Fusarium Boll Rot of Cotton: Pathogenicity and Histopathology

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ABSTRACT

Fusarium moniliforme, F. oxysporum, F. roseum, and *F. solani* decayed wounded green bolls in situ, but differences were noted in the rate of decay and type of tissue affected. Uninjured green bolls were rotted by *F. oxysporum* and *F. roseum*, which initially infected the bracts, and then invaded the capsule base through the receptacle. Disease development was affected significantly by boll age. Only artificially inoculated fruits 33 to 35 days of age or older decayed, even though younger bracts were infected. In the field, the pathogens became established in the bracts soon after bloom, but basal rot was observed only when bolls were 6 to 10 weeks old. Initial invasion of bract tissue occurred as intercellular hyphal growth in the mesophyll parenchyma and epidermis. Necrosis was observed only after intracellular penetration took place. In the latter stage of disease development, all tissues were colonized. A similar pattern of invasion occurred in the receptacle.

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Additional key words: Gossypium hirsutum, senescence, disease resistance.

Throughout the southeastern portion of the cotton belt, *Fusarium* spp. have been associated with decaying cotton bolls more consistently than have other microorganisms (1, 3, 11, 12). They have been regarded as secondary invaders, however, which enter primarily through insect injuries and pericarp lesions caused by *Glomerella gossypii* Edg. and *Xanthomonas malvacearum* (E. F. Sm.) Dows. (5, 7). Recent studies (5, 14) showed that uninjured green bolls may be inhabited by *Fusarium* spp. Cauquil & Ranney (5) demonstrated that certain natural openings into the boll such as involucral nectaries, the calyx pit, the pedicel, and sutural gaps may serve as avenues of ingress.

The bract was suggested as the infection court for *Pellicularia filamentosa* (Pat.) Rogers (13). Invasion of the capsule occurred when the fungus progressed through the bract tissue into the receptacle and subsequently entered the locules. A similar pattern of disease development was prevalent in commercial field-grown bolls in Georgia (12). Only *Fusarium* spp. were isolated consistently from decaying bracts and capsule tissue.

Although the *Fusaria* may enter green bolls using various openings, little is known about the primary pattern of disease development. Our studies emphasized the role of the bract as an infection court as suggested by McCarter et al. (12). Consequently, this paper concerns (i) the pathogenicity of several frequently isolated species of *Fusarium*; (ii) the primary pattern of disease development in green bolls; and (iii) factors affecting boll susceptibility to decay.

MATERIALS AND METHODS.—*General procedures.*—Cotton plants (*Gossypium hirsutum* L. 'Coker 413') were grown in 30-cm (top diam) plastic pots in the greenhouse. Where indicated, plants of the cultivar, Stoneville 7A with the frego bract character, were grown under similar conditions. Inoculations were made using green bolls in situ, with each study being carried out twice. We determined boll age by tagging flowers at anthesis and indicating the date as 0 days.

Fusarium moniliforme Sheld., *F. oxysporum* Schlecht., *F. roseum* Lk. emend. Snyd. & Hans., and *F. solani* (Mart.) Appel & Wr., the species most frequently associated with boll rot in Georgia, were included in pathogenicity tests. All were single spore

isolates obtained from green bolls showing basal decay. Cultures were maintained on Difco potato-dextrose agar (PDA) slants. Inoculum was increased at 30 C in darkness for 6 days on PDA.

After inoculation, all bolls were covered with polyethylene bags which were tied firmly around the pedicel to prevent cross contamination, and the plants were placed in a moisture-saturated atmosphere. Illumination (1,100 ft-c) was provided by incandescent and fluorescent lights on an alternating 12-hr light-dark cycle. Temperatures ranged from 20 to 32 C.

Isolations were made from bolls in greenhouse studies by excising tissue from the lesion margin. Sections were surface sterilized in 0.525% aqueous sodium hypochlorite for 2 min, rinsed in sterile distilled water, and placed on water agar in petri dishes. We made isolations from field-grown bolls after washing them in tap water, followed by a 5-min soak in an aqueous solution of 0.525% sodium hypochlorite and 0.275% Alconox (Alconox, Inc., New York, N.Y.). Tissue sections were then processed as described above.

