# Effect of Harvest Date on Aflatoxin Contamination of Cottonseed

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**ABSTRACT**

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In 1987 and 1988, aflatoxin in cottonseed was three to 12 times higher in seed harvested at the Yuma Valley Agricultural Center near the end of the commercial harvest season than at the beginning in the Yuma Valley of Arizona. Most toxin was detected in seed from tight locks and locks with lint exhibiting bright green-yellow fluorescence. Evidence from laboratory experiments confirmed observations on field-grown cotton that aflatoxin levels may increase in fully mature cottonseed both before and after harvest. Toxin increases occurred over a broad range of temperatures (16–37 C) if the relative humidity was 93% or greater. Early harvest is recommended to improve management of aflatoxin contamination of Arizona cottonseed.

Aflatoxins are toxic metabolites produced by the fungi *Aspergillus flavus* Link:Fr. and *A. parasiticus* Speare during plant infection (13). These natural toxins are potent carcinogens and their presence in foods and feeds is regulated (9). Segments of the cottonseed industry have experienced losses attributable to aflatoxins for more than 25 yr (3,27). *A. flavus* is the predominant cause of aflatoxin contamination of cottonseed (9). In addition to aflatoxin in the seed, *A. flavus* produces kojic acid on the lint (25). Host peroxidases convert the kojic acid to a substance that exhibits bright green-yellow fluorescence (BGYF) (25). Presence of BGYF on lint is an indication that *A. flavus* has infected the lint before boll maturity (2,26).

The cotton growing areas around Yuma, AZ, and El Centro, CA, have some of the highest reported incidences of aflatoxin contamination of cottonseed (28). Improved insect control in these areas has been associated with reduced aflatoxin contamination in some cases (25,31) but not in others (21). Ashworth, however, clearly associated infection by *A. flavus* with pink bollworm (PBW) damage, and we recently found more than 95% of the aflatoxin in two fields in the Yuma Valley in bolls with PBW exit holes (7,12). Relatively little toxin is formed in bolls produced near the end of the season and most toxin occurs in bolls borne near the soil (2,30). Therefore, management of cotton crops to avoid losses attributable to aflatoxin contamination should include provisions to limit PBW damage to bolls forming early in the season (12). In addition, Russell et al (31) demonstrated that aflatoxin contamination was lowered by reductions in late-season irrigation.

Host-free periods have become essential to insect management in western cotton growing regions, and this has necessitated earlier harvest dates (20,34). Furthermore, the importance of winter vegetable crops in the Yuma and Imperial areas, quality loss associated with unfavorable late season weather, and costs associated with extended seasons have enforced the trend toward shorter season cotton (15,33). This study was undertaken to determine the impact of timely harvest on aflatoxin contamination of cottonseed in the Yuma Valley.

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## MATERIALS AND METHODS

**Field tests.** In both 1987 and 1988 at the Yuma Valley Agricultural Center near Yuma, AZ, 0.8 ha of cotton were planted in mid-March on a silty clay loam soil in rows on 100-cm centers. A randomized complete block design was used with two treatments and six replicates in 1987 and three treatments and eight replicates in 1988. The treatments were the harvest dates. In 1987, cv. Deltapine (DP) 90 was planted throughout the plot. In 1988, four rows of DP 90 were alternated with four rows of DP 61 in each block. Plots were furrow-irrigated seven times with the final irrigation 12–15 days before the first harvest. Insecticidal sprays to control the pink bollworm were not applied until 10–15% of the bolls were infested in 1987 and 25–30% were infested in 1988 to ensure high levels of pink bollworm damage. Bolls forming early in the season were tagged while immature. In 1987, 60 20- to 30-day-old bolls were tagged in each replicate plot on 14 July. In 1988, 60 bolls of DP 90 and 60 bolls of DP 61 were tagged in each replicate plot on 14 July. Tagged bolls were harvested at either the beginning (15 September 1987 or 23 August 1988), middle (28 September 1988), or end (27 October 1987 or 25 October 1988) of the commercial harvesting period in the Yuma Valley. Harvested bolls were dried for 3 days at 60 C. Locks from the bolls were sorted by condition into fully fluffed or tight (insect damaged) categories and delinted either with a small laboratory gin (fluffed locks) or by hand (tight locks). In 1988, the fluffed locks were divided before ginning into two groups based on the presence of bright green-yellow fluorescence (BGYF). Weather information over the period of the tests was obtained through the Arizona Meteorological Network station located within 200 m of the field plot.

**Laboratory tests.** A highly aggressive and toxigenic strain of *A. flavus*, AF 13, isolated from soil in the Yuma Valley in 1988 (11), was used in all studies. Active cultures were maintained in the dark at 30 C on 5% V-8 juice, 2% agar. For long-term storage, plugs (3 mm in diameter) of sporulating cultures were submerged in 5 ml of sterile distilled water and kept at 8 C.

