

## Seasonal Formation of Aflatoxins in Cottonseed Produced in Arizona and California

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### ABSTRACT

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Open cotton bolls were hand-harvested at 10- to 15-day intervals throughout two consecutive growing seasons and assayed for aflatoxins. In both years, the percentages of total toxins (70.43% the first year and 78.97% the second year) and toxin levels were highest in seed maturing before 1 September. These toxins were detected in 67% of the total seed harvested the first year and in 55% of the seed harvested the second year. A 500-fold variation in toxin levels was observed in one field between seasons. Maximum field-to-field variation was 230-fold. Levels of toxin were less in early-flowering, second-year, perennial cotton than in annual cotton grown in parallel plots. The critical period for toxin formation was a 30- to 45-day interval commencing about the date of initial boll opening.

Additional key words: *Aspergillus flavus*

Contamination of cottonseed by aflatoxins B<sub>1</sub> and B<sub>2</sub> is a chronic problem

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in some production areas of Arizona and the Imperial Valley of California (5,8). The high temperatures found in these production zones are ideal for development of *Aspergillus flavus* (2), boll and seed invasion by the fungus (6), and aflatoxin formation in the seed (2). Ashworth and McMeans (1) and McMeans and Ashworth (9) found that fruits lower on the plants were more likely to have aflatoxin contamination than those higher on the plants.

Short stapled or Upland cotton (*Gossypium hirsutum* L.) is the major species grown in Arizona and California, where aflatoxins are commonly detected in seed. The commercial cultivars have indeterminate growth habits in that squares (preflowers) and flowers are

produced along with vegetative growth of the plant and may continue to be produced until the growing season is terminated by chemical defoliation or frost. The average initial flowering date in these areas is about 15 June. After fertilization, bolls reach full size within about 21 days, then an additional 21-35 days are required for complete maturation and boll opening. Boll opening occurs vertically up the plant and laterally outward from the main axis until about November (4). The average date for initial boll opening at the bottom of the plant in aflatoxin-prone areas is about 1 August. Bolls from stub cotton mature earlier. Stub or perennial cotton refers to the practice in which cotton plants are ratooned at the end of the normal growing season, protected in the winter, then allowed to regrow in the spring. The advantage of this method of growing cotton over an annual regime is that the plant has a longer production season, bolls open earlier, and yields are much higher. This study was undertaken to define the chronological formation of aflatoxins during two consecutive production seasons in commercial fields in Arizona and the Imperial Valley of California, where aflatoxin contamination of cottonseed is a common occurrence. Results obtained on second-year perennial

cotton were compared with results on cotton planted annually in parallel plots.

## MATERIALS AND METHODS

**Sampling sites.** First-year sites (12–24 replicates) were established in commercial fields: three in the Imperial Valley of California and eight in Arizona fields. Two fields in Arizona were located at Yuma (YM), two at Buckeye (BK), three at Casa Grande (CG), and one at Phoenix (PX).

Second-year sites (nine replicates) were established in commercial fields: three in the Imperial Valley (IV) of California and 27 in Arizona. Six fields in Arizona were located at YM, six at Gila Bend (GB), four at Harquahala Valley (HV), six at BK, two at PX, two at Maricopa (MC), and one at CG. Stub fields at MC, PX, BK, HV, and GB corresponded to planted fields at these locations. Fields were equally divided between stub and planted.

Bolls (carpels plus seed cotton) were harvested during the first year from a minimum of four single-row sites (four per 8.2 ha), each 4 m long (1/1,000 of 0.41 ha). These sites were established in both the irrigation ditch (upper) and tailwater (lower) ends of cotton fields. During the second year, the number of sampling sites was reduced to four per 16.4 ha. All sampling sites were at least 300 m from the field perimeter. Fifteen to 60 plants in each sampling site were hand-stripped of all open bolls within a 5-day interval around the middle and end of each month beginning on 15 August and continuing whenever matured bolls were present or until all were harvested. Because of earlier boll opening, harvest of stub cotton began after 15 July. A boll was considered mature if carpel sutures were separated and dehiscence had commenced. Bolls that were totally rotted and showed gross signs of *A. flavus*, *A. niger*, *Rhizopus* spp., or other fungi or bacteria were not harvested. These were judged not to be harvestable by the commercial spindle type harvester. All carpels and seed cotton from each site were kept intact and placed in paper bags marked by date, field, and field sampling site and returned to the laboratory for processing. All samples were stored in a building with uncooled forced-air ventilation.

