Relationship of Physical Appearance of Individual Mold-Damaged Cottonseed to Aflatoxin Content

ALVA F. CUCULLU, LOUISE S. LEE and **W.A. PONS, JR.**, Southern Regional Research Center, ARS, USDA, P.O. Box 19687, New Orleans, Louisiana 70179

ABSTRACT AND SUMMARY

Over 700 individual aflatoxin-suspect cottonseed were hand-selected from a heterogenous stockpile of ginned seed. The seed were categorized on the basis of (a) bright greenish-yellow, fluorescence termed cateye, on the linter fibers under ultraviolet light; (b) partially bald seed with part of the linter fibers removed by ginning; (c) a combination of cateye and balding; (d) thin and discolored lint; and (e) bluish, not cateye, fluorescence. Aflatoxin assays on each of the 771 selected seed showed that 142 out of 771 (18%) were contaminated by aflatoxin ($B_1 + B_2$) in the range of 150 ppb–5.75 million ppb. Some 93% of the aflatoxin-contaminated seed was concentrated in categories (a), (b), and (c), with the highest concentration, 61%, in category (b). Eight seed in these three categories contained over 1 million ppb of aflatoxins. The data suggest that removal of cateye and partially bald seed from contaminated lots of cottonseed should be more effective for controlling aflatoxin contamination in cottonseed than removal of cateye seed alone.

INTRODUCTION

The presence of a distinctive bright greenish-yellow (BGY) fluorescence, termed cateye (1,2), on the fiber of seed cotton when viewed under long wave ultraviolet light, is associated with fungal invasion of cotton bolls by *Aspergillus flavus* (3). Ginned seed from such fluorescent locks tend to have variable, but generally high aflatoxin content (3). Cateye on the short linter fibers of ginned cottonseed has also been correlated with high levels of aflatoxin contamination (4,5). The cateye/aflatoxin relationship is not a completely reliable technique for screening ginned seed, since noncateye seed can also contain significant aflatoxin contamination (4,5,6), and some cateye seed are free of aflatoxin contamination (5).

To obtain better information on the relationship between physical characteristics of individual cottonseed and aflatoxin contamination, we segregated and categorized individual seed with cateye fluorescence, as well as with other diverse characteristics. Each individual seed was assayed for aflatoxin contamination. No attempt was made to analyze a predetermined number of seed from any of the given categories. The purpose of this study was simply to find seed containing aflatoxins and to correlate their physical appearance with their aflatoxin content.

MATERIALS AND METHODS

From numerous samples of aflatoxin-suspect, ginned cottonseed assayed in our laboratory over a period of several years, a heterogenous stockpile of aflatoxin-contaminated seed was accumulated. From this stockpile, 771 individual seed were hand selected on the basis of physical characteristics such as (a) cateye fluorescence under long wave ultraviolet light, (b) a balding characteristic where the lint was loosely attached and part of it was completely removed, (c) a combination of cateye plus balding, (d) thin or discolored lint, and (e) a bluish, but not cateye fluorescence. From observations of many cottonseed, the above five categories seemed to be those which were readily distinguishable from normal ginned seed.

Seed were cut with a razor blade, and each kernel was carefully removed and weighed to ± 0.01 g. Kernels were finely chopped and assayed for aflatoxin content by the micro method of Cucullu et al. originally developed for assay of individual peanuts (7). Final extracts were dissolved in 0.1 ml chloroform, and 10 µl of extract was spotted on a thin layer chromatographic (TLC) plate, with four standard spots of 1, 2, 3, and 5 µl of a mixed aflatoxin standard (1.0 µg/ml each of B_1 and G_1, 0.3 µg/ml each of B_2 and G_2). Plates were developed in chloroform-acetone (9 + 1) and assayed by visual comparison with the standards. For seed of extremely high contamination levels, extracts were suitably rediluted and assayed. Since the sensitivity of the method varies with the weight of the kernel, detection limits were ca. 100, 200, and 500 (ppb) or µg/kg for kernels weighing 0.1, 0.05, and 0.02 g, respectively.

TABLE I

Weight Ranges and Aflatoxin Contamination of Individual Selected Cottonseed

Kernel wt range, g[a]	No. of seed[b]	Weighted %		Range in total aflatoxins, ppb	% of total aflatoxin contamination
		In category	Total seed		
			Aflatoxin-positive seed		
<0.01	7	4.9	0.9	8,000-2,800,000	2.4
0.01-0.04	52	36.6	6.7	260-5,750,000	42.4
0.04-0.06	53	37.3	6.9	210-3,200,000	50.0
0.06-0.08	25	17.6	3.2	150-520,000	4.9
0.08-0.10	5	3.5	0.6	150-30,400	0.3
			Aflatoxin-negative seed		
<0.01	30	4.8	3.9	-	-
0.01-0.04	315	50.1	40.9	-	-
0.04-0.06	182	28.9	23.6	-	-
0.06-0.08	70	11.1	9.1	-	-
0.08-0.10	32	5.1	4.2	-	-

[a]To convert to seed weight, multiply by 2.

