# Harvesting Method Effects on Aflatoxin Levels in Arizona Cottonseed

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## ABSTRACT

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During 1976, 1977, and 1978 season in Arizona, a comparison was made between the aflatoxin levels found in seed from first-picked cotton harvested by conventional spindle pickers and aflatoxin levels detected in seed from second- or third-picked cotton harvested by ground-gleaning equipment. Samples from both gins and cotton trailers in the field were analyzed. Levels of total aflatoxins were consistently higher (2–279×) in ground-gleaned seed than in first-picked, spindle-harvested seed in all collections made during the 3-yr study.

*Additional key words: Aspergillus flavus.*

Contamination of cottonseed by aflatoxins is a chronic problem in the lower elevation production areas of Arizona (3), and the Imperial Valley of California (1). Almost all (99%) of the seed cotton produced in these areas is initially harvested by a spindle-type picker (Fig. 1). An additional or second picking may be conducted by the same piece of equipment or by a ground-gleaning machine (Fig. 2) which will not only remove seed cotton remaining on the plant, but any knocked to the ground by wind, rain or initial passage of the spindle picker. This ground-gleaned seed cotton (GGS), which may account for 5–15% of the crop, contains extraneous material, such as burrs, sticks, rocks and dirt. Much of the seed is damaged and linterless, and seed quality is much lower than in first spindle-picked seed cotton (SPS). It was with these observations in mind that we analyzed seed from these two types of harvesting operations to detect differences in total aflatoxins..

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## MATERIALS AND METHODS

Seed cotton samples weighing ~5 kg each were removed from 109 cotton trailers leaving selected fields in 1976, 1977, and 1978. Each sample was a composite of samples taken from arbitrarily

selected spots in the trailer. The samples were returned to the laboratory where they were roller ginned and a single 1-kg fuzzy seed subsample was saved for analysis. Ginned seed samples weighing ~200 kg each were taken from eight seed piles, composed of seed from unidentified fields, at gins known to segregate seed into spindle-harvested or ground-gleaned lots. The seed was returned to the laboratory and 234 1-kg subsamples were saved.

In 1976, each 1-kg subsample of fuzzy seed from both sources was ground by a Wiley mill to pass a No. 18 sieve. In 1977 and 1978, samples were decorticated with a Bauer double-disk mill and the screened meats were ground to pass a No. 18 sieve.

A single 25-g sample of meal was removed from each ground lot of 1976 and 1977 meal and four-to-five 25-g samples were removed from each 1978 sample for alfatoxin analysis.

Each meal sample was analyzed by a modification of Pons' high-pressure liquid chromatographic (HPLC) procedure (5). Extraction procedure modifications included the homogenization of the acetone-$H_2O$-cottonseed meal mixture in a Sorvall Omni-Mixer at an 80V setting on the Power-Stat rheostat for 3 min. The column clean-up portion of Pons' purification step was altered to include 5 g of activated acidic alumina (Brockman Activity II) or silica gel (Merck 60) layered beneath 10 g of $Na_2SO_4$ in a 20 ×400-mm chromatographic tube. The additional column chromatography steps described in Pons' procedure were omitted. Solvent was evaporated under an operating fume hood overnight at room temperature. Samples were resuspended in 1 ml of HPLC solvent and filtered through a 0.2-$\mu$m Gelman Alpha-200 filter for HPLC injection. The normal injection quantity was 25 $\mu$l which provides a minimum theoretical detection level of 5 ng/gm. An example of a typical injection for a sample containing aflatoxins $B_1$ and $B_2$ can be seen in Fig. 3.

TABLE 1. Comparison of mean aflatoxin ($B_1$ and $B_2$) levels detected in spindle- and ground-harvested cottonseed samples coming from trailers in identified commercial fields

