Aflatoxin in Arizona Cottonseed: Lack of Toxin Formation Following *Aspergillus flavus* Inoculation at Sutures

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Aflatoxin assays were conducted on seeds from cotton bolls inoculated with *Aspergillus flavus* in commercial fields in Arizona. Inoculations were at sutures at the initiation of boll opening either in the morning or evening in August over a three-year period. One morning inoculation was followed by a water treatment that simulated rain. Fully fluffed bolls were harvested after two or four weeks, and lint and seed linters were examined for bright-green-yellow-fluorescence (BGYF). Ginned seed were assayed for aflatoxin. While BGYF of lint was detected, no seed linters exhibited BGYF and no seeds from the 140 bolls examined contained aflatoxin. Results imply that boll infection by *A. flavus* occurs before the initiation of boll opening, an observation that is in agreement with recent reports of *A. flavus* infection but contrary to conclusions made in earlier reports.

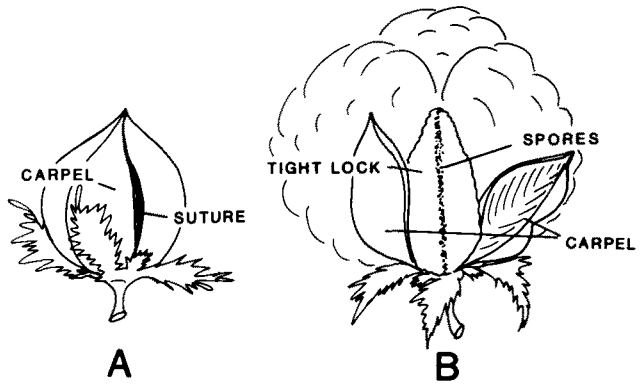

FIG. 1. A. Cotton boll at the initiation of boil opening when carpel walls pull apart, leaving a suture opening. B. Cotton boll showing fully fluffed locks and one tight lock with spores at the position the suture opening occupied at the initiation of boll opening.

Cotton grown in the valley regions of Arizona and southern California is prone to contamination with *Aspergillus flavus*. Infection of cotton bolls by this fungus results in a bright-green-yellow-fluorescence (BGYF) on lint (1) and possible aflatoxin contamination of the seeds from infected locks. Infected locks do not fluff because fungal hyphae weaken fibers (2). Such locks are termed "tight." (Fig. 1 B). Early reports (2-4) postulate entry of the fungus into bolls at suture openings (Fig. 1 A). Differences in the rate of suture openings were assumed to be the reason for differences in boll infection at two locations in California (2). The same authors assessed boll infection by the amount of BGYF on lint from bolls harvested at full maturity following inoculation with *A. flavus* both at initiation of, and after suture opening (3). No toxin assays were conducted. In another study (4), toxin formation was investigated in bolls detached from plants prior to inoculation. Their essentially negative results were attributed to inadequate aeration in such bolls that prevented fungal growth. Gardner et al. (5) inoculated bolls through puncture wounds on plants prior to opening of sutures and assumed that the high toxin levels they observed in seeds paralleled levels that would be found following natural inoculation at the suture openings (Fig. 1 A).

Since the landmark report by Ashworth et al. in 1971 of the relationship of insect wounds on unopened bolls to secondary infection by *A. flavus* (6), little research effort has been expended on infection by *A. flavus* at suture openings. However, due to the earlier reports (2-4) the assumption has persisted that seed infection can occur following inoculation at these natural openings; other authors interpreted their results accordingly (7). Recent reports (8,9) of natural openings early on in boll development as the site of seed infection by *A. flavus* prompted a reexamination of suture openings as another possible natural mode of entry of this fungus into cotton bolls to form toxin in seeds. Knowledge of the time of fungal entry predicates the effectiveness of preventive measures.

EXPERIMENTAL PROCEDURE

Experiments were conducted in commercial fields in low altitude areas near Phoenix during the first two weeks in August in 1984, 1985 and 1986. Bolls with suture openings no greater than three mm were selected. Inoculation was with spores of SRRC 2002, a toxigenic isolate of *A. flavus* obtained from Arizona cotton. Spores were inoculated onto autoclaved rice and incubated for seven days. Spores harvested from the rice were applied to the bolls by various techniques. In 1984 spores were applied by aerosolization. For these experiments rice was inoculated in 125-ml side-arm suction flasks plugged with cotton in both the side-arm and the mouth. Just prior to field inoculations a drilled rubber stopper, with a Pasteur pipette inserted through the opening with the point upward, was substituted for the cotton plug in the mouth of each flask. The side-arm cotton plug was replaced by a rubber bulb so that a sharp squeeze forced spores out of the pipette. The technique allowed direction of spores to suture openings. In 1985 the aerosol inoculations were repeated and, following inoculation, half of the bolls were sprayed with a mist of water that simulated dew or light rain. Fifty bolls were treated each of these years. Bolls were harvested one month following inoculation and lint observed for BGYF

