Fumonisin B_1, B_2, and B_3 Content of Iowa, Wisconsin, and Illinois Corn and Corn Screenings

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Fumonisin B_1, B_2, and B_3 contents of corn from the 1988–1991 crop years and corn screenings from 1989 were assayed. FB_1 ranged from 0 to 14.9, from 0 to 37.9, from 0 to 19.1, and from 0 to 15.8 ppm in corn from 1988, 1989, 1990, and 1991, respectively. FB_2 and FB_3 averaged 0.7 and 0.2, 0.8 and 0.2, 0.9 and 0.3, and 0.8 and 0.4 ppm from 1988, 1989, 1990, and 1991 crop years, respectively. There were statistically significant linear regression correlation coefficients between FB_1 content and FB_2 and FB_3 contents in all crop years. Corn screenings contain about 10 times higher fumonisin contents than intact corn. There was no size-related segregation of fumonisin contents in corn screenings.

INTRODUCTION

The recent concern of fumonisin mycotoxins in animal and human foods prompted this study of corn from the Midwest for fumonisin (FB) contents. The FBs have recently been isolated and characterized by Gelderblom et al. (1988). These researchers have shown that FB_1 is a cancer-promoting agent in rats and have correlated the FBs with the increased incidence of esophageal cancer in parts of South Africa. FB_1 has been demonstrated to cause equine leukoencephalomalacia (ELEM) in purified form (Marasas et al., 1988). Feeding FB_1-containing corn culture material (Kriek et al., 1981) and intravenous dosing of purified FB_1 (Harrison et al., 1990) have been shown to cause porcine pulmonary edema (PPE). Voss et al. (1990) have shown hepatotoxicity and renal toxicity in rats fed corn implicated in field cases of ELEM. *Fusarium moniliforme* corn culture fed to rats caused an increase in the number of placental glutathione *S*-transferase-positive foci in hepatocytes, an early carcinogenic indicator, suggesting a co-initiating activity (Lepebe and Hendrich, 1991). Gelderblom et al. (1991) concluded that liver cancer could be induced by feeding rats 50 mg/kg purified FB_1. Wang et al. (1991) have reported on the disruption of sphingolipid biosynthesis by the fumonisins and suggested that this may be a critical event in the diseases associated with the FBs. Wang et al. (1992) have reported increased serum sphingosine and sphinganine in ponies fed a FB-containing diet.

Surveys of FB contents in feeds associated with animal health problems have appeared (Ross et al., 1991a). FB-contaminated corn has been used for ethanol fermentation without contamination of the alcohol (Bothast et al., 1991). Recently, corn-based human foods from five countries have been surveyed for FB content (Sydenham et al., 1991).

At the time of the 1989 corn harvest and subsequent outbreak of ELEM and PPE, corn samples from the 1988 and 1989 crop years were obtained and analyzed for FBs. When the 1990 and 1991 corn crops became available, samples were assayed for FB levels. Additionally, corn screenings, principally from the 1989 harvest, were obtained to survey for FBs. Corn screenings have been implicated in most field cases of ELEM (Ross et al., 1991a). These were assayed for their FB_1, FB_2, and FB_3 levels.

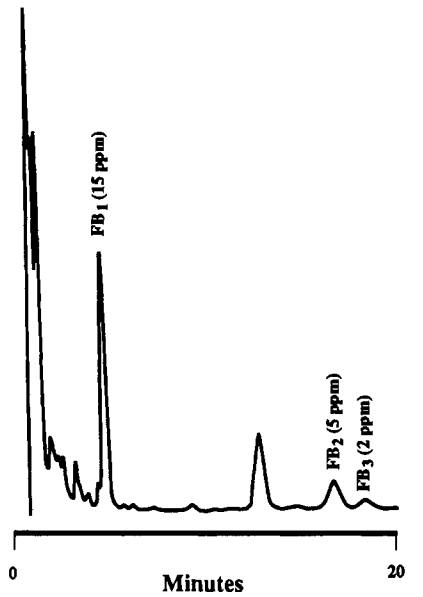

Figure 1. Chromatogram of fumonisins B_1, B_2, and B_3 in corn by fluorescence detection of the *o*-phthalaldehyde derivatives.

