AFLATOXIN CONTAMINATION OF COTTONSEED IN SOUTH TEXAS: THE ROLE OF INSECT INJURY

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Abstract

There was no aflatoxin contamination of cottonseed originating from non-damaged bolls or non-damaged locks from insect-damaged bolls in samples from fields that had not been weathered. In contrast, 61% of seed from insect-damaged locks had high (i.e. >20 ppb) aflatoxin concentrations, with an average concentration of 649 ppb. Cottonseed samples from modules originating from fields that had not been weathered showed a similar association of high aflatoxin concentration with insect injury. High concentrations (35-2,500 ppb) of aflatoxin were found in seed associated with bright green-yellow fluorescent (BGYF) lint, which indicates *Aspergillus flavus* infection occurred prior to boll maturity. High concentrations (27-2,200 ppb) were also found in seed associated with insect-damaged, non-BGYF lint. Concentrations in seed following exposure of mature cotton in the field to rain showers over a period of four weeks were high in both insect-damaged (930-1,400 ppb) and non-damaged (150-280 ppb) locks. Aflatoxin was present in 3% of non-damaged seed obtained from modules made from non-weathered cotton and it was present in 82% of similar samples made from weathered cotton (average content 760 ppb). This study shows that in the absence of weathering, insect injury is the main factor associated with aflatoxin contamination in south Texas.

Introduction

Cottonseed is an important by-product of cotton production used for feed and its contamination with aflatoxin, a carcinogen produced by the fungus, *Aspergillus flavus*, results in a loss of value for what would otherwise be a premium animal feed. Aflatoxin is a chronic contaminant of cottonseed grown in the Rio Grande Valley and Coastal Bend areas of south Texas. As reported by one south Texas oil mill, the approximate annual loss for south Texas is $550,000. This loss is not directly felt by growers and, indeed, few growers in south Texas are aware of the problem.

In Arizona, aflatoxin contamination occurs in two phases: before boll maturity, as a consequence of insect damage (primarily pink bollworm), and after maturity, when bolls are exposed to high humidity or rain (Cotty, 1991). *A. flavus* infection prior to boll maturation is indicated by the presence of bright green-yellow fluorescence (BGYF) on lint (Ashworth and McMeans, 1966), with insect injury serving as the means of entry for the pathogen (Henneberry *et al.*, 1978; Lukefahr and Martin, 1963). Infection can also occur in non-damaged bolls following boll maturation, in which case, BGYF does not develop (Gardner *et al.*, 1974). Thus, the presence of BGYF can indicate insect damage as a predisposing factor for aflatoxin contamination.

Knowledge of the factors responsible for aflatoxin contamination could lead to changes in crop production practices for its management. Since the cotton growing areas in south Texas have different insect pests and climates than Arizona or the Imperial Valley of California (another area with aflatoxin problems), there is a need for information for this geographic area. The main objective of this study was to determine whether aflatoxin contamination of cottonseed in south Texas was associated with insect injury to bolls. The contribution of weathering (in particular, exposure to frequent rain storms over the course of three weeks) of mature cotton in the field to contamination was also monitored, to a limited extent.

Materials and Methods

Sampling - 1995

Cotton was sampled from modules and gin carts at seven gins in San Patricio county from August 25 to September 14, 1995. Modules stored in fields next to the growing area were also sampled. Locks were examined for the presence of BGYF lint using an ultra-violet light. Seed closely associated with BGYF lint (hereafter referred to as "BGYF seed") was segregated from seed associated with non-BGYF lint (hereafter referred to as "non-BGYF seed") and analyzed separately for aflatoxin.

Sampling - 1996

Cotton was sampled from modules at six gins in San Patricio county and one gin in Kleberg county from August 7 to September 13, 1996. Modules stored in fields next to the growing area were also sampled. BGYF seed and seed associated with discolored, non-fluorescent lint (hereafter referred to as "damaged, non-BGYF seed") were segregated and analyzed separately from non-damaged seed.

Cotton was sampled once, within one week of harvest, in 15 fields in Hidalgo, Kleberg, Nueces, and San Patricio counties. These fields were monitored on a weekly basis for insect pests by IPM scouts. Bolls with obvious insect damage were collected separately from intact bolls. In the laboratory, fluffy locks from damaged bolls were segregated from damaged locks. BGYF seed and damaged, non-BGYF seed was segregated from non-damaged seed and analyzed separately.

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Determination of Aflatoxin Content

The quantity of seed samples ranged from 500-1800 g (fuzzy). Samples were ginned, dehulled with a Wiley mill, and ground to pass through a 1-mm-mesh sieve. Fifty-g portions of samples were extracted in 200 ml 80% methanol. Because some of the BGYF or damaged, non-BGYF samples were less than 50 g, extraction volumes were reduced in proportion to sample size. Small samples (1-50 seeds) were dehulled by hand and were extracted with 10 ml. Aflatoxin was quantified by immunoaffinity column chromatography, followed by solution fluorometry. An Aflatest P immunosorbent column (Vicam Company, Somerville, MA) was used and the recommended protocol was followed.

