Screening Cottonseed for Aflatoxins[1]

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Abstract

A rapid screening method for detecting aflatoxins in cottonseed has been developed. Using long-wave ultraviolet light and samples containing aflatoxins, the fibers on a few cottonseed fluoresced a greenish yellow and the ends of some sticks and stems (foreign material) fluoresced with a bluish color. Nine out of 10 of the 2300 samples of cottonseed were correctly screened during the last two years of the study. In only one category, 1–10 ppb aflatoxins, was there an appreciable error in the screening method.

Introduction

In considering problems of contamination in agricultural products, those which are related to sampling and to rapid identification of these contaminants are of utmost importance. The inspection of a commodity, of separating the good from the bad or suspect, can not be emphasized too greatly.

Recent work on mycotoxins in agricultural products, and of cottonseed and peanuts in particular, has brought out the fact that aflatoxin contamination is often concentrated in relatively small amounts of the raw material (1,2,4,7). In other words, most of the cottonseeds and peanuts are good, with only a few contaminated seeds or nuts. These few heavily contaminated seeds or nuts can ruin an entire lot of otherwise good cottonseed or peanuts.

Experimental Procedures

Screening by Fluorescence Observations

During the fall of 1964, the Market Quality Research Division, Agricultural Research Service, U.S. Department of Agriculture initiated a survey to determine the extent of aflatoxin contamination of the 1964 crop of cottonseed in the United States. Samples were analyzed chemically for aflatoxins (6).

Samples of cottonseed from this survey and other sources were examined under long-wave ultraviolet light. No differences were noted on the early precleaned samples with and without aflatoxins. However, when gin-run cottonseed were examined under ultraviolet light, a marked difference was observed between cottonseed with and without aflatoxins. In general, no foreign material or cottonseed fluoresced in lots that were free of aflatoxins. However, in contaminated lots of cottonseed, the ends of sticks and stems and a few seed fluoresced.

The sticks and stems fluoresced with a bluish color whereas the cottonseed fluoresced a greenish yellow. The bluish color on the foreign material is probably related to mold growth other than *Aspergillus flavus*. The greenish yellow, however, is related to *A. flavus*. This relationship was pointed out by Marsh and coworkers who also confirmed the association of the greenish-yellow fluorescence with *A. flavus* and with aflatoxin contamination in cottonseed (1,2).

In our study in 1964, the degree of fluorescence was observed to increase as the aflatoxin content of the cottonseed and cottonseed meal made from seed represented by the sample increased. This led to the establishment of ranges of fluorescence to represent degrees of contamination of cottonseed with aflatoxins.

A grading scheme was proposed to include four degrees of contamination. Grade 0 contained no fluorescent material. In grade 1, one or two seeds and a small amount of foreign material fluoresced. In grade 2, at least 10% of the foreign material fluoresced as well as 3 or 4 seeds. Grade 3 contained a larger amount of fluorescing foreign material and fluorescing cottonseed.

This proposed method of estimating the probable contamination of cottonseed with aflatoxins was explained to two members of our staff. Samples of cottonseed representing fluorescence as observed in the proposed grades were examined and within a few minutes our staff members could match other cottonseed samples against the proposed grades. After examining a few samples, no further reference to these sample grades was necessary. This was fortunate as the fluorescence fades after extended ultraviolet radiation or after the samples remain at room temperature in daylight for several weeks.

A number of samples of cottonseed which fluoresced but which were found to be free of aflatoxins by chemical analysis produced meal which showed contamination when analyzed. This apparent discrepancy was due to the distribution of aflatoxin containing cottonseed as previously mentioned.

Meal samples, because of their small particle size, are more homogeneous and are probably more representative of a lot of seed. Analysis of data for the three years covered in this report showed a better relationship between gin-run seed fluorescence and aflatoxin in meal produced from seed represented by the sample than between fluorescence of the seed sample and aflatoxin content of the seed.

With this method of screening, cottonseed with low levels of aflatoxins would be expected to contain less fluorescing material and would, therefore, be more difficult to grade. That was found to be true (Table

TABLE I

Accuracy of Screening Cottonseed by Fluorescence (Long-Wave Ultraviolet Light) With Cottonseed Meal Produced and Containing Specified Aflatoxin B_1 Contents, Crops of 1965–66 and 1966–67

Aflatoxin content of meal produced from seed represented by sample (ppb, B_1)	Cottonseed samples graded[a]			
	Correctly		Incorrectly	
	No.	%	No.	%
1965–66 Crop year				
0	924	91.0	91	9.0
1– 10	45	55.5	36	44.5
11– 30	67	63.8	38	36.2
31– 70	45	70.3	19	29.7
71–150	22	84.6	4	15.4
151–500	2	100.0	0	0.0
Total	1,105	85.5	188	14.5
1966–67 Crop year				
0	874	92.7	69	7.3
1– 10	5	31.3	11	68.7
11– 30	13	81.2	3	18.8
31– 70	25	92.6	2	7.4
71–150	31	88.6	4	11.4
151–500	34	89.5	4	10.5
501–1500	1	100.0	0	0
Total	982	91.3	94	8.7

[a] Cottonseed samples were graded correctly if meal produced from seed represented by samples in Grade 0 contained no aflatoxin and meal from seed in all other groups contained aflatoxin.

[1] Presented at the AOCS-AACC Joint Meeting, Washington, D.C., March 1968.