MATERIALS AND METHODS

Samples. Whole corn was obtained from the Grain Quality Laboratory (GQL), Department of Agricultural Engineering, Iowa State University, through Dr. Charles Hurburgh, for the 1988, 1989, 1990, and 1991 harvests. These samples were a random selection of corn principally from the state of Iowa and were random samples taken from trucks at grain elevators. A subsample of approximately 400 g was ground in a Stein mill to a uniform consistency before analysis. A few samples from Illinois and Wisconsin were included in the 1989 crop year. Corn screenings (160 samples) from 21 locations were sieved into eight different particle sizes. The GQL sized the screenings prior to analysis of FBs.

Analytical Methods. FB_1, FB_2, and FB_3 were analyzed according to the method of Ross et al. (1991a). Briefly, samples were ground to uniform consistency, and 10 g was extracted with 50% acetonitrile for 30–60 min. A 10–25-mL portion of the solvent was filtered through filter paper. Two milliliters of the

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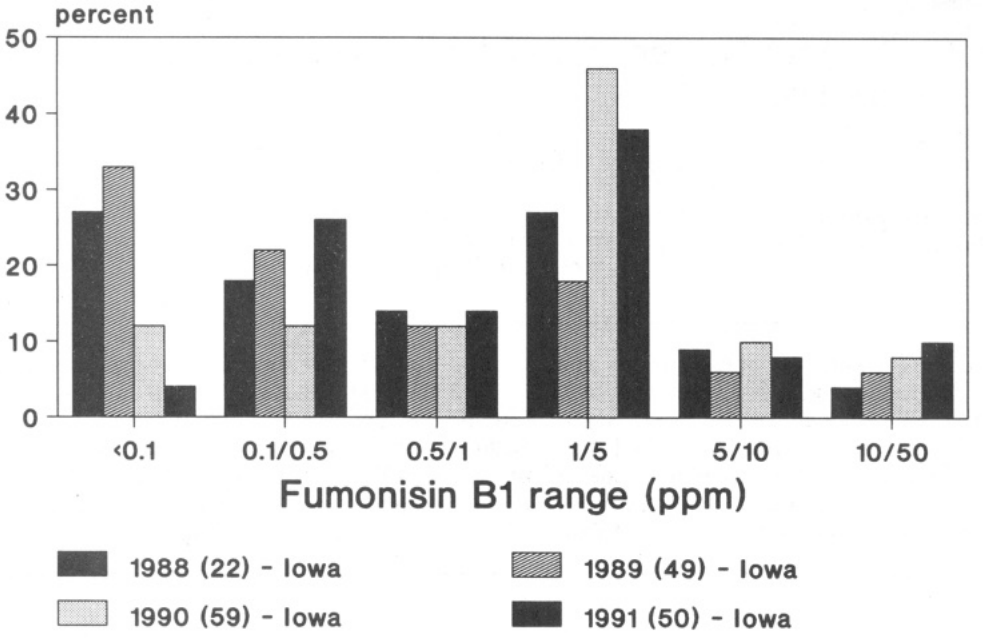

Figure 2. Distribution of fumonisin B_1 in corn from the 1988, 1989, 1990, and 1991 crop years.

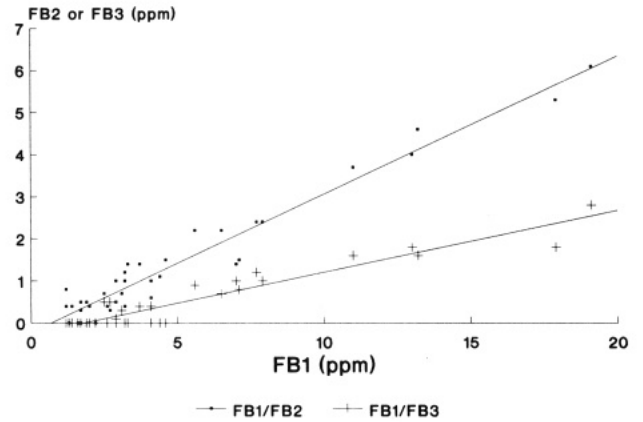

Figure 3. Linear regression of FB_1 on FB_2 and FB_1 on FB_3 in corn from the 1990 crop year.