Results

In 1995, high aflatoxin concentrations (i.e. > 20 ppb) were found in 76% of non-BGYF samples and in 53% of BGYF samples. The distribution of samples into different aflatoxin concentration categories is shown in Figure 1. Aflatoxin concentrations in BGYF seed ranged from 0-218,750 ppb and tended to be higher than that of non-BGYF seed, which ranged from 0-5,000 ppb. BGYF seed was found in 23 out of 29 (79%) module and gin cart samples from San Patricio county. BGYF seed comprised 0.009 - 0.31% of the samples.

In 1996, samples collected before August 21 were not exposed to any significant amount of rainfall. After this date, there was a three-week period of intermittent, heavy rainshowers that impeded the harvest and resulted in a substantial amount of weathering in remaining fields. Only 3% of the non-damaged seed samples obtained from modules that were made prior to this rainy period had high aflatoxin concentrations, while 53% of BGYF samples and 38% of damaged, non-BGYF samples had high concentrations (Figure 2). The concentration of many of these contaminated samples exceeded 200 ppb.

In contrast, 82% of non-damaged samples from modules made after the onset of the rainy period had high aflatoxin concentrations, while 60% of the BGYF and 79% of damaged, non-BGYF samples were high (Figure 3). The severity of contamination tended to be higher for the BGYF and damaged, non-BGYF samples, in comparison with non-damaged samples.

With bolls collected from fields that had not been weathered, aflatoxin was low (i.e. <20 ppb) in seed from non-damaged bolls and from the fluffy locks of insect-damaged bolls. High aflatoxin concentrations in seed from tight locks of insect damaged bolls occurred in 7 out of 12 fields sampled (Table 1). BGYF seed was found in only four fields. In the 12 fields, boll weevil damaged ranged from 1-31% of green squares over the course of the season, while cotton bollworm damage ranged from 1-15%. There were no other boll pests observed.

There were three fields in San Patricio county that were sampled after the onset of the rainy period. One field, sampled August 29, had 1,200 ppb aflatoxin in seed from tight locks of insect-damaged bolls, while seed from fluffy locks of these bolls and from non-damaged bolls had low aflatoxin concentrations. This field was reported to be under heavy weevil pressure. The other fields, under different weevil pressures, were sampled September 13 and were so severely weathered that it was not possible to segregate insect-damaged bolls into fluffy locks and tight lock samples. In the field under light weevil pressure, samples from non-damaged bolls had 280 ppb aflatoxin, while damaged bolls had 930 ppb. The field under heavy weevil pressure had 150 ppb in non-damaged bolls and 1,400 ppb in damaged bolls.

Discussion

This study shows that, in the absence of weathering, insect injury is the main factor leading to aflatoxin contamination in south Texas. High levels of aflatoxin were found in many BGYF and damaged, non-BGYF samples, while non-BGYF samples were usually free of aflatoxin. Similarly, Ashworth and McMeans (1966) reported a range of 6,500-11,200 ppb aflatoxin in BGYF seed, but only a range of 2-30 ppb in non-BGYF seed. The incidence of BGYF seed observed during the 1995 and 1996 seasons was low, confirming the low incidence reported in 1994 (Isakeit and Dunlap, 1995). Ashworth *et al.* (1968) found that BGYF accounts for an average of 0.3% of samples.

In the 1996 season, the sampling protocol was altered to include seed from insect-damaged, non- BGYF lint in a separate category. Such samples included brown or yellow lint. These samples also tended to have high aflatoxin concentrations. Since such samples were not segregated from non-damaged seed during the 1995 study, it is possible that some non-BGYF samples in Figure 1 with high aflatoxin concentrations could also contain contaminated damaged, non-BGYF seed. Some of these samples were likely also weathered and the contribution of this factor to the severity of contamination is not known.

Cotty and Lee (1989) reported high aflatoxin levels in some non-damaged locks adjacent to pink bollworm-damaged locks, indicating the spread of *A. flavus* and production of aflatoxin in these parts of the bolls. In this study, if damaged locks were contaminated, fluffy locks from those bolls were not. One possible explanation is that under the environmental conditions in the 1996 season, the fungus did not colonize beyond the damaged portion of the boll. Colonization of non-damaged locks from contaminated, damaged locks adjacent to them may occur under other environmental conditions.

The association of aflatoxin with seed of tight locks has implications for the use of gin trash for feed. These tight locks tend to be concentrated in the gin trash and create a

risk for aflatoxin contamination. The aflatoxin content of a sample of gin trash obtained early in the 1996 season was 75 ppb.

The insect pests of bolls reported in 1995 in the Coastal Bend include the boll weevil, tobacco bollworm, tobacco budworm, stinkbug and cricket. In 1996, the major pests were the boll weevil, followed by the tobacco bollworm and tobacco budworm. The populations of these pests critical for creating an aflatoxin problem are not known. Cotty and Lee (1989) pointed out that control thresholds for the pink bollworm were often higher than the populations that could cause a contamination problem. They suggested post-harvest removal of damaged locks prior to ginning as a management approach, rather than lowering action thresholds for insect control. There is currently a boll weevil eradication program in progress in the Coastal Bend. If this program is successful, it would be interesting to see what the effect the elimination of the boll weevil will have on aflatoxin contamination over the long term.

