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Aflatoxin B -induced alterations in uptake and distribution of ^{65}Zn-$ZnCl_2$ by four *Zea mays* cultivars and toxin effects on root and stem elongations

GERALD C. LLEWELLYN *, NANCY S. SLACK *, WILLIAM V. DASHEK **[1], JOHN D. REYNOLDS *, MICHAEL B. MOLL **, SARA M. MCCOWEN *

Department of Biology — Virginia Commonwealth University *; Richmond Virginia 23284 and West Virginia University ** — Morgantown, WV 26506, USA

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Abstract

Aflatoxin B_1 has been recovered within seemingly healthy, intact seeds, which suggests that its transport from contaminated soil to the fruit can occur. Previously, we examined the effects of 19.7 $\mu g \cdot ^{-3}$ mixed aflatoxins (AFTs) on the abilities of three *Zea mays* cultivars to remove Zn^{++} from Perlite and the influence of Zn^{++} on the cultivars' capabilities to both take-up and distribute AFTs. Here, we report both 2.5 and 5.0 μg aflatoxin B_1 (AFB_1)$\cdot cm^{-3}$ influences on time-dependent, uptake and organ distribution of ^{65}Zn-$ZnCl_2$ from liquid culture by *Zea mays*, cvs. 'Early Yellow', 'Silver Queen', 'Early White' and 'Golden Queen'. In addition, time-dependent seed germination as well as root and stem elongation responses to AFB_1 are described. Neither 2.5 nor 5.0 μg $AFB_1 \cdot cm^{-3}$ affected the cultivars' abilities to germinate. Whereas an analysis of variance revealed significant differences for the combination cultivars, AFB_1 concentration and time regarding root elongation, such analysis for stem elongation yielded only a significant interaction between AFB_1 concentration and one cultivar at a time course's completion. As for ^{65}Zn-$ZnCl_2$ uptake, an ANOVA indicated that there were significant differences for the combination organs, cultivars and AFB_1 concentration. Certain results suggest *Zea mays* cultivar variability in susceptibility to exogenous AFB_1. The significance of these findings is discussed.

Key words: Zea mays, cultivars, ^{65}Zn-$ZnCl_2$, uptake

INTRODUCTION

Aflatoxins are toxic secondary metabolites which are produced by both *Aspergillus flavus* and *A. parasiticus*. These toxins, especially aflatoxin B_1 (AFB_1) can be carcinogenic, mutagenic, teratogenic, and toxic

[1] Present address: Department of Biology, Atlanta University, Atlanta GA 30314, USA.

(W o g a n 1965). Many agricultural commodities, especially corn, can serve as substrates for natural contamination by the fungi (G o l d b l a t t 1969). Aflatoxins exert either little or no effect upon seed germination, but subsequent development may be inhibited as different cultivars of corn appear to vary in their susceptibility to aflatoxin (C r i s a n 1973). If the plant is susceptible, this may affect its normal physiological functions, such as uptake of trace elements.

In 1977, a severe aflatoxin contamination from *A. flavus* infection in the southeastern United States corn crop resulted in destruction of many fields through plowing under of both stover and grains (M c M i l l i a n et al. 1978). Because both *A. flavus* and *A. parasiticus* can infect crops prior to harvesting (L i l l e h o j and Z u b e r 1975), this practice may prove quite dangerous.

Aflatoxin has been found within seemingly healthy, intact seeds, which suggests that transport of the toxin from contaminated soil to the fruit can occur (A n d e r s o n et al. 1975). In this connection, M e r t z et al. (1980) showed that corn seedlings grown in Hoagland's solution adulterated with AFB_1 could translocate the toxin from roots to the leaf-stem. If soil microflora do not rapidly degrade the aflatoxin contained within the plowed under stover and grains, the possibility that the roots of the next crop will both absorb and subsequently transport the aflatoxin to the stems and leaves exists. This could present hazards to both the consumer's health and the plant's growth and development.

Previously, we (L l e w e l l y n et al. 1982) examined the effects of mixed aflatoxins (AFTs) on the abilities of three cultivars of *Zea mays* to take up Zn^{++} from Perlite and the effect of Zn^{++} on the capabilities of the cultivars to both take-up and distribute AFTs. For that examination, only one AFTs concentration was used. Here, we report the influence of two, lesser concentration of AFB_1 on the uptake of ^{65}Zn-$ZnCl_2$ from liquid culture. In contrast to our previous report, this preliminary investigation utilized four additional *Zea mays* cultivars.