filtered extract was diluted with 5 mL of water and cleaned up on a Waters C_{18} Sep-Pak. The FB fraction was eluted with 70% acetonitrile. The FB fraction was derivatized with *o*-phthaldehyde (OPA), and 10 μL was chromatographed on a Perkin-Elmer 3-cm C_{18} analytical column. The fluorescing derivatives were detected by a Perkin-Elmer LS-3 fluorescence spectrometer (excitation, 335 nm; emission, 440 nm). Peak area was quantitated and correlated with a standard curve. Standard FB_1 and FB_2 were obtained from the Division of Food Science and Technology, Pretoria, South Africa, and later from Sigma Chemical Co. (St. Louis, MO). FB_3 was obtained from Ron Plattner (USDA, ARS, NRRC, Peoria, IL). Recoveries were estimated for FB_1, FB_2, and FB_3, in the concentration ranges 0.8–60.0, 1.4–2.5, and 0.3–2.0 ppm, respectively. Recoveries ranged from 81 to 101%, from 74 to 96%, and from 75 to 96% for FB_1, FB_2, and FB_3, respectively. The minimum quantitation levels were 0.25, 0.5, and 0.5 ppm for FB_1, FB_2, and FB_3, respectively.

Statistics. Corn screening FB_1 levels were analyzed by ANOVA using the SAS system (Cary, NC). Duncan's multiple-range test was performed to determine if there was statistically significant segregation of FB by corn screening size. Linear regression analysis was performed within each harvest year for correlations between FB isomers.

RESULTS AND DISCUSSION

A representative chromatogram of the OPA peaks for the FBs is presented in Figure 1. Confirmation by gas chromatography/mass spectrometry of FBs was performed on a few selected samples according to the method of Wilson et al. (1990).

Table I. Fumonisin Concentration in Corn

			μg of FB/g of corn		
year	*n*		FB_1	FB_2	FB_3
1988	22	$X \pm s$	2.5 ± 3.6	0.7 ± 1.3	0.2 ± 0.5
		median	0.7	0	0
		range	0–14.9	0–5.7	0–2.1
1989	44	$X \pm s$	2.9 ± 7.3	0.8 ± 2.1	0.2 ± 0.7
		median	2.3	0	0
		range	0–37.9	0–12.3	0–4.0
1990	59	$X \pm s$	3.3 ± 4.2	0.9 ± 1.4	0.3 ± 0.6
		median	1.7	0.5	0
		range	0–19.1	0–6.1	0–2.8
1991	50	$X \pm s$	2.9 ± 3.9	0.8 ± 1.1	0.4 ± 0.5
		median	1.5	0.4	0.3
		range	0–15.8	0–4.4	0–2.3

The results of the survey of corn from the 1988, 1989, 1990, and 1991 harvest are presented in Table I and Figure 2. The average FB content in corn was not very different among harvest years with large standard deviations due to the wide range of values observed. Only a limited supply of 1988 samples was still available in late 1989 when PPE was first reported widely in Iowa (Osweiler et al., 1992). The median values may be more representative for the distribution of FB in corn. Using medians as a comparison, the problem year of 1989 stands out with the highest median for FB_1. The ranges of FBs were smaller in the nonproblem years compared to the problem year of 1989. The amounts of FB_2 and FB_3 followed the contents of FB_1 in all samples (see correlations below).

The distribution of FB_1 is presented in Figure 2. In 1988, a low PPE incidence year, more than 50% of the corn samples contained less than 0.5 ppm of FB_1. In contrast, in the 1990 corn, also a low PPEM and ELEM incidence year, almost 50% of the samples were in the 1–5 ppm of FB_1 range. The percentage of samples with FB_1 greater than 5 ppm was not significantly different among harvest years in the samples we had available to survey. In the 1990 and 1991 crop years, the number of samples with FB_1 contents greater than 10 ppm appeared to be greater than in the 1989 problem year. However, the