Insect injury to the developing boll allows the fungus to grow within the seed and produce toxin in it over a long period of time. A small percentage of highly-contaminated BGYF seeds can cause a significant contamination problem. For example, one sample had three BGYF seeds with a concentration of 218,750 ppb. The presence of these three seeds, 0.009% of the total, will cause 1½ pounds of non-contaminated cottonseed to exceed the allowable aflatoxin limit.

The results of the 1996 study shows that weathering can be an important factor for post-maturation contamination of cottonseed with aflatoxin. This was also reported by Ashworth *et al.* (1968) to account for some of the contamination in Arizona. Weathering will vary from year to year and this probably accounts for some of the variation seen in severity of aflatoxin contamination in a growing area.

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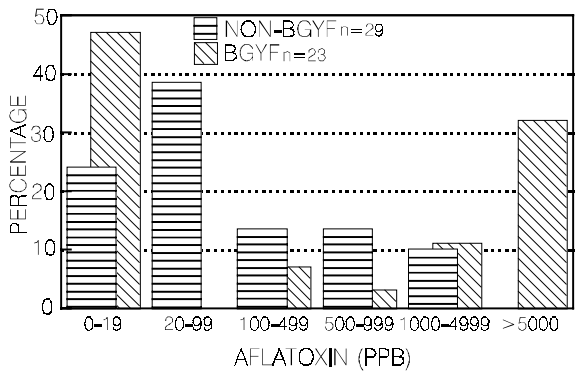

Figure 1. Distribution of BGFY and non-BGYF cottonseed samples collected from modules in San Patricio county in 1995 into different aflatoxin concentration categories.

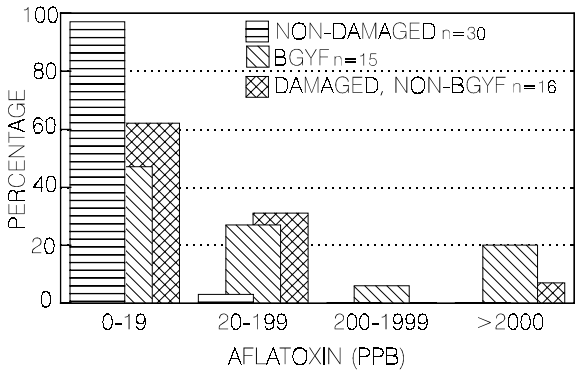

Figure 2. Distribution of insect-damaged (divided into BGFY and non-BGYF) and non-damaged cottonseed samples into different aflatoxin concentration categories. Samples were collected from modules in the Coastal Bend area in 1996, prior to the onset of a sustained rainy period.

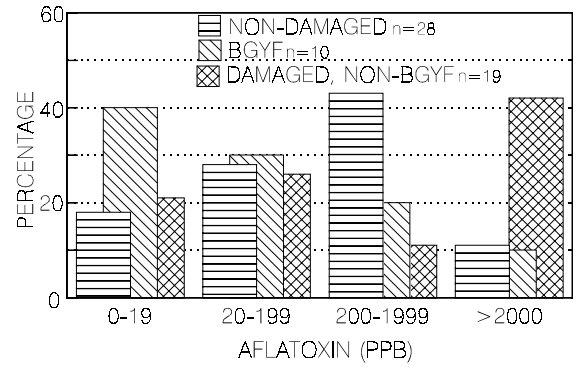

Figure 3. Distribution of insect-damaged (divided into BGFY and non-BGYF) and non-damaged cottonseed samples into different aflatoxin concentration categories. Samples were collected from modules in the Coastal Bend area in 1996, after the onset of a sustained rainy period.

Table 1. Aflatoxin contamination in seed from tight locks of insect-damaged bolls, in relation to duration and severity of insect damage observed in fields during the 1996 season.

Field[1]	Boll Weevil 1st Obs.[2]	Boll Weevil % Damage[3]	Cotton Bollworm 1st Obs.[2]	Cotton Bollworm % Damage[3]	Aflatoxin (ppb)[4]
H-W	56	1-31	70	1-7	3*
H-E	63	2-20	79	1-6	1300*
K-H	58	2-17	72	1-5	2
K-S	65	1-15	71	1-5	4
K-B	63	1-8	63	1-2	20
K-D	52	3-15	63	1	9
K-C	65	2-16	72	2-3	150
K-M	71	2-28	77	1-4	28
N-O	64	5-26	64	2-5	670*
N-J	72	1-18	72	2-4	310*
N-K	37	5-16	37	1-9	18
N-T	72	4-24	72	1-15	20

[1] First letter indicates county: H=Hidalgo, K=Kleberg, N=Nueces.
[2] First observation, days prior to cottonseed sampling.
[3]% Damaged green squares, range observed over the season, when examined at weekly intervals.
[4] Concentration in cottonseed from tight locks of insect-damaged bolls, asterisk indicates BGYF found in the sample.