Aflatoxin Contamination in Corn Samples Due to Environmental Conditions, Aflatoxin-Producing Strains, and Nutrients in Grain Grown in Costa Rica

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Aflatoxins are considered a potential hazard to public health, due to their toxicity and carcinogenicity. Under favorable environmental conditions, insect attack, and substrate, aflatoxin-producing fungal species can grow in certain foods and feeds. The total aflatoxin distribution due to environmental conditions (temperature, relative humidity, and rainfall), collection phase (early, middle, and late), microbiological analysis of aflatoxin-producing strains, and nutrients (minerals, moisture, and carbohydrates) was evaluated in samples of corn grown in Costa Rica. A multiple regression analysis design determined that total aflatoxin levels were significantly correlated ($p \leq 0.05$) with the presence of *Aspergillus flavus* in the grain and temperature conditions. Levels of aflatoxin were highly correlated ($p \leq 0.01$) among minerals (Mg^{2+}, Zn^{2+}, and Ca^{2+}) and the polynomial effect of their interactions. Collection phase had a significant effect on aflatoxin levels ($p \leq 0.05$) due to differences in harvest and storage conditions, as well as agricultural practices in each region. Also, the effect of xylose, fructose, and glucose/mannose content in corn grain on the level of aflatoxin was not significant ($p \leq 0.05$). However, glucose/mannose had an effect of multicollinearity.

Keywords: *Aflatoxin; corn; Costa Rica*

INTRODUCTION

Aspergillus flavus and *Aspergillus parasiticus* are toxin-producing strains of aflatoxin. These fungal species have been found during growth, harvest, and storage of different foods and feeds (Wood, 1989). As a result, human beings and animals are exposed to aflatoxin by consuming contaminated food (Hansen, 1990). This contamination cannot be totally eliminated from the products by prevailing methods, which makes aflatoxin an unavoidable contaminant.

Dutton (1988) and Llewellyn et al. (1988) reported that aflatoxin may be categorized as secondary metabolites and their production is influenced by factors such as catabolic activity, reduced coenzyme level, energy charge, and metal ions. Ellis et al. (1991) identified three main factors (biological, chemical, and environmental) associated with the biosynthesis of aflatoxin which have been shown to have a direct relationship with aflatoxin formation.

Mold strain is an important biological factor because *A. flavus* and *A. parasiticus* are the predominant species producing aflatoxin (Anderson et al., 1975; Karunaratne et al., 1990; Brown et al., 1991; Ellis et al., 1991). Among the chemical factors, some studies have shown that nutrients and substrate are important for aflatoxin production, which was induced by a high level of carbohydrates and a low level of proteins (Wiseman and Buchanan, 1987; Thalmann, 1990; Ellis et al., 1991). According to Wiseman and Buchanan (1987), carbohydrates provide the two carbon precursors for toxin synthesis. Corn is therefore a good substrate for aflatoxin production because of its high carbohydrate content and low nitrogen content.

The biosynthesis of aflatoxin is affected by some metals such as cadmium (Cd^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), and zinc (Zn^{2+}) (Marsh et al., 1975; Wilson et al., 1983; Failla et al., 1986; Stossel, 1986; Ellis et al., 1991). Interest in the production of aflatoxin in corn has focused on zinc as trace element because it is specifically required for aflatoxin synthesis (Failla et al., 1986; Stossel, 1986). It has also been reported that calcium content may reduce aflatoxin contamination of peanuts (Mixon et al., 1984).

Several environmental factors influence the production of aflatoxin in the field and during storage when the conditions are favorable. Some studies have shown that water activity, relative humidity, temperature, light, and pH affect aflatoxin production (D'Aquino et al., 1986; Montani et al., 1988; Karunaratne et al., 1990; Ellis et al., 1991). Temperature is an important factor of growth for *A. flavus* and *A. parasiticus*. In fact, Payne et al. (1988) showed that high temperature seems to be a critical factor affecting the infection process, and Karunaratne et al. (1990) observed that the relationship between initial spore load and aflatoxin production was temperature dependent.