Pathogenicity studies.—Wound inoculations were carried out with the four species of *Fusarium*. Fifteen mature bolls were inoculated with each isolate. The boll pericarp was swabbed with 95% ethyl alcohol and permitted to dry. We introduced inoculum, consisting of spores and mycelium along with PDA, into two nonadjacent locules of each boll by puncturing the pericarp with the eye end of a sterile sewing needle and maneuvering it to deposit the inoculum within the cavity. Fifteen bolls receiving sterile PDA served as controls. The bolls were observed over a 9-day period, after which isolations were made.

Entire bolls and bracts were sprayed with a spore-mycelium suspension to determine the primary method of fungal penetration and disease development and the effect of boll age on susceptibility. *Fusarium oxysporum* and *F. roseum* were chosen since they produced symptoms generally representative of each of the other species in the wound-inoculation studies.

Cultures were flooded with sterile distilled water, and we freed the spores by gently rubbing the colony surface with a sterile glass rod and decanting the suspension. Inoculum consisted of mycelial fragments and, primarily, microconidia of *F. oxysporum*, whereas mycelial fragments and macroconidia were present in suspensions of *F. roseum*. Inoculum density was determined with a hemacytometer and adjusted to 1×10^4 conidia/ml. The bolls and attending bracts were sprayed with 95% ethyl alcohol and allowed to dry. Sixty bolls and their bracts in each of three age categories were atomized uniformly until runoff occurred. Controls consisted of 20 bolls in each age group sprayed with sterile distilled water. Observations of disease development were made 3, 5, and 9 days after inoculation. Isolations were made throughout the period.

Histopathology of boll penetration.—Tissue segments were taken sequentially from (i) advancing lesions on the bract; (ii) the point of juncture between the bract and receptacle; and (iii) the receptacle and basal portion of the capsule showing initial decay. Isolations were made from portions of lesions to determine the presence of the pathogen, and the remaining lesion tissue was killed and fixed. Plant tissue was killed and fixed in Formalin-acetic acid-alcohol (8). Vacuum provided by a standard laboratory water aspirator was used to thoroughly infiltrate the fixing solution into larger tissue sections. Dehydration was carried out in a tertiary-butyl alcohol series, and the tissue was then embedded in paraffin (8). Larger tissue segments were kept under vacuum (275 mm of mercury) to improve paraffin infiltration. Small sections were cut 16 μ in thickness with a rotary microtome, whereas larger sections were cut at the same thickness using a sliding microtome. Tissue sections were mounted on slides, the paraffin was removed in xylene, and sections were rehydrated in 70% ethyl alcohol and stained in trypan blue (0.02% in lactophenol) to provide semipermanent slides.

RESULTS.—*Bract colonization by Fusarium spp. in the field.*—During 1969 and 1970, isolations were made from bracts of 50 bolls/week (cultivar Atlas 67) which were tagged at flowering and sampled through boll dehiscence. The predominant *Fusarium* spp. were similar to those previously listed.

Although bract invasion varied somewhat in different years due to the stand environment, the occurrence of the *Fusaria* generally increased as bolls aged. During the initial 3 weeks after anthesis,