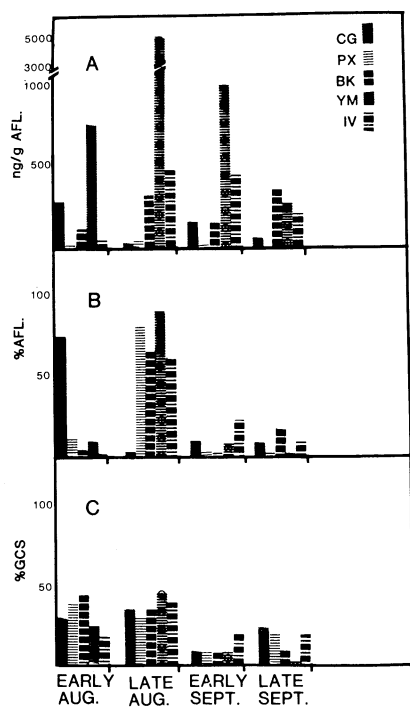
**Boll processing.** Seed cotton (attached lint and seed) was separated from the adjoining carpels for each group of bolls harvested from each replicate in each sampling site. Separated seed cotton was ginned with a laboratory roller gin. Seeds were decorticated in a Bauer double-disk lab mill (The Bauer Bros. Co., Springfield, OH). Linters and hulls were separated from the seed meats or kernels by screening and then discarded. The remaining kernels were ground in the Bauer mill to a flour that would pass a no. 18 screen. In both years, the quantity of

ground cottonseed available in any given sample for aflatoxin analysis ranged from 5 to 350 g. When available, multiple 25-g samples were analyzed for each sampling site. When sample sizes were smaller than 25 g, the whole amount was analyzed. Aflatoxin analysis was by Pon's high-performance liquid chromatography method (10). For statistical analysis, all values for aflatoxin were transformed using a square root transformation ( $x + 1$ ) to obtain normally distributed values. All percentages are reported in terms of actual measured aflatoxin levels.

About 45,000 bolls were harvested from first-year plots and 956 samples of ground fuzzy seed were analyzed. In the second year, about 68,000 bolls were harvested and 1,210 samples of fuzzy cottonseed were analyzed.

## RESULTS

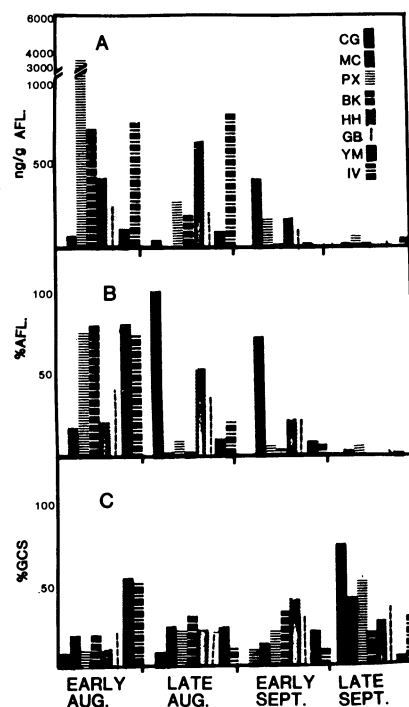
The averages of values obtained at each location for each year on levels of toxins (ng/g of seed), percentage of total toxins, and percentage of ground



**Fig. 1.** Seasonal sampling of planted cotton during the first growing season in Arizona and California. Field locations: Arizona—Casa Grande (CG), Phoenix (PX), Buckeye (BK), Yuma (YM); California—Imperial Valley (IV). (A) Total aflatoxins are average levels of toxins (ng/g of seed) at each location. (B) Percentage of aflatoxin (% AFL) elaborated by location for sampling date was determined from the equation:  $[(\text{ng/g for B1} + \text{B2 in 25-g subsamples GCS of sample B}) / (\text{total wt in g of GCS sample B}) + (\text{ng/g} \dots X) / (\text{wt} \dots X)] \times 100 / (\text{total ng of B1} + \text{B2 detected for all replicates of all sampling dates})$ . (C) GCS percentages were determined from the equation  $[(\text{GCS wt for harvest date}) / (\text{total GCS wt for all dates})] \times 100$ .

cottonseed harvested at each location at each sampling period are presented in Figures 1–3. Second-year results are separated into those obtained on planted and stub cotton. Levels of aflatoxin and percentage of total toxins are averaged across all sampling sites established in commercial fields (Table 1). Total aflatoxins increased until early or late August, then levels declined. Regression analysis indicated a significant quadratic relationship between transformed toxin data and time.

