Separation of Aflatoxin-Contaminated Cottonseed Based on Physical Characteristics of Seed Cotton and Ginned Seed

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ABSTRACT AND SUMMARY

Samples of aflatoxin-contaminated stored ginned cottonseed and of freshly harvested seed cotton and companion ginned seed were examined under long wave ultraviolet (UV) and visible light to develop physical criteria for separating aflatoxin-contaminated seed from sound seed. Seed locks characterized by bright greenish-yellow, or cateye, fluorescence when viewed under long wave UV light were separated from samples of 12 varieties of freshly harvested seed. As a result, 80-100% (mean 96%) of the aflatoxin contamination that was concentrated in 2-9% (mean 6%) of the seed weight was effectively removed. The cateye fluorescence separation technique was slightly less effective for removing aflatoxins from companion freshly harvested, ginned samples, but most of the aflatoxins were concentrated in only 0.5-3% (mean 2%) of the seed weight. This approach was relatively ineffective for stored ginned seed, probably due to deterioration of the cateye fluorescence.

INTRODUCTION

Growing conditions in the lower desert areas of the Southwest are such that cotton bolls can be invaded in the field by the ubiquitous mold *Aspergillus flavus* (1), resulting in extremely high levels of aflatoxin contamination in a small proportion of the seed (2,3). Thus cottonseed processors are faced with the problem of contaminated lots of sound ginned seed, although only a few seed contain high levels of aflatoxins. If contaminated seed could be removed before processing, the resultant meal would be free of aflatoxins. Correlation of unique physical characteristics of either seed cotton or ginned seed with aflatoxin contamination would aid in devising techniques for physical segregation of contaminated cottonseed. Cucullu et al. (2) have reported correlations between physical characteristics and aflatoxin contamination in over 700 individual ginned and stored cottonseed. Their results indicate that the following characteristics are associated with extremely high aflatoxin contamination levels: (a) bright greenish-yellow (cateye) fluorescence under long wave UV light, usually related to *A. flavus* attack (4,5); (b) partially bald seed; and (c) a combination of cateye plus partially bald seed. In contrast, Clark et al. (6) found that removing cateye fluorescent seed from four lots of stored, ginned cottonseed had little, if any, effect on reducing aflatoxin contamination.

The present study was undertaken to determine the effectiveness of separating cottonseed on the basis of physical characteristics suggested by several investigators (2-5) as a means of reducing aflatoxin contamination in stored ginned cottonseed and freshly harvested seed cotton and ginned seed.

MATERIALS AND METHODS

Stored Seed

A 16 kg sample of contaminated ginned cottonseed was obtained from a large seed pile stored for about 1 yr, under high temperature and humidity conditions in the Imperial Valley of California. The sample was riffled successively to provide three 1 kg subsamples for examination. Each subsample was examined first in long wave UV light to separate seed which exhibited either cateye fluorescence (7) or a combination of the cateye and partially balding characteristics. The remaining seed were examined under visible light

TABLE I

Aflatoxin Distribution in Stored Ginned Cottonseed Separated into Physical Categories

	Subsamples[a]					
	A (104 μg/kg)[b]		B (173 μg/kg)		C (118 μg/kg)	
Seed category	Wt[c], %	Aflatoxins[d], %	Wt, %	Aflatoxins, %	Wt, %	Aflatoxins, %
Cateye in UV	0.1	-	0.1	0.5	0.1	-
Cateye and partially bald	0.1	0.3	0.1	25.5	0.1	-
Partially bald	2.6	45.1	3.7	31.6	3.7	17.5
Thin lint	2.3	1.2	1.5	2.1	0.8	1.8
Yellow lint	0.7	22.2	1.1	14.4	0.4	-
Cracked seed	1.1	0.7	0.6	-	1.6	-
Total	8.1	69.5	7.1	74.1	6.7	19.3
Remaining seed						
Wt %	91.9		92.9		93.3	
Aflatoxins, %	30.5		25.9		80.7	
Aflatoxin content, μg/kg	32		45		9.5	

[a]1 kg subsamples from 16 kg seed sample.
[b]Total aflatoxins (B_1 + B_2) in subsamples.
[c]Wt percent of 1 kg subsample in category.
[d]Percent of total aflatoxins (B_1 + B_2) in each category.

to separate nonfluorescent seed with a partially bald characteristic, seed with thin or yellow lint, and cracked seed. Three screenings were performed on each of the three subsamples.

