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Aflatoxins in Cottonseed: Effects of Pink Bollworm Control[1]

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ABSTRACT

In 1971 and 1973 field tests were conducted to determine if control of the pink bollworm [*Pectinophora gossypiella* (Saunders)] at or below 5% infestation would reduce the incidence of bright greenish yellow (BGY) fluorescence of cottonseed (*Gossypium hirsutum*) that is associated with the fungus *Aspergillus flavus* and the accumulation of aflatoxins. Control of the pink bollworm by application of insecticides on a 5 or 7-day schedule resulted in a decrease in the BGY fluorescence of cottonseed and in the accumulation of aflatoxins. The reduced pink bollworm infestation did not insure that the present FDA guideline of 20 ppb for aflatoxins in cottonseed would be attained.

Additional index words: Boll rot, Insecticides.

ASHWORTH et al. (4) demonstrated that damage by the pink bollworm [*Pectinophora gossypiella* (Saunders)] predisposed cottonseed (*Gossypium hirsutum* L.) to invasion by the fungus *Aspergillus flavus* Link and was also associated with increased accumulations of aflatoxins. Based on yield, Watson and Fullerton (14) reported that control of the pink bollworm in Arizona was unwarranted until 20% of the green bolls were infested. Later, this was adjusted to 5 to 15%, depending upon the occurrence of boll rots in the area involved (9). In the Imperial Valley of California, where boll rots frequently occur, the economic

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Table 1. Total number of pink bollworm larvae in bolls from fields under two insect control programs; 1971 and 1973, Brawley, Calif.

1971				1973			
		Total no. of larvae				Total no. of larvae	
Date bolls collected	No. of bolls/ treatment	Economic control	Complete control	Date bolls collected	No. of bolls/ treatment	Economic control	Complete control
20 July	100	6	0	3 July	200	63	0
26	100	39	0	11	400	206	0
30	100	54	0	18	400	416	0
3 Aug.	100	48	0	25	400	231	0
9	100	30	0	1 Aug.	600	118	0
13	100	31	0	8	600	15	0
16	100	17	0	15	600	1	1
20	100	13	0	22	600	5	0
23	100	25	0	29	600	16	3
27	100	97	0	5 Sept.	600	12	5
31	100	213	1	12	600	14	0
3 Sept.	100	232	1	19	600	13	2
10	100	128	0	26	600	7	0
13	100	24	1	10 Oct.	600	2	0
20	100	16	4				
1 Oct.	100	108	20				

infestation level may be 5 to 7% (12). Our studies show that seed cotton harvested in the manner described in this paper, from commercial fields and grown under the recommended intensity of pink bollworm infestation control, often exceed the present FDA guideline of 20 ppb aflatoxins. Russell et al. (11) observed aflatoxin concentrations of 214 and 620 ppb when pink bollworm infestation levels were maintained at 10 and 16%, respectively; these values were significantly lower than that of the untreated control (1,302 ppb). These infestation levels were excessive for this boll-rot area in view of the economic control recommendations (9, 12). This paper reports studies to determine if pink bollworm infestation below the 5% level would reduce the incidence of bright greenish yellow fluorescence (BGY) of cottonseed, that is indicative of *A. flavus* infection, and the subsequent accumulation of aflatoxins to acceptable levels of 20 ppb, the present FDA guideline.

MATERIALS AND METHODS

Cottonseed, cultivar D&PL-16, was planted the last week of March in 1971 and 1973 in two fields approximately 0.4 km apart in Imperial County, Calif. In 1971 and 1973, plots were 8 and 12 rows wide, respectively, 12.8 m long, and replicated eight times. Two pink bollworm infestation levels were imposed: 1) complete insect control (CIC), less than 5% infestation; and 2) economic insect control (EIC), 5% or greater infestation. Beginning on 23 June 1971, before first bloom, 17 insecticide applications were made on approximately a 5-day schedule in the CIC field. In 1973, beginning on 29 June, 15 insecticide applications were made on approximately a 7-day schedule in the CIC field. The EIC fields were sprayed at various times; the first of 11 applications was made on 14 July 1971, and in 1973, the first of eight applications on 15 July. One insecticide application for the control of thirps was made on 1 June 1973 on both the CIC and EIC fields. Insecticides[3] were applied at 454 g of active material/ha by ground machine or by aerial application when the field soils were wet.

In 1971, pink bollworm infestations were determined during the growing season by examining 100 bolls, 15 to 25 days of age at 3 to 7-day intervals. In 1973, employing the unpublished methods of R. E. Fye (6) larvae and moths were determined at weekly intervals in 200 to 600 bolls/field. In 1971, the date of last irrigation was 23 September, defoliants were applied on 12 October, and again on 21 October; all open bolls in a row were hand harvested. In 1973, the date of last irrigation was 14 September, both fields were defoliated on 18 October, and on 9 November, all open bolls in a row were harvested by hand.