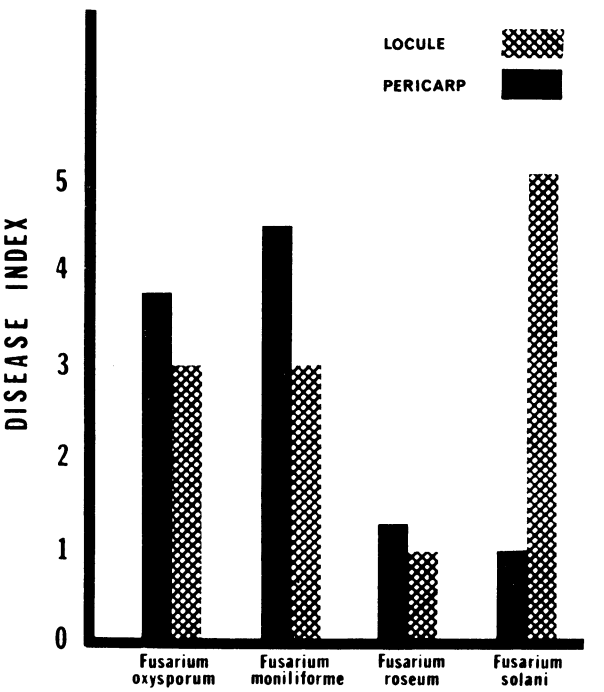

Fig. 1. Internal and external decay of cotton bolls 9 days after wound inoculations with four *Fusarium* spp. 0 = no decay; 1-4 = increasing degrees of rot; 5 = complete rot.

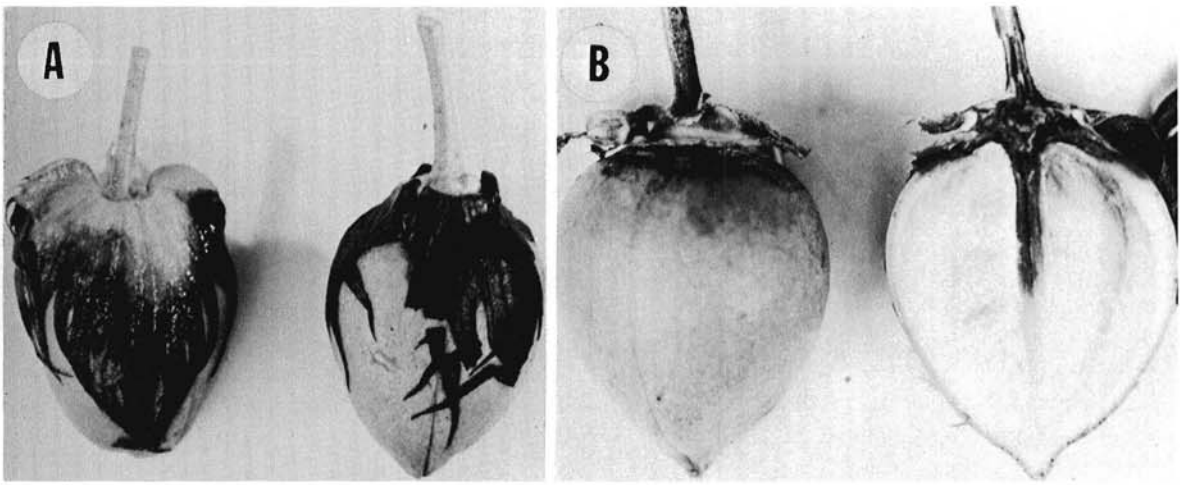

Fig. 2. Stages in the development of basal boll rot caused by *Fusarium oxysporum* following spore inoculation in situ. **A)** Partial bract necrosis (left) and complete bract necrosis (right). **B)** Initial decay of basal portion of the capsule showing entire boll (left) and opened boll (right).

Fusarium spp. were isolated from 0 to 59% of the bracts and necrosis was limited to the margin. After 4 to 6 weeks, they were detected in 42 to 84% of the bracts, and reached a maximum ranging from 75 to 100% from 7 weeks to boll dehiscence. During this period, lesions expanded progressively toward the receptacle. Basal rot was observed only when bolls were 6 to 10 weeks old, and samples of decaying carpel wall and placenta tissues consistently yielded *Fusarium* spp. in culture.

Pathogenicity studies.—To evaluate rot severity in wound-inoculated bolls, disease index ratings were assigned on the following basis: 0 = no decay; 1 = decay at the point of inoculation; 2 = partial decay of inoculated carpel; 3 = complete decay of the inoculated carpel; 4 = decay extending into the adjacent carpels; and 5 = complete decay of adjacent carpels.