In recent years, studies on corn production in Costa Rica showed the presence of aflatoxin. This is a serious problem because of the importance of corn as a source of income for small farmers and its use as the principal component in tortillas, biscuits, chips, and other corn-based food items (Mora, 1988; Echandi, 1987; Morales, 1990). The objective of this study was to evaluate the level of aflatoxin contamination in corn samples grown at three locations in Costa Rica as a function of

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environmental conditions, aflatoxin-producing strains, and nutrients (minerals, moisture, and carbohydrates).

MATERIALS AND METHODS

Corn Samples. Samples of corn of the 1992 crop were obtained from three different regions in Costa Rica: Brunca, Huetar Atlantica, and Huetar Norte. For the purpose of this study, two handling processes (farm harvesting and CNP managerial), three agencies per region, and three collection phases (2 months each) were used as sampling criteria. Phases were designated *early* (August–September), *middle* (October–November), and *late* (December–January). Representative dried ground samples were collected by the CNP (National Production Council) and shipped from Costa Rica to the United States (Alabama A&M University) by INCIENSA (National Institute of Research on Nutrition and Health). Each dried ground sample was stored in a sealed plastic bag at 4 °C prior to analysis.

Aflatoxin Analysis. A modified version of Wilson and Romer's (1991) high-pressure liquid chromatography (HPLC) aflatoxin method was used for analysis of aflatoxin content in the corn samples. Aflatoxin extracts were prepared by extracting 25 g samples with 50 mL of acetonitrile–water (9+1) by shaking for 30 min and filtering through Whatman No. 4 filter paper. An aliquot of 5 mL was transferred to a 10 mL culture tube, and the extract was passed through a Mycosep multifunctional cleanup (MFC) column (Mycosep no. 224). Aliquots of samples (200 μL) of purified extract were then used for derivatization with 700 μL of derivatization solution (water–trifluroacetic acid–acetic acid, 7:2:1) at 55 °C for 30 min. Aflatoxin content in derivatized extracts was determined with a fluorescence detector at 360 nm excitation, 440 nm emission, by HPLC.

Microbiological Analysis. Counts of fungal populations present in the corn grain were performed by plating serial lof dilutions on rose bengal salt agar for *A. flavus* as selective media using standard procedures (R. A. Shelby, personal communication, 1993). Colonies were expressed as log colony forming units (CFUs) per gram of maize.

Carbohydrates. Xylose, glucose, fructose, and mannose were determined using a modified version of the HPLC method by Dunmire and Otto (1977). Samples of corn (10 g) were extracted with ethanol–water (1+1, 100 mL) by heating in a sonic bath (Bransonic 5200, Cole-Parmer, Chicago, IL) at 50 °C for 30 min. Hexane (50 mL) was used to remove fat from extracts. Aliquots of these extracts were centrifuged (15 °C for 15 min at 2000 rpm) and further purified by application to an acrosidic LC-PVDF 0.45 μm cartridge. HPLC (Series 4 HPLC, Perkin-Elmer, Norwalk, CT) separations were performed on an amino bonded Supelcosil LC-NH_2 column (25 × 4.6 cm; Supelco Inc., Bellefonte, PA) and UV detector (LC-95 spectrophotometer, Perkin-Elmer, Norwalk, CT) at 190 nm, with a solvent system of acetonitrile–water (75:25) at 2 mL/min.

Minerals. Calcium, magnesium, and zinc contents were determined by inductively coupled plasma atomic emission spectrometry (ICI-AES plasma 400) by acid digestion procedures of the sample (Stewart et al., 1974). Samples of corn (0.4 g) were digested with 5 mL of mixed digestion reagent (350 mL of H_2SO_4 concentrate–0.42 g of Se–14 g of $LiSO_4$–420 mL of H_2O_2, 30%) and diluted to 50 mL.