TABLE II

Accuracy of Screening Cottonseed by Fluorescence (Long-Wave Ultraviolet Light) With Aflatoxin Contamination of Meal Produced, Crops of 1964–65, 1965–66 and 1966–67

Estimated contamination of meal produced from cottonseed		Cottonseed samples graded[a]			
		Correctly		Incorrectly	
Grade	Description	No.	%	No.	%
1964–65 Crop year					
0	No aflatoxin	480	90.0	54	10.0
1	May contain aflatoxin suspect	129	43.0	170	57.0
2	Contains greater amounts of aflatoxins	66	72.0	26	28.0
3	Contains more aflatoxins than Grade 2	18	86.0	3	14.0
	Total	693	73.3	253	26.7
1965–66 Crop year					
0	No aflatoxin	716	93.4	61	6.6
1–	Borderline-no aflatoxins	208	87.4	30	12.6
1+	Contains aflatoxins	173	65.0	24	35.0
2	Contains more aflatoxins than Grade 1+	8	72.7	3	27.3
3	Contains more aflatoxins than Grade 2	0	0.0	1	100.0
	Total	1,105	85.5	188	14.5
1966–67 Crop year					
0	No aflatoxin	644	98.0	13	2.0
1–	Borderline-no aflatoxin	230	76.9	69	23.1
1+	Contains aflatoxins	106	89.8	12	10.2
2	Contains more aflatoxins than Grade 1+	2	100.0	0	0.0
	Total	982	91.3	94	8.7

[a] Cottonseed were graded correctly if meal produced from seed represented by samples in Grade 0 contained no aflatoxin and meal from seed in all other groups contained aflatoxin.

I); of the cottonseed which produced meal samples containing between 1 and 10 ppb of aflatoxin B_1, only half were screened correctly. (Since the yield of cottonseed meal is slightly less than half the weight of the seed, this category would include cottonseed with 0 to 5 ppb of aflatoxin B_1.) As the contamination with aflatoxins increased, the accuracy of screening improved. Screening was most accurate, however, in grading samples that contained no aflatoxins.

Screening the 1964–65 Crop

In 1964–65, more than half of the 946 samples of cottonseed were graded as having no aflatoxins in meal produced from the seed (Table II). Nine out of ten of these observations were correct. In grade 1, the suspect grade, slightly more than one half of the samples contained no aflatoxins. For this reason, the grade was further divided into two sub-grades for examination of the 1965 crop.

When the questionable grade 1 was not considered, seven out of eight samples were screened accurately, indicating a good relationship between seed sample fluorescence and aflatoxin contamination of meal. When all samples were considered, three out of four were graded accurately.

Examination of the 1965 Crop

A total of 1293 samples of cottonseed representing the 1965 crop of cottonseed was screened for aflatoxins. Meal produced from cottonseed represented by these seeds was also analyzed by chemical analysis.

The percentage of samples of cottonseed and of cottonseed meal containing aflatoxins was about the same in 1965 as in 1964. However, the intensity and amount of fluorescing material appeared to be less than was observed in the previous year. Only 12 samples of cottonseed were screened as grade 2 or higher. An average of 15 out of 16 samples of the cottonseed with no aflatoxins in the meal produced was screened correctly on the 1965 crop (Table II).

Screening the 1966 Crop

Since the survey of aflatoxins in cottonseed was continued for a third year, the screening program was also continued. There were 1076 samples of cottonseed from the 1966 crop screened for aflatoxin contamination (Table II). Of all samples graded as containing no aflatoxins, 98% were screened correctly. Of the nearly 300 which fell in the suspect 1 category, three out of four were screened correctly as containing no aflatoxins. In the grade 1+ category, nearly 9 out of 10 were screened correctly. The two samples which fell in grade 2 were graded correctly.

Of the 943 samples which contained no aflatoxins, in the 1966 crop, 874 or about 93% were graded correctly (Table I). This is slightly better than the 91% correctly screened in 1965. In the 1 to 10 ppb B_1 category, only one third of the samples were screened correctly as compared with one half the previous year.

In the other categories, however, our screening was somewhat more efficient on the 1966 crop. We were correct more than 9 times out of 10.

What are the advantages of this screening method? It provides a rapid on-the-spot evaluation of a given lot of cottonseed that has proved in all but one category to be correct 9 times out of 10. It allows the processor to divert suspect seed for further analysis to determine the disposition to be made of the lot. The suggested use of sorting equipment may be an answer for disposition of contaminated seed (2).

In the meantime, the processor can be fairly certain that this simple rapid screening procedure will assist him in diverting possibly contaminated cottonseed. It will definitely lessen the probability of commingling good cottonseed with contaminated cottonseed.

REFERENCES

1. Ashworth, L. J., Jr., and J. L. McMeans, Phytopathology *56*, 1104–1105 (1966).
2. Ashworth, L. J., Jr., J. L. McMeans, J. L. Pyle, C. M. Brown, J. W. Osgood and R. E. Ponton, Phytopathology *58*, 102–107.
3. Bollenbacher, K., and P. B. Marsh, Plant Dis. Reptr. *38*, 375–379 (1954).
4. Dickens, J. W., "Summary of the Proceedings of the Mycotoxin Research Seminar," U.S. Department of Agriculture, June, 1967, p. 6.
5. Marsh, P. B., and E. E. Taylor, Plant Dis. Reptr. *42*, 1368–1371 (1958).
6. Pons, W. A., Jr., and L. A. Goldblatt, JAOCS *42*, 471–475 (1965).
7. Whitten, M. E., Cotton Gin and Oil Mill Press *67*, 7–8 (1966).

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