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Aflatoxins in Cottonseed: A Comparison of Two Cultivars[1]

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ABSTRACT

Two cotton cultivars, S-1322 and Deltapine-16, (*Gossypium hirsutum* L.) were compared in field plots in Imperial Co., Calif. in 1973, to determine causes of differences in severity of seed infection by *Aspergillus flavus* Lk. ex Fr. and in accumulation of aflatoxins. S-1322 was not as susceptible as Deltapine-16 because rapid moisture loss from the bolls creates an unfavorable environment for growth of *A. flavus*, and more bolls escape the pink bollworm [*Pectinophora gossypiella* (Saunders)] because of its flowering characteristics.

Additional index words: **Mycotoxins, Boll rot, Hard cottonseed, Impermeable seedcoat, Seed index, Linters.**

BROWN et al. (5) demonstrated that cultivars of cotton (*Gossypium hirsutum* L.) differ in severity or frequency of seed infection by *Aspergillus flavus* Lk. ex Fr. and in the amount of aflatoxin contamination. A survey was initiated in 1969 to find cotton cultivars that may be tolerant or resistant to seed infection by *A. flavus*. Of 74 cultivars tested, all have been susceptible; however, S-1322 (Yugoslav) contained fewer infected seed, as indicated by fiber fluorescence (1, 15), and contained less aflatoxin (unpublished data). This paper reports differences between resistant S-1322 and a more susceptible cultivar, Deltapine-16 (DPL-16), that affect susceptibility to infection by *A. flavus* and the accumulation of aflatoxins.

MATERIALS AND METHODS

Cultivars S-1322 and DPL-16 were planted the last week in March 1973, on 1.06-m centers at the Imperial Valley Conservation Research Center, Brawley, Calif. Plots were 16 rows wide, 18.3 m long, and were replicated eight times. Fifteen insecticide applications were applied commencing prior to first bloom on approximately a 7-day schedule, beginning on 29 June.[3] Insecticides were applied at the rate of 508 g active material/ha by ground machine or by airplane when fields were wet. The last irrigation was applied 14 September and defoliants were applied on 18 October and 9 November.

Earliness was determined by hand harvesting open, air-dried bolls from the entire length of one row in each plot, six times for DPL-16 and seven times for S-1322. From bolls tagged on the day of bloom, boll size and percent boll set were determined throughout the growing season from 4 m of row in each replication. Earliness was again determined from these tagged bolls. Tagged bolls were harvested 7 to 9 November.

Carpel thickness was determined with a binocular microscope equipped with a micrometer disc. One carpel segment from each of 25 bolls per plot was stored in a formalin-aceto-alcohol solution. Two thickness measurements were made midway between the carpel base and apex at the thinnest point in from each margin.

The rate of moisture loss was determined from 10 mature intact bolls per plot. Bolls that cracked when slight finger pressure was applied were considered to be mature. Bolls were harvested on 17 October and dried in the laboratory under 15 100-watt incandescent lamps on 0.5-m centers 0.34 m above the bolls. Air temperature was 40 C at the boll level.

All bolls from the center three rows of each plot (three subsamples) were hand harvested on 7 November. Seed cotton from each subsample was sorted under long wave high intensity ultraviolet light. Those locks that fluoresced a bright greenish yellow (BGY) were removed and examined visually for evidence of pink bollworm [*Pectinophora gossypiella* (Saunders)] damage. A cotton lock consists of seed that are held together by the fiber and each borne in a locule of the ovary. The fluorescent locks were ginned and then examined under uv light. The BGY fluorescent seed were removed, counted and analyzed for aflatoxins (17). The nonfluorescent seed from fluorescent locks were mixed into the corresponding ginned nonfluorescent seed sample. The nonfluorescent seed cotton from the nonfluorescent locks was ginned and three 50-g seed samples from each subsample analyzed for aflatoxins. Pink bollworm damage was determined on 250 gin run fuzzy seed from each subsample (9). Percent linters was determined from 50 g of seed from each subsample (18), and 300 of these acid delinted, visually sound seed were used to determine seed index (weight of 100 seed). Impermeable seed coat was determined by placing 100 gin run fuzzy seed from each subsample between moist filter paper in petri plates, 10 seeds per plate. Seeds that did not take up water were considered to have impermeable seed coats. The number of seminaked seed was determined visually by examining 300 seed from each subsample.

