Absorption of Aflatoxin by Lettuce Seedlings Grown in Soil Adulterated with Aflatoxin B_1

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The objective of this study was to determine whether seedling plants accumulate aflatoxin B_1 (AFB_1) from soils that had been contaminated with the toxin. The results of this study are of major interest due to the large amount of aflatoxin-contaminated plant material that is often disposed of by returning it to the soil. Two-day-old lettuce seedlings were transplanted to a loam soil that had been adulterated with AFB_1. Following a growth period of 7–12 days, aflatoxin was recovered from leaf–stem and root tissue. The amount of AFB_1 recovered represented only a small percentage of the total toxin that had been previously added to the soil; nevertheless, the uptake by seedlings suggests that absorption of the toxin could constitute a health hazard to consumers of agricultural commodities grown on soils that had been contaminated with aflatoxin.

Following the discovery that aflatoxins are products of certain isolates of *Aspergillus flavus* and *Aspergillus parasiticus*, it was generally assumed that the principal hazards from aflatoxin contamination occurred as a consequence of improper field curing or improper storage of agricultural commodities. However, it is now recognized that *A. flavus* and *A. parasiticus* can severely infect preharvested standing crops, especially maize. In 1977 and 1980 the maize crop in parts of the southeastern United States was so severely damaged by drought, insects, and *A. flavus* infection that many fields of corn were destroyed by incorporating them into the soil. Furthermore, aflatoxin-contaminated commodities are from time to time introduced into agricultural soils (Dean, 1979). Absorption of aflatoxin by plants grown in contaminated soils could present a health hazard to the consumer and seriously affect the growth, development, and productivity of plants.

Studies conducted in our laboratory have demostrated that aflatoxin B_1 (AFB_1) can be absorbed by the root system of maize seedlings grown in Hoagland's solution adulterated with AFB_1 (Mertz et al., 1980). So that it could be determined whether plants are able to absorb aflatoxin from soil, lettuce seedlings were grown on sterile and nonsterile Shelby loam soil adulterated with AFB_1.

MATERIALS AND METHODS

Lettuce seeds (*Lactuca sativa* cv. Grand Rapid) were germinated on moist filter paper under constant illumination for 48 h. Thirty seedlings were then transferred to Petri dishes (10 × 35 mm) containing 1.5 g of sterile or nonsterile Shelby loam soil (21.4% sand, 50.5% silt, and 28.1% clay) adulterated with AFB_1. Sterilization of the soil was accomplished by placing the soil in an atmosphere saturated with propylene oxide for 48 h. The soil was then placed under the hood for 2 h to permit residual gas to be dissipated. The exposure of a solution of aflatoxin to propylene oxide has no effect on the aflatoxin molecule nor was there any effect on the growth of lettuce seedlings following the soil treatment. The seedlings were grown in an environment chamber at a constant temperature of 23 °C and a photoperiod of 13 h of light. The light intensity was 200 W/m^2 at the plant level. The seedlings were watered daily. The Petri dishes were placed in a pan and covered with clear plastic to prevent the soil from drying out.

In an initial study, aflatoxin was introduced into the soil by adding 60 mL of an ethanolic solution of AFB_1 equivalent to 125 μg of AFB_1/1.5 g of soil. After the suspension of soil was stirred for 10 min, the ethanol was removed by evaporation and the soil dried for 12 h at 60 °C. In subsequent experiments 1.5 g of soil was added to 50 mL of an aqueous solution of AFB_1. After being stirred for 10 min, the suspension was centrifuged for 10 min at 27000 g. The adulterated soil was dried for 48 h at 60 °C and ground with a pestle and mortar. The amount of aflatoxin bound to the soil was calculated by determining the concentration of aflatoxin extracted from the supernatant fraction by chloroform following centrifugation.