After 9 days, *F. moniliforme, F. oxysporum, F. roseum,* and *F. solani* caused decay in all bolls when injected into the locules. There was, however, a difference in the rate and type of decay caused by the different species (Fig. 1). Both *F. moniliforme* and *F. oxysporum* caused extensive decay of the pericarp extending into the adjacent carpels. Internal decay was less extensive, being confined to the locule which was inoculated. *Fusarium roseum* and *F. solani* had little effect on the pericarp. *Fusarium solani* decayed both inoculated and adjacent carpels, whereas *F. roseum* caused decay only at the point of inoculation.

When entire bolls and bracts were sprayed with a spore-mycelium suspension, the primary pattern of boll decay was initiated in the bracts and progressed through the capsule base. Both *F. oxysporum* and *F. roseum* invaded and decayed bolls in a similar manner, and were isolated consistently from inoculated bolls. Earliest symptoms, 3 to 4 days after inoculation, were small expanding lesions located along the bract margin, particularly at the apices of the toothed areas. Lesion enlargement continued toward the proximal portion of the bract, and 6 to 7 days after inoculation, the entire organ became necrotic (Fig. 2-A). Invasion of the capsule took place through the receptacle, with the affected tissues showing a dark blue-green discoloration (Fig. 2-B). Frequently, the fungus moved from the receptacle into the pedicel. The rate of decay was accelerated considerably in dehiscing bolls.

To determine the effect of host age on the rate of disease development due to *F. oxysporum* and *F. roseum*, bolls grouped into three age categories (juvenile, 10 to 20 days old; intermediate-aged, 21 to 30 days old; and mature, 31 to 40 days old) were evaluated according to the following index: 0 = no symptoms; 1 = bract margin necrotic; 2 = 50% of bract necrotic; 3 = 100% of bract necrotic; 4 = basal boll rot; and 5 = entire boll rotted.

Boll age significantly affected the rate of disease development by both *F. oxysporum* and *F. roseum* (Table 1). Basal boll rot occurred primarily in mature bolls 33 to 40 days old, where *F. oxysporum* and *F. roseum* decayed 30 and 25% of the bolls, respectively. With intermediate-aged and juvenile bolls, disease development was slower and was limited generally to the bracts. The pathogenicity of *F. roseum* was affected more by host age than was *F. oxysporum*, since the former was unable to cause a significant degree of necrosis in juvenile bracts.

Bract necrosis occurred in control plants and increased as the incubation period lengthened (Table 1). However, the degree of bract decay was always significantly greater when plants were inoculated with either species of *Fusarium*, and basal boll rot was not observed in noninoculated green bolls. Members of the genera *Alternaria, Aspergillus, Cladosporium,* and *Helminthosporium* and unidentified bacteria were frequently isolated from necrotic bracts of the controls, but the *Fusaria* were predominantly isolated

TABLE 1. Influence of boll age on rot development in situ after inoculation with spore-mycelium suspensions of *Fusarium oxysporum* and *F. roseum*

Days of incubation	Boll maturity (age in days)								
	Juvenile (10-20)			Intermediate-aged (21-30)			Mature (31-40)		
	3	5	9	3	5	9	3	5	9
	Disease index[a]								
Pathogen									
F. oxysporum									
Inoculated	0.6[b]	1.1	1.9	1.8	2.3	3.0	1.5	2.5	3.4
Control	0.4	0.9	1.2	0.8	1.5	2.3	1.4	2.2	2.9
F. roseum									
Inoculated	0.5	1.0	1.4	1.3	1.8	2.6	1.6	2.5	3.2
Control	0.4	0.9	1.5	0.5	1.3	2.2	1.0	1.9	2.6

[a] Disease index: 0 = no symptoms; 1 = bract margin necrotic; 2 = 50% of bract necrotic; 3 = 100% of bract necrotic; 4 = basal boll rot.

[b] Disease indices for inoculated and control bolls for each species are significantly different ($P = .05$, Duncan's multiple range test) at equivalent incubation periods with the exception of *F. roseum* on juvenile bolls. All disease indices between age groups of inoculated bolls are significantly different ($P = .05$) at equivalent incubation periods.

from inoculated bracts and bolls. The presence of these microorganisms and senescence of bract tissue apparently accounted for the development of necrosis in the noninoculated plants.