[b]771 seed, 142 aflatoxin-positive, 629 aflatoxin-negative.

TABLE II

Aflatoxin Ranges of Contaminated Cottonseed Kernels

Number of seed[a]	Aflatoxin ranges of contaminated kernels, ppb	% of contaminated seed
29	100-1,000	20.4
37	1,000-10,000	26.1
36	10,000-100,000	25.4
24	100,000-500,000	16.9
8	500,000-1 million	5.6
8	> 1 million	5.6

[a]142 contaminated cottonseed.

RESULTS AND DISCUSSION

Only 18.4% of the 771 selected individual cottonseed contained detectable aflatoxins, but these ranged from ca. 150 ppb to 5.75 million ppb, with a mean total aflatoxin level of 257,000 ppb. Eight of the 142 positive kernels had over 1 million ppb, ranging from 1.13-5.75 million ppb.

Kernel Weight vs. Aflatoxins

Grouping the positive and negative kernels by weight range (Table I) shows that ca. 74% of the contaminated kernels were in the 0.01-0.06 g range, corresponding to 0.02-0.12 g on a ginned, fuzzy seed basis. The aflatoxin detected in these seed represented ca. 92% of the total aflatoxins present in the 142 contaminated kernels. For the 629 negative kernels, 64% were also in the 0.01-0.06 g weight range (Table I). Since normal cottonseed kernels range from ca. 0.04-0.08 g (8), physical separation of kernels on the basis of weight or size would not seem to be an effective method for segregating aflatoxin-contaminated cottonseed. A similar conclusion was reached by Koltun et al. (2) from seed density vs. aflatoxin relationships on a limited number of individual cateye cottonseed.

Aflatoxin Ranges

Grouping the contaminated kernels by range of aflatoxin contamination (Table II) shows a fairly uniform percent distribution, 17-26%, in the 4 ranges of 100-500,000 ppb. Less than 12% of the kernels contained over 500,000 ppb, but only a few of these highly contaminated kernels in a sample of cottonseed could result in a meal of significant aflatoxin contamination. Calculations based on the mean kernel aflatoxin contamination level found in this study—257,000 ppb—and the mean kernel weight—.01045 g—showed that of the ca. 1.50 million kernels required to produce 100 lb of cottonseed meal, only 78 (0.005%), 392 (0.03%), and 1,962 (0.13%) of these contaminated kernels would result in meal of 20, 100, and 500 ppb of aflatoxins, respectively. This observation emphasizes that effective physical techniques for segregating cottonseed of high aflatoxin contamination levels could be a practical and effective means for materially reducing aflatoxin contamination in cottonseed meals.

TABLE III

Aflatoxin Content of Individual Cottonseed Categorized by Physical Appearance

Seed category	Seed		Aflatoxins in positive seed		Positive seed in category, %	Category in positive seed[a], %
	No.	Positive	Range, ppb	Mean, ppb		
Cateye fluorescence	80	38	220,000-2.70 million	454,000	47.5	26.8
Balding only	596	86	150-3.20 million	186,800	14.4	60.6
Balding + cateye	19	8	160-5.75 million	125,400	42.1	5.6
Thin and discolored lint	52	10	250-750,000	148,600	19.2	7.0
Blue fluorescence	24	0	-	-	-	-
Total	771	142			18.4	

[a]No. of positive seed in each cateogry/total no. of positive seed.

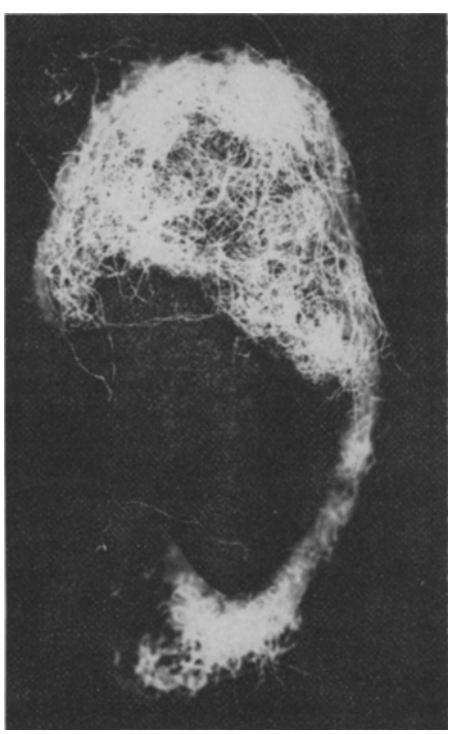

FIG. 1. Photograph of typical balding cottonseed; 500,000 ppb total aflatoxins.

