Relationship of BGYF Spots Detected on the Sides of Trailers and Modules of Seed Cotton to Aflatoxins in Ginned Seed

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ABSTRACT

Sides of 146 trailers and 60 modules packed with freshly harvested seed cotton from commercial fields were examined at night using a long-wave ultraviolet lamp for bright-green-yellow fluorescent (BGYF) spots caused by contamination of cotton lint with *Aspergillus flavus*. During ginning, a 22.7 kg (50 lb sack) of seed was removed from each trailer or module for determination of aflatoxin content. For both carriers, as the number of BGYF spots increased, the level of toxin in ginned seed increased. Assays of seed from 90% of the trailers and 64% of the modules with 0-1 BGYF spots were below 20 μg/kg.

INTRODUCTION

Presence of a bright-green-yellow fluorescence (BGYF) on cotton lint viewed in long-wave ultraviolet (UV) light has been associated with invasion of the bolls by *Aspergillus flavus* (1). Invasion of cottonseed in the boll by this fungus can cause aflatoxin contamination (2). Subsequent studies showed that removal of seed with BGYF linters from samples of ginned seed or removal of BGYF seed cotton from samples of nonginned seed cotton effectively reduced toxin levels in the ginned seed to nondetectable levels (3). However, analysis of seed from BGYF-positive locks showed that not all seed contained toxins (4). This indicated that fungi responsible for the formation of the BGYF compound on cotton locks are not invariably toxigenic or, if toxigenic, do not always invade seed to form aflatoxins. The cottonseed industry is seeking a rapid, practical and effective screening procedure for the diversion of seed contaminated with aflatoxin from uncontaminated seed. The current study was undertaken in an attempt to relate the incidence of BGYF spots in commercially produced seed cotton, before ginning, with aflatoxin content of ginned seed. This involved a large-scale study in which freshly harvested seed cotton contained in either trailers or modules was examined at gins for BGYF spots and samples of ginned seed were removed and analyzed for aflatoxin content.

EXPERIMENTAL PROCEDURES

Trailers and modules were examined at night for BGYF spots with a generator-powered, hand-carried UV lamp. BGYF counts and sample collections were made by individuals with only rudimentary training in the use of UV and BGYF identification. As ginning proceeded, 22.7 kg (50 lb sack) of ginned seed was collected at the gin stand. One sack was collected during ginning of seed cotton from each trailer or module. Samples were collected after the carrier was about half emptied.

Ginned seed from each sack were dehulled in a Bauer mill, meats were separated from hulls and ground to pass a 2 mm screen. Screened meats were hand blended. About 10 kg of meats were obtained from each sack. From each sample of ground meats, 4, 25 g subsamples were analyzed for aflatoxins (5). High performance liquid chromatography (HPLC) was used for quantitation (6).

RESULTS AND DISCUSSION

One hundred forty-six trailers and 60 modules were examined at 4 gins. Samples represented part of the harvest from fields planted by 17 growers in an area extending from Casa Grande to Gila Bend, AZ. The compacted modules contained ca. 7,000-8,000 kg (8-9 tons) of seed cotton, whereas the trailers contained ca. 1/3-1/2 this weight although the area viewed on the trailers was several times greater. Results (Table I) show a high correlation between number of BGYF spots observed on sides of trailers or modules and aflatoxin detected in seed. Carriers with only a few BGYF spots on seed cotton contained seed with low to nondetectable levels of aflatoxin. As the number of spots increased the level of toxin in ginned seed increased.

Aflatoxin results are given as numbers of samples or percentage of samples with toxins below 20 μg/kg (Table I). The greater the number of fluorescent spots per trailer, the fewer trailers with less than 20 μg/kg of aflatoxins. Of the 25 trailers with 7 or more BGYF spots, only 5 (20%) had

TABLE I

Aflatoxin Content of Seed and Bright-Green-Yellow Fluorescent Spots on Lint of Seed Cotton in Trailers and Modules

