Pathogenicity Studies of Organisms Involved in the Cotton Boll-Rot Complex

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ABSTRACT

Pathogenicity studies of 36 organisms associated with the cotton boll-rot complex were conducted to determine organisms capable of directly penetrating the pericarp of the cotton boll. *Bacillus subtilis, Diplodia gossypina, Glomerella gossypii,* and *Xanthomonas malvacearum* are primary pathogens, capable of direct penetration. *Myrothecium roridum* penetrated the pericarp directly, indicating that this organism is also a primary pathogen of the cotton boll. The degree of internal rot produced by each of the primary pathogens was similar in vitro and in vivo. Phytopathology 60:158-160.

Cotton boll-rot organisms are most active during periods of hot humid weather in the Yazoo-Mississippi Delta. These organisms cause reduction in yield and quality of lint, and also contaminate seeds. Among the different organisms isolated from bolls and generally accepted as causal agents of the "rot complex", few are known as primary pathogens (1, 2, 4). Only *Xanthomonas malvacearum* (E. F. Sm.) Dows. (3, 9, 10) and *Glomerella gossypii* Edg., alone (5, 7, 9) or in conjunction with *X. malvacearum* (10), are reported to penetrate directly the pericarp of the cotton boll.

In vitro and in vivo studies with *Diplodia gossypina* Cke. and *Bacillus subtilis* Cohn. indicate that both organisms are primary pathogens of the cotton boll (3). Studies herein reported are a continuation of the above to determine whether additional organisms associated with the boll-rot complex could directly penetrate the pericarp of the cotton boll.

MATERIALS AND METHODS.—Green, healthy bolls, 6-7 weeks old, were collected from field plants of *Gossypium hirsutum* L. 'Stoneville 7A'. The surfaces of the bolls (with bracts removed) were sterilized for 3 min each with a 1:1,000 solution of $HgCl_2$ and with a 1.3% solution of sodium hypochlorite, and rinsed twice in sterile water. They were placed individually in sterilized half-pint fruit jars containing water to maintain a high relative humidity. The pedicel of each boll was immersed in the water (3). Injection and contact inoculation methods were used. Inoculum was prepared by growing the fungal organisms on potato-dextrose agar and bacterial organisms on Bird's modified potato-carrot-dextrose agar (6) for 6-10 days at 28°C. The inoculum was injected through the boll valve, using the eye end of a sewing needle manipulated to leave the inoculum inside the boll. With the contact method for fungal inoculations, a mycelial plug 5 mm in diam was placed on an adhesive bandage 22 mm in diam and placed on the surface of the boll valve. For bacteria, the bandage was moistened with a bacterial suspension prior to inoculation. Fifty bolls were inoculated for each treatment. A 50-boll check for each method of inoculation was included to determine presence of internal infection (4). Bolls were incubated at 28-30°C for 2-3 weeks. Rot development in control samples was 38%. Since this level of internal infection was present in the sample of bolls, reisolations from infected bolls were made to confirm the cause of rot. Thirty-six organisms associated with the cotton boll-rot complex were tested (Table 1).

An in vivo evaluation of *Myrothecium roridum* Tode was made in the greenhouse, using the contact method of inoculation. One hundred inoculated bolls were separately covered with a polyethylene bag to maintain a high relative humidity. A similar number of bolls was included in the check. Pathogenicity ratings were made 18 days after inoculation.

Field studies were also conducted with Stoneville 7A. Forty bolls were inoculated with each organism by injection and by contact. Forty bolls were used as a control for each method of inoculation. Bolls were covered with polyethylene bags to maintain a high relative humidity. Evaluations were made 2-3 weeks after inoculation. Reisolations were made from rotted bolls

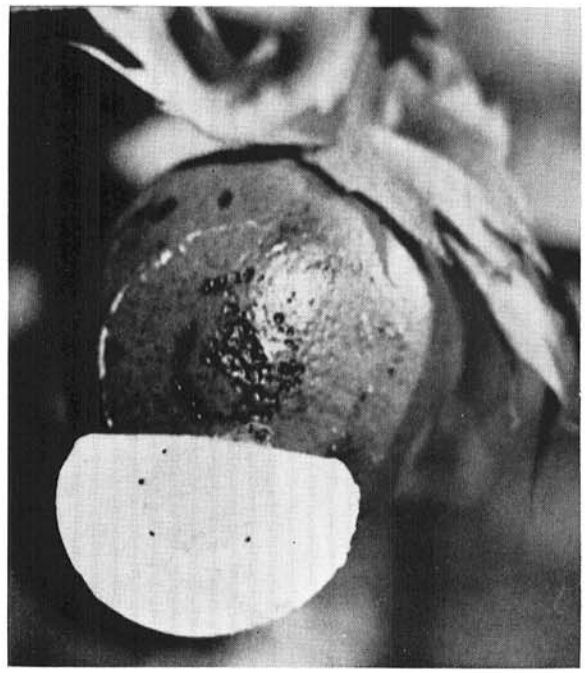

Fig. 1. Necrosis of cotton boll wall under bandage 5 days after contact inoculation with *Myrothecium roridum* in the greenhouse.