The susceptibility of the cultivar, Stoneville 7A with the frego bract character, was determined since it has shown promise in control of boll rot in the field (9). Inoculation with *F. oxysporum* caused symptoms similar to those previously described. Boll age was a significant factor in decay, since basal rot was found only on bolls 33- to 35-days of age or older.

Histopathology of boll penetration.—Both *F. oxysporum* and *F. roseum* invaded and decayed tissues in a similar manner; consequently, their activities are discussed jointly. Lesion development was initiated from small necrotic areas on the toothed bract margin of intermediate-aged and mature bolls. The epidermal and mesophyll cells of this region were often wholly or partially destroyed, and provided a food base for *Fusarium* spp. The etiology of the lesions present before inoculation was not determined. However, such necrosis is common on maturing field-grown and greenhouse-grown bolls.

Longitudinal sections from the margins of expanding bract lesions revealed that hyphae had extended beyond the necrotic zone into apparently healthy tissue. External hyphal growth in this region was observed in close association with the cuticle (Fig. 3-B). Internal hyphal growth was subcuticular, extending between the epidermal cells and within the mesophyll parenchyma (Fig. 3-C, F). The rate of invasion was apparently facilitated by the structure of the mesophyll, which lacked a defined palisade layer and consisted of loosely packed parenchyma cells with large intercellular air spaces. Necrosis was not evident until cell penetration had occurred. Intracellular hyphae were detected initially in epidermal cells (Fig. 3-D), mesophyll, and vascular parenchyma. At this time, there was also a greater abundance of intercellular mycelium throughout the mesophyll.

Growth of intercellular hyphae at the expanding lesion margin usually circumvented the vascular bundles, moving between adjacent parenchyma cells. Thick-walled vascular elements such as vessels and tracheids were invaded only during the latter stage of disease development (Fig. 3-E).

Fungal invasion of the receptacle from the bract was not impeded by any apparent anatomical barrier, since the bract mesophyll was continuous with the collenchyma and parenchyma of the receptacle (Fig. 3-H). Invasion of this area provided the fungus access to other parts of the boll such as the carpel wall, placenta, and locks, inasmuch as the receptacle serves as a common point of juncture. Initial invasion of receptacle collenchyma occurred as intercellular hyphal growth (Fig. 3-G). Necrosis coincided with intracellular penetration of the collenchyma, parenchyma, and vascular elements. Invasion of the basal portions of the placenta and carpel wall occurred in a similar sequence.

DISCUSSION.—Our studies show that *F. oxysporum* and *F. roseum* invade and decay green bolls by initially colonizing the bracts and penetrating the capsule base through the receptacle. The pattern of disease development corresponds with the most prevalent type of decay found in commercial fields throughout Georgia (12). Although *Fusarium* spp. do not penetrate the pericarp directly, they are not strictly wound pathogens, as they may invade uninjured bolls through the bract and receptacle. Other microorganisms were isolated from necrotic bracts, but unlike the *Fusaria*, they were unable to penetrate and decay unopened green bolls.

The most important factor limiting rot development, both in field and greenhouse studies, was boll age. Bolls 33 to 35 days of age or older were more susceptible than were younger fruits. We feel that the basis for resistance in young bolls is primarily physiological, and that rot is associated with senescence. The possible role of gossypol in boll rot resistance was suggested by Bell (4). Bolls 35 to 45