under ultraviolet light (1). Bolls were ginned individually and the lintered seed were also examined for BGYF. Seeds (20 or 30 from each boll) were assayed for aflatoxin as a composite sample by the method used by Lee et al. (7).

Postulating that rate of boll opening might be slower at night, resulting in slow drying that would maintain moisture in the lock at a level required for fungal growth, 1986 inoculations were performed both at daybreak and just prior to sunset. Twenty bolls were inoculated at each time. Spores harvested from rice grown in a standard Erlenmeyer flask were gently inserted under the carpel wall at suture openings with a small paint brush filled with spores. Fully fluffed bolls were harvested two weeks following inoculation and handled in the same manner as in previous years.

RESULTS AND DISCUSSION

BGYF was detected on lint from some of the water-treated bolls in 1985 and on bolls inoculated just before sunset in 1986 (Table 1). The overnight temperature on this evening was 35.5 C, the highest average overnight temperature ever recorded in the Phoenix area. None of the seeds from bolls harvested in each of the three years exhibited BGYF on the short linter fibers that remain attached to seeds following ginning (Table 1). Only faint traces of BGYF were detected on lint from the non-wetted bolls both in 1984 and in 1985. No toxins were detected in any of the seeds from the 140 bolls harvested in each of the three years (Table 1).

TABLE 1.

Type of Inoculation, BGYF and Aflatoxin in Bolls Inoculated at Suture Openings

Type of inoculation	Bolls with: BGYF Lint/ Total bolls	Seeds having BGYF linters	Seeds having aflatoxin
Aerosol (no water)	0/75[a]	0	0
Aerosol (followed by water)	10/25	0	0
Direct application (morning)	0/20	0	0
Direct application (evening)	6/20	0	0

[a]Faintly visible fluorescence detected in two bolls.

Toxin formation is predicated on fungal infection of seed. Low toxin levels were detected in seeds following inoculation of developing bolls by a soil-spore mixture (7). Higher toxin levels were detected in seeds following drill inoculation of unopened bolls (10,11). Since toxins were detected in these field studies, it follows that fungal growth did progress from inoculation sites to seeds. A recent study reports initiation of toxin formation between six and 10 days following inoculation and offers microscopic evidence of fungal penetration into seeds six days following inoculation (11). The 1969 study (3) reported a rapid drying of bolls from 52-54% moisture at suture opening to 5-6% moisture when bolls were nearly fluffed. Results of the present study where BGYF was detected on lint corroborate those reported earlier (2,3). However, both the lack of BGYF on seed linters and the lack of aflatoxin in seed indicate lack of fungal growth from the inoculation site on into the seed area. *A. flavus* probably requires the high moisture level encountered in unopened bolls for maximum fungal growth and subsequent seed invasion. Rapid opening of bolls (from 1 to 3 days following cracking of sutures) and the concomitant drying probably do not allow sufficient moisture for such growth.

Most field fungi require air, especially for sporulation. Therefore, the author's observation on several fluffed bolls with one tight lock (with *A. flavus* spores on the tight lock at the location of the crack in the carpel wall where normal suture opening occurred) probably are due to fungal outgrowth to this natural opening (Fig. 1 B) where air enhanced sporulation. It follows that the sporulation observed at these suture openings is a result of an earlier fungal infection and was not the source of infection.

While the present study did not address all of the environmental factors that could aid fungal entry at suture opening, it was conducted in a potentially potent aflatoxin area of Arizona at the time when *A. flavus* propagules in air have been shown to be dramatically high (7). Lack of aflatoxins in seeds following boll inoculation at suture openings strengthens the hypothesis that boll infection by *A. flavus* to cause toxin in seeds occurs before the initiation of boll opening either through natural openings during early boll development (8,9) or by secondary infection following boll injury, probably insect vectored (6,11).

ACKNOWLEDGMENTS

The National Cottonseed Products Association provided travel funds for L. S. Lee to Arizona; L. V. Lee Jr. provided technical assistance in 1986; J. Brockmann provided art work.

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[Received April 16, 1987]