RESULTS AND DISCUSSION

Results confirm the serious problem of aflatoxin contamination in corn grown in Costa Rica. The levels of aflatoxin measured in the corn samples per region (Brunca, Huetar Norte, and Huetar Atlantica) were 11.92, 14.23, and 17.26 ppb, respectively. Mean total aflatoxin levels were high (14.47 ppb), with minimum and maximum values of 0.00 and 76.32 ppb, respectively (Table 1). Chemical analysis of the total samples (36) showed that aflatoxin B_1 was present in 81% of the samples and aflatoxin B_2 in 56% of the samples. The average aflatoxin B_1 and B_2 levels for the three regions were 11.23 and 0.69, 13.06 and 1.17, and 16.34 and 0.94 ppb, respectively.

Table 1. Levels of Moisture Content, Total Aflatoxins, Aflatoxin B_1, Aflatoxin B_2, and *A. flavus* Found in Corn Grown in Costa Rica for Human Consumption

region[a]	phase[b]	moisture (%)	aflatoxins			*A. flavus* (log/CFU)
			total (ppb)	B_1 (ppb)	B_2 (ppb)	
1	1	22.26	4.51	4.51	0.00	2.35
1	1	20.90	10.30	9.70	0.61	3.55
1	1	20.71	20.15	19.66	0.49	3.43
1	1	22.55	4.24	4.24	0.00	0.00
1	1	19.80	2.73	2.73	0.00	2.35
1	1	18.23	11.23	11.07	0.16	2.35
1	1	24.36	6.57	5.85	0.72	0.00
1	1	23.00	0.00	0.00	0.00	0.00
1	1	22.42	6.92	6.44	0.48	2.82
1	1	22.29	0.00	0.00	0.00	0.00
1	2	19.58	76.32	70.50	5.82	2.65
1	2	21.64	0.00	0.00	0.00	2.35
2	2	24.35	3.15	3.15	0.00	0.00
2	2	22.00	5.70	4.20	1.50	0.00
2	2	23.00	0.00	0.00	0.00	0.00
2	2	23.00	23.39	24.90	0.49	3.30
2	2	22.00	68.44	62.60	5.48	3.25
2	2	21.20	11.60	8.91	2.69	2.35
2	2	22.00	23.39	18.59	4.80	2.82
2	2	16.50	7.91	7.24	0.65	0.00
2	2	22.00	15.96	15.96	0.00	3.60
2	2	21.20	0.54	0.54	0.00	2.35
2	2	24.03	12.48	12.48	0.00	0.00
2	2	23.03	2.38	1.97	0.41	2.95
2	2	20.30	26.11	25.01	1.10	3.19
2	2	22.00	23.09	21.60	1.49	2.65
2	2	21.00	0.00	0.00	0.00	3.25
2	2	13.50	1.56	1.56	0.00	0.00
3	3	22.20	0.00	0.00	0.00	0.00
3	3	14.00	18.95	18.52	2.35	2.35
3	3	23.00	37.14	35.37	2.35	2.35
3	3	13.50	11.54	10.60	0.00	0.00
3	3	13.50	7.29	6.66	2.82	2.82
3	3	13.50	62.49	58.49	0.00	0.00
3	3	13.50	0.67	0.67	0.00	0.00
3	3	13.50	0.00	0.00	0.00	0.00

[a] 1, Brunca; 2, Huetar Norte; 3, Huetar Atlantica. [b] 1, early (Aug–Sept); 2, middle (Oct–Nov); 3, late (Dec–Jan).