Fluorescent spots per trailer or module	Number of trailers	Trailers with less than 20 μg/kg aflatoxin (%)	Number of modules	Modules with less than 20 μg/kg aflatoxin (%)
0	61	55 (90)	41	26 (63)
1	27	24 (89)	9	6 (67)
2	13	10 (77)	4	2 (50)
3	12	7 (58)	4	1 (25)
4	2	2 (100)		
5	2	1 (50)		
6	4	2 (50)	1	0 (0)
7 or over	25	5 (20)	1	0 (0)

less than 20 μg/kg of aflatoxins in the ginned seed. Only 14 trailers of the 146 examined had more than 10 BGYF spots. Trailers with a high count of spots on one side usually had about the same number on the other side. The highest toxin level detected was in seed ginned from a trailer with 12 spots on one side and 14 on the other. The average toxin content on meats from this trailer was 1371 μg/kg; standard deviation (SD) between the 4 subsamples was 236.07 with a coefficient of variation (CV) of 17%. Two other trailers with 12 and 14 spots on one side and 10 and 15 on the other had seed with 284 and 49 μg/kg with SD of 67.34 and 55.17 and CV of 24 and 112. One exception to the trend were the trailers with the highest number of BGYF spots (19 and 18); seed had only 97 μg/kg of toxins, SD=107.11, CV=110. Six trailers had seed with low levels of toxins when no BGYF spots were detected. Such toxin-containing seed cotton could have come from the center of these trailers.

Results from module sampling paralleled those from trailers though BGYF spots on modules were more difficult to count. Dust on the sides and the apparent compression of fluorescent fibers so that spots could not be seen easily made observations difficult. Moreover, because of compacting of seed cotton to form the modules, the sides of the modules represented less of the total volume than did the respective sides of the trailers. No module had over 7 BGYF spots. Only one contained 7 spots and another 6. In all cases, ginned seed with levels of toxin below 20 μg/kg (and often ND) were from modules with a single or no fluorescent spots, although toxin levels in 18 modules with 0-1 fluorescent spots were above 20 ppb.

A Pearson correlation between number of BGYF spots on trailers and toxin in seed was 0.41, which is significant at a 0.0001 level, whereas a similar correlation on modules was 0.29, significant at only a 0.05 level. These results show that night or simulated nighttime examination for BGYF spots could be used as a practical procedure to divert trailers with seed potentially high in toxin. The procedure is not as effective for modules.

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The Determination of Light Petroleum Residues in Refined Oils and Fats

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ABSTRACT

A rapid, direct injection gas liquid chromatographic (GLC) method for determining residual light petroleum in edible vegetable oils has been developed. The response is linear at levels between 0.05-0.5 mg hexane/kg oil. A sample containing 0.2 mg hexane/kg oil was analyzed for repeatability, giving a standard deviation of 0.008 mg/kg, equilvalent to a coefficient of variation of 4%. Separation of pentane, hexane, heptane, octane and decane was obtained by this method. A survey of 23 samples of freshly refined vegetable oils obtained from 13 U.K. refiners in 1981 showed that these all contained less than 0.05 mg hexane/kg oil.

INTRODUCTION

Light petroleum (hexane fraction) is a preferred solvent for the extraction of oil from oilseeds. Unavoidably, residual solvent is in both the oil and the meal after extraction. For example, previous investigators have reported levels of 310 mg kg^{-1} (1) and 550-3500 mg kg^{-1} (2) in crude soybean oil.

In the refining process, residual solvents are predominantly removed at 2 stages—bleaching and deodorizing. Some will be adsorbed onto the bleaching earth and the remainder drawn off with other volatile matter during deodorization. Temperatures of up to 270 C and a vacuum of up to 0.3 torr are applied during processing. After this treatment, residual solvent should be completely removed.

Hirayama and Imai (1) have reported "none detected" for residual hexane in deodorized oils. Their limit of detection was ca. 1 mg kg^{-1}.

The aim of the current study was to optimize analytical techniques and to obtain a lower detection limit. The method was then used to determine the residual levels of solvent remaining after refining, to provide evidence for the Seed Crushers' and Oil Processors' Association for submission to the (U.K.) Ministry of Agriculture, Fisheries and Food in connection with the Proposed EEC Directive on Extraction Solvents (3).

Many workers have described the determination of solvents in oilseed meals (4-8), in oils (1,2,12-17) and in other foods and biological tissues (9-11). The analytical methods described in the above papers can be classified into three groups: (a) headspace gas analysis; (b) solvent extraction and (c) direct injection into a gas chromatograph (GC). These methods are described below.

Headspace Gas Analysis

A suitable amount of sample is weighed into a septum vial, and the vial is sealed and placed in an oven. Low-boiling-point hydrocarbons pass into the headspace above the sample until equilibrium is achieved. Aliquots of the headspace gas are injected onto a GC column and peak areas compared with standards prepared in a similar manner.