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Brief Articles

AFLATOXINS IN COTTONSEED AS AFFECTED BY THE PINK BOLLWORM

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ABSTRACT

Pink bollworm [*Pectinophora gossypiella* (Saunders)] larval exit holes increased infection by *Aspergillus flavus* Link and aflatoxin accumulation in seed from all zones of the cotton plant (*Gossypium hirsutum* L.). The average aflatoxin level in seed from the bottom, middle, and top zones of the plant was at least 1,000 times greater in 1970, when the pink bollworm was common, than in 1965 when the insect was absent. In order to determine total aflatoxins in cottonseed from pink bollworm infested areas, it becomes necessary to harvest seed from the entire plant as infection by *A. flavus* and aflatoxin accumulation are no longer limited to the bottom third of the plant. Control of the pink bollworm will be necessary if the aflatoxin problem is to be minimized.

Additional index words: **Mycotoxin, Boll rot, Cottonseed.**

AFLATOXINS are known carcinogens and may occur in cottonseed (*Gossypium hirsutum* L.) as a result of infection by the fungus *Aspergillus flavus* Link. When contaminated cottonseed or meal are used as animal feeds the occurrence of these toxins is undesirable. Ashworth et al. (6) found that the larval exit holes of the pink bollworm [*Pectinophora gossypiella* (Saunders)] predispose cotton bolls to infection by *A. flavus* and the accumulation of aflatoxins. Prior to the occurrence of the pink bollworm, in Imperial County, California in 1965 (11), field infection of cottonseed by the fungus *A. flavus* and the accumulation of aflatoxins were confined primarily to the bottom third of the plant (8). The purpose of this study was to determine if, under conditions of a pink bollworm infestation, this relationship remains valid.

MATERIALS AND METHODS

Seed cotton samples (2.3 kg) from commercial or experiment station fields were handpicked from the bottom, middle, and top thirds of the plant from a total of eight fields in Imperial County, California and in Maricopa County, Arizona in 1970. All cotton bolls in a single row were picked until the requisite amount was harvested. The same cotton variety was grown at all test locations; however, cultural practices varied depending upon the grower. The seed cotton samples were visually examined under long-wave ultraviolet light and those locules which fluoresced a bright greenish-yellow were removed from the nonfluorescent portion of the sample. The fluorescent locules were visually examined for pink bollworm larval damage. The samples of fluorescent and nonfluorescent seed cotton were ginned separately. The ginned seed from the fluorescent locules were again visually examined under long-wave ultraviolet light and those seeds which did not fluoresce were incorporated into the corresponding nonfluorescent seed sample. Three 100 gm seed samples from each nonfluorescent sample were acid delinted (7) and 300 seeds from each visually examined for pink bollworm larval damage. Three hundred acid delinted seeds with visually intact seed coats from each nonfluorescent seed sample were cultured on malt salt agar for the determination of internal *A. flavus* infection (3). Three 50 gm subsamples from each nonfluorescent seed sample and the fluorescent seed were analyzed for aflatoxins according to the methods of Pons and Goldblatt (12).

RESULTS AND DISCUSSION

In 1970, pink bollworm damage was progressively greater in fluorescent locules harvested from the bottom, middle, and top thirds of the plant (Table 1). The percent of pink bollworm-damaged locules was 72.3, 83.8, and 91.0 from the bottom, middle, and top thirds of the plant respectively. Pink bollworm damage to the nonfluorescent seed in 1970 also increased from the bottom to the top of the plant and was 5.0, 10.0, and 11.4% from the bottom, middle, and top thirds of the plant respectively (Table 1). The increased damage in the top of the plant may have resulted from an increase in the pink bollworm population in late season or a breakdown in the insect control program.

In 1970, there were no significant differences associated with boll position on the plant with regard to the percent infection by *A. flavus* of fluorescent or nonfluorescent seed (Table 2). The percent of fluorescent seed ranged from 3.9 in the bottom third to 1.7 in the top third with a mean of 3.1 for all three plant zones. The percent *A. flavus* infection in the nonfluorescent seed ranged from 7.3 in the bottom third to 4.1 in the top third with a mean of 6.1 for all three plant zones. It has been shown that a close relationship exists be-

Table 1. Mean percent of pink bollworm damage in fluorescent locules and nonfluorescent seed in three zones of the cotton plant in 1970.

Sample source	Pink bollworm damage	
	Fluorescent locules	Nonfluorescent seed
	%	
Top	91.0	11.4
Middle	83.8	10.0
Bottom	72.3	5.0
L.S.D. 5%	6.3	3.4
1%	8.7	4.7

Table 2. Mean internal *A. flavus* seed infection occurring in three zones of cotton plant in 1970.