Extraction Procedure. The root tissue was separated from the leaf and stem tissue and washed free of soil. The stem and leaf tissue were bulked into one component. The tissues were dried for 12 h at 60 °C, weighed, and extracted for 24 h in chloroform. Prior to the extraction with chloroform, the plant material was ground in a mortar with a few milliliters of water. The chloroform extract containing aflatoxin was cleaned up by column chromatography using 10 g of activated silica gel, 60–200 mesh, according to the procedure outlined in Association of Official Analytical Chemists (1975). Briefly, the chloroform extracted was layered on a silica gel column and eluted first with 150 mL of hexane, followed by 150 mL of ether. The aflatoxin was then recovered in 150 mL of chloroform–methanol (97:3).

Aflatoxin Identification and Quantitation. Authentic AFB_1 and unknowns were chromatographed on TLC plates prepared with silica gel G (0.25 mm). The plates were activated for 1 h at 110 °C and developed for 15 cm in unlined tanks by using chloroform–acetone (85:15). The aflatoxin was visualized under long-wave UV. For quantitation TLC plates were scanned by using a Densicord densitometer, Model 530, modified for fluorodensitometric analysis according to the method of Pons et al. (1966). Confirmation of the identify of AFB_1 was made by overspotting with trifluoroacetate (TFA) (Przybylski, 1975). All procedures were carried out under reduced laboratory light to minimize photochemical changes on TLC plates.

RESULTS

So that it could be determined whether aflatoxin can be absorbed from soil, 30 2-day-old lettuce seedlings were transferred to 1.5 g of sterile and nonsterile loam soil adulterated with 125 μg of AFB_1. After 7- and 10-day growth periods, the leaf–stem and root tissues were pooled separately from five replications and extracted for AFB_1. A fluorometric TLC scan of the chloroform–methanol

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Table I. Absorption of AFB_1 by Lettuce Seedlings Grown in Loam Soil[a]

	μg of AFB_1/g dry wt for			
	day 7		day 10	
tissue	sterile	non-sterile	sterile	non-sterile
leaf	3.7	0.7	4.6	0.7
root	10.1	3.1	1.8	0.04

[a] Thirty 2-day-old lettuce seedlings were transferred to 1.5 g of sterile and nonsterile Shelby loam soil adulterated with 125 μg of AFB_1. After a growth period of 7 or 10 days, the seedlings were extracted for AFB_1. The data represent the total uptake from the pooled tissue of five replications.

(97:3) fraction from 7-day-old leaf tissue revealed that AFB_1 was the major fluorescing material isolated. The recovery of aflatoxin from seedlings grown in sterile and nonsterile soil is shown in Table I. Seedlings grown for 7 days in sterile soil accumulated ~5-fold more aflatoxin in the leaf–stem tissue than seedlings grown in nonsterile soil. Root tissue accumulated ~3-fold more aflatoxin from sterile soil than from nonsterile soil. After 10 days, aflatoxin continued to increase in the leaf–stem tissue from seedlings grown in sterile soil with no change noted in leaf–stem tissue from seedlings grown in nonsterile soil. After 10 days, aflatoxin declined in the root tissue from seedlings grown in both sterile and nonsterile soil. Whether this decline represents a metabolic breakdown of AFB_1 or loss by leaching of toxin from the root system is unknown.

So that the extent to which aflatoxin is adsorbed by Shelby loam soil could be determined, 1.5 g of soil was added to an aqueous solution with various concentrations of AFB_1. The soil suspension was then centrifuged and the supernatant fraction extracted with chloroform to determine the amount of aflatoxin adsorbed by the soil. Lettuce seedlings were grown for 11–12 days to determine the amount of AFB_1 absorbed from the adulterated soil.

Table II illustrates that all concentrations of AFB_1 ranging from 50 to 400 μg/1.5 g of soil were adsorbed from the aqueous solution in excess of 97%. Lettuce seedlings grown for 11 days in adulterated soil accumulated increasing concentrations of AFB_1 with increasing concentrations of soil borne aflatoxin. In a second study where seedlings were grown for 12 days, a similar trend was observed; however, far less toxin was absorbed than in the preceding study.

