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# Cotton Cultivar and Boll Maturity Effects on Aflatoxin Production[1]

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## ABSTRACT

**Commercial cotton cultivars (*Gossypium hirsutum* L.) were evaluated for aflatoxin production in 1974, 1975, and 1976 crop seasons. Bolls were inoculated at different levels of maturity with *Aspergillus flavus* Link. In 1974, the fungus produced aflatoxin in bolls of all 11 cultivars infected, but 'Acala SJ-1' and 'Acala 1517' were better subtrates for the production of aflatoxin than 'Delcott 277', 'Stoneville 213', 'Coker 711', 'Stoneville 731-N', or 'Deltapine SR-1'. In each of the 3 years, the fungus produced the greatest amount of aflatoxin in bolls of most cultivars inoculated at 30 days post-anthesis, but less in 20-day inoculated bolls and the least in 40-day inoculated bolls. 'Stripper 31' was a notable exception in 1975 because *A. flavus* produced most aflatoxin in bolls of this cultivar inoculated 40 days post-anthesis. In 1976, 20-day and 30-day post-anthesis-inoculated bolls were harvested 10, 15, 20, 25, and 30 days after inoculation. Aflatoxin was found in closed bolls 10 to 20 days after inoculation. The amount of aflatoxin increased with each subsequent time interval. The presence of aflatoxin in closed bolls demonstrated that bolls do not have to open to have toxin formation. A single pink bollworm [*Pectinophora gossypiella* (Saunders)] exit hole, which serves as the entrance hole for the fungus, allows enough oxygen penetration into the boll for aflatoxin formation.**


***Additional index words:* *Gossypium hirsutum* L., Cottonseed, *Aspergillus flavus*, Genotype-environmental interaction, Inoculation, Host-plant resistance, Carcinogen.**

AFLATOXIN, a common fungal metabolite of *Aspergillus flavus* Link, is a highly toxic hepatocarcinogen. The problem of aflatoxin contamination is widespread (12). It involves most of the major seed crops and is particularly serious in corn (*Zea mays* L.) (13) and peanut (*Arachis hypogaea* L.) (9). In cotton (*Gossypium hirsutum* L.), *A. flavus* is one of the boll-rotting fungi that induce fiber and seed decay and the accumulation of aflatoxin in seeds (2, 3, 4, 5). Aflatoxin contamination of cottonseed in the field is especially prevalent in the southwestern U. S. cotton-growing region (1, 8, 14). Infestation by the pink bollworm [*Pectinophora gossypiella* (Saunders)] appears to be the major factor in field infection (6, 7). The exit holes made by the mature larvae of the pink bollworm serve as the entrance point of the fungus. An increase in the severity of pink bollworm infestation increases the opportunity for fungus infection and aflatoxin contamination of the seed. Stephenson and Russell (15) reported isolating *A. flavus* from *Lygus hesperus* Knight (lygus bug), *Chlorochroa savi* Stal (stink bug), and from cotton plant floral bracts and leaves. It was suggested that squares and developing bolls were infested with *A. flavus* spores carried by lygus and stink bugs and that the fungus subsequently gained entry into the bolls via necrotic tissue surrounding the pink bollworm exit holes and the wound.

The initial objective of the study was to determine if differences in resistance could be found in existing commercial cotton cultivars. When we found wide variations in incidence and levels of aflatoxin production within cultivars in the 1974 season, it was then necessary to modify our objective to that of developing a better understanding of the host-fungus relationship to help reach our primary objective.

