

## Aflatoxin in Cotton After Harvesting

A. C. Griffin and H. W. Schroeder

Research Physicist, Agricultural Research Service, U.S. Department of Agriculture, U.S. Cotton Ginning Research Laboratory, Stoneville, MS 38776; and Research Leader, Agricultural Research Service, U.S. Department of Agriculture, Transportation and Marketing Research Unit, College Station, TX 77840.

We thank C. A. Collins for making tests for aflatoxins, and the many individuals and organizations who furnished cotton for these experiments.

We also thank the USDA-ARS Southern Regional Research Center in New Orleans, Louisiana, for supplying the quantitative standard containing aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>.

The authors also express appreciation to J. W. Halloin for critical review and suggestions during preparation of this manuscript.

Accepted for publication 31 May 1977.

---

### ABSTRACT

GRIFFIN, A. C., and H. W. SCHROEDER. 1978. Aflatoxin in cotton after harvesting. *Phytopathology* 68:119-122.

Three harvest-storage treatments were applied to seed cotton in the Yazoo-Mississippi Delta area to determine their effects on development of aflatoxins in cottonseed during the period between harvesting and ginning. The experimental treatments were: (i) cotton harvested wet with dew and stored without drying, (ii) cotton harvested wet with dew and gin-dried before storage, and (iii) cotton harvested after evaporation of the dew and stored without further drying. The

development of aflatoxins in cottonseed during the interval between harvesting and ginning was minimized by ginning the damp-picked cotton by the end of the third day after harvesting and by ginning the dry-picked cotton by the end of the fourth day. The gin-drying treatment controlled aflatoxin production in the seed of stored seed cotton, but was considered impractical as a cotton-production process.

Aflatoxins develop in many raw agricultural products if favorable conditions of temperature, moisture, and *Aspergillus flavus* or *A. parasiticus* are present. Ashworth et al. (2, 3), McMeans et al. (8), Marsh et al. (6, 7), and Simpson et al. (10) reported the occurrence of fungi and aflatoxin in cottonseed and fiber before harvest. Methods were developed for removing or detoxifying aflatoxins in cottonseed as a part of the milling process (5). However, Goldblatt (4) stated that aflatoxins are best avoided by preventing their development.

In a series of small-scale experiments, Sorensen et al. (11) stored seed cotton at five moisture levels and found that aflatoxins were produced in cottonseed at moistures as low as 15% (wet basis) with temperatures under 35 C. Guidelines have been published for preventing mycotoxins in farm commodities (1). However, the information on the protection of cottonseed at the farm and gin level is general in nature and contains no data on the relationship of aflatoxin production in cottonseed to harvest and storage conditions.

In the Yazoo-Mississippi Delta area cotton usually is harvested with spindle-type mechanical harvesters in an 8- to 10-wk period during October, November, and December. Most cotton producers wait until the dew has evaporated and the relative humidity has fallen to about 50% before starting the daily harvest. The cotton is collected in a trailer and hauled to a local gin for ginning. Trailers containing cotton harvested in late afternoon may be left in the field or stored under a farm shed overnight and hauled to the gin the next morning. Thus,

the cotton usually is ginned within 24 hr after harvest. It is unlikely that aflatoxins will occur in cottonseed harvested and ginned in this manner (6, 7, 10).

However, exceptions to these practices do occur: (i) cotton sometimes is harvested by contract pickers who often harvest on a round-the-clock basis; (ii) when rain has been forecast during the harvest period, a grower may decide to begin picking during early morning hours and continue at night rather than risk cotton loss owing to rain storms; (iii) during peak harvest periods, when harvest often exceeds the daily capacity of the local gin, ginning may be delayed; (iv) breakdown of mechanical picker, trailer, or gin may cause unscheduled delays; and (v) the recent practice of storing harvested cotton as compacted 10-bale free-standing modules in the field or on a gin yard to await a convenient time for ginning involves a planned delay, often of 1 wk or more.

