Special Report

Occurrence of Fungi and Mycotoxins Associated with Field Mold Damaged Soybeans in the Midwest

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ABSTRACT

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Abnormally warm, humid weather from 18 September to 7 October 1986 resulted in a delayed soybean harvest throughout the northern half of Illinois and portions of Iowa, Michigan, Minnesota, Missouri, and Wisconsin. Twenty-four soybean samples from Illinois, Iowa, and Michigan from soybean seed either refused or heavily discounted by grain elevators were analyzed for percent damage, fungal infection and germination, and oil and protein content; free fatty acid content in oil; and mycotoxins including diacetoxyscirpenol (DAS), deoxynivalenol (DON), HT-2 toxin, ochratoxin, T-2 toxin, zearalenone, zearalenol, and aflatoxins B_1, B_2, G_1, and G_2. The percent damage ranged from 4.7 to 25.8%, and germination ranged from 7.5 to 39.0%. Seeds were heavily infected with *Alternaria alternata* (6.3–63.0%), *Fusarium graminearum* (13.0–51.3%), and *Phomopsis* spp. (12.5–49.0%). Other fungi, including *Cercospora kikuchii* and *Aspergillus* spp., were identified in 2.0–15.5% of the seeds. Protein, oil, and free fatty acid content were in expected ranges. Zearalenone was detected in whole soybeans, hulls, meal, and oil. Zearalenol was detectable in hulls and meal. DON was detected in whole soybeans, hulls, meal, and oil. DAS was detected in whole soybeans, hulls, and meal. T-2 (primarily HT-2) was detected in whole soybeans, hulls, meal, and oil. Ochratoxin was not detected and aflatoxin B_1 was found in hulls of only three of 24 samples, with the greatest amount detected being 5.8 ηg/g.

Additional keywords: *Glycine max*

Abnormally warm, humid weather from 18 September to 7 October 1986 resulted in a delayed soybean (*Glycine max* (L.) Merr.) harvest throughout the northern half of Illinois and in portions of Iowa, Michigan, Minnesota, Missouri, and Wisconsin. Soybeans that were mature or nearly so during this time showed extensive damage from field molds such as *Alternaria alternata* (Fr:Fr.) Keissl., *Fusarium graminearum* Schwabe (*Gibberella zeae* (Schwein.) Petch), *Phomopsis longicolla* T.W. Hobbs, other *Phomopsis* spp., and *Cercospora kikuchii* (Matsumoto & Tomoyasu) M.W. Gardner. Soybean producers experienced difficulty in marketing these soybeans because of their seed coat discoloration. Damage by *A. alternata* was characterized as a gray-black discoloration of the seed coat and by *F. graminearum* as a reddish pink discoloration. Local grain merchandisers either did not accept these damaged soybeans or discounted them $0.03 to $0.04/kg ($0.90 to $1.20/bu). This problem was exacerbated by the poorly understood changes in soybean grading standards implemented on 1 October 1987 by the U.S. Federal Grain Inspection Service via revisions in the line slides used to grade for damage.

Initial research identified seed infection by *A. alternata* and *F. graminearum*. Because it is unusual to find significant infections by these fungi in soybean grain in Illinois, it was important to determine their effect on quality factors such as protein, oil, and free fatty acid content for soybean processors. Field fungal infections, primarily *Phomopsis*, reduce the quality of soybean oil and flour (6). Also, because *F. graminearum* produces mycotoxins in soybeans (11,18), it was important to assess the potential of mycotoxins in field mold damaged soybeans. The quality factors and mycotoxin contamination in soybeans naturally infected with *A. alternata*, *F. graminearum*, and *Phomopsis* spp. in 1986 was assessed so that processors could ascertain their true value and so that those feeding contaminated soybean products could avoid mycotoxin-induced animal losses.

MATERIALS AND METHODS

Seed samples were acquired in two ways. Seven samples from Illinois were collected through the Dekalb and Kane County Cooperative Extension offices. A set of 17 examples was acquired through the American Soybean Association, which contacted growers in northern Illinois, Frankenmuth and Mason, Michigan, and Wapella and Mt. Pleasant, Iowa. All samples represented soybeans that had been refused or heavily discounted by local grain merchants. Sample size in both instances was approximately 12.5 kg. Samples were split using a Boren divider, and 7.5 kg was shipped to Malcomb Gorngross at the Pilot Soybean Processing Plant, Texas A&M University, College Station, Texas. The remaining 5 kg were used for determination of moisture, damage by Federal Grain Grading Standards, germination, fungal infection, free fatty acid content, and mycotoxin analysis. The initial seven samples were analyzed for fungal infection, protein, oil, and free fatty acid content. Crude oil samples were obtained by mechanical extrusion and solvent extraction (1). Damage determinations were done by the federally licensed Champaign-Danville Grain Inspection Department Inc., Danville, Illinois, using official U.S. Standards for Grain of the Federal Grain Inspection Service.