**First season.** Percent toxins were highest in seed harvested from both states in August (Fig. 1). Fields in the YM area of Arizona had seed with the highest toxin levels detected in early August (102 and 1,340 ng/g). One field yielded seed with the highest level of toxins detected in this study (6,756 ng/g). This high level of toxin was detected in seed harvested in the second (late August) sampling period. In the third sampling in early September, an average toxin level of 1,100 ng/g was detected. The percentage



**Fig. 2.** Seasonal sampling of planted cotton during the second growing season in Arizona and California. Field locations: Arizona—Casa Grande (CG), Maricopa (MC), Phoenix (PX), Buckeye (BK), Harquahala Valley (HH), Gila Bend (GB), Yuma (YM); California—Imperial Valley (IV). (A) Total aflatoxins are average levels of toxins (ng/g of seed) at each location. (B) Percent of aflatoxin (% AFL) elaborated by location for sampling date were determined from the equation:  $[(\text{ng/g for B1} + \text{B2 in 25-g subsamples GCS of sample B}) / (\text{total wt in g of GCS sample B}) + (\text{ng/g} \dots X) / (\text{wt} \dots X)] \times 100 / (\text{total ng of B1} + \text{B2 detected for all replicates of all sampling dates})$ . (C) GCS percentages were determined from the equation  $[(\text{GCS wt for harvest date}) / (\text{total GCS wt for all dates})] \times 100$ .

of total aflatoxins represented by these values was highest in the second sampling period (49%) than in either the first or in the third sampling period. More seed were harvested in the second sampling period; 45% of the ground cottonseed (GCS) were harvested at YM in late August, 20% in early August, and 25% in early September. The same general pattern of higher toxins in late August emerged from data obtained in all other Arizona fields except those in the CG area. All three of the CG fields produced

more toxins in early August (300 ng/g) than in late August (17 ng/g), yet the GCS value was slightly higher in the second sampling than in the first.

Although there was little variation in the amount of seed harvested (GCS) from field to field, there was considerable field-to-field variation in toxin levels. The three CG fields, during the first sampling period, yielded toxin levels of 96, 140, and 666 ng/g. In early September, one of these fields had a toxin level of 456 ng/g and the other two had none.

**Second season.** The planted field in the PX area of Arizona was the most contaminated, with a toxin level of 3,221 ng/g (Fig. 2). This level was detected during the first sampling period in just 12% of the seed and represented 80% of the total aflatoxins. The only field in Arizona with toxin levels that did not fit the pattern of increased contamination in August was at MC. A toxin level of 399 ng/g was detected in 13% of the GCS in seed harvested there in early September.

Field-to-field variation of toxin levels in planted cotton could be assessed in the YM, GB, HH, and BK areas of Arizona. The BK fields yielded seed with similar toxin levels in the first sampling period (715 and 737 ng/g); the HH fields varied (86 and 718 ng/g) as did those at GB (43, 211, and 492 ng/g). Toxins in the six fields at YM ranged from 63 to 100 ng/g. In the second sampling period, four of the YM fields had no toxins, whereas two had 230 and 480 ng/g of toxins.

Field-to-field variations in GCS were not great within one sampling period in an area, but there was a noted difference in this value at different sampling times. At CG, MC, and PX, percent GCS was greater in late September than at any other time.

Data from California paralleled the general pattern observed in Arizona; the three fields in the IV had more seed (GCS) with toxins harvested in early August than were harvested either in late August or in early September. Field-to-field variation was large, whereas GCS harvested during the first sampling period was fairly uniform; toxin levels ranged from 98 to 1,840 ng/g. During the second sampling period, values ranged from 1,865 to 8 ng/g. In this second (late August) sampling period, the highest

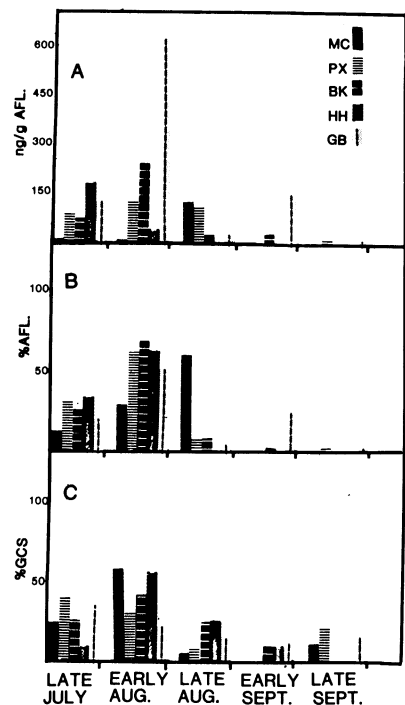
level of toxin (1,865 ng/g) was from seed that represented only 5% of the GCS. High levels of toxin were detected even in early September; the IV field that had a high toxin level in early and late August also had a high level of toxin in early September (2,346 ng/g). Again, these samplings yielded a concentration of toxin seed in a small amount of GCS; this 2,346 ng/g of toxin was detected in just 9% of the GCS. Conversely, no toxins were detected in seed from the second field in the IV area in September, and 10 ng/g were detected during this sampling period in the third field.