We undertook this work to examine the effects of delayed ginning on the development of aflatoxins in cottonseed harvested, stored, and ginned under real, but adverse circumstances.

### MATERIALS AND METHODS

**Harvesting and storage treatments.** — Trailer-stored and house-stored cotton were used in 1970, but in 1971, 1972, and 1973 only spindle-picked cotton was used to evaluate the effects of the time of day of harvest and gin-drying on the development of aflatoxins in cotton stored in trailers before ginning. The three treatments were: (i) cotton was picked wet with dew and stored for 7 days before ginning; (ii) cotton was picked wet with dew, dried through two stages of gin-type drying at 150 C, and redeposited without cooling on a trailer for 7 days of

0032-949X/78/000017: \$03.00/0

Copyright © 1978 The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, MN 55121. All rights reserved.

storage before ginning; and (iii) cotton picked after 1300 hours from the same field as for treatments i and ii was stored 7 days before ginning. Cotton picked after 1300 hours is called "field dried" in this report.

Cotton for each experiment was harvested from the same field on the same day. Each experiment was performed five times during the harvest period of each year. A three-bale load (2,000 kg), the normal capacity of the trailers, was used for each replication of each treatment.

**Temperature monitoring.** — Six thermocouples were inserted into each trailer load of cotton as it arrived at the laboratory (treatment i and iii), or immediately after reloading if the cotton passed through the driers (treatment ii). Each thermocouple was 1.2 m beneath the surface of the load; a thermocouple was located 1 m from each corner of the trailer on the diagonal line from front to rear in opposite corners, and on the centerline 2 m from the front and rear walls of the trailer. Temperatures were observed daily using a portable read-out device.

**Sampling.** — Bulk samples of about 1.5 kg of cotton were collected each day from near each thermocouple location within each trailer. The sample pairs from center, right, and left sides were combined to form three bulk samples that were promptly ginned without precleaning or drying on a 20-saw laboratory gin to provide samples of cottonseed for moisture and aflatoxin assays. Cottonseed samples used in the 1970 experiment were collected from cotton stored for 54 to 104 days in our seed-cotton storage house. These cottons were from random harvestings and were stored at ambient conditions until used in other experiments.

The cottonseed for aflatoxin assays received special handling. Immediately after ginning, 200 g of cottonseed were dried to below 8% moisture in an electric oven at 50 C. Drying inactivated the fungi so that the amount of aflatoxin present at sampling would remain unchanged until the chemical assays were made. Seeds were dehulled in a laboratory mill with the grinding surfaces set as far apart as adjustment would permit. The mill was opened and cleaned with compressed air between samples. The kernels were sealed in plastic bags and stored in a dry place to await analysis.

**Aflatoxin assay procedure.** — The cottonseed were tested for aflatoxin using the procedure developed by Velasco (12). The quantity of aflatoxin was estimated by comparing the fluorescence of the test specimen with that of known concentrations prepared from a standard mixture of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. Assays were performed on duplicate specimens from each daily collection of bulk samples.

**Moisture tests.** — The cottonseed-moisture content was determined by drying 50 g of cottonseed for 5 hr in an electric oven at 104 to 110 C (9). Moisture content was calculated on the wet-weight basis.

**Determination of internal mycoflora.** — Fifty samples of 50 seed each from the 1970 experiment were acid delinted, surface disinfected by immersion for 1 min in 1% sodium hypochlorite solution, rinsed in sterile distilled water, and plated on malt-salt agar (2% malt extract and 7.5% sodium chloride) at the rate of 25 seed/petri plate. The plates were incubated 7 days at 25 C. Fungi were identified and the seeds supporting fungal growth were counted.