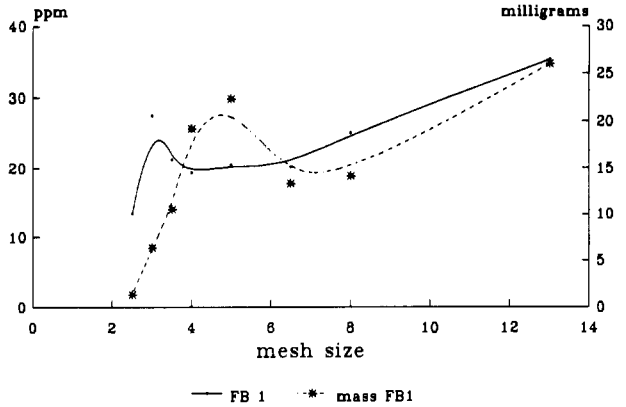

Figure 4. Distribution of FB_1 in corn screenings from 1989. Left Y coordinate is concentration (micrograms of FB_1 per gram of corn). Right coordinate is mass on each screen size (milligrams of FB_1 per screen). n = 21 at each screen size.

Table II. Correlation of FB_1 with FB_2 and FB_3 in Corn by Crop Year

isomer	crop year	r	slope
FB_1/FB_2	1988	0.9549	0.3591
	1989	0.9761	0.2885
	1990	0.9756	0.3199
	1991	0.9636	0.2588
FB_1/FB_3	1988	0.9446	0.1435
	1989	0.9603	0.0960
	1990	0.9443	0.1454
	1991	0.8807	0.1085

concentrations in the 1990 and 1991 years were at the low end of the ≥10 ppm group, while in the 1989 crop the distribution of FB_1 in this 10–50 ppm was much broader. Ross et al. (1991) have used 10 ppm of FB_1 as a reference point for subdividing problem and nonproblem feeds for horses. This is not assumed to be a safe or a toxic level at this time. There are too few studies to suggest a toxicity level (Ross et al., 1991b; Wilson et al., 1992).

The correlations between FB_1 and FB_2 and between FB_1 and FB_3 were determined for these naturally occurring contaminations by *F. moniliforme*. These are presented in Figure 3 and Table II. Remarkably, the correlation coefficient was very close to 1 for both comparisons in each harvest year. Apparently, the fungi are producing proportional amounts of each FB in field exposures. Corn culture material produced in our laboratories do not follow this correlation (data not shown).

Corn screenings were examined to determine if the FBs were segregated into a particular particle size. Many of the reported cases of ELEM are associated with the feeding of corn screenings (Wilson et al., 1990). The distribution of FB_1 is presented in Figure 4. The average FB_1 concentration in screenings (20.8 ± 21.3 ppm) was approximately 10 times higher than in whole corn. The FB_1 content ranged from 0.1 to 239 ppm. There did not appear to be a major size-associated segregation of FB_1 in these screenings. The ANOVA indicated that the smallest and the largest particle sizes had significantly different FB_1 concentrations, but no other significant differences were observed. When the masses of FB corn screenings at each size were compared, there did appear to be a slight concentration of FB material in the midsize corn screenings. The same distribution and level of significance were observed for FB_2 and FB_3 contents because the correlation between FB_1 and the other two isomers, FB_2 and FB_3, was still valid for these naturally contaminated corn screenings. It does not appear that corn screenings could be further fractionated by size to decrease the FB content. The use of corn screenings in feed formulas, for livestock and companion animals, is a wide practice and a multimillion dollar part of the corn industry. Thus, FB contents of corn screenings will continue to be a source of concern.

In conclusion, the FBs were found in all crop years surveyed. On the basis of the average content of FBs, it was difficult to differentiate a crop year that will cause higher levels of ELEM and PPE from nonproblem years. The crop median of FB content may be a better indicator. There was a strong linear correlation between concentrations of the FB isomers found in naturally contaminated corn and in corn screenings. Additional research is required to determine the significance of these levels of FB in corn.

ACKNOWLEDGMENT

This work was supported by the Iowa Corn Promotion Board and the Food Safety Consortium. This is Journal Paper J-14974 of the Iowa Agricultural and Home Economics Experiment Station, Ames, IA, Projects 2955 and 2406, the latter a contributing project to North Central Regional Project NC-129.

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Received for review August 7, 1992. Accepted November 10, 1992.