Stub cotton had less aflatoxin than its planted counterpart (Fig. 3, Table 1). The highest level of toxin (637 ng/g) was detected at GB. The three fields in that area had toxin levels of 0, 503, and 1,407 ng/g. Levels from all other locations were lower than 250 ng/g. With the exception of three fields, about half of the toxin produced during the season was detected in samples from the second bi-weekly August sampling period. About 93% of the total aflatoxins in 62% of the total GCS could be accounted for in the combined late July and early August sampling periods.

## DISCUSSION

In addition to high toxin formation in August, two other salient points emerge from this study: variation in toxin levels from field to field within an area and season-to-season variation in a field. The PX field yielded a high toxin level (>3,000 ng/g) in the second season, yet in the first season, this field had only 6 ng/g, a 500-fold difference. Weather factors could account for these seasonal differences, or if *A. flavus* contamination is insect-vectored, a season-to-season change in insect populations might have been the cause of toxin variations. The 230-fold field-to-field variation of toxins encountered in California in the second season had to be due to factors other than weather because all fields in an area would be exposed to about the same weather conditions.

Even though toxin levels were low in stub cotton, the seasonal occurrence of aflatoxins in the stub cotton paralleled the findings of the first-season planted cotton in that significantly more aflatoxins were detected during the



**Fig. 3.** Seasonal sampling of perennial (stub) cotton during the second growing season in Arizona. Field locations: Arizona—Maricopa (MC), Phoenix (PX), Buckeye (BK), Harquahala Valley (HH), Gila Bend (GB). (A) Total aflatoxins are average levels of toxins (ng/g of seed) at each location. (B) Percent of aflatoxin (% AFL) elaborated by location for sampling date were determined from the equation: [(ng/g for B1 + B2 in 25-g subsamples GCS of sample B) (total wt in g of GCS sample B) + (ng/g . . . X) (wt . . . X)] × 100 / (total ng of B1 + B2 detected for all replications of all sampling dates). (C) GCS percentages were determined from the equation [(GCS wt for harvest date) / (total GCS wt for all dates)] × 100.

**Table 1.** Statistical analysis of results from aflatoxin analyses obtained on cotton harvested in two consecutive growing seasons

Sampling time	Mean aflatoxin levels (ng/g)			Percent of total aflatoxins			Mean of transformed value <sup>a</sup>		
	First year		Second year	First year		Second year	First year		Second year
	Planted	Planted	Stub	Planted	Planted	Stub	Planted	Planted	Stub
Late July	...	...	82.88	...	...	19.89	...	...	7.86
Early Aug.	219.53	459.89	222.83	11.13	50.18	53.48	10.32	15.92	11.01
Late Aug.	1,169.80	263.79	50.97	59.30	28.79	12.23	23.57	11.32	6.01
Early Sept.	367.80	180.26	50.64	18.64	19.67	12.15	14.40	7.71	4.15
Late Sept.	215.60	12.47	9.33	10.93	1.36	2.24	10.91	2.52	2.79

<sup>a</sup> Transformed value =  $\sqrt{1 + \text{aflatoxin value}}$  obtained from mean averaging over field means.

second sampling period than in the first, not completely supporting previous observations that seeds from bolls closest to the ground contain the most toxins (1,9). Every stub field was paired with and was physically next to a planted field. Because of early maturation, dehiscent bolls were available from stub cotton as early as 15 July. If late July values are added to those of early August, >60% of GCS was accounted for in these periods. In the planted counterparts from fields at MC, PX, HH, and GB, only 18% of the GCS was accounted for by bolls opened by early August. The early maturation of stub cotton is probably the key to these differences in aflatoxin contamination. Because more than half of the stub cotton matured before late August, these bolls escaped the severe weather and fungal contamination encountered then. Maximum increases in populations of *A. flavus* have been reported in air samples collected between 25 July and 15 August (7). Late August usually has high night and day temperatures that have been correlated with high aflatoxin levels in seed (11). Because stub cotton flowered and developed earlier than its planted counterpart, highest temperatures did

not coincide with maximum boll production. Insect populations, documented vectors of *A. flavus* contamination of cotton (3), might be underdeveloped in late July or early August, the time of maximum boll development in stub cotton.

Development of later maturing cultivars might help alleviate the aflatoxin problem in planted cotton. Levels of toxin detected in late September were negligible even though the GCS from that period was greater than that obtained in any of the three previous periods. Conversely, results from stub cotton mediate toward development of cultivars that would mature before August, when toxin formation is maximal.

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