The Pilot Soybean Processing Plant produced hulls, meal, and crude unrefined oil. These materials were returned to the University of Illinois campus, and three subsamples of each sample were used for mycotoxin analysis. Hull and meal samples were ground with a Stein mill and extracted with 9 + 1 acetonitrile + 4% KCl following the method of Stoloff et al (16). Extracts were assayed directly by high pressure liquid chromatography (HPLC) fluorescence for aflatoxin as described by Beebee (2), for ochratoxin A as described by Cohen and LaPointe (4), and for alpha-zearalenol

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and zearalenone as described by Chang and De Vries (3).

Initial purification of samples used for analysis of the trichothecene toxins DON, DAS, T-2, and HT-2 was done following procedures described by Romer (12). Hydrolysis of trichothecene-ester groups and subsequent capillary gas chromatographic analysis was accomplished as described by Rood (13,14). Confirmation of metabolites by gas chromatography–mass spectrometry was obtained on an Extranuclear 300 series mass spectrometer using positive ion chemical ionization with methane as the reagent gas.

Moisture content was determined by drying three replicates of 100 seeds each to constant weight in an oven at 105 C. Soybean grades were determined by the Champaign-Danville Grain Inspection Service. Percent fungal infection and germination were determined by surface sterilizing seeds in 0.5% NaOCl for 3 min, rinsing in sterile distilled water, and germinating seeds on sterile Kimpac pads at 100% relative humidity for 5 days at 25 C. The data were averaged for the three subsamples of each lot. Correlation coefficients were determined using the SAS general linear model.

RESULTS AND DISCUSSION

The percent germination, infection by *F. graminearum* and *Phomopsis,* percent damage as determined by USDA grading standards, and free fatty acids in oil extracts are given in Table 1. Correlations (R^2) for fungi and percent damage using the general linear model were 0.0003 for *A. alternata,* 0.188 for *Phomopsis,* 0.262 for *F. graminearum,* and 0.029 for all other fungi. While damage levels ranged between 2.4 and 25.8%, free fatty acid, protein, and oil contents were within the normal range for USDA No. 2 yellow soybeans. Free fatty acids exceeding 0.75% contribute to "off-odors and flavors" in soybean oil or meal products. While USDA damage levels were relatively high and would cause these soybeans to be downgraded or heavily discounted, the normal levels of protein, oil, and free fatty acids suggested that the discoloration damage determination did not indicate the true economic value of soybeans. Moisture levels in all lots were in the 11–12.5% range. Aflatoxins B_2, G_1, and G_2 and ochratoxin A were not detected. Aflatoxin B_1 was found in hulls of three samples, with 5.8 ηg/g being the greatest amount detected.

Zearalenone, deoxynivalenol (DON), T-2, and diacetoxyscirpenol (DAS) were found in whole soybeans (Table 2); and zearalenone, zearalenol, DON, T2, and DAS were found in soybean hulls, meal, or oil (Table 3).

In whole soybeans, zearalenone was detected in 19 of 24 samples, with levels ranging from 80 to 1,720 ηg/g. Zearalenone was found in 16 of 17 hull samples, five of 17 meal samples, and two of three oil samples. Highest concentrations were in hulls and lowest in oil. Zearalenol was not detected when whole soybeans were analyzed but was found in 11 of 17 hull samples and two of 17 meal samples, with each of these representing the highest concentration (680 and 1,200 ηg/g, respectively) in hull samples. Zearalenol was not detected in the oil samples.

DON was detected in 16 of 24 whole soybean samples and in 15 of 17 hull samples, 14 of 17 meal samples, and one of three oil samples analyzed. Concentration ranged from not detectable to 490 ηg/g in whole soybeans, from <10 to 420 ηg/g in hulls, from <5 to 600 ηg/g in meal, and from not detectable to 30 ηg/g in oil.