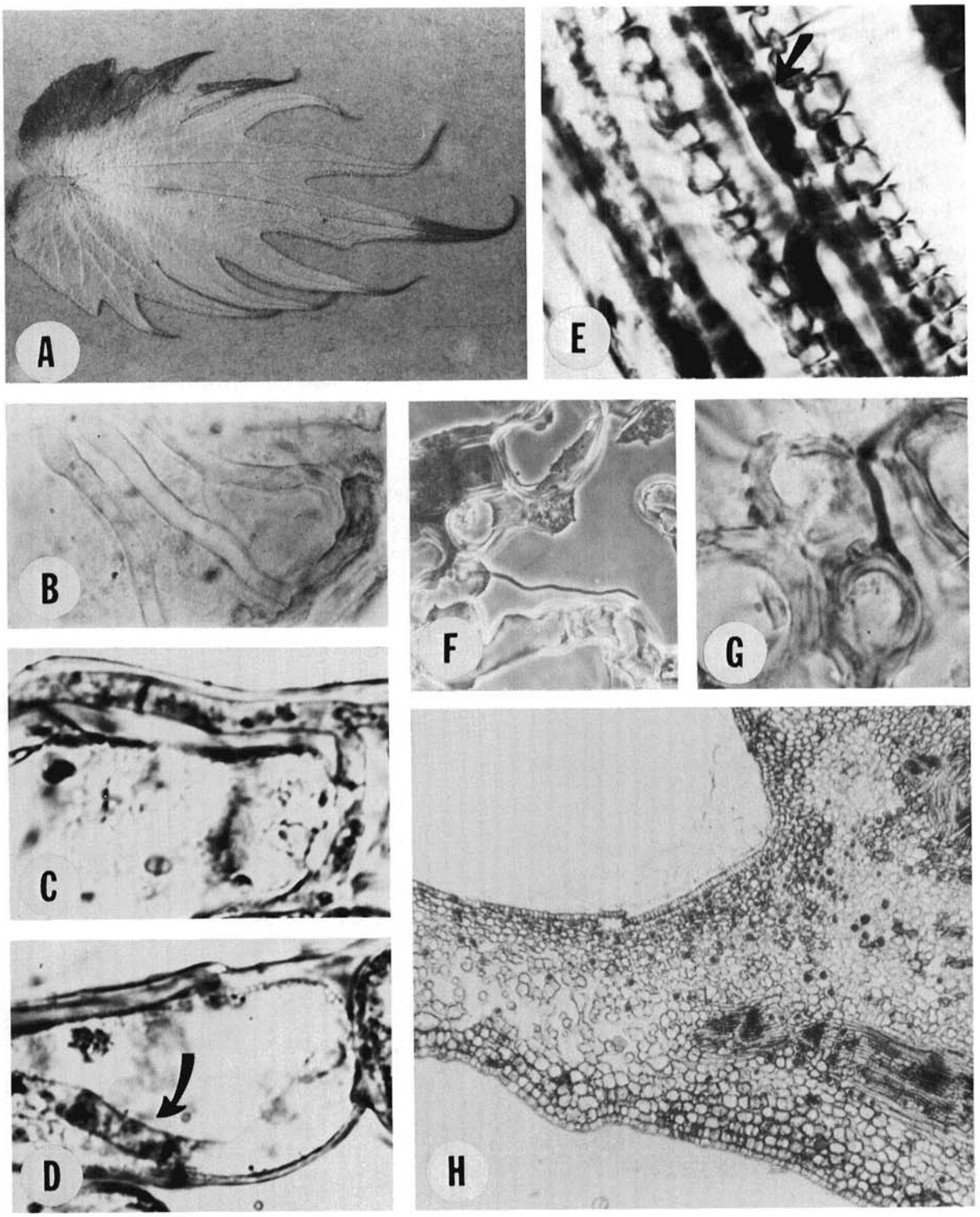

Fig. 3. Initial development of cotton boll rot: necrosis and histopathology of bract and boll invasion. **A)** Necrosis of apices of toothed bract margin caused by *Fusarium roseum*; **B)** Mycelium of *F. oxysporum* associated with the cuticle surface at bract lesion border; **C)** Subcuticular and intercellular hypha of *F. oxysporum* in young bract lesion; **D)** Intracellular hypha of *F. oxysporum* initiating necrosis of an epidermal cell; **E)** Hyphal growth of *F. oxysporum* in vessels within bract; **F)** Intercellular hypha of *F. oxysporum* in bract mesophyll; **G)** Intercellular hypha of *F. oxysporum* in collenchyma of receptacle; **H)** Longitudinal section of a boll receptacle and bract showing continuity of bract mesophyll (left) and collenchyma and parenchyma (right).

days old inoculated with *Verticillium albo-atrum* produced only 10 to 30% as much gossypol as did 15- to 25-day-old bolls. Davis (6) has shown that gossypol in root bark homogenates was toxic to 14 formae speciales of *F. oxysporum*.