All attempts to extract aflatoxin from the soil using various organic solvents such as chloroform, methanol, and chloroform–methanol (80:20) failed to free the bound toxin.

DISCUSSION

The guideline level of aflatoxin in foods for human consumption is 20 ppb (Bullerman, 1979). When levels of toxin are found in agricultural commodities so high that it is not possible to bring the toxic concentration within acceptable limits by the addition of uncontaminated commodities, the foodstuff is frequently disposed of by use as mulch for vegetable crops or incorporated into agricultural soils as a fertilizer. Furthermore, where standing crops are so severely contaminated with aflatoxin caused by *A. flavus* infection, the stover and grain are incorporated back into the soil. In the present study, it was demonstrated that lettuce seedlings grown in soil adulterated with AFB_1 can absorb and transport the toxin into the leaf tissue. The amount of AFB_1 absorbed from the soil on a gram dry weight basis of plant tissue represented only a very small percentage (0.2–0.8%) of the total toxin bound by the soil

Table II. Absorption of Aflatoxin from Nonsterile Loam Soil Adulterated with AFB_1[a]

μg of AFB_1/ 1.5 g of soil	μg of AFB_1 bound	μg of AFB_1/g dry wt of leaf	% recovered/g dry wt
Seedlings Grown for 11 Days			
100	99.0	0.2	0.20
150	145.5	0.6	0.41
200	198.0	1.7	0.85
300	294.0	1.3	0.44
Seedlings Grown for 12 Days			
50	49.5	trace	
150	148.5	0.2	0.13
300	297.0	0.1	0.03
400	395.0	0.3	0.08

[a] Thirty 2-day-old lettuce seedlings were transferred to 1.5 g of nonsterile loam soil adulterated with AFB_1. After a growth period of 11-12 days, the seedlings were extracted for AFB_1. The data represent the total uptake from the pooled tissue of three to five replications.

(Table II); nevertheless, recovery of aflatoxin from the seedlings suggests that absorption of toxin could constitute a health hazard to consumers of agricultural commodities grown on contaminated soil. Additionally, the absorption of toxin could have deleterious effect on the growth, development, and productivity of plants. In the present investigation it was only at the highest concentration of aflatoxin (~300–400 μg/1.5 g of soil) that any noticeable effect was observed on growth of lettuce seedlings (slightly smaller and chlorotic leaves). A more harmful effect of aflatoxin-contaminated soils may be through changes in the rhizosphere microorganisms that are associated with plants growth (Subrahmanyam and Rao, 1974).

Nothing is known about the fate of aflatoxin introduced into agricultural soils; however, an examination of Table II reveals that loam soil has the capacity to adsorb large amounts of aflatoxin. Angle and Wagner (1980) were able to extract B_2 and G_2 from a silt loam soil adulterated with B_1; however, in the present study aflatoxin resisted extraction with organic solvents. It is not known what component of the soil is responsible for binding of the toxin; however, it has been demonstrated that certain montmorillonitic clays can bind more or less irreversibly AFB_1 from Sorensen buffer at pH 6.5 (Masimango et al., 1979). In the Shelby loam soil used in the present study, 28% or 429 mg/1.5 g of soil is montmillonite; therefore, the clay fraction and the organic component of the soil are undoubtedly responsible for the absorption of AFB_1. Although the toxin appears to be irreversibly bound by the loam soil, and examination of Table I reveals that there was ~5 times more toxin absorbed by seedlings grown in sterile soil than by the seedlings grown in nonsterile soil. It is unlikely that residual propylene oxide affected either uptake or breakdown of aflatoxin. Propylene oxide had no effect on the aflatoxin molecule nor did seedlings grown in soil treated with the oxide exhibit any growth differences compared with those grown in the presence of low levels of aflatoxin. These data show that not only can plants absorb the bound toxin but also it may be in part biologically degraded in the nonsterile soil.