Seed cotton was sorted under long-wave, high-intensity ultraviolet light and those "locks" that fluoresced a BGY were removed and examined for visual evidence of pink bollworm damage. The fluorescent locks were ginned and the fluorescent seeds were removed, counted, and analyzed for aflatoxins (10). The nonfluorescent seeds from the fluorescent locks were incorporated into the ginned nonfluorescent seed portion of the sample. Three 50-g nonfluorescent seed samples from each plot were analyzed for aflatoxins.

RESULTS AND DISCUSSION

Fye's method for the determination of pink bollworm infestation is based on the total number of larvae, whereas the data of Watson and Fullerton (14) and the control recommendations of Moore and Watson (9) and Sharma (12) are based on the number of infested bolls. In the CIC plots, based on the total number of larvae in the bolls examined, with the exception of the 20 Sept. and 1 Oct. 1971 observations, the pink bollworm infestations were maintained well below the 5% level in both years (Table 1). In the EIC fields, attempts to maintain a 5% infestation level were not successful in either test year. In 1971, the 5% infestation level was exceeded for the entire growing season and in 1973, the infestation level was exceeded early in the growing season but for the remainder of the year was less than the 5% level.

The occurrence of cotton fiber with BGY fluorescence, which is associated with infection by *A. flavus* (1, 8), was significantly reduced in fields under the CIC program (Table 2). Reduction in the number of fluorescent locks in the CIC fields was due primarily to control of the pink bollworm, as indicated by the significant number of fluorescent locks in the EIC fields with pink bollworm damage (Table 2). Of the fluorescent locks, 61 and 79% (29.1 and 13.8 fluorescent locks/kg of seed cotton) showed visual evidence of pink bollworm damage in 1971 and 1973, respectively. The number of observed pink bollworm damaged locks was undoubtedly low due to difficulty in locating the damage. Control of other insects may also have been a contributing factor (13). With regard to BGY fluorescence in the CIC fields, pink boll-

[3] N'-(4 chloro-o-tolyl)-N, N-dimethylformamidine or 3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate. The mention of specific chemicals is for identification only and does not imply endorsement by the USDA.

Table 2. Effect of two insect control programs on the mean fluorescent locks and the fluorescent locks with pink bollworm damage/kg of seed cotton and the fluorescent seeds/1,000; 1971 and 1973, Brawley, Calif.

	Fluorescent locks/ kg of seed cotton		Fluorescent locks/ kg of seed cotton with pink bollworm damage		Fluorescent seed/ 1,000	
Year	Economic	Complete	Economic	Complete	Economic	Complete
1971	48.6	2.2**	29.1	0.3**	25.3	1.2**
1973	17.4	0.3**	13.8	.03**	11.0	0.1**

** Significant at the 0.01% level.

Table 3. Effect of two insect control programs on the mean aflatoxin (B_1 + B_2) content of cottonseed; 1971 and 1973, Brawley, Calif.

	Fluorescent seed computed for weight of seed harvested		Fluorescent seed		Nonfluorescent seed	
Year	Economic	Complete	Economic	Complete	Economic	Complete
			ppb			
1971	1,939	52**	63,356	56,943	229	35*
1973	523	Tr**†	56,596	789*	29	ND**‡

*,** Significant at the 0.05 and 0.01% levels, respectively.
† Trace. ‡ Nondetected.

worm control was not as satisfactory as the infestation levels in Table 1 would indicate, as 14 and 10% (0.3 and 0.03 fluorescent locks/kg of seed cotton) of the fluorescent locks showed visual evidence of damage in 1971 and 1973, respectively. As a result of the reduction in the number of fluorescent locks, there was a corresponding significant decrease in the number of fluorescent seed in the CIC fields in both test years (Table 2).

Control of the pink bollworm on a 5 or 7-day insecticide spray schedule reduced the occurrence of fluorescent locks and seed by reducing the number of exit holes of the mature larvae and limiting the sites for *A. flavus* infection (4). Exit holes also predisposed bolls to infection by *A. niger* and *Rhizopus* spp., resulting in premature boll opening and exposure of the fiber to infection by *A. flavus* and the accumulation of aflatoxins in the seed (4). These data agree with other studies that showed corresponding decreases in fluorescence as the pink bollworm infestation was reduced (4, 7).