MATERIALS AND METHODS

SEED GERMINATION

Four cultivars of captan fungicide-treated *Zea mays*, 'Silver Queen' (SQ), 'Golden Queen' (CQ), 'Early Sunglow Yellow' (EY), 'Early Sunglow White' (EW), were obtained from Wilson's Feed and Seed Company (Richmond, VA, USA).

One hundred and twenty randomly selected seeds of each cultivar were placed into sterile Petri dishes containing 10 cm^3 sterile distiled water. Seeds were germinated within a Freas growth chamber in con-

tinuous light supplied by two fluorescent tubes whose combined intensities were 300 ft. candles. The humidity within the chamber was $63 \pm 5\%$ as determined with a wet/dry bulb hygrometer.

To determine the effects of AFB_1 (Calbiochem, LaJolla, CA) on seed germination, twenty seeds of each cultivar were randomly selected for each of the treatment groups — high (5.0 μg $AFB_1 \cdot cm^{-3}$ water) and low (2.5 μg $AFB_1 \cdot cm^{-3}$ water) toxin as well as the control (sterile distilled water). The seeds were placed into sterile Petri dishes containing 10 cm^3 of the appropriate treatment solution. Seeds were germinated under constant light at $26 \pm 2^\circ$ C and germination was scored after 24 h. Germination is defined as radicle emergence as visualized without a microscope.

SEEDLING GROWTH

Root lengths were measured every 24 h for four days when over onehalf of the seedlings were selected for treatment on the basis of their size and freedom from natural mold growth. Holders were constructed of two 4.5 × 26.0 cm pieces of styrofoam. Absorbent paper was cut and placed between the seedlings and the styrofoam to act as a wick. Two holders were placed into each clear, plastic storage box (31.0 × 16.5 × × 8.0 cm) containing 300 cm^3 sterile distilled water which contained Ca^{++} to prevent membrane leakage. This amount of medium was maintained throughout the experiment. Sterile Hoagland's nutrient solution was not used because it contains a variety of cations which could obscure a Zn^{++} effect and because it usually resulted in mold contamination in our experimental design. To protect the roots from light, aluminum foil was wrapped around the lower third of the box and also placed between the holders.

Three days after being in the holder, most of the seedlings possessed an established root system, at which time the wicks were removed and the water decanted. Thirty seedlings of approximately equal size from each cultivar were placed into three treatment groups — 5.0 μg $AFB_1 \cdot \cdot cm^{-3}$, 2.5 μg $AFB_1 \cdot cm^{-3}$, and control.

AFLATOXIN AND ZINC ADMINISTRATION

A stock AFB_1 (Calbiochem, LaJolla, CA) solution consisting of 10,000 μg $AFB_1 \cdot 5$ cm^{-3} acetone (to solubilize AFB_1) and 2,000 cm^3 distilled water was prepared. The solution was warmed gently and stirred. To prevent exposure to light, the flask was wrapped in aluminum foil and then refrigerated.

To the storage boxes was added 300 cm^3 of the stock solution for the 5.0 μg $AFB_1 \cdot cm^{-3}$ group, 150 cm^3 stock and 150 cm^3 sterile distilled

water for the 2.5 μg $AFB_1 \cdot cm^{-3}$ group, and 300 cm^3 of sterile distilled water and 0.25% acetone (equivalent to that in the experimentals) for the control group. Sterile distilled water was added daily to maintain the 300 cm^3 level. To aid mixing and aeration, the entire box was gently rotated. The seedlings were treated for five days at which time another set of boxes containing 0.25 cm^3 (25 μCi) of ^{65}Zn-$ZnCl_2$ (spec, act. 1-10 $Ci \cdot g^{-1}$ Zn^{++}, New England Nuclear, Boston, MA) in 300 cm^3 water was prepared. The seedlings in their holders were transferred to the boxes containing the radionuclide. The water level was maintained in the same manner as with the toxins for three days.

On the sixteenth day, the seedlings were removed from the boxes and rinsed thoroughly. Then, they were dissected into root, seed, stem and leaves. The stems and leaves were separated at the first node above the coleoptile. Wet and subsequent dry weights were taken of all the organs. To obtain wet weights, organs were blotted prior to weighing. Dry weights were recorded following drying of organs for 24 h at 65° C.

