tion. Those samples stored in clear bottles did, however, exhibit a significant rise in flavor intensity scores which did not appear to be altered by the addition of antioxidants.

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Distribution of Aflatoxin-Containing Cottonseed within Intact Locks

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ABSTRACT

Location of aflatoxin-containing seeds within locks of *Aspergillus flavus* contaminated bolls was determined. Of the 141 seeds examined from 22 intact locks, 78 exhibited bright greenish yellow fluorescence (BGYF) on the linters. Twenty-four seeds contained aflatoxins ranging from 0.231 to 151.3 μg of toxin per gram of seed. Twenty-one of these aflatoxin-positive seeds had linters exhibiting BGYF, and three had nonfluorescent linters. With one exception, aflatoxin contamination was concentrated in only one or two highly contaminated seeds in the apex half of tight locks, and the rest of the three to five seeds were negative. Explanations for this type of infection are discussed.

INTRODUCTION

Aflatoxin contamination in freshly harvested cotton is associated with lint that is bright greenish yellow fluorescent (BGYF) when viewed in long wave ultraviolet (UV) (1). In 1977 Lee et al. reported on the elimination of aflatoxin contamination by removal of all BGYF locks before ginning (2). In 1978 Lee and Cucullu reported on the aflatoxin content of individual cottonseed separated from BGYF locks by ginning (3). They found the highest concentration of aflatoxin in five seeds with BGYF linters. No attempt was made in that study to determine whether the five seeds were from the same lock or whether one or two seeds from several locks were contaminated.

The present study was undertaken to determine both the distribution and location of aflatoxin contaminated seeds within a lock. Such information will aid in establishing the mode of infection of cottonseed by toxin-causing fungi in the field.

EXPERIMENTAL PROCEDURES

Approximately 1 kg of Hopeville cotton was hand-harvested at the University of Arizona Experiment Station at Yuma, AZ, in the late fall of 1978, a year of high aflatoxin contamination (4). The field had a high incidence of pink boll worm infection; humidity under the canopy was also high. Peduncles and carpels were harvested with the intact bolls. Bolls and separated locks were examined in long wave UV and inspected for insect damage. Bolls containing any locks with BGYF, separated BGYF locks, or locks with lint that appeared brown-organge in UV were separated from the bulk of the sample. When possible, locks were identified with particular bolls. Seeds from locks separated for study were hand-ginned and their position in each lock recorded. Presence or absence of BGYF linters (fuzz) was noted; whole fuzzy seeds were then weighed and analyzed individually for aflatoxins (5). Separated lint adjacent to several aflatoxin-positive seeds was also analyzed by a slight modification of the same procedure. In this modification the lead acetate precipitation was eliminated. Lint from one seed that contained all four toxins was cultured on Blakeslee's Malt Agar (BMA). Dry spores of the cultured mold were inoculated onto autoclaved cottonseeds and seeds were incubated for seven days at 27 C and assayed for aflatoxins (6).

RESULTS AND DISCUSSION

The 22 locks separated because of fluorescence of any part of the bolls in UV (Table I) comprised 3% of the sample. Nineteen of the 22 locks were tight; three were fluffy locks (E-2, E-3, E-4) from a boll with one tight BGYF lock (E-1). This tightness could have been caused by mold infection (7,8). Only 12 locks could be identified with four bolls (C, D, E, and M). Because there were no fluffy fibers to cling to carpel walls, tight locks were easily separated and under normal field conditions could have fallen to the

TABLE I

Distribution of Contaminated and Noncontaminated Seeds in 22 Separated Cotton Locks[a]