The significant reduction in numbers of fluorescent seed from the CIC fields did not insure a reduction in the aflatoxin concentration from those seed, even though 22 times more fluorescent seed occurred in the EIC field in 1971 (Table 3). Only in 1973 was there a significant reduction in aflatoxins in the fluorescent seed associated with the CIC fields.

While aflatoxins are generally associated with fluorescence, the percentage of fluorescent seed is not proportional ro the aflatoxin content of the sample (1). When the aflatoxin concentrations of fluorescent seed were computed for the weight of the total seed harvested, concentrations were reduced more than 35 and 10,000 times in 1971 and 1973, respectively (Table 3). While these reductions are highly significant, only in 1973 was the aflatoxin concentration less than the present 20 ppb FDA guideline for cottonseed.

Following boll opening and drying of the fiber, rains may rewet fiber and seed, promoting seed-to-seed infection and an increase in total aflatoxins (2). In this case, lint fluorescence does not develop. During the time open bolls were on the plants, total rainfall was 3.05 and 0.66 cm in 1971 and 1973, respectively, and resulted in aflatoxin accumulation in the nonfluorescent seed. Concentrations of aflatoxins in nonfluorescent seed were *significantly* reduced in fields under the CIC program in both test years (Table 3). The reduced aflatoxin concentrations may be due to exposure of fewer nonfluorescent seed to infection by reducing the sites of initial infection, that is, the number of fluorescent locks and seed. Control of primary infection thus would be doubly important in years when fall rains occurred, as aflatoxins in the nonfluorescent seed alone could exceed the present FDA guideline.

The data indicate that control of the pink bollworm does not insure a reduction of the aflatoxin concentration sufficient to meet the present 20 ppb FDA guideline. The aflatoxin concentrations are such that if economic and effective pink bollworm control methods are found, agronomic methods such as bottom defoliation, skip-row planting (3), selection of varieties that are less prone to *A. flavus* infection (5), or a combination of these, may once again be of benefit.

REFERENCES

1. Ashworth, L. J., Jr., and J. L. McMeans. 1966. Association of *Aspergillus flavus* and aflatoxins with a greenish yellow fluorescence of cottou seed. Phytopathology 56:1104-1105.
2. ———, ———, J. L. Pyle, C. M. Brown, J. W. Osgood, and R. E. Ponton. 1968. Aflatoxins in cotton seeds: Influence of weathering on toxin content of seeds and ou a method for mechanically sorting seed lots. Phytopathology 58:102-107.
3. ———, ———, and C. M. Brown. 1969. Infection of cotton by *Aspergillus flavis*: Epidemiology of the disease. J. Stored Prod. Res. 5:193-202.
4. ———, R. E. Rice, J. L. McMeans, and C. M. Brown. 1971. The relationship of insects to infection of cotton bolls by *Aspergillus flavus*. Phytopathology 61:488-493.
5. Brown, C. M., L. J. Ashworth, Jr., and J. L. McMeans. 1975. Differential responses of cotton varieties to infection by *Aspergillus flavus*. Crop Sci. 15:276-277.
6. Fye, R. E. Cotton Insects Research Laboratory, Tucson, Ariz. Personal communication.
7. Lukefahr, M. J., and D. F. Martin. 1963. Evaluation of damage to lint and seed of cotton caused by the pink bollworm. J. Econ. Entomol. 56:710-713.
8. Marsh, P. B., M. E. Simpson, R. J. Ferretti, T. C. Campbell, and J. Donoso. 1969. Relation of aflatoxins in cottonseeds at harvest to fluorescence in the fiber. J. Agric. Food Chem. 17:462-467.
9. Moore, L., and T. F. Watson. 1974. Insect control — 1974. Circular Q-11. Coop. Ext. Serv. Agric. Exp. Stn. Univ. of Arizona. Tucson, Ariz.
10. Pons, W. A., Jr., and L. A. Goldblatt. 1965. The determination of aflatoxins in cotton seed products. J. Am. Oil Chem. Soc. 42:471-475.
11. Russell, T. E., L. W. Stephenson, and G. F. Ryan. 1974. Progress in controlling aflatoxin contamination of cotton seed. Beltwide Cotton Prod. Res. Conf. Proc. p. 29.
12. Sharma, R. K. 1974-75. Pest control recommendations for Imperial County field crops. Univ. of California, El Centro Court House Circ. 117.
13. Stephenson, L. W., and T. E. Russell. 1974. The association of *Aspergillis flavus* with hemipterous and other insects infesting cotton bracts and foliage. Phytopathology 64:1502-1506.
14. Watson, T. F., and D. G. Fullerton. 1969. Timing of insecticidal applications for control of the pink bollworm. J. Econ. Entomol. 62:682-685.