## RESULTS

In 1971, samples of cotton for laboratory testing were collected from the center of the load on days zero through seven. On day seven samples also were collected from the corners of the load. The most consistent contamination with aflatoxins was found in cottonseed from the picked-wet/nondried treatment at 22% cottonseed moisture. Samples from the center of the load, which heated to 63 C, did not show the presence of aflatoxins. The corner samples collected on day seven were positive for aflatoxins. These data indicated that the part of the load between the hot damp core and the dry cooler surface would be more likely to yield aflatoxin-bearing samples than other parts of the load. The 1972 and 1973 sample-collecting plan included such samples.

**Cottonseed moisture content.** — Moisture content of the cottonseeds varied with the harvesting and gin-drying treatments. The cotton that was picked with dew had seed moistures of 20.8% (Table 1), with individual loads averaging from 19.5 to 23.8%. One load averaged only 15.4%, probably because the harvest crew started late so that most of the dew had evaporated before the harvest of this lot was completed.

The gin-drying treatment reduced the moisture content of cotton going into storage; moisture content for the 10 lots after drying averaged 14.4%. Thus, an average of about 6% (20.8 to 14.4%) of moisture was removed by two passes through shelf-type driers operating at 150 C at the air/cotton mixpoint. In two samples, the data did not indicate the expected moisture removal, but in the others the amount of moisture removed illustrates the relative ease with which moisture, such as rain or dew, can be removed from the surface of cotton and cottonseed.

Field-drying also lowered fiber- and cottonseed-moisture content. The average cottonseed-moisture content for the field-dried cottons was 12.9% — about 1.5% lower than that of the picked-wet/gin-dried cottons.

TABLE 1. Effect of harvest and conditioning procedure on cottonseed-moisture content of cotton stored in trailers for 7 days.

Replication	Picked-wet and nondried	Picked-wet and gin-dried <sup>b</sup>	Field-dried <sup>c</sup>
5	15.4 <sup>a</sup>	9.6 <sup>a</sup>	8.1
8	19.5	17.5	10.6
6	19.8	15.8	16.7
4	19.8	10.5	13.4
2	20.8	19.0	12.7
3	21.1	14.2	14.9
7	22.0	16.5	13.7
10	22.4	14.9	13.8
9	23.2	10.4	10.4
1	23.8	15.4	14.4
Mean	20.8	14.4	12.9

<sup>a</sup>Dew evaporated from the field before harvesting of these loads was completed.

<sup>b</sup>Two-stage, hot-air, gin-drying system; mixpoint temperature was 150 C.

<sup>c</sup>Harvested after 1300 hours.

Thus, cotton entered the 7-day storage period with cottonseed-moisture content averaging about 21, 14, and 13%, respectively, for the picked-wet/nondried, picked-wet/gin-dried, and field-dried treatments.

**Load temperature.**—Each load that was picked with dew and stored without drying heated spontaneously (Fig. 1), because of bacterial and fungal growth. The average temperatures of these loads was 26 C at the beginning of storage, and was 50 C on the 3rd day. The temperature continued to increase and reached 54 C on the 7th day. The highest cotton temperature recorded for this treatment was 63 C.

Cotton that was gin-dried before storage averaged 50 C as it passed from the drying system directly to the storage trailer. The initial storage temperatures ranged from 45 to 54 C; the temperature of each of these loads fell continuously toward ambient and averaged 27 C on the 7th day.

The field-dried cotton entered storage at temperatures ranging from 29 to 41 C. Some of these loads heated