RADIONUCLIDE COUNTING

To quantify the amount of radioactivity, plastic vials containing the organs were placed into a Beckman Gamma 9000 counter (Fullerton, CA). Each organ was counted three time for three minutes and the mean of the replicate counts determined following bockground subraction. The window was 470 to 570 KEV to include the gamma peak. Subsequent to counting, the dry weight of segments was determined to 0.001 g following drying to constant weight at 40 ± 1° C. Initially, the data were expressed as $cpm \cdot g^{-1}$ dry weight and then as percent of that recovered from the leaf, seed, stem and root.

ANALYSES

The culture media aflatoxin concentrations were verified by partitioning the media against chloroform and subsequent thin layer chromatography of the extract as well as a visual dilution technique which is sensitive to 2 ppb (H o r o w i t z et al. 1975). The data were analyzed by an analysis of variance (SAS User's Guide, 1982).

RESULTS AND DISSCUSION

SEED GERMINATION

Alfatoxin B_1 did not statistically alter the abilities of the cultivars to germinate at either 2.5 or 5.0 $\mu g \cdot cm^{-3}$ (data not shown).

STEM AND ROOT ELONGATION

Although root lengths of both toxin-treated and control 'Early Yellow' seedlings (Table 1) increased throughout the time course, the final root lengths for both toxin-trated seedlings were less than that of the control. The reductions were ∼40 and 3% for the 2.g and 5.0 μg $AFB_1 \cdot cm^{-3}$-treated seedlings, respectively.

Table 1 also presents the mean root lengths for 'Silver Queen' seedlings which were treated with either 2.5 or 5.0 μg $AFB_1 \cdot cm^{-3}$. Root

Table 1

Effect of aflatoxin on root elongation[a]

Treatment, μg $AFB_1 \cdot cm^{-3}$	Time							
	24 H		48 H		72 H		96 H	
	length	% difference	length	% difference	length	% difference	length	% difference
'Early Yellow'								
0.0	0.08 ± 0.07	0.0	0.65 ± 0.46	0.0	1.79 ± 0.94	0.0	3.53 ± 0.98	0.0
2.5	0.07 ± 0.08	−12.5	0.67 ± 0.48	+3.0	1.57 ± 0.85	−12.5	2.21 ± 1.13	−37.5
5.0	0.06 ± 0.06	−25.0	0.75 ± 0.48	+16.0	2.16 ± 0.92	+20.5	3.44 ± 1.32	−2.5
'Silver Queen'								
0.0	0.07 ± 0.07	0.0	0.37 ± 0.28	0.0	1.02 ± 0.62	0.0	1.71 ± 0.91	0.0
2.5	0.04 ± 0.05	−43.0	0.38 ± 0.25	+3.0	1.16 ± 0.57	+14.0	2.72 ± 1.07	+59.0
5.0	0.06 ± 0.07	−14.0	0.59 ± 0.37	+60.0	1.44 ± 0.91	+41.0	2.65 ± 1.45	+55.0
'Early White'								
0.0	0.05 ± 0.06	0.0	0.36 ± 0.44	0.0	1.04 ± 0.85	0.0	1.39 ± 0.98	0.0
2.5	0.03 ± 0.05	−40.0	0.30 ± 0.31	−17.0	1.48 ± 1.02	+30.0	2.27 ± 1.42	+63.0
5.0	0.04 ± 0.05	−20.0	0.19 ± 0.24	−43.0	0.67 ± 0.66	−36.0	1.03 ± 1.00	−26.0
'Golden Queen'								
0.0	0.08 ± 0.08	0.0	0.60 ± 0.46	0.0	1.34 ± 1.16	0.0	2.32 ± 0.86	0.0
2.5	0.06 ± 0.07	−25.0	0.52 ± 0.35	−13.0	1.38 ± 0.73	+3.0	3.47 ± 1.04	+50.0
5.0	0.04 ± 0.05	−50.0	0.42 ± 0.24	−30.0	1.23 ± 0.49	−8.0	1.81 ± 0.59	−22.0

[a] Data are mean root lengths in cm and standard deviations for 18 replicate plants.

lengths increased in semi-linear fashion for both the 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$-treated seedlings as well as control. Root lengths at 5.0 μg $AFB_1 \cdot cm^{-3}$ were greater than that of the control at 72 and 96 h. The maximum differences occurred at 96 h for both toxin concentrations, 1.59- and 1.55-folds for 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$, respectively.

