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Survey of 1977 Crop Year Preharvest Corn for Vomitoxin

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Fifty-two preharvest corn samples were collected in mid-October 1977 from 26 farms in a four-county area of northwest Ohio. Vomitoxin ranging from 0.5 to 10 μg/g was found in 24 of the samples. Analysis for vomitoxin was by gas-liquid chromatography. Preceding harvest in northwest Ohio, unusual wet conditions prevailed, making it favorable for *Fusarium* growth. *Gibberella zeae*-infected kernels ranged from 2 to 50% of the kernels analyzed for 44 corn samples, and 8 corn samples showed no infection.

Gibberella zeae, the perfect stage of *Fusarium graminearum* (1), may occur on field corn as a reddish mold that usually begins observable growth at the ear tip. The condition is commonly referred to as *Gibberella* or pink ear rot. Ingestion of corn infected with *G. zeae* by humans or animals may cause mycotoxicoses, resulting from a variety of toxins produced by this fungus (2). One problem of concern, especially to hog farmers, is the reluctance of swine to eat corn infected by *Gibberella*.

In the field, *G. zeae* corn infection is favored by low temperatures with concomitant high moisture conditions. These conditions may result in harvest delay, allowing prolonged growth of the fungus with concomitant higher production of toxin. Large-scale outbreaks of *G. zeae* infection in the U.S. corn belt occurred in 1966, 1972, and 1975. The northwest region of Ohio appears to be more susceptible than others to *Gibberella* ear rot, with outbreaks reported in 1970, 1972, 1975, and 1977. The most severe of these occurred in 1975 when a major portion of the corn was refused by swine herds.

Corn contaminated with *F. graminearum* from the 1972 crop of northwest Ohio, which swine refused to eat, was shown to contain the trichothecene vomitoxin (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) (4). Later, vomitoxin was shown to be the etiological agent responsible for both refusal and vomiting exhibited by swine (5). In late September of 1977, reports were received from county extension officers of northwest Ohio that a portion of the corn intended for hogs was contaminated with *G. zeae*. Hence, a survey was organized with the aid of county extension officers to collect corn samples with obvious *Fusarium* contamination to be analyzed for mycotoxins from farms located in the counties of Defiance, Fulton, Paulding, and Williams. Rainfall through September and the first 4 days of October was above average, making conditions favorable for *Gibberella* growth. However, harvest was not delayed, and corn was being combined in mid-October while we were selecting samples for the survey. Therefore, conditions were conducive to obtaining information on the presence of vomitoxin in corn contaminated with *G. zeae* at harvest. The histories of corn samples from the 1966 and 1972 crops, which were analyzed for refusal factors, were complicated because harvest had been delayed for 1 to 2 months because of wet and cool conditions. In addition, the corn from the 1972 crop was not dried immediately after harvest because of energy shortages for operating dryers; this introduced a storage variable. We believe that the present survey is the first valid study of field corn for the trichothecene vomitoxin.

MATERIALS AND METHODS

Collection of corn samples. Samples of corn from 26 farms were picked from two diagonally opposite locations in each field. Fields were entered 20 rows in from the edges, and 100 ears from each were examined visually for *Gibberella* ear rot. The first 20 to 25 ears that showed *Gibberella* rot were collected. If no ear rot was visible for the first 100 ears, then 20 to 25 of the ears were picked at random. The sampling was done 12 and 13 October 1977. Corn was shelled 14 October, placed in plastic bags, and blended by shaking. Specimens of the grain were removed for microbial and mycotoxin analyses. Moisture contents of the blended corn samples (250 g) were determined with a Motomco Moisture Meter (model 919).

Microbial analyses. From each blended corn sample were selected 50 whole kernels, which were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 min and washed with sterile water. The sterilized kernels were plated on potato-dextrose agar (five ker-

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nels to a plate) and incubated at 28°C for 7 days. On the basis of cultural and morphological characteristics, the infecting internal fungi were identified.

Vomitoxin analysis. A gas-liquid chromatography (GLC) method, using an internal standard, was employed for vomitoxin quantitation. Vomitoxin standard was obtained from rice fermentations of *F. graminearum* as previously reported (5) and was crystallized from ethyl acetate to a 154°C mp (uncorrected Fishers John apparatus). The internal standard diacetoxyscirpenol (DAS) was obtained from Makor, Israel, Jerusalem Ltd., and was used with no further purification.