Seed source	*A. flavus* infection		
	Fluorescent seed	Nonfluorescent seed	
	%		
Top	1.7	4.1	L.S.D. 1%
Middle	3.9	7.0	
Bottom	3.9	7.3	
Total	9.5	18.5	8.2
L.S.D. 5%	NS*	NS*	

* Not significant.

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Table 3. Mean aflatoxin B_1 + B_2 content from fluorescent cottonseed computed for the total weight of seed harvested and from nonfluorescent seed in 1970.

Seed source	Aflatoxins B_1 + B_2	
	Fluorescent seed	Nonfluorescent seed
	ppb	
Top	37	92
Middle	841	660
Bottom	1,968	1,732
L.S.D. 5%	1,143	1,102
1%	1,586	

Table 4. A comparison of the percent of samples with alflatoxins and the aflatoxin B_1 content from fluorescent seed from 1965, prior to the occurrence of the pink bollworm, and from 1970, when the insect was common throughout the test area.

Sample source	No. of samples		Samples with aflatoxins		Aflatoxin B_1 from fluorescent seed	
	1965	1970	1965	1970	1965	1970
			%		ppb	
Top	48	8	6	100	2.5	2,898
Middle	60	8	3	100	Trace*	27,505
Bottom	60	8	52	100	43.5	46,321
L.S.D. 5%						24,532
1%						34,047

* Less than 1 ppb.

tween the bright greenish-yellow fluorescence of fiber and infection by *A. flavus* (1, 10, 13). Thus, it was felt that the occurrence of fluorescence was indicative of infection by the fungus. The seed, however, remain prone to infection following boll opening if the fiber is rewet (1), but without development of the characteristic fluorescence (9). As infection of the nonfluorescent seed by *A. flavus* is dependent on the fiber being rewet, the number of seeds involved will vary from year to year (1, 4).

Total *A. flavus* infection of nonfluorescent seed from all three plant zones was significantly greater at the 1% level than the total fluorescent infection. There was no significant difference between fluorescent and nonfluorescent *A. flavus* seed infection in the three plant zones due to differences in rainfall that occurred at the various test locations (Table 2). The *A. flavus* infection occurring in the nonfluorescent seed resulted in significant accumulations of aflatoxins among the three plant zones and was approximately equal to the concentration from fluorescent seed when computed for the weight of the total seed harvested (Table 3).

A significant increase in the accumulation of aflatoxins B_1 plus B_2 from the fluorescent seed occurred in the bottom third of the plant when computed for the total weight of seed harvested (Table 3). The aflatoxin B_1 concentration from the fluorescent seed in the bottom and middle thirds of the plant were significantly greater than in the top third (Table 4). As a result of pink bollworm larval exit holes, cotton bolls are susceptible to infection not only by *A. flavus*, but also other fungi, principally *A. niger* and *Rhizopus* Sp. and this results in the premature opening of the boll and exposure of the fiber to attack by *A. flavus* (6). Ashworth et al. (2) reported that field infection by *A. flavus* was often associated with imperfectly opened bolls, such as often occur with pink bollworm damage. This poor dehiscence prolongs the time bolls are susceptible to infection. Unless there is damage to the carpel the unopened bolls are protected from infection by *A. flavus* (2). Thus, the occurrence of the pink bollworm resulted not only in an increase in *A. flavus* infection and aflatoxins in the bottom third of the plant but also in the middle and top thirds where boll rots normally do not occur.

The impact of the increased infection resulting from pink bollworm injury to the cotton boll was readily apparent with an aflatoxin B_1 concentration in the fluorescent seed at least 1,000 times greater in 1970 than in 1965 in all three plant zones (8). In 1970 all samples contained aflatoxins associated with the fluorescent seed, but in 1965 only 52, 3, and 6% of the samples from the bottom, middle, and top thirds of the plant, respectively, contained aflatoxins.

These data show that in areas where the pink bollworm occurs, infection by *A. flavus* and aflatoxin accumulation in cottonseed are no longer confined to the bottom third of the plant. Pink bollworm injury may also result in additional infection and aflatoxin accumulation in the nonfluorescent seed when precipitation occurs prior to harvest. Any increase in the infection and aflatoxins of the nonfluorescent seed reduces the likelihood of effective methods for reducing aflatoxins by mechanical removal of the fluorescent seed (5). Methods for effective and economic pink bollworm control will be necessary if the aflatoxin problem in cottonseed is to be minimized.

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