Five μg $AFB_1 \cdot cm^{-3}$ inhibited root elongation of 'Early White' by ∼25% at 96 h but 2.5 μg $AFB_1 \cdot cm^{-3}$ stimulated it approximately 1.63

times. A similar pattern was observed for 'Golden Queen'. Two and half μg $AFB_1 \cdot cm^{-3}$ stimulated root length 1.50-fold at 96 h but 5.0 μg $AFB_1 \cdot cm^{-3}$ inhibited it by ∼22%.

The results of a statistical analysis of the root length data are depicted in Table 2. There are significant differences for the combination cultivars, AFB_1 concentration and time.

Table 2

Summary of statistical analysis of the effects of aflatoxin on root elongation

Sources*	df	Sum of squares	Mean square	F-value	Pr > F
TRT A	3.0	48.0	16.0	30.32	0.0001
TRT B	2.0	5.0	2.5	4.75	0.0089
Time	3.0	613.9	204.6	387.55	0.0001
TRT A-TRT B	6.0	35.1	5.9	11.08	0.0001
TRT A-Time	9.0	31.1	3.5	6.55	0.0001
TRT B-Time	6.0	6.5	1.1	2.04	0.0577
TRT A-TRT B-Time	18.0	40.4	2.2	4.25	0.0011
Error	751.0	396.5	0.5		
Total (corrected)	798.0	1176.5			

* Sources: TRT A — cultivars, TRT B — AFB_1 concentrations, Time — 24, 48, 72, 96 h.

The effects of AFB_1 addition upon stem elongation of 'Early Yellow' seedlings are displayed in Table 3. The stem elongation data are expressed as seedling height above the holders (distance between the seedling's tip and that portion at the top of the holder). Both 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$ decreased stem elongation. At the time course's completion, these decreases were ∼14 and 23% for 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$, respectively. In contrast to these results, both 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$ almost completely suppressed stem elongation of 'Silver Queen'.

Addition of 2.5 μg $AFB_1 \cdot cm^{-3}$ to a culture medium at 3 days resulted in inhibition of stem elongation of 'Early White' seedlings at every time examined except at day 7. The percent inhibition at completion of the time course was ∼8. Whereas inclusion of 5.0 μg $AFB_1 \cdot cm^{-3}$ into the medium initially reduced stem elongation, this elongation surpassed (day 7) and approximated (days 8 and 9) that of the control as the time course progressed.

Table 3 also shows the influence of AFB_1 addition on 'Golden Queen' elongation. Both 2.5 and 5.0 μg $AFB_1 \cdot cm^{-3}$ inhibited stem elongation by 96 h. The percent inhibitions were ∼16 for the former and ∼23 for the latter concentrations. Whereas addition of 5.0 μg $AFB_1 \cdot cm^{-3}$ initially stimulated seedling height, supplementation of the medium with 2.5 μg $AFB_1 \cdot cm^{-3}$ exhibited little effect during the first two days after addition.

Table 3

Effect of aflatoxin on stem elongation[a]