Although *Fusarium* spp. generally decayed wounded bolls more effectively than uninjured bolls, *F. roseum* reacted in an opposite manner. Decay of wounded bolls was limited to the inoculated carpel, whereas the inoculated, uninjured bolls often decayed more extensively. In uninjured bolls, "youth resistance" retarded the activity of *F. roseum* more effectively than that of *F. oxysporum* (Table 1). If such resistance is associated with gossypol synthesis, mechanical injury, which also stimulates the production fo the compound (4), may have had an adverse effect on *F. roseum* in the wound inoculation tests.

In addition to serving as an infection court in green boll rot, bracts may be important in decay during dehiscence (2, 10). Infected bracts provided a convenient source of inoculum, particularly when appressed to the pericarp surface. We have observed a high frequency of boll rot in cases where the fungus grows directly from the bract through the opened sutures, and then into the locks. Luke & Pinckard (10) reported a significant reduction boll rot incidence in the field when bracts were removed.

Knowledge of the method of disease development and factors affecting it are essential to a program of breeding for resistance and disease escape. Since basal boll rot due to *Fusarium* spp. is the most prevalent type in Georgia, the use of high gossypol lines along with bract modification may show promise in control.

Although the frego bract is readily attacked by *F. oxysporum*, yield increases due to boll rot control by this character have been reported (9). The susceptibility of the frego bract to other boll rot organisms is not known. However, disease control seems possible, due to the growth habit of the modified bract which causes it to twist away from the boll and minimizes contact with the pericarp surface as well as altering the microclimate in that area (9). Such features were nullified in our inoculation tests.

LITERATURE CITED

1. ARNDT, C. H. 1950. Boll rots of cotton in South Carolina in 1949. Plant Dis. Reptr. 34:176.
2. BAEHR, L. F., & J. A. PINCKARD. 1970. Histological studies on the mode of penetration of boll rotting organisms into developing cotton bolls. Phytopathology 60:581 (Abstr.).
3. BAGGA, H. S. 1968. Fungi associated with cotton boll rot and their frequency. Plant Dis. Reptr. 52:582-584.
4. BELL, A. A. 1967. Formation of gossypol in infected or chemically irritated tissues of Gossypium species. Phytopathology 57:759-764.
5. CAUQUIL, J., & C. D. RANNEY. 1967. Studies on internal infection of green cotton bolls and the possibility of genetic selection to reduce incidence of boll rot. Miss. State Univ. Tech. Bull. 54. 24 p.
6. DAVIS, D. 1964. Host fungitoxicants in selective pathogenicity of Fusarium oxysporum. Phytopathology 54:290-293.
7. EDGERTON, C. W. 1912. The rots of the cotton boll. Louisiana Agr. Exp. Sta. Bull. 137. 113 p.
8. JOHANSEN, D. A. 1939. Plant micro-technique. McGraw-Hill Co., New York. 523 p.
9. JONES, J. E., & J. A. ANDRIES. 1969. Effect of frego bract on the incidence of cotton boll rot. Crop. Sci. 9:426-428.
10. LUKE, W. J., & J. A. PINCKARD. 1970. The role of the bract on boll rots of cotton. Cotton Growing Rev. 47:20-28.
11. MARSH, P. B., M. E. SIMPSON, B. M. WADDLE, & D. C. HARRELL. 1965. Observations on cotton boll rot at Florence, South Carolina in 1964. Plant Dis. Reptr. 49:138-142.
12. MC CARTER, S. M., R. W. RONCADORI, & J. L. CRAWFORD. 1970. Microorganisms associated with cotton boll rots in Georgia. Plant Dis. Reptr. 54:586-590.
13. PINCKARD, J. A., & W. J. LUKE. 1967. Pellicularia filamentosa, a primary cause of cotton boll rot in Louisiana. Plant Dis. Reptr. 51:67-70.
14. RONCADORI, R. W. 1969. Fungal invasion of developing cotton bolls. Phytopathology 59:1356-1359.