Sample collection was carried out from August to January (1992–1993) by the National Production Council (CNP) in Costa Rica. The 6 month period was divided into three phases: early, middle, and late. The results showed that the collection phase had a significant effect on aflatoxin levels ($p \leq 0.05$). The mean values of total aflatoxin for early, middle, and late phases were 6.67, 16.89, and 17.18 ppb, respectively (Table 1). It was noted that aflatoxin levels in the early phase were lower than in the other two phases. This phase accounted only for samples from the Brunca region, which showed lower levels of aflatoxin contamination. It seems that differences in harvest and storage conditions as well as the agricultural practices in each region also influence the amount of aflatoxin production in the crop. F. Bolaños (Department of Quality Control, CNP, personal communication, 1993) noted that Costa Rican farmers used different handling processes before and during harvest and that the Huetar Atlantica region had the poorest agricultural practices (Table 1).

Fungal microflora in corn showed that *A. flavus* was found in 58% of the samples (Table 1), which means that, in this particular study, this factor seemed to be an indicator of toxin levels. The levels of *A. flavus* found in the corn samples were significantly different ($p \leq 0.05$) (Table 2).

Three environmental factors (temperature, relative humidity, and rainfall amount) were identified for each

Table 2. Analysis of Variance of the Model for Aflatoxin Content in Corn as a Function of Nine Independent Variables

source of variation		degrees of freedom (DF)	square sum (SS)	probability (PR > F)
model	Mg		2732.80	0.0008[b]
	Zn		1982.62	0.0032[b]
	Mg × Mg		2862.31	0.0006[b]
	Mg × Zn × Ca		1817.54	0.0046[b]
	Mg × Ca	9	1502.72	0.0090[b]
	A. flavus		891.88	0.0389[a]
	temperature		878.91	0.0402[a]
	phase		763.60	0.0506[a]
	glucose/mannose		519.05	0.1090
error		26	4900.85	

[a] Data are significant at 0.05 level of probability (PR). [b] Data are highly significant at 0.01 level of probability (PR).

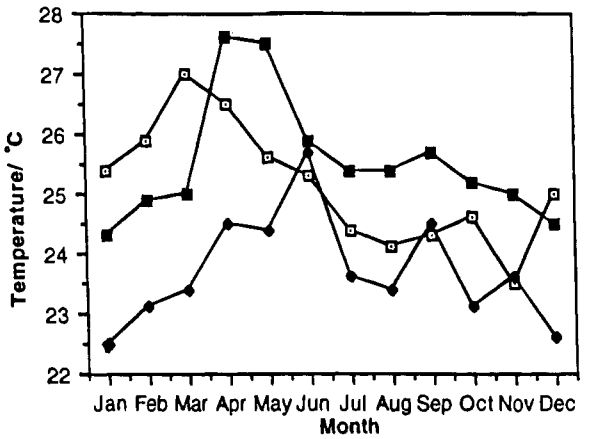

Figure 1. Average temperature conditions in Costa Rica during harvest and collection season per region: (⊡) Brunca; (◆) Huetar Atlantica; (■) Heutar Norte.

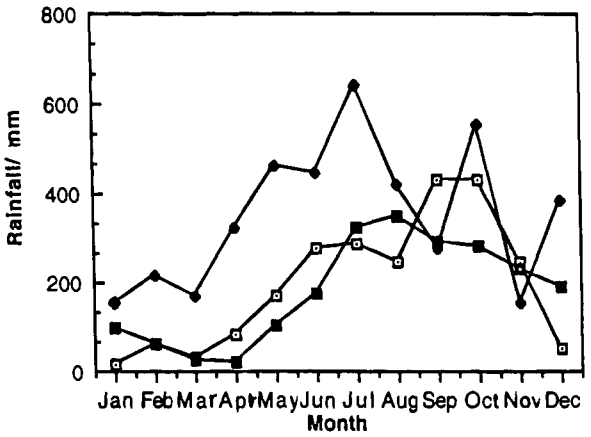

Figure 2. Average rainfall conditions in Costa Rica during harvest and collection season per region: symbols as in Figure 1.