Treatment, μg $AFB_1 \cdot cm^{-3}$	Days														
	1	2	3	4[b]		5		6		7		8		9	
				H[c]	%D	H	%D	H	%D	H	%D	H	%D	H	%D
'Early Yellow'															
0.0	0.78±0.56	1.46±0.78	2.02±1.02	3.05±1.23	0.0	3.57±1.45	0.0	4.09±1.59	0.0	4.67±1.68	0.0	4.95±1.90	0.0	5.22±2.27	0.0
2.5	0.78±0.56	1.46±0.78	2.02±1.02	2.75±1.31	−8.9	3.14±1.58	−12.0	3.84±1.69	− 6.0	4.08±1.58	−13.0	4.32±1.81	−12.7	4.49±2.09	−14.0
5.0	0.78±0.56	1.46±0.78	2.02±1.02	2.46±1.61	−19.4	2.80±1.70	−22.0	3.29±1.84	−19.6	3.47±1.79	−25.7	3.84±1.72	−22.5	4.02±1.76	−23.0
'Silver Queen'															
0.0	0.17±0.31	0.48±0.56	0.56±0.61	1.73±0.59	0.0	1.95±0.77	0.0	2.71±1.08	0.0	3.32±1.37	0.0	3.75±1.61	0.0	3.94±1.71	0.0
2.5	0.17±0.31	0.48±0.56	0.56±0.61	0.65±0.83	−62.0	0.69±0.87	−64.7	0.76±0.90	−72.0	0.67±0.80	−70.0	0.64±0.83	−83.0	0.69±0.89	−82.5
5.0	0.17±0.31	0.48±0.56	0.56±0.61	0.52±0.51	−70.0	0.55±0.48	−71.8	0.54±0.46	−80.0	0.61±0.53	−81.7	0.77±0.77	−79.5	0.76±0.89	−80.7
'Early White'															
0.0	0.38±0.53	1.48±1.36	1.82±1.62	5.32±2.42	0.0	6.63±3.54	0.0	7.08±4.18	0.0	6.75±4.31	0.0	8.44±5.81	0.0	8.91±6.34	0.0
2.5	0.38±0.53	1.48±1.36	1.82±1.62	3.95±1.71	−25.8	4.93±2.72	−25.7	5.70±3.33	−19.5	7.32±4.62	+8.5	7.91±5.40	−6.3	8.27±5.90	−7.2
5.0	0.38±0.53	1.48±1.36	1.82±1.62	4.30±1.32	−19.2	5.68±1.66	−14.3	6.44±2.25	−9.0	7.61±2.91	+13.0	8.29±3.25	−1.8	8.51±3.58	−4.6
'Golden Queen'															
0.0	0.36±0.47	1.47±1.49	1.69±1.63	3.19±1.96	0.0	3.83±2.05	0.0	4.76±2.55	0.0	7.41±2.69	0.0	8.42±2.43	0.0	9.02±2.75	0.0
2.5	0.36±0.47	1.47±1.49	1.69±1.63	3.33±1.34	+4.0	3.57±1.26	−6.8	4.02±1.69	−15.6	5.80±3.02	−21.8	6.76±3.55	−19.7	7.61±3.91	−15.6
5.0	0.36±0.47	1.47±1.49	1.69±1.63	3.96±1.60	+24.0	4.47±1.92	+16.0	4.99±2.33	+5.0	6.32±3.55	−14.8	6.72±3.75	−20.0	7.00±3.94	−22.4

[a] Data are mean stem lengths in cm and standard deviations for 18 replicate plants.
[b] Toxin treatment began on day 4. Prior to day 4 data were pooled for all three groups.
[c] H = height in cm.

Table 4

Summary of recovery of radioactivity from [$^{65}ZnCl_2$] in organs of seedlings of *Zea mays* cultivars

Treatment, μg $AFB_1 \cdot cm^{-3}$	Root	Seed	Stem	Leaf
	'Early Yellow'			
0	244,543.2± 35,386.6	15,388.2± 5450.8	16,077.8± 2673.3	1416.8± 485.2
5	224,267.5± 35,621.0	11,928.4± 6113.6	18,099.6± 2249.6	1752.1± 794.9
10	208,833.6± 39,636.0	8,900.5± 2954.8	7,974.8± 2195.4	1439.3± 482.7
	'Silver Queen'			
0	256,349.9± 76,367.1	14,642.5± 9890.2	7703.9± 3360.6	2232.8± 654.4
5	171,447.6± 49,018.2	3514.7± 2273.8	1087.1± 459.9	a
10	110,815.0± 34,730.3	4031.9± 2417.6	1301.6± 382.8	a
	'Early White'			
0	376,638.2±144,345.5	67,439.5±36,514.1	64,676.9±15,740.1	10,077.3±6714.7
5	213,259.3± 61,086.8	21,333.6±13,137.6	32,666.0±11,819.0	4833.8±2665.3
10	194,459.6± 36,836.5	50,925.6±20,629.7	87,063.2±33,367.1	3866.8±2160.9
	'Golden Queen'			
0	223,761.3± 59,324.6	42,863.7±12,383.4	58,679.0±17,626.8	1853.5± 484.6
5	163,190.5± 22,487.9	36,375.7±16,143.3	44,549.1±19,079.7	2369.4± 454.9
10	148,140.0± 20,920.1	56,053.9±11.047.0	76,969.9±27,685.0	2267.4± 899.9

[a] No leaves obtained. Data are means and standard deviations for 10 replicate plants.