Physical Segregation

The 771 selected individual cottonseed were categorized according to unique physical characteristics as shown in Table III. Seed with cateye fluorescence on the linter fibers constituted ca. 27% of the 142 aflatoxin positive seed. Within the cateye category, 48% of the seed were aflatoxin positive and 52% were negative. These percentages are nearly identical to those found by Koltun et al. (2) on assays of 36 cateye seed. The positive cateye seed tended to be high in aflatoxin content, the mean value of 454,000 ppb being the highest of the five categories. Four seed in this category had over 1 million ppb of aflatoxins. No aflatoxins were detected in 24 seed with bluish, but not cateye, fluorescence.

The highest percentage, 61%, of the 142 contaminated seed was in the balding category. Even though only 14% of the balding seed contained aflatoxin, their aflatoxin content also tended to be high, with a mean level of over 186,000 ppb. Three seed in this category had over 1 million ppb of aflatoxins.

Seed with both cateye fluorescence and balding characteristics were only 6% of the positive seed, but 42% of the seed in this category contained aflatoxins, with a mean level of 125,000 ppb. The highest kernel contamination, 5.75 million ppb, was found in this category.

We theorize that fungal invasion of cotton bolls in the field involves mold attack of the seed, with consequent weakening of the short linter fibers, a portion of which are completely removed during ginning. A typical partially bald seed is illustrated by the photograph in Figure 1. The kernel of this seed contained 500,000 ppb of aflatoxins. This weakening of the linter fibers probably accounts for the 7% of the 142 contaminated seed which had thin and discolored lint (Table III), with mean kernel aflatoxin contamination over 148,000 ppb.

Only aflatoxins B_1, or $B_1 + B_2$, were detected in the 142 contaminated seed, indicating contamination by *A. flavus* rather than by *A. parasiticus* (9). Although most of the seed contained four to five times as much B_1 as B_2, anomalies were noted. Five seed from one location contained more B_2 than B_1. The B_2 content ranged from 2 million ppb to 200 ppb, and the ratio of B_2 to B_1 varied from 30:1 to 2:1. These results were confirmed by preparatory TLC of the presumptive B_2 spot, and elution from the plate. The absorption spectra of the presumptive B_2 material was identical to that of authentic aflatoxin B_2.

Information developed in the present work suggests that about 93% of 142 aflatoxin-contaminated cottonseed selected from a heterogeneous stockpile of seeds were characterized either by cateye fluorescence, by a partially bald characteristic, or by a combination of these two characteristics. Removal of such seed from lots of aflatoxin-contaminated cottonseed should materially reduce aflatoxin contamination. Work is under way on the evaluation of this approach with several reasonably large lots of seed and will be reported in a subsequent communication.

ACKNOWLEDGMENT

We wish to thank L. Ashworth, H. Gardner, Jr., G. Harper and M. Whitten for cottonseed samples.

REFERENCES

1. Marsh, P.B., K. Bollenbacher, J.P. San Antonio, and G.V. Morola, Textile Res. J. 25:1007 (1955).
2. Koltun, S.P., H.K. Gardner, Jr., F.G. Dollear, and E.T. Rayner, JAOCS 51:178 (1974).
3. Marsh, P.B., M.E. Simpson, R.J. Ferretti, T.C. Campbell and J. Donoso, J. Agr. Food Chem. 17:462 (1969).
4. Ashworth, L.J., Jr., J.L. McMean, J.L. Pyle, C.M. Brown, J.W. Osgood, and R.E. Ponton, Phytopathology 58:102 (1968).
5. Whitten, M.E., Cotton Gin Oil Mill Press 67:7 (1966).
6. Clark, S.P., and C.M. Cater, 52:124A JAOCS (1975).
7. Cucullu, A.F., L.S. Lee, R.Y. Mayne and L.A. Goldblatt, Ibid. 43:89 (1966).
8. Tharp, W.H., in "Cottonseed," edited by A.E. Bailey, Interscience Publishers Inc., New York, NY, 1948, Chapter IV.
9. Hesseltine, C.W., O.L. Shotwell, M. Smith, J.J. Ellis, E. Vandergraft, and G. Shannon, "Proceedings of the First US-Japan Conference on Toxic Microorganisms," edited by M. Herzberg, UJNR Joint Panels on Toxic-Microorganisms and the U.S. Department of the Interior, 1970, p. 202.

[Received July 6, 1976]