| Year and field | Spindle-harvested seed | | | Ground-recovered seed | | |
|---|---|---|---|---|---|---|
| | Samples[a] | N.D.[b] | Aflatoxin ($\bar{x}$ ng/g [ppb])[c] | Samples[a] | N.D.[b] | Aflatoxin ($\bar{x}$ ng/g [ppb])[c] |
| 1976 | | | | | | |
| A | 12 | 10 | 9 | 12 | 1 | 144 |
| C | 20 | 7 | 54*[d] | 20 | 1 | 489 |
| D | 10 | 2 | 14* | 10 | 5 | 26 |
| E | 20 | 20 | ...* | 20 | 3 | 215 |
| 1977 | | | | | | |
| A | 20 | 3 | 202* | 20 | 0 | 7,283 |
| B | 20 | 5 | 182* | 20 | 0 | 2,398 |
| C | 20 | 5 | 361* | 20 | 0 | 2,398 |
| 1978 | | | | | | |
| A | 63 | 53 | 7* | 41 | 7 | 467 |
| B | 183 | 172 | 3* | 11 | 0 | 637 |
| C | 249 | 158 | 24* | 52 | 5 | 157 |
| D | 70 | 64 | 2* | 105 | 60 | 26 |
| E | 68 | 43 | 15* | 66 | 15 | 47 |

[a] Number of 2-kg samples analyzed.
[b] Number of samples analyzed having no detectable aflatoxin.
[c] Includes N.D. as zero in calculation.
[d] * = Significantly different from ground harvested mean, $P = 0.05$ according to Student's $t$-test.

## RESULTS AND DISCUSSION

At elevations in Arizona below 549 m (1800 feet), cottonseed from seed cotton harvested by ground-gleaning harvesters contained significantly more aflatoxin than did first-picked SPS. In 1976, mean aflatoxin levels were low in first-picked SPS sampled