Of these stem data, only the interaction between AFB_1 concentration and 'Silver Queen' cultivar was statistically significant (statistical analysis not shown).

RECOVERY OF RADIOACTIVITY FROM ^{65}Zn-$ZnCl_2$

Roots. The reductions in recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ at 2.5 μg $AFB_1 \cdot cm^{-3}$ were 8.3, 33.1, 43.4, and 27.1% for cultivars, 'Early Yellow', 'Silver Queen', 'Early White' and 'Golden Queen', respectively (Table 4). In contrast, the diminutions in recoveries at 5.0 μg $AFB_1 \cdot cm^{-3}$ were 14.6, 56.8, 48.4 and 33.8% for the same cultivar sequence.

Seeds. Whereas 22.5, 76.0, 68.4 and 15.1% decreases in radioactivity from ^{65}Zn-$ZnCl_2$ were observed for cultivars 'Early Yellow', 'Silver Queen', 'Early White' and 'Golden Queen', respectively, at 2.5 μg $AFB_1 \cdot cm^{-3}$, the supressions for 'Early Yellow', 'Siver Queen' and 'Early White' were 42.2, 72.5, nad 24.5%, respectively at 5.0 μg $AFB_1 \cdot cm^{-3}$ (Table 4). A diminution in the recovery of ^{65}Zn-$ZnCl_2$ was not seen for 'Golden Queen'. Only 'Early Yellow' exhibited a concentration-dependent response to AFB_1. The significance of these distributions within the remains of the seeds (endosperm largely depleted) remains to be established since the seed at the plant developmental stage employed herein constitutes a 'source' rather than a 'sink'.

Stems. At 2.5 μg $AFB_1 \cdot cm^{-3}$ the inhibitions in recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ were 86.0, 49.5 and 24.1%, respectively, for 'Silver Queen', 'Early White' and 'Golden Queen' (Table 4). A reduction in recovery was not seen for 'Early Yellow'. Alfatoxin B_1 (5.0 $\mu g \cdot cm^{-3}$)--induced changes in recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ were 50.4 and 83.1% suppressions for 'Early Yellow' and 'Silver Queen' and 1.35- and 1.31-fold promotions in 'Early White' nad 'Golden Queen'. Two and a half μg $AFB_1 \cdot cm^{-3}$-induced alterations in recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ included 1.24- and 1.27-fold enhancements for 'Early Yellow' and 'Golden Queen' and 52.0% depression for 'Early White' (Table 4). Leaves were not observed for 'Silver Queen'. At 5.0 μg $AFB_1 \cdot cm^{-3}$, a 61.6% decline and a 1.22-fold rise in radioctivity occurred for 'Early White' and 'Golden Queen', respectively. As previously mentioned, leaves were separated from the stem at the first node above the coleoptile.

Table 5 summarizes the statistical analysis of AFB_1 effects upon ^{65}Zn-$ZnCl_2$ uptake. The ANOVA indicated that there were statistically significant differences for the combination organs, cuttivars and AFB_1 concentration.

Table 6 presents the percent radioactivity recovered within the organs. The majority (65-90%) of the radioactivity from ^{65}Zn-$ZnCl_2$ recovered within untreated seedlings occurred within the root. With the

Table 5

Summary of statistical analysis of the effects of aflatoxin on ^{65}Zn-$ZnCl_2$ uptake

Sources*	df	Sum of squares	Mean square	F	Pr > F
Organs	3.0	280.4	93.5	656.88	0.0001
TRT A	3.0	12.2	4.1	28.62	0.0001
TRT B	2.0	8.9	4.5	31.21	0.0001
Organs-TRT A	9.0	10.3	1.1	8.03	0.0001
Organs-TRT B	6.0	15.3	2.6	17.96	0.0001
TRT A-TRT B	6.0	4.6	0.8	5.45	0.0001
Organs-TRT A-TRT B	18.0	5.6	0.3	2.44	0.0017
Error	325.0	46.3			
Total (corrected)	370.0	383.6			

* Sources: TRT A — cultivars, TRT B — AFB_1 concentrations, Organs — seed, leaf, root, stem.

exception of 5.0 μg $AFB_1 \cdot cm^{-3}$ for 'Early White' (23% reduction) and 'Golden Queen' (20% suppression), AFB_1 did not alter the % radioactivity recovered within the root to any appreciable extent.