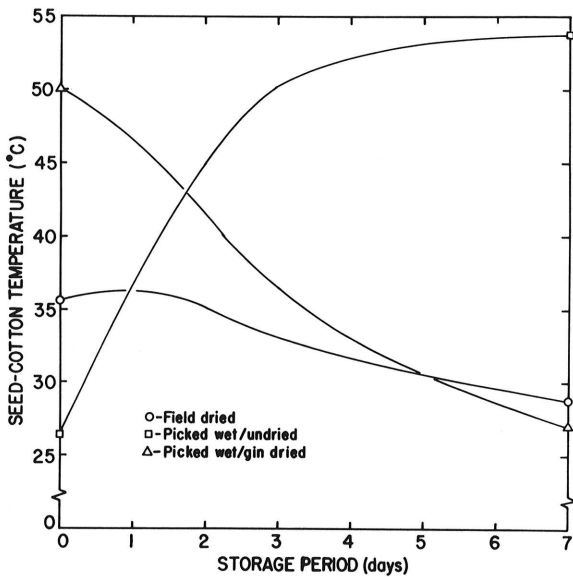


Fig. 1. Daily temperature change of seed cotton with different moisture contents during storage in harvesting trailers for 7 days. Sensors were located 1.2 m from top of the load and 2.0 from the end walls.

slightly (2 C) during the 1st day of storage, but the temperature of all loads slowly declined thereafter and averaged 29 C on the final day.

**Internal fungi.**—*Aspergillus flavus* was isolated from 11 of the 50 samples and the percentage of infested seeds ranged from 2 to 6%. Other species of fungi (*Aspergillus* and *Penicillium*) were isolated from only five samples but did not exceed 4% in any sample. In contrast, all other genera of fungi were isolated from an overall average of 26% of the seed; 74% of the seed were free of internal fungi.

Nine of the *A. flavus* isolates were tested on cottonseed and peanut media prepared by autoclaving fresh cottonseed and peanut kernels. Each produced aflatoxins, and six of the nine produced amounts high enough to classify them as highly toxigenic.

**Aflatoxin assay.**—None of the cottonseed contained aflatoxin at the beginning of the storage period (Table 2). After 1 day of storage, two samples gave weak responses when tested for aflatoxin, but samples collected the next day from these loads did not contain aflatoxin. It was not until after 3 days of storage that the samples began to show consistent, strong, positive tests for aflatoxin.

Seven of the 10 loads picked-wet/nondried contained aflatoxin after 3 days of storage, and five tested positive after 4 days of storage. The decline in the number of loads testing positive after 3 and 4 days of storage was attributed to the sampler missing the localized pockets of infested seeds. By the end of the storage period, however, the contamination was so prevalent that the samples from all loads except one contained aflatoxin. The one load of this treatment that did not contain aflatoxin after 7 days was the load with cottonseed moisture of 15.4%.

From the field-dried treatment, three of the loads tested positive for aflatoxin after 4 days of storage, and six tested positive after 7 days. All of the aflatoxin-bearing loads from the field-dried treatment had cottonseed-moisture contents in the 12.0-16.9% range.

These data indicate that seed cotton in trailers may be stored up to 3 days with minimum risk of aflatoxin contamination. After 3 days of storage, aflatoxin was detected in some samples of wet-picked cotton but no aflatoxin was detected in field-dried samples until after 4 days.

Heat drying was more effective than field drying for controlling aflatoxin development. The heat-drying treatment prevented aflatoxin formation in nine of the 10 loads. The one heat-dried load that tested positive for

TABLE 2. The effect of harvesting and conditioning treatments and cottonseed-moisture content on the number of loads of cotton that tested positive for aflatoxin during 7 days of storage.

Cottonseed moisture content (%)	Loads of cotton testing positive for aflatoxins											
	Picked-wet/nondried			Picked-wet/gin-dried <sup>a</sup>			Field-dried <sup>b</sup>					
	Loads (no.)	Days elapsed		Loads (no.)	Days elapsed		Loads (no.)	Days elapsed				
		3	4	7		3	4	7		3	4	7
17.0 and over	9	7	5	9	2	0	1	1	0	...	...	...
12.0-16.9	1	0	0	0	5	0	0	0	7	0	3	6
11.9 or less	0	...	...	...	3	0	0	0	3	0	0	0
Total	10	7	5	9	10	0	1	1	10	0	3	6

<sup>a</sup>Two-stage, hot-air, gin-drying system with a mixpoint temperature of 150 C.

