Influence of Diurnal Temperature Cycles on Infection of Cotton Bolls by Aspergillus flavus

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ABSTRACT

Excised cotton bolls were inoculated with an aflatoxin-producing strain of *Aspergillus flavus* (ATCC #26994), and were placed in growth chambers for 7 or 3 days with short (2-hour) or long (10-hour) diurnal maximum temperature cycles. The maximum and minimum temperatures were 30-32 C and 16-18 C, respectively. The percent bright greenish-yellow (BGY) fluorescence of locks and seeds, as well as seed infection of BGY-fluorescent seeds, increased as the duration of the daily maximum temperature of 30 C increased, and/or as the number of diurnal maximum temperature cycles of 30 C increased. Aflatoxin did not accumulate in *A. flavus*-infected seed, regardless of treatment.

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The influence of environmental factors on aflatoxin production by *Aspergillus flavus* Link. in cotton (*Gossypium hirsutum* L.) and other crops has been reviewed (4). Aflatoxins in cottonseed are associated with the bright greenish-yellow fluorescence (BGY) of the lint (1), which is especially prevalent in the hot desert regions in the southwestern United States (5). Low-temperature boll rot fungi are prevalent in the central valley of California (summer average 26 C), whereas high-temperature boll rot fungi, especially *A. flavus,* are prevalent in hotter southern valleys of California (summer average 32 C) (6). The high incidence of *A. flavus* boll rot in southern California was favored by higher relative humidity (RH) and slower rates of boll drying at temperatures optimal for growth, infection, and aflatoxin accumulation (2). Gardner et al. (5) reported a high incidence of *A. flavus* infection of cottonseed in Safford, Arizona, but no BGY or aflatoxins were present although toxicogenic strains were isolated. They suggested that cooler daily maximum temperatures coincident with boll opening were the principal factors limiting aflatoxin accumulation in this region of Arizona. Schroeder and Hein (9) found that aflatoxin production of four isolates of *A. flavus* on three substrates was maximal between 20 and 35 C. Subsequently, they studied effects of diurnal temperature cycles on toxin production by *A. parasiticus* Speare in Czapek's broth (10). Cycles with high temperature maxima of 40, 45, and 50 C reduced growth, production, and accumulation of toxins compared with similar cultures held at a constant 25 C. However, cycles with low-temperature maxima of 20, 15, and 10 C had no effect on fungal growth or the production of aflatoxins.

In this paper, we used simulated field conditions to study the influence of diurnal temperature cycles on growth, infection, and toxin accumulation by *A. flavus* in cotton seeds and lint.

MATERIALS AND METHODS.—Mature cotton bolls that split when squeezed slightly were excised from greenhouse-grown plants. Bolls were inoculated with a toxicogenic isolate of *A. flavus* (ATCC #26994), grown 5-7 days on malt salt agar (MSA) at room temperature (approximately 23 C). The inoculum was a conidial suspension of 10^6 conidia/ml in sterile water containing 0.05% Tergitol-NPX. Inoculum was introduced into the base of the opened boll and fibers with a sterile plastic syringe (20 ml) and 0.57 mm diameter needle (23-gauge), and each boll was suspended inside a glass jar by attaching the peduncle to a wire on the lid. In the incubation jars, the moisture evaporating from the bolls quickly established and maintained a RH of 100%.

The jars with bolls were incubated in Plexiglas growth chambers that received natural sunlight and were equipped with diurnal temperature control systems (±0.5 C). Bolls were incubated for 3 or 7 days with 2 hours (short) or 10 hours (long) duration of the maximum temperature cycles. The maximum and minimum temperatures were 30-32 C and 16-18 C, respectively. After incubation, the bolls were removed from the incubation jars and dried in a forced air oven at 55-60 C.