Wilcoxon's signed rank test was used to test for significant differences between aflatoxin determinations (19).

RESULTS AND DISCUSSION

BGY fiber fluorescence of locks and seed is associated with infection by *A. flavus* and aflatoxin accumulation (1, 15). Significantly lower levels of BGY fiber fluorescence of locks and seed and aflatoxin occurred in S-1322 than in DPL-16 (Table 1). After the fiber dries, fluorescence does not develop (14), but rains may rewet fiber and seed and promote seed-to-seed infection and an increase in aflatoxin contamination (2). Only trace amounts of precipitation occurred in 1973 during the time open bolls were on the plants. Thus, low amounts of aflatoxin were found in the non-fluorescent seed (Table 1). These data demonstrate that differences exist between S-1322 and DPL-16 in susceptibility to infection by *A. flavus*, as indicated by fiber fluorescence and aflatoxin development in the seed.

The above conclusion is supported by observations from 1969 and 1972 tests. In 1969, the test was conducted at Imperial, Calif., insecticides were applied nine times, and plants were harvested 3 to 4 October. Significant differences occurred in number of BGY fluorescent and pink bollworm damaged seed per 1,000 and aflatoxins B_1+B_2 from BGY fluorescent and nonfluorescent seed computed for the total weight of seed harvested (Table 1).

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[3] N'-(4 chloro-*o*-tolyl)-*N*, *N*-dimethylformamidine (chlordimeform). This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation by the USDA nor does it imply registration under FIFRA.

Table 1. A comparison of the BGY fiber fluorescence of locks, locks and seed with pink bollworm damage, seed per 1,000, and aflatoxins in seed from two cotton cultivars, Imperial Co., Calif.

Year	Location	Cultivar	Fluorescent locks per kg of seed cotton	Fluorescent locks per kg of seed cotton with pink bollworm damage	Fluorescent seed per 1,000	Pink bollworm damaged seed per 1,000	Aflatoxins ($B_1 + B_2$) ppb		
							BGY fluorescent seed	Nonfluorescent seed	BGY fluorescent plus nonfluorescent seed computed for weight of seed harvested
1969	Imperial	S-1322			6.8**	58.0			27*
		DPL-16			26.3	189.9			260
1972	Brawley	S-1322			0.03**	1.7			2
		DPL-16			0.23	3.8			3
1973	Brawley	S-1322	0.24**	0.09**	0.05**		6,852**	ND†	1**
		DPL-16	0.68	0.38	0.38		47,636	4	33

*,** Differences between cultivars significant at 0.05 and 0.01 levels of probability, respectively. † Nondetected.

In 1972, the tests were conducted at Brawley, Calif., insecticides were applied 10 times, and plants were harvested 2 August. Significance occurred only in number of BGY fluorescent seed per 1,000, however trends for pink bollworm damaged seed and aflatoxins were similar to those in 1969 and 1973 (Table 1).

Exit holes of pink bollworm larvae predispose cotton bolls to infection by *A. flavus* and result in increased contamination with aflatoxins (4, 12). The insecticide schedule was only partially successful in reducing the pink bollworm infestation as exit holes were observed in fluorescent locks of both cultivars (Table 1). DPL-16 had significantly more bollworm damaged fluorescent locks; however, X-ray examination did not disclose any damaged nonfluorescent seed from the two cultivars. The significant increase in fluorescent locks damaged by the pink bollworm indicated that this insect was one of the major factors that contributed to the differences in number of fluorescent seed and concentration of aflatoxin in the two cultivars. This agrees with data from 1969 and 1972 (Table 1).