<sup>b</sup>Harvested after 1300 hours.

aflatoxin had a cottonseed-moisture content of 17.5%. The five loads that were heat-dried to the 12.0 - 16.9% cottonseed-moisture range tested negative for aflatoxin, but six of the seven field-dried loads in the same cottonseed-moisture range tested positive for aflatoxin. These data suggest that the temperature of the air in the driers had a beneficial effect by preventing aflatoxin development.

#### DISCUSSION

Even though passing wet-picked cotton through two stages of 150 C drying in the gin reduced moisture and prevented aflatoxin development in nine of the 10 loads, it is not likely to be done commercially because of the extra time and expense required, and because it would interfere with normal ginning of the crop. These results indicate that cotton producers should delay daily harvesting until the dew has evaporated and damp cotton should never be stored. Damp-harvested, and also field-dried cottons should be ginned by the 3rd day to minimize the production of aflatoxin in seed cotton after harvest.

#### LITERATURE CITED

1. ANONYMOUS. 1968. Preventing mycotoxins in farm commodities. U.S. Dep. Agric. Res. Serv. ARS 20-16. 7 p.
2. ASHWORTH, L. J. JR., J. L. MCMEANS, and C. M. BROWN. 1969. Infection of cotton by *Aspergillus flavus*: time of infection and influence of fiber moisture. *Phytopathology* 59:383-385.
3. ASHWORTH, L. J. JR., J. L. MC MEANS, and C. M. BROWN. 1969. Infection of cotton by *Aspergillus flavus*: the influences of temperature and aeration. *Phytopathology* 59:669-673.
4. GOLDBLATT, L. A. 1971. Control and removal of aflatoxin. *J. Am. Oil Chem. Soc.* 48:605-610.
5. MANN, G. E., L. P. CODIFER, JR., H. K. GARDNER, JR., S. P. KOLTUN, and F. G. DOLLEAR. 1970. Chemical inactivation of aflatoxins in peanut and cottonseed meals. *J. Am. Oil Chem. Soc.* 47:173-176.
6. MARSH, P. B. and M. E. SIMPSON. 1976. Influence of some field conditions on the occurrence of aflatoxins in cottonseeds at harvest. Pages 22-30 in *Public-Supported Cotton Research: Proc. Conf. Collab. South Agric. Exp. Stns. New Orleans, LA. 2-3 April 1973*. Published as ARS-S-70, U.S. Govt. Printing Office, Washington, D.C.
7. MARSH, P. B., M. E. SIMPSON, G. O. CRAIG, J. DONOSO, and H. H. RAMEY, JR. 1973. Occurrence of aflatoxins in cotton seeds at harvest in relation to location of growth and field temperatures. *J. Environ. Qual.* 2:276-281.
8. MC MEANS, J. L., L. J. ASHWORTH JR., and W. A. PONS, JR. 1968. Aflatoxins in hull and meats of cottonseed. *J. Am. Oil Chem. Soc.* 45:575-576.
9. SHEPHERD, J. V. 1972. Standard procedures for foreign matter and moisture analytical tests used in cotton ginning research. Pages 8-10 in *U.S. Dep. Agric. Handb.* 422. 13 p.
10. SIMPSON, M. E., P. B. MARSH, G. V. MEROLA, R. J. FERRETTI, and E. C. FILSINGER. 1973. Fungi that infect cottonseeds before harvest. *Appl. Microbiol.* 26:608-613.
11. SORENSEN, J. W. JR., N. K. PERSON, JR., L. H. WILKES, and H. W. SCHROEDER. 1969. Quality of cottonseed from seed cotton stored for various lengths of time prior to ginning. *Progress Rep., Dept. Agric. Eng., Texas A&M University. College Station, Texas.* 23 p.
12. VELASCO, J. 1972. Determination of aflatoxin using microcolumns layered with florisil. *J. Am. Oil Chem. Soc.* 49:141-142.