region during the period of corn production in Costa Rica (Figures 1–3), and their effect on the level of aflatoxin contamination in the corn samples was evaluated. These factors were measured daily and reported as an average value per month in each region during the corn harvesting season and collection phase. The effect of rainfall and relative humidity on aflatoxin levels during sampling was not significant ($p \leq 0.05$) (Table 2). This may be due to the insufficient variation of the data values from August to December (Figures 2 and 3). However, rainfall and relative humidity showed a drastic variation during the harvesting season (March through August), which could have produced physiological stresses on the crops. It is difficult to conclude if these two parameters affected the levels of aflatoxin in corn due to their variation before the harvesting season. Studies done on the effect of environmental conditions on aflatoxin contamination of corn showed that, when the conditions were favorable, the occurrence of aflatoxin was highly related to these factors (Shotwell et al., 1984; Lokesha et al., 1987; Chiou et al., 1990; Ellis et al., 1991).

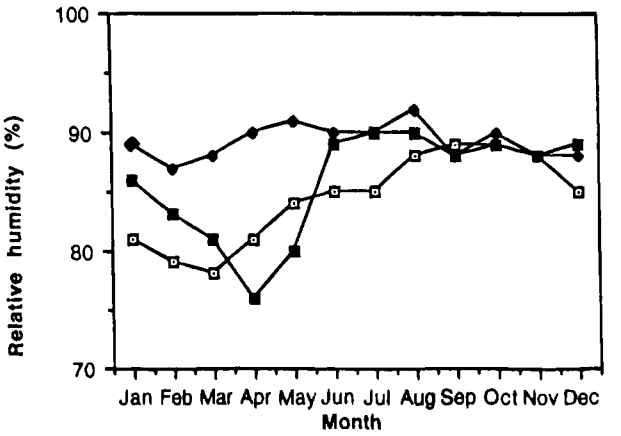

Figure 3. Average relative humidity conditions in Costa Rica during harvest and collection season per region: symbols as in Figure 1.

Studies on the effect of temperature on aflatoxin production consider it an important factor for growth of *A. flavus* (Ellis et al., 1991). In this study, the aflatoxin level was significantly ($p \leq 0.05$) affected by the effect of temperature (Table 2). Average temperature conditions were 23.7 °C with a range of 20.6–25.5 °C (Table 1).

Table 3 shows the nutrients in corn grain grown for human consumption in Costa Rica. The response of aflatoxin levels in corn to mineral content—magnesium (Mg^{2+}), zinc (Zn^{2+}), the polynomial effect of magnesium, the interaction Mg × Ca, and the three-way interaction of Mg × Zn × Ca—was highly correlated ($p < 0.01$) (Table 2). Previous studies established that aflatoxin biosynthesis was increased by zinc content (Failla et al., 1986). The increased aflatoxin production in corn could be attributed to the availability of zinc in the soil and corn grain. The present study demonstrated that zinc and magnesium are correlated with aflatoxin production by *A. flavus*. On the other hand, the interaction of the polynomial effect of magnesium, the interactions Mg × Ca and Mg × Zn × Ca, has a correlated effect on aflatoxin levels. An explanation of this effect could be the differences in the process of mineral absorption by the plant, the interaction between minerals, the concentrations of these minerals in the soil, and the dynamics of nutrient uptake. Also, corn samples were collected from regions in Costa Rica with very high acidity in the soil (Bertsh, 1987; F. Bolaños, personal communication, 1993). Some studies showed that the pH of the soil is an important factor in the concentration of different nutrients and in the regulation of aflatoxin levels (Rusul and Marth, 1987; Cotty, 1988; Ellis et al., 1991; Jones et al., 1991).