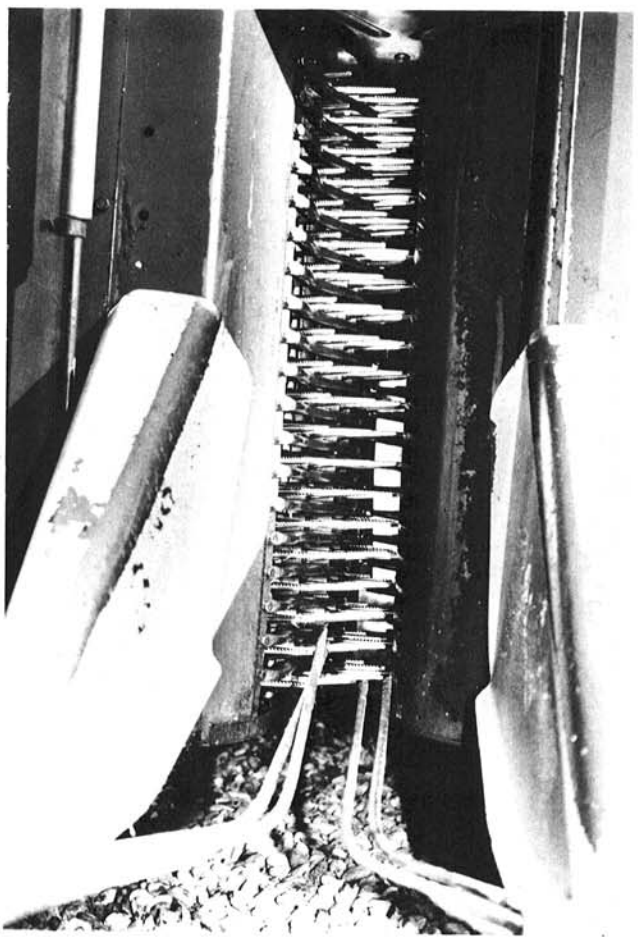

**Fig. 1.** Close-up of the intake portion of a spindle-type cotton harvester.

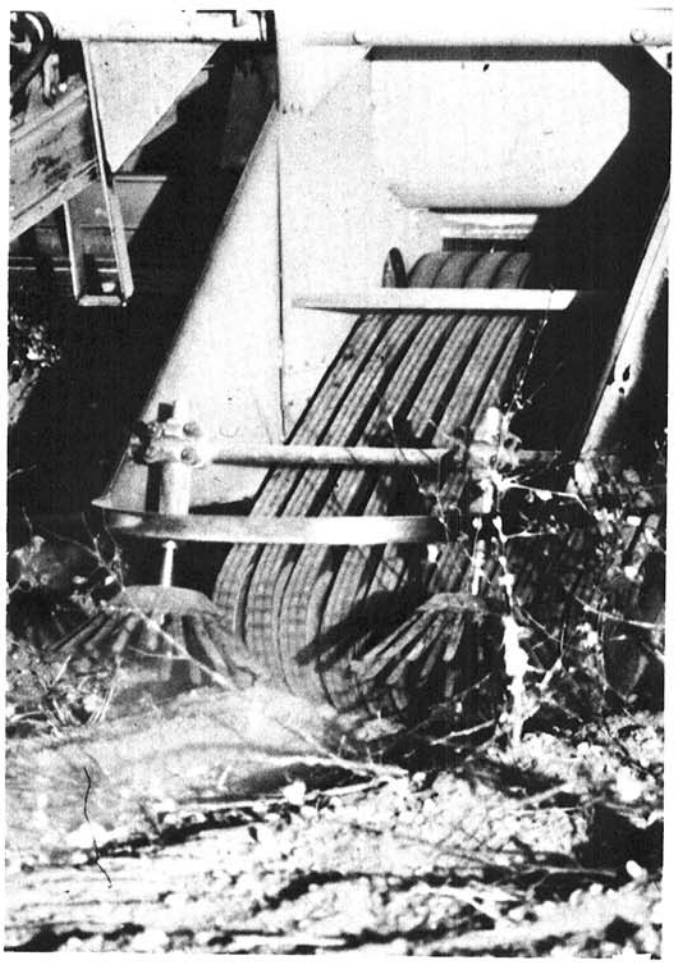

**Fig. 2.** Close-up of the intake portion of a ground-cleaning-type cotton harvester.

TABLE 2. Comparison of mean aflatoxin ($B_1 + B_2$) levels detected in gin seed piles of spindle- and ground-harvested fuzzy seed from unidentified fields

| Year and gin | Spindle-harvested seed | | | Ground-recovered seed | | |
|---|---|---|---|---|---|---|
| | Samples[a] | N.D.[b] | Aflatoxin (x ng/g [ppb])[c] | Samples[a] | N.D.[b] | Aflatoxin (x ng/g [ppb])[c] |
| 1976 | | | | | | |
| A | 17 | 10 | 5 | 17 | 7 | 90 |
| 1977 | | | | | | |
| A | 30 | 2 | 314*[d] | 30 | 0 | 2,079 |
| B | 40 | 1 | 514 | 40 | 0 | 1,132 |
| C | 30 | 0 | 4,399*[d] | 30 | 0 | 104,734 |

[a]Total number of 2-kg samples analyzed.
[b]Number of samples analyzed having no detectable aflatoxin.
[c]Includes N.D. as zero in calculation of mean.
[d]* = Significantly different from ground harvested mean, $P = 0.05$ according to Student's *t*-test.

from identified fields, as well as from gins which segregate fuzzy seed by picking sequence and type of harvester used (Tables 1 and 2). But, levels in GGS were 2–279 times higher in field samples and 11 times higher in samples from the single gin location.

Analysis of field and gin samples for 1977 reflected a higher overall incidence of seed contamination than was experienced in 1976. The disparity between aflatoxin levels in first SPS and GGS seed was again significant. GGS seed from identified fields contain 11–16 times more aflatoxin than did the first SPS. GGS seed from three gins contained 2–30 times the levels detected in SPS.

Samples were obtained from identified fields, but not from gins in 1978. Overall aflatoxin levels were comparable to those observed in 1976 and a similar significant difference between SPS and GGS was observed. GGS contained 3–212 times the levels of aflatoxin as SPS.

The first SPS from identified fields in all years tended to yeild a higher percentage of aflatoxin-negative samples than did GGS from the same fields. The same trend was evident in samples from gin seed piles where pile composition was unknown but unlikely to contain both SPS and GGS from the the same fields at sampling time.

Few gins in Arizona segregate seed according to harvesting method and equipment; SPS and GGS are therefore mixed at the gin before shipment to oil mills or feeders of whole cottonseed.

If it is assumed that GGS constitutes 11% and SPS 89% of the total harvested seed (4), and that GGS has an average of 50 times more aflatoxin than SPS as calculated from Table 1, then if a given quantity of SPS contained 10 ppb aflatoxin, the corresponding GGS would contain 500 ppb. Combining the two sources (89:11, v/v) would then yield an adjusted level of 64 ppb, which is 44 ppb above the FDA quideline of 20 ppb for food and feed commodities.

The previous discussion has been concerned with whole seed. In the process of making cottonseed meal (CSM), linters and hulls are first removed and oil is extracted from the remaining meats. The components (linters, hulls, and oil) contain little or no aflatoxin (6) and constitute 50% of the seed weight. Consequently, when the aflatoxin-free portions of the seed are removed, the remaining meal contains twice as much toxin as the whole seed. Therefore, cottonseed meal processors must receive whole seed containing <10 ppb in order to produce meal which will meet a 20 ppb guideline.