T-2 tetraol (primarily HT-2) was detected in 17 of 24 whole soybeans, 17 of 17 hulls, 13 of 17 meal samples, and three of three oil samples. The concentration ranged from undetectable to 1,070 ηg/g in whole soybeans, 10 to 4,610 ηg/g in hull samples, not detectable to 1,420 ηg/g in meal samples, and 90 to 1,050 ηg/g in oil samples.

DAS was identified in five of 20 whole soybeans, 12 of 17 hulls, six of 17 meal, and none in oil samples. Concentration ranged from undetectable to 230 ηg/g for whole soybeans, undetectable to 130 ηg/g in hulls, and undetectable to 130

Table 1. Percent infection by *Fusarium graminearum* and *Phomopsis* spp. warm germination, free fatty acid in oil extracts, and damage in 1986 midwestern soybean lots rejected or heavily discounted by grain merchants

Sample no.	State of origin	Germination (%)	*F. graminearum* (%)	*Phomopsis* (%)	Free fatty acid in oil[a]	Damage[b] (%)
2581	IL	39	22.5	28.3	0.50	6.0
2582	IL	37.5	22.5	19.5	0.38	5.9
2583	IL	26.5	28.3	17.8	0.43	20.9
2584	IL	23.5	38.0	18.3	0.61	25.8
2575	IL	23.5	22.0	22.8	0.36	2.4
D	IA	18.0	28.3	40.5	0.40	2.4
2578	IL	17.5	21.5	30.5	0.41	12.5
2580	IL	17.0	42.0	18.8	0.54	25.3
2576	IL	15.5	34.8	16.5	0.52	23.6
B	IL	15.0	34.3	12.5	0.42	12.5
2585	IL	14.5	21.5	34.5	0.41	12.0
2577	IL	13.0	31.0	25.0	0.37	3.6
F	MI	12.5	36.0	19.3	0.41	4.7
C	MI	12.0	18.3	25.5	0.49	4.0
A	IL	11.5	51.3	16.3	0.57	14.1
2574	IA	7.5	31.5	48.5	0.50	8.1
FLSD ($P = 0.05$)		12.0	15.3	15.1		

[a] Free fatty acid expressed as oleic acid. Results are the average of triplicate determination.
[b] Percent damage as determined by USDA grading standards.

Table 2. *Fusarium* mycotoxins (ηg/g) found in whole soybeans from soybean lots discounted or refused by grain merchants in Illinois, Iowa, and Michigan

Sample no.	Zearalenone	Zearalenol	DON[a]	T-2[b] equivalents	DAS[c]
1	80	ND[d]	160	40	30
2	140	ND	200	35	ND
3	ND	ND	130	30	15
4	140	ND	490	ND	ND
5	290	ND	240	20	40
2574	ND	ND	ND	ND	ND
2577	ND	ND	ND	190	ND
2580	227	ND	250	440	230
2581	1,670	ND	130	ND	ND
2582	260	ND	ND	ND	ND
2583	1,110	ND	100	570	ND
2584	1,720	ND	360	370	70
2585	490	ND	70	ND	ND
A	1,440	ND	400	1,070	ND
C	490	ND	ND	ND	ND
D	440	ND	ND	ND	ND
F	ND	ND	ND	210	ND
X	210	ND	ND	ND	ND
Y	ND	ND	50	ND	ND
Z	330	ND	ND	80	ND

[a] Deoxynivalenol.
[b] T-2 tetraol after base hydrolysis (primarily HT-2).
[c] Diacetoxyscirpenol.
[d] ND = not detected.

ηg/g in meal. Correlation coefficients for mycotoxins and percent damage for whole soybeans was $R^2 = 0.204$ for whole soybeans, 0.007 for DON, 0.681 for T-2, and 0.183 for DAS. Correlation coefficients for percent *F. graminearum* infection and mycotoxins in hulls were $R^2 = 0.369$ for zearalenone, 0.379 for zearalenol, 0.649 for DON, 0.614 for T-2, and 0.533 for DAS; in meal, $R^2 = 0.342$ for zearalenone, 0.391 for zearalenol, 0.505 for DON, 0.595 for T-2, and 0.082 for DAS.