G. zeae-contaminated corn samples (300 g) were extracted with 600 ml of butanol. The corn residue remaining was mixed with 600 ml of 40% CH_3OH–water two times in a blender; combined aqueous methanol extracts were evaporated to dryness in a forced-air oven at 70°C. The residue redissolved in CH_3OH was mixed with 10 g of silica gel; it was then taken to dryness and added to the top of a 10-g silica gel column presaturated with $CHCl_3$. The column was eluted sequentially with 500 ml of $CHCl_3$, 500 ml of 5% CH_3OH–$CHCl_3$, 200 ml of 10% CH_3OH–$CHCl_3$, and 100 ml of CH_3OH. All elutates were analyzed for vomitoxin qualitatively by thin-layer chromatography or GLC. The eluates were evaporated to dryness (forced-air oven, 70°C) and the residues were redissolved in CH_3OH (5 ml). Fifty microliters of each methanol solution was spotted on thin-layer plates (silica gel 60 F-254; EM Reagents, Elmsford, N.Y.). The plates were developed in $CHCl_3$–CH_3OH (8:2, 1 drop of water), dried at room temperature, and visually examined under shortwave ultraviolet light. Vomitoxin quenches fluorescence and appears as a dark spot at R_f 0.6. On spraying the plates with *p*-anisaldehyde reagent and heating at 110°C, vomitoxin appears as a yellow spot under visible light. The chloroform and methanol eluates from the silica gel column did not give a spot at the R_f of vomitoxin, nor did a yellow color develop with spray reagent. The residue obtained from the 5% CH_3OH–$CHCl_3$ eluate of five of the samples gave the characteristic yellow spot. Those residues from the column eluates (5% CH_3OH–$CHCl_3$) of the 47 samples that gave no yellow spot with the spray reagent were rechromatographed on a 10-g silica gel column. The column was eluted with 200 ml each of the solvent and solvent combination previously used, and each eluate was again analyzed for vomitoxin by thin-layer chromatography. No vomitoxin spot could be detected by the spray reagent, and the 5% CH_3OH–$CHCl_3$ eluates contained spots at the same R_f as vomitoxin. Each residue obtained from these 5% CH_3OH–$CHCl_3$ eluates was dissolved in methanol (5 ml). A 100-μl fraction of each methanol solution was added to a vial containing 48 μg of DAS, taken to dryness, and converted to trimethyl silyl ethers (TMS) with 50 μl of TBT (Pierce Chemical Co.) at 120°C for 15 min. The TMS solution was injected into a Bendix model 2500 gas chromatograph equipped with hydrogen flame-ionization detectors and a glass U-shaped column (1.82 m by 2 mm) packed with 3% OV 101 on 100–200 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Column temperature was programmed from 160 to 250°C at 5°C/min. Peak areas and retention times were measured by an integrator digital computer (Modcomp II, Modular Computer System). Vomitoxin retention time (11.81 min) relative to the TMS derivative of the internal standard DAS (13.12 min) was 0.9. Those samples that showed this relative retention time but fell above or below the desired weight ratio of 0.5/1 to 1.5/1 of vomitoxin/DAS were rechromatographed by using greater or lesser amounts of solution until the proper weight ratio range was obtained. The amount of vomitoxin present was calculated from the ratio of peak areas by the internal standard method (3). A standard curve of the TMS derivative of weight ratios of vomitoxin/DAS versus peak area ratios gave a linear curve over the ranges of 0.5/1 through 12/1. Uncontaminated corn samples (100 g) spiked with vomitoxin at levels of 1, 5, 10, and 20 μg/g were extracted as before but with the butanol extraction step omitted; recovery was 65% for 1 μg/g and +92% for levels of 5, 10, and 20 μg/g. Determinations were carried out in duplicate.

GLC-mass spectrum confirmation. Sample peaks that showed a retention time of 0.9 relative to DAS were confirmed as vomitoxin by obtaining their mass spectra on a Bendix model 2600 gas chromatograph operated in conjunction with a Dupont (CEC) 21-492 mass spectrograph (GLC-MS) (voltage, 70 eV) and comparing with an authentic sample of the TMS derivative of vomitoxin.

RESULTS AND DISCUSSION

Fifty-two preharvest corn samples were collected in mid-October from 26 farms in a four-county area of northwest Ohio. An incidence of 46% vomitoxin contamination was found in these samples. The 24 corn samples that analyzed positively for vomitoxin ranged from 0.5 to 10.7 μg/g and came from 18 farms from which a total of 36 samples were collected. The levels of vomitoxin found in the corn samples are listed in Table 1, along with the mean for each county. Also listed in Table 1 are moisture levels of each of the samples, variety of corn when known, percentage of *G. zeae*-infected kernels, and the percentage of *G. zeae* infection of ears as visually estimated in the field.