Freshly Harvested Seed

Approximately 1 kg of each of seed cotton and 2.3 kg of companion ginned seed from 12 varieties of freshly harvested cotton were obtained from the University of Arizona Agricultural Experiment Station, Yuma, AZ. Samples were taken from cotton harvested in late October 1975, stored in the dark at room temperature, and examined from 1-5 mo after harvesting. Ginned seed samples were mixed and quartered to 500 g subsamples, which were examined under UV to separate seed with cateye, with cateye plus partially bald character, and with orange and white fluorescence. The seed remaining were examined in visible light to separate those with partially bald character, thin lint, yellow lint, and cracked seed. These screenings were conducted on half of the 12 variety samples. The remaining six variety samples were screened to remove only seed with cateye fluorescence.

The entire 1 kg sample of seed cotton from each variety was examined under UV to separate seed locks with either cateye or a combination of cateye and orange fluorescence. Locks in each category and locks remaining after the separation were ginned separately in a small laboratory gin.

Aflatoxin Assay

Seed samples (450 g or more) resulting from the physical separations above were decorticated in a Bauer mill. The kernels were separated from the hulls, ground in a Waring Blendor, and thoroughly mixed. Three independent aflatoxin assays were conducted on samples of ground meats by the method of Pons et al. (8). The values were averaged and calculated to a whole seed basis, assuming a 1:1 weight relationship between kernels and hulls. Smaller seed samples from the physical separations (0.5-5.0 g) were cracked, soaked overnight in aqueous acetone, and assayed for aflatoxins (8). Suitable modifications in the sample-to-solvent ratio of the assay method were made to obtain adequate sensitivity.

In all cases, total μg of aflatoxins in each seed category were calculated from the aflatoxin assays, μg/kg, and from the seed weight in the appropriate category. These data were used to calculate the aflatoxin content of the original samples and the distribution of aflatoxins among the categories.

RESULTS AND DISCUSSION

Stored Seed

Physical separations of three subsamples of stored ginned seed, 107-173 μg/kg total aflatoxins, into six categories removed only 19-74% of the aflatoxin contamination, representing 7-8% of the total seed weight (Table I). The major portion of aflatoxin contamination, 18-45%, was concentrated in the nonfluorescent, partially bald category, but very little of the contamination was removed in the two cateye fluorescent categories. The aflatoxin levels of the residual seed (92-93% of seed weight) were undesirably high (32-95 μg/kg). These results are in essential agreement with those reported for physical separations of individual stored, ginned seed, where the major contamination category was also partially bald and nonfluorescent seed (2). These results, and those reported by Clark et al. (6), suggest that the cateye fluorescence characteristic of *A. flavus* invasion (4,5) may be unstable and thus is not a reliable indicator of aflatoxin contamination in stored, ginned seed. Marsh et al. (9) have shown that cateye fluorescence is rapidly degraded when moist cotton fiber is exposed to long wave UV. Information developed in the present study also confirmed the

TABLE II

Aflatoxin Distribution in Freshly Harvested and Ginned Cottonseed Separated into Physical Categories