These results showed that zearalenol, DON, T-2 tetraol, and DAS were produced in infected soybean seeds in the field, and confirmed the report of Wicklow et al (18) and the production of DAS and zearalenol. These mycotoxins were produced in nonsterilized soybean seeds, in contrast to Richardson et al (11), who reported that heat sterilization was required for trichothecenes (T-2) and zearalenone production. The levels of *Fusarium*-produced mycotoxins would be high enough to cause clinical symptoms in animals (10). Zearalenone and zearalenol, which is three times as estrogenic as zearalenone (5), were found in the highest concentrations in the hulls and contained an estrogenic equivalent of zearalenone at 13.1 μg/g (zearalenone at 11.3 μg/g + [zearalenol at 3 × 0.6 μg/g]). Based on a 20% dietary component of hulls in a dairy cattle ration, decreased fertility and abortion could occur (7). Young swine given diets containing as much as 18% protein from soybean meal that may contain up to 8% hulls could receive an estrogenic dose of 0.58 μg/g, which is lower than the estimated minimum toxic dose of 1–2 μg/g (9), although the dose may be high enough to cause breeding problems based on guidelines for feeding mycotoxin-contaminated feeds developed by the Illinois Animal Poison Information Center, Department of Veterinary Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign.

Gas chromatographic–mass spectrometry results indicated that DAS, DON, and HT-2 were the most frequent trichothecenes present and supported the observation of Richardson et al (11). Limited data suggest that HT-2 is equal to T-2 in toxicity (11). Based on the levels present, typical dietary concentration would be less than 2 μg/g, which is safe for beef animals and is above the 1 μg/g no-effect level for swine (8). Based on typical diets for broiler chickens, laying hens, or turkeys, feeding the most contaminated sample would result in dietary exposure to approximately 0.4 μg/g of HT-2, a level lower than that required to cause oral lesions or reduced feed efficiency (10). However, these levels are high enough to cause oral lesions in Muscovy ducklings (15).

The effects of these mycotoxins on humans are unknown, although exposure via hulls, a fiber source, or meal in infant formulas or soya milk preparations may be of concern. Based on concentrations observed, it is unlikely that human exposure via milk, meat, or eggs from animals fed these soybeans would be a significant hazard because animals metabolize and excrete these compounds (17).

Our results with a limited number of samples showed that USDA damage ratings were not an indicator of mycotoxin contamination potential, nor did they predict oil quality as measured by free fatty acid content. Research regarding the true loss in value based on USDA damage ratings is needed. Based on typical dietary rations, mycotoxin intoxication would not occur in beef, poultry, or swine, except in young animals. However, the effects of a combination of mycotoxins are unknown.

Table 3. *Fusarium* mycotoxins (ηg/g) found in soybean hulls, meal, or oil from soybeans harvested in 1986 processed in a pilot processing plant and discounted or refused by grain merchants in Illinois, Iowa, and Michigan

Sample no.	Type	Zearalenone	Zearalenol	DON[a]	T-2[b] equivalents	DAS[c]
2574	Hull	ND[d]	ND	30	10	ND
	Meal	ND	ND	30	ND	ND
	Oil	ND	ND	ND	90	ND
2575	Hull	800	ND	10	10	10
	Meal	ND	ND	30	20	ND
2576	Hull	5,980	540	190	870	40
	Meal	220	ND	80	190	20
2577	Hull	720	ND	50	280	ND
	Meal	ND	ND	120	270	ND
2578	Hull	3,480	360	40	270	ND
	Meal	180	ND	80	130	ND
2580	Hull	11,260	580	380	1,000	70
	Meal	400	ND	500	530	<5
2581	Hull	4,560	370	60	220	<10
	Meal	ND	ND	80	70	ND
2582	Hull	3,100	320	70	220	<10
	Meal	ND	ND	100	<5	ND
2583	Hull	4,040	500	220	1,260	<10
	Meal	ND	ND	120	430	ND
2584	Hull	10,940	680	260	1,260	130
	Meal	760	180	600	1,210	130
	Oil	590	ND	30	1,050	ND
2585	Hull	2,260	ND	60	320	<10
	Meal	ND	ND	<5	30	<5
A	Hull	8,120	1,200	420	4,610	100
	Meal	340	180	460	1,420	ND
	Oil	270	ND	ND	800	ND
B	Hull	2,720	260	90	670	<10
	Meal	ND	ND	90	160	<5
C	Hull	1,300	340	<10	120	ND
	Meal	ND	ND	<5	<5	ND
D	Hull	1,940	240	50	140	ND
	Meal	ND	ND	20	<5	ND
E	Hull	2,640	ND	60	1,200	<10
	Meal	ND	ND	30	80	ND
F	Hull	820	ND	30	820	<10
	Meal	ND	ND	<5	120	30

[a] Deoxynivalenol.
[b] T-2 tetraol after base hydrolysis (primarily HT-2).
[c] Diacetoxyscirpenol.
[d] ND = not detected.

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