TABLE 1. Pathogenicity evaluation of 36 contact-applied organisms involved in the cotton boll-rot complex in the Yazoo-Mississippi Delta

Organism	Rot development[a]			
	In vitro		In vivo	
	Internal	pericarp	Internal	pericarp
Alternaria tenuis Auct.	—	+	—	—
Ascochyta gossypii Woron.	—	+	—	—
Aspergillus flavus Lk. ex Fr.	—	+	—	—
A. fumigatus Fres.	—	+	—	—
A. luchuensis Inui	—	+	—	—
A. nidulans (Eidam) Wint.	—	+	—	—
A. niger v. Tiegh.	—	+	—	—
A. ochraceus Wilhem	—	+	—	—
A. repens (Cda.) de By.	—	+	—	—
A. ustus (Bainier) Thom & Church	—	+	—	—
A. versicolor (Viull.) Tiraboschi	—	+	—	—
Bacillus subtilis Cohn.	+	+	+	+
Botryosphaeria berengeriana de N.	—	+	—	—
B. ribis Gross. & Dug.	—	+	—	—
Cercospora gossypina Cke.	—	+	—	—
Choanephora cucurbitarum (Berk. & Rav.) Thaxt.	—	+	—	—
Cladosporium herbarum Lk. ex Fr.	—	+	—	—
Diplodia gossypina Cke.	+	+	+	+
D. natalensis P. Evans	—	+	—	
Fusarium moniliforme Sheldon	—	+	—	—
F. roseum (Lk. ex Fr.) Snyd. & Hans.	—	+	—	—
F. solani (Mart.) Appel & Wr.	—	+	—	—
Glomerella gossypii Edg.	+	+	+	+
Macrophomina phaseoli (Maubl.) Ashby	—	+	—	—
Myrothecium roridum Tode	+	+	+	+
Nigrospora sp.	—	+	—	—
N. sphaerica (Sacc.) Mason	—	+	—	—
Penicillium luteum Sopp.	—	+	—	—
Physalospora rhodina (Berk. & Curt.) Cke.	—	+	—	—
Phytophthora parasitica Dast.	—	+	—	—
Rhizopus stolonifer (Ehr. ex Fr.) Lind	—	+	—	—
Thielaviopsis basicola (Berk. & Br.) Ferr.	—	+	—	—
Trichoderma viride Pers. ex Fr.	—	+	—	—
Trichothecium roseum Lk. ex Fr.	—	+	—	—
Xanthomonas malvacearum (E. F. Sm.) Dows.	+	+	+	+
Verticillium albo-atrum Reinke & Berth.	—	+	—	—

[a] + = Rot developed; — = no rot.

to determine if the organism used in each inoculation was present.

RESULTS AND DISCUSSION.—In both laboratory and field studies, *B. subtilis, D. gossypina, G. gossypii, M. roridum*, and *X. malvacearum* penetrated the boll valve directly from contact inoculation, and the internal rot was similar to that resulting from injection inoculation. This shows that these organisms are primary pathogens of the cotton boll. The remaining 31 organisms tested in this study did not penetrate the endocarp when applied by contact (Table 1), but all caused a complete rot of the pericarp, and in most instances a complete internal rot when the injection method of inoculation was used. In case of in vivo control bolls, there was no rot with contact method, but 20% of the bolls rotted with the injection method.

Contact inoculation with *M. roridum* in the laboratory produced extensive external and internal damage, appearing as a black circular spot below the bandage at the point of inoculation. The boll was covered by numerous jet black sporodochia in 14-16 days.

In the greenhouse, 2 days after contact inoculation with *M. roridum,* the region of the boll wall under the bandage became dark. This area enlarged considerably 5 days after inoculation (Fig. 1). The fungus gradually penetrated the endocarp and destroyed the locule. The inner wall between the two locules was also penetrated by the fungus, thus destroying adjacent locules. Internally the rot progressed rapidly, destroying lint and seed, turning the entire boll dark brown. The entire boll rotted and black sporodochia covered the boll 16-18 days after inoculation. There was no rot in control bolls.

M. roridum has been reported to cause boll-rot from wound inoculation (8). This study shows that it can also directly attack green cotton bolls.

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