Seed category	Deltapine 16		Acala 1517-70		Stoneville 213		Acala SJ-1		McNair 511		Coker 310	
	463 μg/kg[a]		149 μg/kg		127 μg/kg		80 μg/kg		37 μg/kg		30 μg/kg	
	Wt, %[b]	Aflatoxin, %[c]	Wt, %	Aflatoxin, %	Wt, %	Aflatoxin, %	Wt, %	Aflatoxin, %	Wt, %	Aflatoxin, %	Wt, %	Aflatoxin, %
Cateye in UV	0.7	25.3	1.6	98.7	2.4	7.6	0.7	66.2	0.8	100	1.5	94.2
Cateye and partially bald	2.1	71.1	0.1	1.3	1.0	92.4	0.4	33.8	0.1	-	0.2	5.8
Other categories[d]	15.3	3.6	7.0	-	9.0	-	7.2	-	4.7	-	9.8	-
Remaining seed[e]	81.9	-	91.3	-	87.6	-	91.7	-	94.4	-	88.5	-

[a] Aflatoxin content ($B_1 + B_2$) of approximately 500 g sample.
[b] Wt percent of 500 g sample in category.
[c] Percent of total aflatoxin content of sample in category.
[d] Partially bald only, orange or white in UV, thin lint, yellow lint, cracked, bald seed.
[e] Remaining seed had nondetectable aflatoxins, < 1 μg/kg.

TABLE III

Reduction of Aflatoxins in Freshly Harvested Cottonseed by Removal of Cateye Fluorescent Ginned Seed

Cotton variety	Aflatoxins in total samples, μg/kg[a]	Fluorescent seed		Remaining seed		
		Wt, %	Aflatoxin, %	Wt, %	Aflatoxin, %	Aflatoxin content, μg/kg
*S. Okra	19	1.0	64.0	99.0	36.0	7
Acala SJ-1	80	1.1	80.0	98.9	20.0	16
*Dalcott 277	61	1.9	100	98.1	-	ND[b]
Deltapine 16	463	2.8	96.4	97.2	3.6	17
Stoneville 213	127	3.4	100	96.6	-	ND
Acala 1517-70	149	1.7	100	98.3	-	ND
Coker 310	30	1.7	100	98.3	-	ND
*Pima S-4[c]	48	0.5	21.8	95.5	77.2	38
*DP-66	270	0.5	100	95.5	-	ND
McNair 511	37	0.9	100	99.1	-	ND
*Coker 711[d]	6	1.4	23.0	98.6	77.0	5
*DP-61	ND	-	-	-	-	-

[a]Total aflatoxins (B_1 + B_2) in 500 g samples of ginned seed.

[b]Nondetectable, < 1 μg/kg.

[c]Seed essentially lint free.

[d]Glandless seed.

[e]*Seed categorized only as fluorescent or nonfluorescent.

TABLE IV

Reduction of Aflatoxins in Freshly Harvested Seed Cotton by Removal of Cateye Fluorescent Locks

Cotton variety	Aflatoxins total sample[a], μg/kg	Seed from fluorescent locks[b]		Seed from remaining locks[b]		
		Wt, %	Aflatoxins, %	Wt, %	Aflatoxins, %	Aflatoxin content, μg/kg
S. Okra	416	7.9	100	92.1	-	ND[c]
Acala SJ-1	205	3.4	100	96.6	-	ND
Dalcott-277	189	5.1	95.0	94.9	5.0	10
Deltapine 16	161	8.0	99.0	92.0	1.0	2
Stoneville 213	158	2.2	99.3	97.8	0.7	1
Acala 1517-70	136	8.7	99.2	91.3	0.8	1
Coker 310	109	3.1	98.9	96.9	1.1	1
Pima S-4[d]	70	8.5	100	91.5	-	ND
DP-66	65	2.8	100	97.2	-	ND
McNair 511	33	8.2	100	91.8	-	ND
Coker 711[e]	25	2.1	79.5	97.9	21.5	5
DP-61	20	7.0	81.2	93.0	18.8	4

[a]Total aflatoxins (B_1 + B_2) in approx. 500 g seed sample.

[b]After laboratory ginning.

[c]Nondetectable.

[d]Seed essentially lint free.

[e]Glandless seed.

rapid degradation of fluorescence when either cateye fluorescent seed cotton or ginned seed was exposed to long wave UV or direct sunlight.