The *G. zeae*-infected kernels ranged from 2 to 50% for 44 corn samples, whereas 8 showed none. However, the visual estimation of infection at time of collection ranged from 1 to 100% for the 52 samples. Those eight samples which showed no *G. zeae* were heavily infected with other *Fusarium* spp. Most of the 52 samples were heavily contaminated with the common fungi: *Trichoderma, Penicillium, Alternaria, Cladosporium, Nigrospora,* and *Helminthosporium.*

Of the 24 samples that analyzed positively for vomitoxin, 10 were selected at random for GLC-MS confirmation of the peaks associated with vomitoxin. Each sample was run in the same

TABLE 1. *Gibberella zeae infection and vomitoxin levels in northwest Ohio 1977 corn*

County	Farm no.	Sample no.	% *G. zeae* infection		Vomitoxin (μg/g)	Moisture (%)	Variety
			Ears[a]	Kernels			
Paulding	1	1	95	22	ND[b]	22.1	NK[c]
		2	100	22	ND	25.2	
	2	3	95	16	9.3	25.2	NK
		4	100	22	4.6	27.4	
	3	5	50	20	ND	21.7	NK
		6	50	4	ND	22.0	
	4	7	10	18	5.8	22.2	NK
		8	60	24	7.8	24.6	
	5	9	76	4	ND	27.2	NK
		10	80	10	ND	27.6	
	6	11	75	4	ND	32.4	NK
		12	93	10	ND	32.9	
Mean			71	15	2.3	25.9	
Williams	7	13	26	10	7.1	35	NK
		14	24	6	3.9	28.5	
	8	15	18	2	4.4	25.3	NK
		16	10	8	8.6	26.7	
	9	17	70	10	ND	23.1	Pioneer 37/80
		18	45	4	ND	22.7	
	10	19	72	18	1.4	24.0	DeKalb XL 64a
		20	33	22	7.9	23.7	
	11	21	75	0	ND	30.1	Crow HL 620 high lysine
		22	96	0	ND	28.2	
	12	23	94	20	ND	33.6	Crow HL 450 high lysine
		24	89	20	1.1	34.0	
Mean			54	10	2.9	27.9	
Defiance	13	25	72	10	2.9	21.2	Landmark 6/11 modified single cross
		26	90	0	ND	20.1	
	14	27	16	26	10.7	28.6	Landmark 7/47 single cross
		28	9	16	6.7	25.8	
	15	29	38	22	ND	23.2	Landmark 6/11 modified single cross
		30	72	36	7.7	22.8	
	16	31	72	12	2.5	21.7	Parker single cross
		32	52	0	ND	21.7	
	17	33	14	10	ND	27.5	Landmark 722 three way
		34	17	14	ND	22.2	
	18	35	92	16	ND	23.0	Landmark 722 three way
		36	70	26	ND	25.1	
	19	37	40	8	ND	23.8	Landmark 7/47 single cross
		38	11	46	6.6	23.8	
Mean			48	17	2.7	23.6	
Fulton	20	39	34	50	4.4	23.6	DeKalb 64
		40	10	18	4.3	29.0	
	21	41	9	10	ND	27.2	DeKalb 25
		42	15	8	2.5	23.3	
	22	43	20	4	1.1	25.7	NK
		44	1	4	ND	26.1	

TABLE 1—*Continued*

County	Farm no.	Sample no.	% *G. zeae* infection		Vomitoxin (μg/g)	Moisture (%)	Variety
			Ears[a]	Kernels			
	23	45	1	2	5.8	26.4	DeKalb 35
		46	2	0	ND	26.8	
	24	47	4	0	ND	34.6	DeKalb 432
		48	5	0	ND	32.1	
	25	49	50	6	2.2	24.7	Pioneer 3780
		50	52	14	ND	25.6	
	26	51	52	2	0.5	24.6	Pioneer 3780
		52	56	0	ND	22.6	
Mean			22	8	1.5	26.6	

[a] Visual estimate made in the field of ears possessing *Gibberella* infection.
[b] ND, Not detected.
[c] NK, Not known.

manner as for GLC analysis, i.e., the same amount of sample and TMS reagent. The spectrum of the selected peak was compared with the spectrum of the authentic TMS vomitoxin derivative. All of these samples analyzed for vomitoxin. In addition, eight samples that showed no *G. zeae* or vomitoxin were analyzed for the trichothecenes T-2 and DAS by GLC-MS. Sample 22, which showed a peak with the same retention time as DAS, could not be confirmed at a level of 1 μg/ml. No T-2 or DAS could be detected in these eight samples at six times the level used in the GLC procedure for vomitoxin analyses by GLC-MS.

Of the 20 samples that analyzed negative for vomitoxin, five were selected at random for GLC-MS confirmation. Again all these were run at six times the level concentration for GLC analyses. Four showed no vomitoxin at these levels. However, sample 29 showed a small amount of vomitoxin, estimated to be present at 0.07 μg/g. This survey for vomitoxin in preharvest *G. zeae*-contaminated corn, in a hog-producing area which has a 7-year documented history of swine refusing to eat corn, indicates its importance as one of the major toxins.

Perhaps even higher levels of vomitoxin might have occurred had a rainy period delayed harvest. Since many samples showed positive *G. zeae* contamination but analyzed negative for vomitoxin, it may be that some members of this species may not produce the toxin under preharvest conditions. Our limited survey prevented drawing conclusions with respect to comparative susceptibility of corn varieties.

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