## MATERIALS AND METHODS

*Field Experiment.* Experiments were conducted in a field near Raleigh, N.C., in 1974, 1975, and 1976 crop seasons. Eleven, six, and two cultivars were planted, respectively. The field experiment involved a split plot design with two replications of two rows in 1974, five rows in 1975 and 30 rows in 1976. After initiation of flowering, blooms were tagged daily over a 3-week period. Boll age was expressed as number of days post-anthesis. A battery-driven electric hand drill was used to drill one hole (3 mm in diam. and 5 mm deep) into bolls at 20, 30, and 40 days post-anthesis. Dry spores of *A. flavus* were inoculated into the hole with an inoculation needle. (Dr. Tom Russell of the Univ. of Arizona developed this inoculation technique to simulate the pink bollworm exit hole. He kindly provided us with the dry spores of *A. flavus*, which were a mixture of 2,000 different isolates.) Bolls of all cultivars were inoculated at the same date for each level of maturity. The inoculated bolls developed in the field and were picked by hand at 5 to 10-day intervals after inoculation, up to full maturity. The number of samples harvested at each date varied because of boll drop, boll rot and insect damage. Sample size is indicated by cultivar and boll age in Tables 1 to 3. The samples were stored individually in plastic bags in a freezer prior to analysis for aflatoxin content.

*Analysis of Aflatoxin.* For 1974 samples, each inoculated boll was tested for aflatoxin contamination according to AOAC Method (11). For 1975 samples, the amount of aflatoxin in each inoculated boll was determined quantitatively by thin-layer chromatography according to the AOAC Method (11). For 1976 samples, the amount of aflatoxin was determined by using Holaday's mini-column method (10). The amount of aflatoxin in 1975 and 1976 samples was expressed as $\mu$g aflatoxin per boll.

## RESULTS AND DISCUSSION

Results of the first year's study are shown in Table 1. The fungus produced aflatoxin $B_1$ and $G_1$ in bolls of all cultivars inoculated. Control bolls, which were drilled but not inoculated, were negative for aflatoxin. 'Acala SJ-1' and 'Acala 1517' had the highest incidence of bolls containing aflatoxin, while 'Deltapine SR-1' and 'Stoneville 731-N' were among the lowest. When fungal infection occurred at different boll ages, a distinctive trend was established with the highest incidence of aflatoxin production in bolls inoculated at 30 days post-anthesis. When a combination test was performed across all cultivars, 72% of the bolls inoculated at 30 days post-anthesis contained aflatoxin. However, only 32% and 36% of the bolls inoculated at 20 days and 40 days, respectively, contained aflatoxin.

In 1975, five cultivars were planted that represented the most susceptible, moderately susceptible, and least susceptible cultivars with respect to aflatoxin production in 1974. 'Pima S4' (*G. barbadense* L.), with a different genetic background from the upland cultivars, was chosen as the sixth cultivar to be tested. The sample size in each cultivar was increased to

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**Table 1. Percentage of cotton bolls containing aflatoxin when infected by *Aspergillus flavus* at different levels of maturity in the 1974 crop season.**

| Cultivar | Days post-anthesis when inoculated: 20 — Bolls with aflatoxin | 20 — Samples | 30 — Bolls with aflatoxin | 30 — Samples | 40 — Bolls with aflatoxin | 40 — Samples | Weighted avg. |
|---|---|---|---|---|---|---|---|
| | % | No. | % | No. | % | No. | % |
| Acala SJ-1 | 80 | 10 | 90 | 10 | 62 | 8 | 79 a* |
| Acala 1517 | 66 | 6 | 100 | 9 | 62 | 8 | 78 a |
| Coker 310 | 28 | 7 | 90 | 10 | 50 | 8 | 60 ab |
| McNair 511 | 25 | 4 | 66 | 6 | 50 | 10 | 50 abc |
| Delcott 277 | 25 | 8 | 50 | 12 | 62 | 8 | 46 bcd |
| Stripper 31 | 33 | 6 | 75 | 4 | 20 | 5 | 40 bcd |
| Deltapine 16 | 37 | 8 | 100 | 2 | 0 | 8 | 28 cd |
| Stoneville 213 | 0 | 7 | 28 | 7 | 50 | 6 | 26 cd |
| Coker 711 | 14 | 7 | 50 | 2 | 22 | 9 | 22 cd |
| Stoneville 731-N | 2 | 8 | 75 | 4 | 0 | 7 | 21 cd |
| Deltapine SR-1 | 12 | 8 | 50 | 2 | 11 | 9 | 16 d |
| Weighted avg. | 32.6 b* | | 72.1 a | | 36.1 b | | |

* Values in "weighted average" column or row followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