Dried locules from individual bolls were examined under a high intensity fluorescent lamp for BGY fluorescence. All seeds were ginned and BGY-fluorescent seed were then separated from the nonfluorescent seed and subsamples were taken from each lot. Subsamples of seeds from the inoculated bolls were delinted in concentrated sulfuric acid, surface-sterilized with 0.5% sodium hypochlorite for 15 minutes and cultured on MSA plus 0.5 μg/ml (ppm) Hg (methylmercury dicyandiamide) (3). Molds which grew from these seeds were identified and counted after 16-18 days. *A. flavus* isolates from infected seed were reisolated on MSA and tested for toxicogenicity on autoclaved nondelinted cottonseed. The remaining fluorescent seed subsamples were analyzed for aflatoxins (8).

RESULTS AND DISCUSSION.—BGY fluorescence of locules and seeds and *A. flavus* infection of BGY-fluorescent seeds was greatest with the long maximum temperature cycle (10 hours) and long incubation period (7 days) (Table 1). The percent fluorescence of both locules and seeds was decreased by either the 2-hour maximum daily temperature or by the 3-day incubation period, with the latter having a considerably greater influence. *A. flavus* infection of BGY-fluorescent seed was also greatly reduced in these two treatments. Combination of the short maximum temperature cycle (2 hours) and the short incubation period (3 days) gave only 8 and 1%, respectively, of BGY fluorescence of locules and seeds. No *A. flavus* infection of BGY-fluorescent seed was detected. These results indicate that the duration of the maximum temperature and the number of daily cycles can regulate both expression of BGY fluorescence and seed infection by *A. flavus* in cotton bolls.

No aflatoxins were detected even though BGY

TABLE 1. Influence of diurnal temperature cycles on locule and seed fluorescence and infection of fluorescent seed from cotton bolls inoculated with *Aspergillus flavus*[a]

Diurnal temperature cycles			Fluorescence		*A. flavus* infection
Temperature max.-min. (C)	Duration max. temp. (hours)	Incubation time (days)	Locule (%)	Seed (%)	Fluorescent seed (%)
30-16	10	7	98	93	43
32-18	2	7	86	75	6
30-16	10	3	79	50	7
32-18	2	3	8	1	0

[a]The average number of bolls inoculated was 66 (range, 54 to 88) for each diurnal temperature cycle.

fluorescence and considerable seed infection occurred; and all isolates from infected fluorescent seeds were toxicogenic in laboratory tests with sterilized nondelinted cottonseed. Ashworth et al. (3) reported similar results, and concluded that inadequate aeration prevented the formation and accumulation of aflatoxins in unopened and imperfectly opened bolls. Since the inoculated bolls remained only slightly open in the closed incubation jars at 100% RH and were dried quickly after incubation, the lack of aflatoxins in our experiments could have resulted from poor aeration. However, other factors which may have been equally important must be considered:

(i) Possibly, the mature cottonseeds were resistant to internal infection and were only infected within the integuments of the seed coat. Mayne et al. (7), using conidial inoculum of *A. parasiticus,* detected less than 0.2 μg/ml of aflatoxins in mature impermeable cottonseed and 78 to 500 μg/ml in mature permeable cottonseeds.

(ii) When the boll opens, the available nutrients decrease rapidly and the fungus, consequently, must enter and respond to the favorable environment quickly. Therefore, lack of energy may limit the potential of *A. flavus* for internal infection and production of aflatoxins (3).

(iii) The virulence of *A. flavus* may be reduced when cultured on inhibitory selective media, such as MSA. Preliminary results from our laboratory show that the toxicogenic strain of *A. flavus,* when cultured on MSA, lacks sufficient energy and/or virulence to internally infect mature cottonseed (Gilbert et al., *unpublished*).

Our results demonstrated the importance of diurnal temperature cycles on the virulence of *A. flavus* under simulated field conditions. They also indicate that long daily periods with temperatures above 30 C, especially at the time of boll opening, may partially explain why aflatoxins are found primarily in cottonseed from the hotter desert regions of the southwestern United States. However, in our tests no aflatoxins were produced, and our conclusion is based solely upon the frequency of occurrence of BGY fluorescence and seed infection by *A. flavus*.

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