Cotton plants of cultivar DP 90 were grown in a greenhouse in 3-L pots containing a 50:50 mixture of Pro-mix (Premier Brands Inc., New Rochelle, NY) and sand. After 21 days, plants were fertilized weekly with 100 ml of 2,000 ppm Miracle-Gro, 15-30-15, (Sterms Miracle-Gro Products, Inc., Port Washington, NY). Flowers were dated at opening.

The effect of relative humidity and temperature on infection of and $B_1$ production in mature bolls by *A. flavus* was tested with greenhouse-grown plants. Fully mature bolls were excised at the pedicel. Intact mature bolls were misted with 3 ml of an aqueous spore suspension containing approximately $3 \times 10^6$ spores per milliliter and incubated at 100% relative humidity (RH) on a screen placed 2 cm above 500 ml of distilled water in a sealed plastic container ($21 \times 30 \times 8$ cm) for 10 days at either 16, 22, 27, 32, or 37 C. In addition, the time course of toxin accumulation in bolls inoculated in this manner was evaluated over a 7-day period at 30 C.

Greenhouse-grown plants were also used in tests on the effect of periodic increases in relative humidity after boll maturity on toxin content of bolls infected before maturity. Bolls were

inoculated before maturity as previously described (12). Immature bolls (34–36 days old) were wounded in a single locule each with a cork borer (3 mm in diameter) (23) to simulate pink bollworm exit holes. Wounds were inoculated with 10 μl of an aqueous spore suspension containing approximately 5,000 spores of *A. flavus*. At maturity, inoculated bolls were excised from the plants and dried at 40 C for 3 days. The bolls were then incubated on a screen above either distilled water or a saturated salt solution in a closed container for 0–12 days at 30 C as above. Saturated salt solutions were selected sensu Karon (22) as follows: $NH_4H_2PO_4$ (93% RH), $(NH_4)_2SO_4$ (81% RH), NaCl (75% RH), and $NH_4NO_3$ (64% RH).

After incubation, bolls were dried at 60 C for 3 days and stored at room temperature with silica gel dessicant until analyzed. Wound-inoculated locules and unwounded locules from the same bolls were analyzed separately. All greenhouse and laboratory experiments were performed twice and contained three to six replicates.

**Aflatoxin analyses.** Seeds were extracted with an acetone-water solution following a modification of the method of the Association of Official Analytical Chemists as described earlier (10,32). The extract was reacted with a zinc acetate-aluminum chloride solution and the liquid phase was separated from the seeds by filtration. Aflatoxin was extracted from the filtrate with methylene chloride and then further purified with thin-layer chromatography (TLC). Aflatoxin $B_1$ was quantified based on direct densitometric measurement of fluorescence on TLC plates.

**Statistical analyses.** Analyses were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Data from the 1988 field study were subjected to a two-way ANOVA with cultivar and harvest date as class variables. All multiple comparisons were first subjected to an analysis of variance. Simple linear regressions were calculated with the least squares method.

## RESULTS

**Field tests.** In 1987, more than 28,000 ppb of $B_1$ was found in seed from locks that did not fully fluff out at maturity (tight locks) and were harvested near the end of the commercial harvest season. In contrast, only 8 ppb was detected in seed from fluffed locks that were harvested 42 days earlier. The mean concentration in total seed from the earlier harvest, 83 ppb, was significantly ($P = 0.05$) lower than that for the later harvest, 1,023 ppb. In both harvests, seed from tight locks contained more than 200-fold greater concentrations of $B_1$ than seed from fluffed locks. In 1988, cultivar did not affect toxin accumulation in seed, and the interaction of cultivar and harvest date was not significant. Data for DP 90 and DP 61 were therefore combined in subsequent analyses. The toxin content of seed from both tight and fluffed locks increased between 23 August and 28 September but did not change significantly ($P = 0.05$) between 28 September and 25 October (Table 1). The average toxin content of the crop increased just threefold in 1988 (Table 1) compared with 12-fold in 1987. The log of the toxin concentration in the seed at harvest in 1988 was significantly ($P = 0.01$) correlated with the number of days postanthesis that the bolls were held in the field. However, the coefficients of determination were very low ($R^2 = 0.13$–$0.29$).

During both years, the maximum temperature at the Yuma Valley Agricultural Center ranged from 27 to 44 C during the harvest period. The minimum temperature ranged from 16 to 28 C. In 1987, measurable rain occurred between harvests on 3 days with rainfall of 6, 1, and 1 mm. In 1988, rain occurred once (9 mm) between the first two harvests and once (6 mm) between the second and last harvest.

**Laboratory tests.** Seed in intact bolls that were spray-inoculated in the laboratory after maturity and incubated at 100% RH became readily infected with *A. flavus* and contaminated with aflatoxins. Aflatoxins were detected in the seed within 24 hr after inoculation and the concentration of aflatoxins increased logarithmically with incubation time for 7 days (linear regression log aflatoxin vs. time, $P = 0.001$, $R^2 = 0.77$). Aflatoxins were produced in mature bolls at all temperatures tested. After 10 days, the level of toxin in bolls incubated at between 27 and 37 C did not differ significantly ($P = 0.05$). The highest level (29 μg/g) was in bolls incubated at 37 C and the lowest (6 ng/g) in bolls incubated at 16 C. Toxin accumulation increased with incubation temperature from 16 to 37 C (linear regression, log aflatoxin vs. temperature, $P = 0.001$, $R^2 = 0.48$).