The relationship between aflatoxin and moisture content was found to be not significantly ($p \leq 0.05$) different among the total number of samples. The average level of moisture in the samples was 20.15% (Table 1). This may be related to the traditional blending of the crop in the field, 15 days before the corn

Table 3. Levels of Nutrients in Corn Grown in Costa Rica for Human Consumption

region[a]	phase[b]	Zn (μg/g)	Mg (μg/g)	Ca (μg/g)	xylose (mg/g)	fructose (mg/g)	glucose/mannose (mg/g)
1	1	17.20	1086	176	324.26	323.41	967.80
1	1	19.70	1136	183	172.87	107.15	600.58
1	1	18.20	1033	183	247.78	62.43	524.68
1	1	17.10	1015	181	342.87	144.37	337.90
1	1	17.90	1038	176	189.95	30.78	711.74
1	1	17.80	1111	179	245.97	211.14	914.24
1	1	12.80	981	171	224.68	127.13	60.20
1	1	15.30	1032	166	194.01	86.23	538.00
1	1	15.20	918	193	179.58	151.38	595.42
1	1	15.00	920	178	200.14	174.77	582.13
1	2	21.10	1286	201	287.63	20.48	738.94
1	2	16.10	1049	172	210.16	127.35	566.40
2	2	18.00	983	159	233.65	42.05	345.60
2	2	20.00	1067	162	239.60	31.60	442.80
2	2	18.00	993	161	264.94	128.20	402.18
2	2	18.50	1128	166	224.40	63.20	447.20
2	2	22.00	944	161	240.15	14.77	424.64
2	2	19.30	953	160	217.01	75.04	447.18
2	2	16.60	962	175	319.36	59.91	441.73
2	2	16.60	1071	160	329.17	54.43	510.35
2	2	16.40	1028	159	183.52	108.28	441.18
2	2	16.30	925	171	121.92	94.88	735.24
2	2	17.20	987	187	99.13	28.40	266.10
2	2	14.80	930	199	164.00	115.58	955.00
2	2	16.60	932	177	197.18	126.33	903.82
2	2	18.40	1056	178	206.89	81.58	403.50
2	2	23.00	877	229	178.40	42.99	987.02
2	2	19.40	1000	163	144.72	55.56	665.18
3	3	17.40	1033	166	171.28	43.62	536.43
3	3	20.70	1231	231	135.58	29.78	596.90
3	3	17.10	1005	176	58.54	12.36	301.20
3	3	16.60	1063	158	166.33	126.00	510.72
3	3	15.70	990	308	203.89	48.72	628.40
3	3	17.80	1078	172	223.58	68.75	617.26
3	3	14.20	1047	163	160.87	109.92	464.80
3	3	16.00	985	167	165.37	99.81	547.20

[a] 1, Brunca; 2, Huetar Norte; 3, Huetar Atlantica. [b] 1, early (Aug–Sept); 2, middle (Oct–Nov); 3, late (Dec–Jan).

is collected. It can be said that a range of 13.5–24.36% moisture in the corn does not affect the levels of aflatoxin in corn grown in Costa Rica for human consumption (Table 1).

The effect of carbohydrate (xylose, fructose, and glucose/mannose) content in corn grain on the level of aflatoxin was not significant ($p \leq 0.05$) (Table 2). However, when the effect of glucose/mannose was statistically removed, it greatly affected the other independent variables. These carbohydrates presented an effect of multicollinearity among the independent variables in the statistical model (Viquez et al., 1994). Glucose/mannose were then correlated with the other independent variables; therefore, a marginal or partial effect in the regression coefficient of the best fitting model is due to these carbohydrates. This result is in accordance with the outcome of previous studies such as that of Wiseman and Buchanan (1987), who concluded that aflatoxin levels depended on the concentration of glucose. In this study, the mean value for glucose/mannose was 575.04 mg/g (Table 3).

It can be concluded that most of the corn grown in Costa Rica is contaminated with aflatoxin. The mean value of aflatoxin levels in corn was high (14.47 ppb), and Costa Rican corn for human consumption contained the most toxic aflatoxin, aflatoxin B_1. The infection and/or development of aflatoxin production by *A. flavus* was correlated with zinc, magnesium, and calcium. Among environmental effects, temperature was a stimulatory effect on aflatoxin production by *A. flavus*. Finally, glucose/mannose produced a multicollinearity effect on the incidence of aflatoxin production in corn.

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