Lock	Type of lock fluorescence in UV	Total seed examined from each lock	Number of Af. positive seed with fluorescent linters	Number of Afl. positive seed with nonfluorescent linters	Wt range of seed (g)
A	BGY[d]	8	0/8	–	0.06 - 0.13
B	BGY	7	1/7	–	0.04 - 0.13
C-1	BGY	6	0/6	–	0.04 - 0.07
C-2	BGY	6	0/5	0/1	0.03 - 0.07
D-1[b]	BGY	8	3/8	–	0.09 - 0.12
D-2[b]	BGY	7	2/7	–	0.05 - 0.08
D-3[b]	BGY	7	–	0/7	0.12 - 0.17
E-1	BGY	6	0/3	0/3	0.08 - 0.17
E-2	Non-fl.	7	–	0/7	0.12 - 0.15
E-3	Non-fl.	6	–	0/6	0.11 - 0.16
E-4	Non-fl.	7	–	0/7	0.14 - 0.16
F[c]	Br.-or.[e]	6	3/6	–	0.09 - 0.27
G[c]	BGY	7	6/7	–	0.03 - 0.11
H	BGY	4	–	0/4	0.11 - 0.13
I	Br.-or.	5	–	2/5	0.04 - 0.12
J	BGY	5	2/5	–	0.06 - 0.10
K	Br.-or.	7	–	0/7	0.09 - 0.12
L	BGY	5	1/5	–	0.04 - 0.12
M-1	Br.-or.	6	–	0/6	0.03 - 0.11
M-2	Br.-or.	7	–	0/7	0.04 - 0.09
N	BGY	7	2/6	0/1	0.06 - 0.09
O	Br.-or.	7	1/5	1/2	0.02 - 0.08
		41	21/78	3/63	Av. 0.10 g

[a]Where possible locks were identified with specific bolls (C, D, E, and M).
[b]Presumptive insect damage near the apex.
[c]Boll worm exit hole in mid-section.
[d]BGY: Bright greenish yellow.
[e]Br.-or.: Brown-orange.

ground. In harvesting and shipping, this type of lock may separate from the boll. Thirteen of the tight locks were BGYF and the other six were brown-orange in UV. Loss of typical BGY fluorescence is an indication of sample weathering (9).

Seeds from only ten of the 22 locks contained aflatoxin. Of the 141 seeds analyzed 78 had BGYF linters (Table I). Twenty-four seeds contained aflatoxins; levels ranged from 0.230 to 151.2 μg total aflatoxins per gram of seed. Only three seeds with nonfluorescent fuzz were aflatoxin positive; two from the same lock (I) contained 13.9 and 3.6 μg of the **B** toxins per gram of seed, and the third was the seed with the highest aflatoxin level, 151.2 μg of B_1 and B_2 per gram of seed (lock **O**). All three of these seeds were separated from locks that fluoresced brown-orange in UV. When these locks were broken open, some of the lint not adjacent to these toxin positive seeds exhibited BGYF. Positions of toxin-positive seeds within the locks are shown in Figure 1. These seeds were contained in ten of the 22 locks.

Twenty-three of the contaminated seeds contained only aflatoxins B_1 and B_2. One lock (F) contained a seed (position 6, Fig.1) with the G toxins in addition to the B toxins, indicating probable infection by *A. parasiticus* (10). The seed contained 49.4 μg of B_1, 28 μg of B_2, 36 μg of G_1 and 11 μg of G_2 per gram of seed. Additional confirmation of the infecting fungus as *A. parasiticus* was made by the detection of all four toxins in cottonseed inoculated with the fungus plated from lint adjacent to this seed. Two other seeds (positions 2 and 3, Fig. 1) from this lock contained only the B toxins, indicating probable infection by *A. flavus* (10). Only one lock (G) had more than three contaminated seeds. Six of the seven seeds contained aflatoxins B_1 and B_2, ranging from 3.2 to 57.9 μg per gram of seed. This lock and locks D-1, D-2, D-3 and F showed deterioration that could be associated with insect damage; mold invasion could have been insect related (11). Even though the locks were damaged, most seed did not appear to be insect invaded. Only three seeds (in locks C and F) of the 141 seeds examined showed any evidence of insect damage. No seeds apparently damaged by insects contained toxins.