Fruit may escape pink bollworm damage because of differences in the fruiting patterns of the two cultivars. Dates of first bloom were 2 and 6 June for S-1322 and DPL-16, respectively. Fruiting stopped (cut-out) (20) twice in DPL-16 for periods of 14 days (13 to 26 July) and, 1 week later, 7 days (2 to 8 August); however S-1322 stopped fruiting only once for a period of 7 days (16 to 22 July). The difference in bloom set was also reflected in the date of first open boll, 16 and 30 July in S-1322 and DPL-16, respectively. The increased earliness of S-1322 resulted in more bolls escaping succeeding pink bollworm generations.

Data from adjacent plots under identical cultural practices indicate that a pink bollworm infestation did not occur until after 8 August (13). Lukefahr and Griffin (10) found that pink bollworm larval survival was lower, and fewer bolls became infested, when bolls were older than 30 days.

S-1322 has an open growth habit that facilitates penetration of insecticides, sunlight, and drying of bolls. The increased penetration of sunlight could have raised the soil temperature in the S-1322 plots and reduced the pink bollworm populations (8).

A. flavus infection and aflatoxin accumulation are directly affected by the rate that bolls open and air dry (3). At the time boll opening was determined, bolls from S-1322 were higher on the plants. Although boll moisture was 65% for both cultivars, water loss was more rapid from S-1322. Bolls reached 15% moisture in 23.5 and 31 hours for S-1322 and DPL-16, respectively, and was significant at the 1% level of probability. Aflatoxin production is greatly reduced at 15% seed moisture (6). The carpels of both cultivars were 2.2 mm thick and probably did not affect rate of water loss. While these data were from laboratory observations, they provided an explanation for the reduced aflatoxins in fluorescent seed from S-1322. Of the 11 S-1322 samples that contained fluorescent seed, seven did not contain detectable aflatoxin, which indicated that bolls dried before the fungus penetrated the seed coat.

Table 2. A comparison of some boll and seed characters in two cotton cultivars, Brawley, Calif., 1973.

Cultivar	Dry weight entire boll	Seed index†	Linters	Hollow seed	Seminaked seed	Hard seed
	g					
S-1322	3.80**	9.26**	10.4**	5.5**	8.1**	1.5**
DPL-16	5.52	9.77	13.2	3.4	0	0

** Differences between cultivars significant at the 0.01 level of probability.
† Acid-delinted seed.

Mayne et al. (16) demonstrated the potential value of hard seed (impermeable seed coat) in reducing *A. flavus* invasion and the subsequent accumulation of aflatoxins. Hard seed would be important only in nonfluorescent seed as seed moisture must be reduced to 11% to establish the character (7). The 1.5% hard seed in S-1322 probably did not reduce infection and aflatoxins appreciably in the nonfluorescent seed (Table 2).

The cultivars differed significantly in the mean air-dry weight of bolls from flowers tagged during the growing season (Table 2). Boll weights of both cultivars decreased and the difference between the weights of bolls of the same age from the two cultivars also decreased as the season advanced until, at the end of the growing season, boll weights of both cultivars were approximately the same. Boll weights were 4.46 and 6.74 g on 11 June and 2.78 and 3.17 g on 18 October for S-1322 and DPL-16, respectively.

The observed rate of moisture loss could have been caused by the difference in boll weight. Because this difference was greater early in the boll setting period, DPL-16 bolls could have lost moisture more slowly than S-1322, thus enhancing *A. flavus* infection and aflatoxin accumulation in DPL-16 in the lower plant zone where infection and aflatoxins are known to be highest (11, 12).

Percent linters was significantly lower in S-1322 because this cultivar had more seminaked seed (Table 2). This factor could affect the rate of moisture loss. Seed

index was significantly lower for S-1322 than for DPL-16; however, this difference can be attributed to the significantly higher percentage of hollow seed in S-1322 (Table 2).

The significant difference between cultivars studied in amount of infection of cotton seed by *A. flavus,* as indicated by fluorescence and the accumulation of aflatoxins, was apparently caused by an increased rate of moisture loss and escape from attack by the pink bollworm of bolls from S-1322 compared with DPL-16.

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