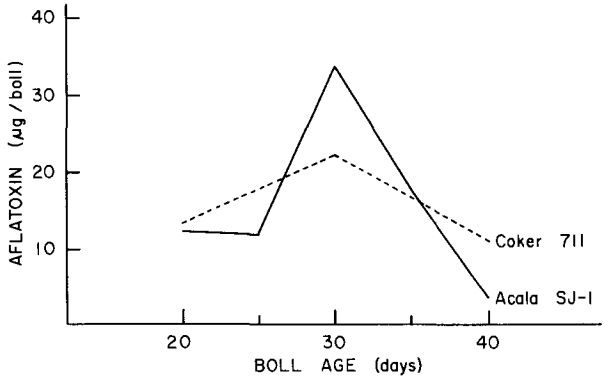


**Fig. 1. Aflatoxin content of fully open cotton bolls inoculated by *Aspergillus flavus* at various days post-anthesis in 1976 crop season.**

**Table 2. Aflatoxin content of cotton bolls from six cultivars infected by *Aspergillus flavus* at different levels of maturity in the 1975 crop season.**

| Cultivar | Days post-anthesis when inoculated: 20 — Aflatoxin | 20 — Samples | 30 — Aflatoxin | 30 — Samples | 40 — Aflatoxin | 40 — Samples |
|---|---|---|---|---|---|---|
| | µg/boll | No. | µg/boll | No. | µg/boll | No. |
| Acala SJ-1 | 38.7 a* | 18 | 71.9 a | 7 | 5.4 b | 9 |
| McNair 511 | 26.4 a | 19 | 39.6 a | 22 | 1.0 a | 7 |
| Pima S-4 | 8.9 b | 11 | 76.8 a | 11 | 0.6 b | 29 |
| Stoneville 731-N | 13.7 b | 16 | 41.2 a | 27 | 6.7 b | 14 |
| Coker 711 | 4.4 b | 11 | 52.1 a | 13 | 1.7 b | 14 |
| Stripper 31 | 2.7 c | 7 | 78.1 b | 7 | 168.2 a | 7 |

* Values followed by the same letter in each row are not significantly different at the 0.05 level according to the Student's "t" test.

permit quantitative comparisons. These results are shown in Table 2. The aflatoxin production followed the same trend as in the 1974 crop season. The fungus in bolls inoculated at 30 days post-anthesis produced the highest amount of aflatoxin in all cultivars tested, except 'Stripper 31'. There was a significant increase in the amount of aflatoxin produced between the bolls inoculated at 20 and 30 days post-anthesis. Also, there was a significant decrease in the amount of aflatoxin produced in bolls inoculated at 40 days post-anthesis compared with that produced in bolls inoculated at 30 days post-anthesis. Only Stripper 31, a short-season cultivar, deviated from this low-high-low trend, the amount of aflatoxin production increasing continuously with boll age. When compared separately at each inoculation date, using an "F" test, cultivars differed significantly at the 0.05 level. However, when cultivars were put in order at each inoculation date, there was little or no correlation between the three inoculation dates. This result indicated a strong interaction between cultivar and the boll age when inoculation occurred. Because of this strong interaction and a large difference in sample size, a comparison between cultivars over the three inoculation dates would not be meaningful.

Results of the 1976 season are shown in Table 3 and Fig. 1. In Acala SJ-1, bolls inoculated at 30 days post-anthesis, showed aflatoxin 10 days later, while still closed (Table 3). The amount of aflatoxin produced increased with subsequent incubation time interval, up to boll opening. There was no increase in the amount of aflatoxin produced when the open bolls were left in the field for 10 more days, i.e., from 35 days to 45 days after inoculation. 'Coker 711' showed a similar pattern, with increased production of aflatoxin as incubation time extended. Bolls inoculated at 20 days post-anthesis behaved similarly to those of bolls inoculated at 30 days post-anthesis, but with reduced levels of toxin. Aflatoxin was produced in closed bolls in both cultivars. Acala SJ-1 bolls were inoculated at 20, 25, 30, 35 and 40 days post-anthesis and Coker 711 bolls were inoculated at 20, 30 and 40 days post-anthesis. The bolls were harvested after they were fully mature and open. When compared statistically, using Student's "t" test at the 0.05 level of probability, the amount of aflatoxin produced in Acala SJ-1 remained unchanged in bolls inoculated at 20 and 25 days (Fig. 1). It then increased significantly and reached its maximum in bolls inoculated at 30 days. At this point, production started to decrease significantly in the bolls inoculated at 35 days and decreased further significantly in bolls inoculated at 40 days. The amount of aflatoxin produced in Coker 711 increased significantly from that in bolls inoculated at