Seeds Whereas neither 2.5 nr 5.0 μg $AFB_1 \cdot cm^{-3}$ altered the percentage of radioactivity recovered within the seed of 'Silver Queen', 5.0 μg $AFB_1 \cdot cm^{-3}$ lowered the percentage by 27% for 'Early Yellow'.

Other AFB_1-induced changes included a 24% decline and 1.18-fold rise at 2.5 μg $AFB_1 \cdot cm^{-3}$ for 'Early White' and 'Golden Queen', respectively. At 5.0 μg $AFB_1 \cdot cm^{-3}$, 'Early White' and 'Golden Queen' showed 1.65- and 1.33-fold promotions in the recoveries of radioactivity.

Stems. Two and half μg $AFB_1 \cdot cm^{-3}$-promoted changes in the recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ were 1.54-, 1.45-, and 1.05-fold enhancements for 'Early Yellow', 'Early White' and 'Golden Queen' respectively, and a 50.0% suppression for 'Silver Queen'. The 5.0 μg $AFB_1 \cdot cm^{-3}$-induced alterations in recoveries of radioactivity were 11.5 and 50% reductions for 'Early Yellow' and 'Silver Queen', respectively, and 2.10- and 1.40-folds accelerations for 'Early White' and 'Golden Queen', respectively.

Leaf. The recoveries of radioactivity from ^{65}Zn-$ZnCl_2$ for the leaf were 20 and 33% reductions from the control for 'Early Yellow' and 'Early White', respectively, at 2.5 μg $AFB_1 \cdot cm^{-3}$. 'Golden Queen' exhibited a 1.57-fold rise in recovery of radioactivity. At 5.0 μg $AFB_1 \cdot cm^{-3}$, the recoveries for leaf were 1.2- and 1.1-folds greater than that of the control for 'Early Yellow' and 'Golden Queen', respectively. 'Early White' exibited a 54% diminution in recovery.

The present results differ from those obtained by Llewellyn et al. (1982) with AFTs for cultivars 'X-Sweet', 'Merit' and 'Trucker's White'. In that investigation, a higher amount of label from ^{65}Zn-$ZnCl_2$ was recovered within the stem than within either the root-seed or leaf when

Table 6

Percent radioactivity recovered in root, seed, stem, and leaf

Treatment, μg $AFB_1 \cdot cm^{-3}$	Root		Seed		Stem		Leaf	
	%	difference	%	difference	%	difference	%	difference
	'Early Yellow'							
0	88.2± 6.0	0.0	5.2±3.0	0.0	6.1± 3.2	0.0	0.5±0.3	0.0
2.5	85.0± 6.4	−3.6	5.2±3.0	0.0	9.4± 4.9	+54.0	0.4±0.3	−20.0
5.0	90.2± 5.4	+2.3	3.8±2.0	−27.0	5.4± 4.2	−11.5	0.6±0.4	+20.0
	'Silver Queen'							
0	90.5± 6.3	0.0	5.2±4.0	0.0	3.6± 2.9	0.0	0.7±0.6	0.0
2.5	93.0± 7.5	+3.9	5.2±5.8	0.0	1.8± 2.2	−50.0	a	
5.0	92.8± 6.7	+3.1	5.4±5.4	+4.0	1.8± 1.6	−50.0	a	
	'Early White'							
0	76.5± 9.8	0.0	10.0±6.8	0.0	11.1± 4.9	0.0	2.4±2.7	0.0
2.5	74.8±16.9	−2.2	7.6±6.8	−24.0	16.0±12.4	+45.0	1.6±1.3	−33.3
5.0	59.0±10.5	−22.9	16.5±6.0	+65.0	23.4±11.1	+111.0	1.1±0.6	−54.2
	'Golden Queen'							
0	65.4±11.5	0.0	13.6±6.5	0.0	20.3± 9.5	0.0	0.7±0.4	0.0
2.5	61.6±12.2	−5.9	16.0±8.0	+17.5	21.3± 9.0	+6.5	1.1±1.0	+57.0
5.0	52.7±10.1	−19.4	18.1±5.3	+33.0	28.4±11.1	+40.0	0.8±0.6	+15.0

[a] Leaves not obtained; data were calculated from cpm/g dry weight recovered in four organs; data are means and standard deviations for 10 replicate plants.

the seedlings were not irrigated with AFTs. The data within Table 4 demonstrate that more radioactivity was recovered within the root-seed than within either the stem or leaf in AFB_1's absence. This result agrees with those of Turner (1970) who found that main of Zn^{++} accumulation within *Agrostis tenuis* was the root suggesting that it may protect against Zn^{++} toxicity (Turner 1970, Turner and Marshall 1971).