Freshly Harvested Ginned Seed

In striking contrast to the results obtained with stored seed, physical separations of ginned seed from six varieties of freshly harvested cotton that contained 30-463 μg/kg of aflatoxins (Table II) removed 96-100% of the aflatoxin contamination in two cateye fluorescent categories, representing 0.9-3% of the total seed weight. Six other physical categories contained very little aflatoxins. Residual seed, 97-99% of the total sample, contained nondetectable (< 1 μg/kg) aflatoxin levels in 5 of the 6 samples, and 17 μg/kg in the sample with the highest (463 μg/kg) aflatoxin, Deltapine 16. Based on these results, the remaining six variety samples of ginned seed were separated into cateye fluorescent and nonfluorescent seed. Data for the entire 12 ginned seed samples separated into these two categories are given in Table III. Aflatoxin reductions were ineffective with the Pima S-4 variety, which is a bald seed, and with the Coker 711 glandless seed, which had a low aflatoxin content (6 μg/kg). No aflatoxins were present in the subsample of the DP-61 variety. For the other nine seed samples containing 19-463 μg/kg of aflatoxins, 64-100% of this contamination was concentrated in the fluorescent seed, which represented only 0.5-3% (mean 2%) of the seed weight. The residual aflatoxin levels of the remaining seed ranged from nondetectable to 17 μg/kg.

Freshly Harvested Seed Cotton

Removal of seed locks characterized by cateye fluorescence on the cotton fiber was found to be an effective approach for reducing aflatoxin contamination in freshly harvested seed cotton (Table IV). Separation of cateye fluorescent seed locks, containing seed of 20-416 μg/kg of aflatoxins, was uniformly successful in removing 81-100% of the aflatoxin contamination. Residual seed had aflatoxin levels ranging from nondetectable to 10 μg/kg. However, seed losses ranged from 2-9% (mean 6%) and were about three times greater than those found for separation of cateye fluorescent ginned seed (Table III), where seed losses ranged from 0.5-3% and averaged 2%. This approach was also successful for reducing aflatoxins in Pima S-4 bald seed and the Coker 711 glandless varieties, where the separations on the basis of cateye fluorescence of ginned seed was not effective (Table III).

Removing cateye fluorescent seed locks from freshly harvested seed cotton may well be the most expedient approach for physical separation of aflatoxin-contaminated seed. This approach may be more effective for irrigated cotton grown at high ambient temperatures, represented by the samples used in this study, than for rain-grown cotton in other production areas (10) or weathered seed cotton (11).

REFERENCES

1. Halisky, P.M., W.C. Schnathorst, and D.C. Erwin, Calif. Agr. 15:6 (1961).
2. Cucullu, A.F., L.S. Lee, and W.A. Pons, Jr., JAOCS 53:21 (1976).
3. Ashworth, L.J., Jr., and J.L. McMeans, Phytopathology 56:1104 (1966).
4. Marsh, P.B., and M.E. Simpson, Plant Dis. Rep. 52:671 (1968).
5. McMeans, J.L., and L.J. Ashworth, Jr., Phytopathology 56:899 abst. (1966).
6. Clark, S.P., C.M. Cater, and G.A. Harper, JAOCS 52:124A (1975).
7. Marsh, P.B., K. Bollenbacher, J.P. San Antonio, and G.V. Merola, Text. Res. J. 25:1007 (1955).
8. Pons, W.A., Jr., A.F. Cucullu, and A.O. Franz, Jr., J. Ass. Offic. Anal. Chem. 55:768 (1972).
9. Marsh, P.B., M.E. Simpson, R.J. Ferreti, T.C. Campbell, and J. Donosa, J. Agr. Food Chem. 17:462 (1969).
10. Hamsa, T.A.P., and J.C. Ayres, JAOCS 52:444A (1975).
11. Ashworth, L.J., Jr., J.L. McMeans, J.L. Pyle, C.M. Brown, J.W. Osgood, and R.E. Ponton, Phytopathology 58:102 (1968).

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