Toxin contents of both unwounded and wounded locks taken from wound-inoculated bolls increased logarithmically with time in the laboratory at 100% RH and 32 C (linear regression, log aflatoxin vs. time, $P = 0.001$, $R^2 = 0.76$ and $P = 0.001$, $R^2 = 0.46$, respectively). Toxin concentrations increased from 2 ng/g to 22 μg/g in unwounded locks and from 2 to 213 μg/g in wound-inoculated locks during the 12-day experiment. At 30 C, aflatoxin concentration in wound-inoculated locks increased over a 10-day period at 100 and 93% RH but not at 81% RH or lower.

## DISCUSSION

Commercial harvest of cotton typically extends longer than 10 wk in the desert valleys of Arizona and southern California where crops may be left in the field until it is convenient for the grower to harvest (8). Prolonged exposure of mature cotton to conditions in the field may result in increased levels of aflatoxins in the seed. In field tests reported here, concentrations of aflatoxins in seed from bolls formed early in the season increased up to 12-fold over a 42-day period during the commercial harvest season. Similar aflatoxin increases have been observed on wound- and silk-inoculated corn exposed to late season rain (27).

Levels of aflatoxins detected in the field tests reported here exceeded 1,000 ppb in both years. These levels are higher than those expected in commercial crops, which have much lower levels of pink bollworm damage and a mixture of seed from bolls that matured on different dates throughout the season.

Results reported here agree with previous reports on the influence of temperature on aflatoxin production by *A. flavus* (5,18). Aflatoxin concentrations in the crop will increase over a broad range of temperatures (16–37 C in the current study) if humidity requirements are met. In tests reported here, toxin was produced in cottonseed incubated at 93% RH but not at 81%. This agrees with reports on aflatoxin contamination of peanuts, where contamination occurred above but not below 85% RH (14). Seed produced in areas where aflatoxin contamination is severe are often either infected with *A. flavus* or superficially associated with conidia of *A. flavus* (3,24). In laboratory tests reported here, aflatoxin occurred in spray-inoculated bolls after just 24 hr at

**Table 1.** Quantity of aflatoxin $B_1$ detected in seed from fluorescent and nonfluorescent fluffed locks and in seed from tight locks harvested on several dates during 1988

| Lock category[a] | Subcategory | Aflatoxin $B_1$ (ng/g) | | |
|---|---|---|---|---|
| | | 23 August | 28 September | 25 October |
| Tight | | 3,865 | 12,717 | 12,872 |
| Fully fluffed | Nonfluorescent | 257 | 892 | 1,122 |
| | Fluorescent | 2,419 | 11,681 | 6,519 |
| Total | | 2,555 | 7,316 | 8,162 |

[a] For each treatment, 60 immature bolls were tagged on 15 July in each replicate plot. Only tagged bolls were harvested and analyzed for toxin content. Each value is an average of 16 replicates. In each category, values for 28 September and 25 October differ significantly ($P = 0.05$ by Fisher's LSD test) from values for 23 August but not from each other. Values were log transformed before analysis.

100% RH. Incremental toxin increases may be expected during similar periods of high humidity in the field. Aflatoxin increases should occur even more rapidly in seed infected before the humidity period. Therefore, timely harvest and utilization of cottonseed are important components of a sound overall management program directed toward limiting aflatoxin contamination of cottonseed.

Aflatoxin contamination of cottonseed in Arizona occurs in at least two phases. The crop is first contaminated with aflatoxins when *A. flavus* infects bolls damaged by pink bollworms or other means, before maturity (7,20,31). This first phase is associated with BGYF on lint and it causes most of the contamination in the western desert valleys in some years (12). A second phase occurs when mature bolls are exposed to high humidity or rewetting at or after opening (4,16,24). Increases in toxin concentrations associated with this second phase can occur both before and after harvest as the crop is held in modules and seed piles (17,19,29). Bolls forming early in the season become more contaminated than later formed bolls (2,30). Timely harvest should decrease the severity of both phases by reducing aflatoxin increases in early bolls after maturation and by reducing overwintering PBW populations and subsequent PBW damage to the early bolls of the next crop (1,20,34).

The cotton season in the Arizona desert valleys has been shortened considerably since the mid-1960s when aflatoxin contamination of cottonseed was first identified as a serious problem (6,8). Cotton is no longer grown in December and January in the Yuma area, where aflatoxin contamination has historically been most severe (8). Most cotton is harvested before November in this area, and the trend has been toward an even earlier crop to reduce late season expenses and facilitate winter crops (15,33). Lower aflatoxin levels experienced by the industry in the 1980s as compared with the 1960s and 1970s probably can be attributed, at least partially, to the shorter season.


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