When seedlings were irrigated with a solution containing AFTs (Llewellyn et al. 1982), either a higher ('X-Sweet') or nearly equivalent amount of radioactivity was recovered within the root-seed than within the stem. We interpreted this to mean that AFTs may have interfered with ^{65}Zn-$ZnCl_2$ translocation from the root to the stem and leaf. The mechanism by which AFB_1 interfered with the translocation was not examined. Investigations are required to determine whether the interference is either direct or alternatively indirect, e.g., by impairment of leaf metabolism thereby preventing acropetal ion transport. In the present investigation, an AFB_1-promoted accumulation of radioactivity from ^{65}Zn-$ZnCl_2$ within the stem appeared to occur for 'Early White' and 'Golden Queen'. These conflicting results may reflect differences in the procedures which were used for growth. Whereas our previous investigation employed Perlite and AFTs, the present study utilized both a liquid culture and AFB_1.

In summary, certain of the results presented herein support the suggestion of Crisan (1973) that different cultivars of *Zea mays* vary in their susceptibility to exogenous alfatoxin.

As for an agro-economic importance, the present data together with our previous findings (Llewellyn et al. 1982) may be relavant to the farmer who wants to select a corn cultivar that is fairly resistant to the influence of any alfatoxin present within an agricultural soil. The corn seedling must be able to take-up vital trace metals such as Zn^{++} for both proper growth and development. These metals are often supplied within fertilizers, one of the farmer's major expenses, making it important that a plant be able to utilize them efficiently. As has been demonstrated, AFB_1 can reduce the seedling's ability to absorb zinc, the extent to which depends upon the cultivar.

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Zmiany w pobieraniu i rozprzestrzenianiu ^{65}Zn-$ZnCl_2$ pod wpływem aflatoksyny B_1 u czterech odmian Zea mays i toksyczny wpływ na wydłużanie się korzenia i łodygi

Streszczenie

Uzyskano aflatoksynę B_1 z nasion, które wydawały się zdrowe i nieuszkodzone, co sugeruje że była ona transportowana z zanieczyszczonej gleby do owocu. Poprzednio badaliśmy wpływ 19,7 $\mu g \cdot cm^{-3}$ mieszaniny aflatoksyn (AFTs) na zdolność trzech odmian *Zea mays* do pobierania Zn^{++} z Perlitu i wpływ Zn^{++} na zdolność odmian do pobierania i rozprzestrzeniania AFTs. Tutaj podajemy, że aflatoksyna B_1 (AFB_1) w stężeniu 2,5 i 5 $\mu g \cdot cm^{-3}$ wpływa na zależne od czasu pobieranie z płynnej kultury ^{65}Zn-$ZnCl_2$ przez odmiany *Zea mays* i rozprzestrzenianie tego związku w organach. Stosowano odmiany: 'Early Yellow', 'Silver Queen', 'Early White' i 'Golden Queen'. Oprócz tego opisujemy jak zależnie od czasu, AFB_1 wpływa na kiełkowanie nasion oraz na wydłużanie łodygi i korzeni.

Ani 2,5 ani 5 μg $AFB_1 \cdot cm^{-3}$ nie wpływa na kiełkowanie badanych odmian. Podczas gdy analiza wariancji wykazała istotne różnice w wydłużaniu się korzeni zależne od odmiany, stężenia AFB_1 i czasu. Ta analiza wykazała, że wydłużanie się łodygi tylko u jednej z odmian zmieniło się istotnie w badanym czasie. Jak pokazał test ANOVA były istotne różnice w pobieraniu ^{65}Zn-$ZnCl_2$ dla kombinacji organów, odmian i stężeń AFB_1. Niektóre wyniki sugerują, że istnieją różnice między odmianami *Zea mays* co do wrażliwości na egzogenną AFB_1. Dyskutujemy znaczenie tych obserwacji.