The difference in aflatoxin levels between SPS and GGS can be traced to the selective nature of the spindle harvester. The serrated spindles were designed to remove fluffed lint from open bolls. Imperfectly opened bolls that contain partially or unfluffed locules (tight locules) at harvest are difficult to retrieve by the spindle harvester and many remain on the plant or are knocked to the ground. This tight-loculed condition may be caused by damage to the boll by insects, such as the pink bollworm (*Pectinophora gossypilla* Saunders) (PBW), or by microbial invasion of lint enhanced by prolonged exposure to high humidity. Ashworth et al (2) found that in the Imperial Valley of California most of the field infection by *Aspergillus flavus* occurred in those imperfectly

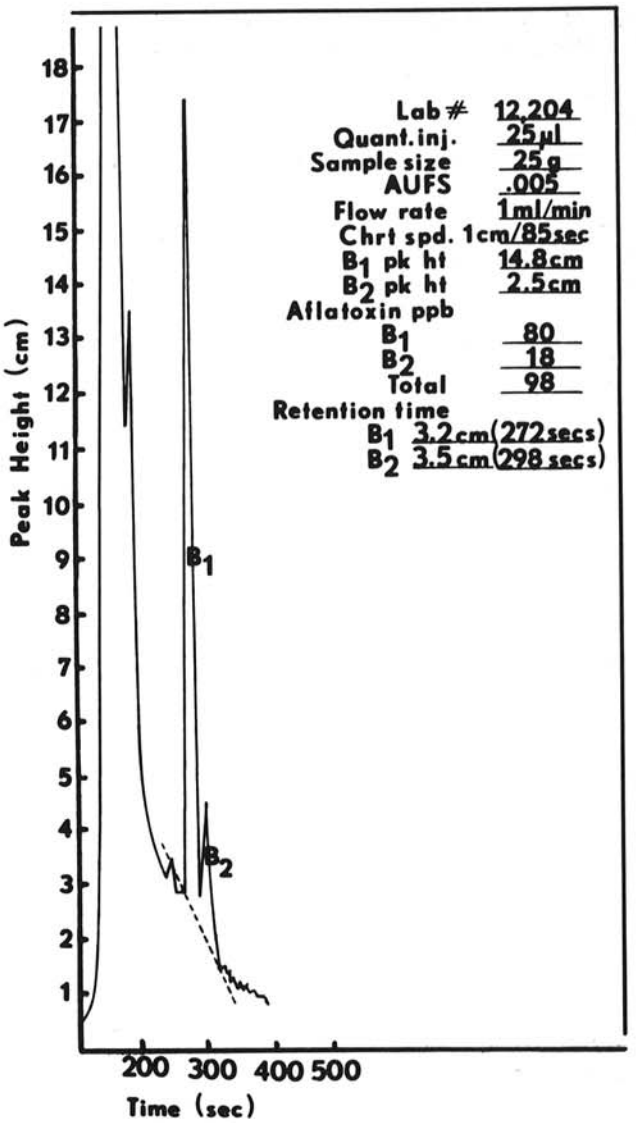


**Fig. 3.** High-pressure liquid chromatograph showing peaks of aflatoxins $B_1$ and $B_2$ typically observed in whole cottonseed extracts (365 nm).

dehisced bolls and their tight locules. It has been determined that ~60–70% of the aflatoxins in seed harvested from Arizona and the Imperial Valley could be found in ~10–20% of the bolls with PBW damage and/or bolls with at least one tight locule (T. Russell, *unpublished*).

The spindle harvester often fails to remove bolls set close to the planting bed. These bolls, because of their proximity to the soil, a source of inoculum and moisture, would be expected to be more prone to contamination by aflatoxin-producing fungi than those higher on the plant. Ground gleaners will retrieve these bolls, thus increasing overall aflatoxin levels.

The results of this study suggest that segregation at the gin of seed harvested by the two methods will result in lower aflatoxin levels in most of the seed. Producers normally do not comingle SPS and GGS cotton at harvest because of the timing of the use of these two types of equipment and the depressing effect lint from GGS cotton has on the grades of better quality SPS lint. A continuation of this segregation after ginning would provide seed with aflatoxin levels that approach a 10–20 ppb level and ultimately make available whole seed for feed and meal with acceptable levels of aflatoxin.

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