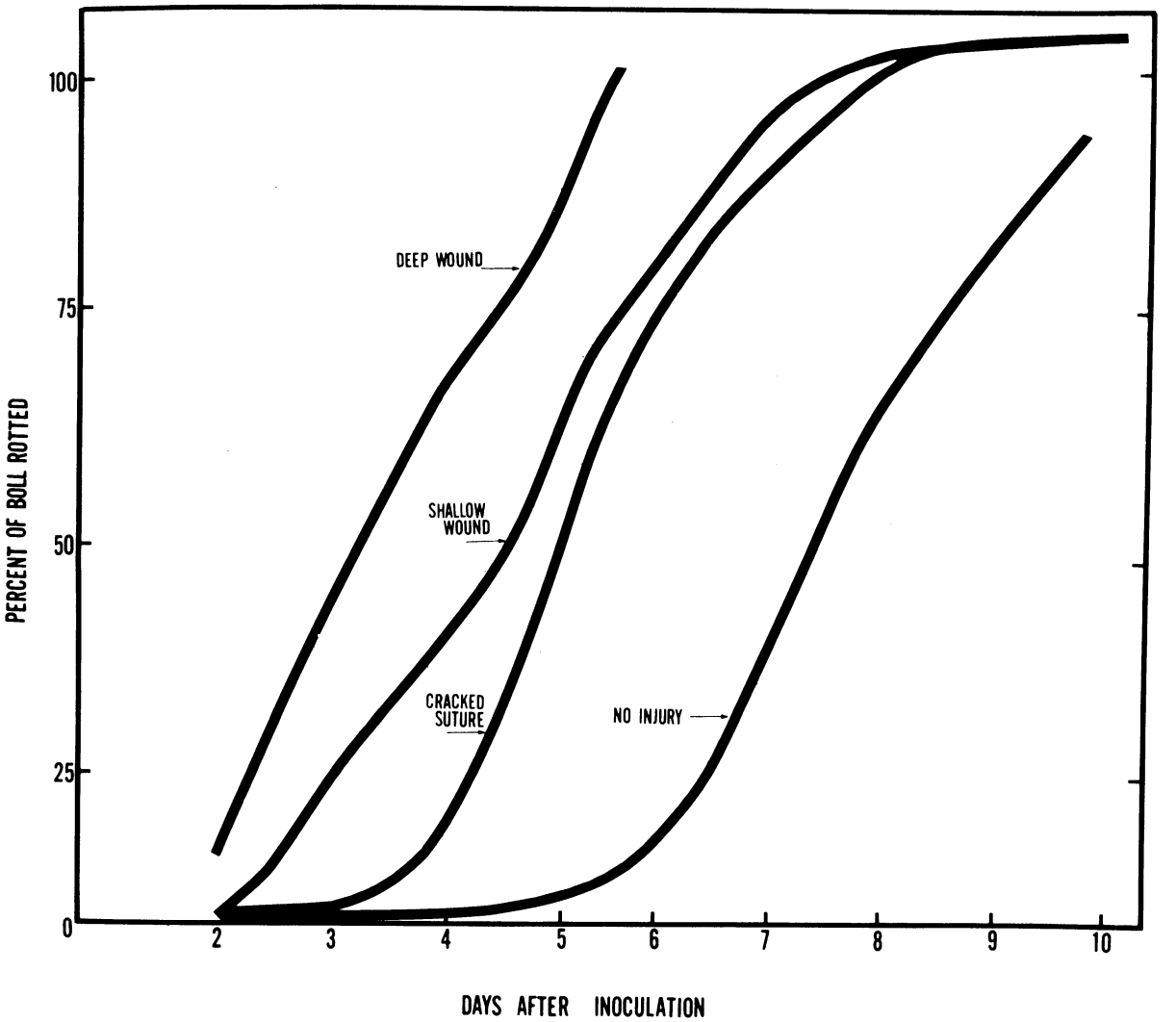

Fig. 1. Influence of the portal of entry on the development of boll rot of cotton caused by *Diplodia gossypina.*

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