Pre-Ripening Damage to Cottonseed by *Aspergillus flavus* Is Not Influenced by Seed Coat Permeability

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Infection of cottonseed (*Gossypium hirsutum* L.) by *Aspergillus flavus* and associated production of aflatoxins are problems in the arid portions of the United States cotton belt. The hard seed (impermeable to water) characteristic confers resistance to these problems in ripened cottonseed. Experiments were done to determine if low seed coat permeability to water or impermeability protect developing seeds from deterioration and aflatoxin formation. No differences were observed in the degree of seed deterioration in the various cotton lines that could be attributed to seed coat permeability. It is likely that, because the impermeable or low permeability phenotypes are expressed only upon seed ripening, these characteristics afford no protection to cottonseed against attack by *A. flavus* during seed development.

KEY WORDS: Aflatoxin, *Aspergillus flavus*, cottonseed, impermeable seed, mycotoxins, seed coat permeability, seed development, seed maturity.

Contamination of cottonseed (*Gossypium hirsutum* L.) with aflatoxin is a major problem in arid portions of the United States cotton belt. Ashworth *et al.* (1) demonstrated that an important aspect of this problem is infection of cotton bolls by *Aspergillus flavus* through exit holes produced by larvae of the pink boll worm, *Pectinophora gossypiella*. Christiansen *et al.* (2) demonstrated that the hard seed coat characteristic (impermeability to water) protects ripened seeds from deterioration. Similarly, cottonseed from lines selected for low seed coat permeability exhibit improved resistance to seed deterioration (3). Mayne *et al.* (4) showed that mature hard seeds are resistant to infection by *A. flavus* and elaboration of aflatoxins. Lee *et al.* (5), however, showed that seeds of a permeable-seeded variety within inoculated bolls were vulnerable to infection and aflatoxin production throughout their development, with highest levels of susceptibility in bolls inoculated about 30 days after flowering. Like the seeds of the "soft" seed coat varieties, seeds of hard-seeded cotton lines are permeable to water throughout their development, and express their impermeable "hard" seed coat trait only upon boll dehiscence and ripening (6). Thus it seems likely that, if the hard seed characteristic affords protection against *A. flavus*, then the resistance of hard-seeded lines to aflatoxin production is manifested after, but not before, boll dehiscence and seed ripening. Experiments were done to determine if seeds of cotton lines demonstrating low permeability or impermeability to water after ripening exhibit resistance to seed deterioration and aflatoxin synthesis prior to ripening.

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EXPERIMENTAL PROCEDURES

Cotton was grown in field plots at the USDA/ARS, Southern Regional Research Center in New Orleans (LA) in 1988. Plants included one commercial cultivar (Deltapine 90), an inbred line selected for seed coat impermeability to water (Rhyne's hard-seeded inbred 16B7), and lines selected within two commercial cultivars (Stoneville 213 and Coker 420) by phenotypic recurrent selection for unusually high or low permeability of ripened seed coats to water.

Plants of each cultivar or line were inoculated 30 days after flowering with spores of an aflatoxin-producing strain of *A. flavus* (AFMC 5A86), through 3-mm holes bored into individual locules as described by Lee *et al.* (5). Plants were maintained in complete randomized blocks. Treatments were replicated five times and each replicate consisted of 4–8 plants. Bolls were harvested after ripening, with bolls from individual plants serving as replicate samples. Each replicate was one analysis of 4–10 bolls combined. Inoculated locules and their adjacent locules were oven dried at 5°C and analyzed separately. Aflatoxin concentrations in seeds were determined by the methods of Lee *et al.* (7). Seeds from non-inoculated bolls on the same plants were used for other seed quality evaluations. These latter seeds were separated from the long fibers, delinted with concentrated H_2SO_4, neutralized in saturated $NaHCO_3$, rinsed with tap water, and air dried. Percentages of normal-appearing seeds (those not exhibiting cracks, holes, shrivelling, or other blemishes) were determined. Seeds were then placed on moist paper towels for one week at 22°C to measure germination. Those that produced a radicle at least 1 cm long were scored as viable. Seeds that failed to imbibe during this week were nicked at the chalaza with a razor blade and subjected to another germination test.

RESULTS AND DISCUSSION

Inoculated locules (locks) from ripened bolls consistently exhibited the "tight lock" characteristic described by Lee *et al.* (5), in which fibers fail to fluff. Conversely, locules adjacent to inoculated ones fluffed normally. Seeds within inoculated locules were quite friable, and had to be handled carefully. Few of these seeds survived acid delinting. Results of aflatoxin determinations, visual examinations (intact seed), and germination tests are summarized in Table 1. Sample-to-sample variability was extremely high (as much as 100% of the means) for all characteristics measured. Thus, there were no statistically significant differences among the cultivars or lines for any of the

TABLE 1

Aflatoxin Concentrations and Seed Quality of Cottonseed from Locules Inoculated with *Aspergillus flavus* Prior to Boll Ripening, and from Adjacent Locules

Variety or line	Aflatoxin concentration (μg/g)[a]		Intact seed (%)		Viable seeds (%)	
	Inoculated locules	Adjacent locules	Inoculated locules	Adjacent locules	Inoculated locules	Adjacent locules
Deltapine	292	4	0	86	0	81
Stoneville						
Low permeability	220	16	12	96	0	61
High permeability	174	10	0	96	0	80
Coker 420						
Low permeability	181	13	8	90	0	47
High permeability	128	7	0	96	0	57
Rhine's Hard Seed	171	5	11	91	0	84
Mean	194	9	5	92	0	68
LSD (0.05)	NS[b]	NS	NS	NS	NS	NS

[a]μg/g = Parts per billion. [b]Not significant.

characteristics. However, even though aflatoxin formation and seed damage did occur in locules adjacent to inoculated ones, toxin formation and seed damage was much less severe in adjacent locules. Clearly, the characteristics of low seed coat permeability or impermeability to water that confer resistance to seed deterioration (2,3) and aflatoxin formation (4) in mature cottonseed afford no protection to developing seeds. Seeds ripen (mature) in bolls at dehiscence. Dehiscence usually occurs 45–50 days after flowering. Because the permeability barrier does not form until the onset of boll dehiscence and seed ripening (6) it is likely to be of little consequence in the resistance to fungal attack in developing seeds.

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