The results of this preliminary study indicate additional research is needed to determine the longevity of aflatoxin in different types of agricultural soils. Until more is known of the fate of aflatoxin in the environment, some restraint should be excercised in the disposal of contaminated commodities by introducing them to agricultural soils.

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Effect of Hybrids with Different Levels of Susceptibility to Second-Generation European Corn Borers on Aflatoxin Contamination in Corn

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Two corn hybrids, *Zea mays* L., grown from two planting dates in Georgia, Missouri, and Iowa were inoculated with two strains of *Aspergillus flavus* Link ex Fr (one a producer of aflatoxins B_1 and B_2 and the other a nonproducer) and a strain of *Aspergillus parasiticus* Spears (producer of aflatoxins B_1, B_2, G_1, and G_2). Half of the plots also were infested with European corn borer (ECB), *Ostrinia nubilalis* (Hübner), egg masses (in four applications of two masses each, spaced 1 day apart). Infestations were started 3 days after full silk. Corn hybrid B86 X SC213 was resistant to sheath-collar and stalk feeding by second-generation ECB, and Oh43 X W182E was susceptible. A higher incidence of *A. flavus* group isolates occurred in ECB larvae collected from the susceptible hybrid than from the resistant hybrid. Hybrid differences in levels of aflatoxin, however, exhibited significant intralocation variation, but the location by hybrid interaction was significant. The hybrid susceptibility to aflatoxin contamination was location dependent, and the specific environment at a corn-growing site is a critical component among the host of factors that are related to preharvest aflatoxin contamination.

Kernel damage in corn, *Zea mays* L., by larvae of second-generation European corn borers, *Ostrinia nubilalis* (Hübner) (ECB), has been implicated in the fungal infection by *Aspergillus flavus* Link ex Fr (Lillehoj and Hesseltine, 1977). Hybrid differences also have been noted for aflatoxin contamination in preharvest corn (Zuber and Lillehoj, 1979).

Resistance to leaf feeding by first-generation ECB has not been as difficult to locate as resistance to sheath-collar feeding by second-generation ECB (Guthrie et al., 1971). Only two agricultural experiment station inbred lines of corn (B52 and B86) have been identified with high degrees of resistance to second-generation borers, whereas inbred SC213 has a low level of resistance. More than 95% second-generation larval mortality occurs within 3 days after egg hatch on resistant inbred lines (Guthrie et al., 1971).

The primary purpose of the current study was to compare the ECB *A. flavus* group interaction in a hybrid resistant to second-generation ECB with a susceptible hybrid.

MATERIALS AND METHODS

Two hybrids (B86 X SC213 and Oh43 X W182E) were grown at three locations: Ankeny, IA, Portageville, MO, and Tifton, GA. The field experiment at each location involved two replications of a split-plot design with main blocks containing planting dates (May 1 and June 1, Ankeny, IA; April 1 and May 1, Portageville, MO, and Tifton, GA). Hybrids were associated with plots within main plots. The subplot for each hybrid contained 12 treatments: (1) *A. flavus* inoculum (nonproducer of aflatoxin) placed in silks, (2) *A. flavus* (nonproducer) in silks plus ECB, (3) *A. flavus* (nonproducer) in leaf axils, (4) *A. flavus* (nonproducer) in leaf axils plus ECB, (5) *A. flavus* (producer of aflatoxin) in silks, (6) *A. flavus* (producer) in silks plus ECB, (7) *A. flavus* (producer) in leaf axils, (8) *A. flavus* (producer) in leaf axils plus ECB, (9) *Aspergillus parasiticus* (producer) in silks, (10) *A. parasiticus* (producer) in silks plus ECB, (11) *A. parasiticus* (producer) in leaf axils, and (12) *A. parasiticus* (producer) in leaf axils plus ECB. Fungal spores with varied aflatoxin producing potential included *A. flavus* (nonproducer) NRRL 1957, *A. flavus* (producer of aflatoxins B_1 and B_2) NRRL 3357, and *A. parasiticus* (producer of aflatoxins B_1, B_2, G_1, and

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