**Table 3. Aflatoxin content of bolls inoculated at 20 days and 30 days post-anthesis in 1976 crop season.**

| Boll condition | Time of inoculation, post-anthesis | Time of harvest, post-inoculation | Acala SJ-1 | | Coker 711 | |
|---|---|---|---|---|---|---|
| | | | Aflatoxin | Samples | Aflatoxin | Samples |
| | days | | µg/boll | No. | µg/boll | No. |
| Closed | 20 | 20 | 0.2 c* | 12 | 0 d* | 10 |
| Closed | | 25 | 3.0 b | 14 | 3.1 bc | 15 |
| Closed | | 30 | 14.4 a | 15 | 1.4 cd | 19 |
| Closed | | 35 | – | | 5.1 b | 18 |
| Open | | 45 | 12.3 a | 93 | 13.1 a | 24 |
| Closed | 30 | 10 | 0.9 d | 19 | 0 c* | 15 |
| Closed | | 15 | 4.9 c | 20 | 1.3 c | 18 |
| Closed | | 20 | 14.9 b | 19 | 9.7 b | 21 |
| Open | | 35 | 34.7 a | 35 | 22.4 a | 68 |
| Open | | 45 | 33.4 a | 52 | – | |

* Values followed by the same letter in each column, within time of inoculation, are not significantly different at 0.05 level according to Student's "t" test.

20 days to that of bolls inoculated at 30 days and decreased from the 30-day-to-40-day bolls. There was no difference in aflatoxin production between Acala SJ-1 and Coker 711 in bolls inoculated at 20 days. But in bolls inoculated at 30 days, there was significantly more aflatoxin produced in Acala SJ-1 than Coker 711. The situation reversed in bolls inoculated at 40 days. This again indicated strong interaction between cultivar and the boll age when inoculation occurred.

Visual examination of the seeds in the bolls was made after the introduction of the fungal spores. Bolls were observed following inoculation at 20 and 30 days post-anthesis. The seeds began to show signs of fungal infection (or invasion) 2 days after the bolls had been inoculated. By 15 days after inoculation, some of the seeds from the bolls inoculated at 20 days had completely disintegrated. In bolls inoculated at 40 days, the more mature seed coat appeared to slow down the process of fungal invasion into the seeds. Signs of fungal penetration were not visible until 5 days after treatment. The fungal infection was limited to the locule in which the hole had been drilled and where the fungal spores had been introduced. The fungus spread throughout the inoculated locule and invaded the seed. It appeared that normal development in the locule ceased after fungal growth began. When the inoculated bolls matured and opened, all locules in the boll fluffed out except the inoculated locule, which remained tight.

The findings of the 1976 field experiments are contrary to the previous belief that aflatoxin in cotton bolls is formed only after the bolls have opened. These results indicate that the aflatoxin is formed while the bolls are closed. It is conceivable that a pink bollworm exit hole, which serves as the entrance hole for the fungus, could permit enough oxygen penetration into the boll for aflatoxin formation.

The results of these experiments suggest that increased availability of substrates or the presence of a particular substrate in the bolls 30 days post-anthesis versus bolls 20 days post-anthesis could account for the difference in aflatoxin production. However, the possibility cannot be overlooked that oxygen tension inside the 20-day-old bolls is low enough to act as a limiting factor in aflatoxin production. In bolls inoculated at 40 days post-anthesis, additional time was required for fungal invasion of the seed coat. Also, incubation interval for fungal development was reduced to about 15 days before boll opening. The seed coat penetration factor, short incubation period, less favorable substrate and failure of toxin levels to increase after boll opening may account for the low levels of aflatoxin production in the 40-day-old bolls.

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