The average weight of all seeds analyzed was 0.10 g. Based on this weight the lower limit for quantitation was 0.01 μg total toxins per gram of whole seed. There was little difference in the average weight of aflatoxin-positive

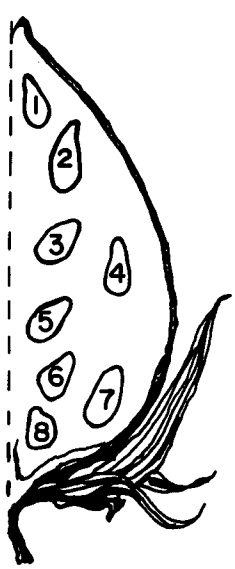

FIG. 1. Number and location of aflatoxin-positive seeds in locks; three, position 1; six, position 2; three, position 3; four, position 4; position 5; two, position 6; one, position 7; and one, position 8.

seeds (0.09 g) and aflatoxin-negative seeds (0.11 g). Weights of seed varied more between locks than within locks (Table I). Aflatoxin-containing seed reflected this homogeneity. Contaminated seeds were as often the heaviest seed in the lock as the lightest. In lock F the *A. parasiticus* contaminated seed was the lightest seed (0.09 g), and the heaviest seed (0.27 g) was one of those contaminated with *A. flavus*. The seeds from this lock were among the heaviest of those assayed. This boll was possibly formed during mid-season when heavier bolls are produced (12) and become contaminated with *A. parasiticus* perhaps through the nectaries (13). *A. flavus* infection could have occurred just at dehiscence when the seeds in positions 2 and 3 would be close to the suture. Sun et al. (8) reported that in inoculated bolls development ceases when extensive mold damage occurs. Weights of seeds from boll E corroborate this. Only three seeds from lock E-1 had BGYF linters, indicating mold contamination. These seeds were the lightest (0.08 g to 0.09 g). Weights of other seeds in that lock and seeds from locks E-2, 3, and 4 ranged from 0.11 g to 0.16 g. Locks E-2, 3, and 4 were examined only because of the fluorescence of one lock in that boll. Lock O, with the highest contaminated seed (151.2 μg of B_1 and B_2 per gram of seed), contained seed weighing 0.03 g to 0.07 g, and the high toxin seed weighed 0.06 g.

Lint ginned from seven seeds with BGYF linters contained trace amounts of aflatoxin B_1 (<0.01 μg per gram of lint). Five of these lint samples were from the single lock (G) that contained six aflatoxin positive seeds. The barely detectable toxin on cotton lint corroborates the results of Mayne et al. (6), who found cotton lint a poor substrate for *A. flavus* production of aflatoxin. However, the wet fiber in the unopened boll seems to be an excellent substrate for *A. flavus* production of kojic acid. The BGYF compound is subsequently formed by the action of peroxidase from the fiber on kojic acid (9). Detection of BGYF in all parts of most locks examined and aflatoxin in seeds near the apex of the locks differs from the report of fungal contamination in tight locks by Ashworth et al. (14). They reported fungal invasion of only the lower third of tight locks.

Results of this study show that aflatoxin contamination of seeds within infected locks is highly selective. This selectivity could be explained if mold infection occurred during fertilization when insect-carried mold spores could have intermingled with pollen. Hepperly et al. offered experimental evidence for this type of mold infection in *Ananas comosus* (15) and Edgerton hypothesized this mode of infection of cottonseed (16). Contamination of two adjacent seeds from a lock with *A. flavus* and a third from the same lock contaminated with *A. parasiticus* suggests infection at different times and confirms the possibility of infection by two fungal species. Infection of a single lock with both *A. parasiticus* and *A. flavus* is unusual, particularly because *A. parasiticus* is rarely found in cotton in Arizona.

Insects were probably the vector for contamination of six seeds from a single lock (17). Seed viability or seed coat permeability (18) could play an important part in aflatoxin contamination. No attempt was made in this study to measure either of these biological properties. These were naturally contaminated samples and, since all bolls were harvested at the same time, no conclusion could be made about seed maturity at the time of infection. The distribution of the few highly contaminated seeds in all positions in the lock does, however, preclude the supposition that seed at the base of the lock where nectaries in the bracts might allow microorganisms to penetrate the fruit (13) would be more prone to aflatoxin contamination.

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