Distribution and Occurrence of Mycotoxins in 1993 Kansas Wheat[1]

D. M. TRIGO-STOCKLI, S. P. CURRAN, and J. R. PEDERSEN[2]

ABSTRACT

Wheat samples from various locations in Kansas were tested for mold invasion by plating on agar medium and then analyzing for the presence of deoxynivalenol, zearalenone, and aflatoxin B_1 using thin-layer chromatography. In eastern Kansas, 29 of 30 samples (96%) were invaded with *Fusarium graminearum* and the mean percentage of invaded kernels was 33% in northeast, 16% in east central, and 15% in southeast districts. The highest level of invasion observed was 75% on a sample from northeast Kansas. Deoxynivalenol was detected in 23 of 116 samples analyzed (20%) and was correlated highly ($r = 0.839$, $P < 0.001$) with the level of invasion by *F. graminearum*. Zearalenone was detected only in one sample from northeast Kansas. Cool, wet weather during 1993 was an important factor affecting invasion and toxin formation by *F. graminearum*. The level of mycotoxin in 1993 Kansas wheat was relatively low.

Cereal Chem. 72(5):470–474

Mycotoxins are compounds that are produced by fungi and can cause illness or even death when food or feed containing them are consumed (CAST 1989). Mycotoxins often occur in grain crops in the field before harvest when certain weather conditions prevail.

The crop year from July 1992 through June 1993 in Kansas was characterized by above normal total precipitation. For most of the spring and summer of 1993, heavy and frequent rains saturated fields in central and eastern Kansas (Roozeboom 1993). Such unusually high precipitation favors the growth of many fungi, including *Fusarium* species, that produce toxins.

One of the *Fusarium* species favored by such weather conditions is *Fusarium graminearum* Schwabe (*Gibberella zeae* (Schw.) Petch), which causes wheat scab or head blight. Scab results from infection of individual spikelets at or soon after flowering, when they are most susceptible. Infected spikelets are killed, and the fungus then may girdle the rachis so that the head above that point dies. A distinct salmon-pink ring of fungus develops at the base of the glumes. Direct yield losses from scab are usually minor but can reach 25–40% in severely diseased fields (Willis 1984). Of greater concern is the ability of the causal fungi to produce deoxynivalenol (vomitoxin), zearalenone, and other toxins.

Deoxynivalenol (DON) is one of the most common trichothecene toxins produced by *F. graminearum*. It causes feed refusal and emesis in pigs and experimental animals (Marasas and Nelson 1987). In the United States, zearalenone commonly occurs with DON in cereal crops (Bennett and Shotwell 1979, Wood 1992). Zearalenone, an estrogenic metabolite, induces feminization at dietary concentrations of >1 ppm, whereas higher concentrations interfere with conception, ovulation, implantation, fetal development, and the viability of newborn animals (CAST 1989). Aflatoxins also have been reported in wheat (Hesseltine et al 1966, Shotwell et al 1969, Kurata 1990). They are produced primarily by some strains of *Aspergillus flavus* Link: Fr. and most strains of *A. parasiticus* Speare.

As part of the North Central NC-129 project, a survey on the occurrence of molds and mycotoxins on major grains in Kansas was conducted. The objective of this study was to determine the distribution and the extent of mold invasion and mycotoxin occurrence in 1993 Kansas wheat.

[1]Contribution 94-556-J. Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

[2]Research associate, assistant professor, and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan.

MATERIALS AND METHODS

Wheat Samples

Wheat samples (276) from a yield and quality survey (Kansas Agricultural Statistics 1993) were obtained. Survey samples were collected proportionally to the acreage grown in each area of the state.

Determination of Fungal Invasion

Kernels were surface-disinfected by shaking for 1 min in 2% NaClO (Clorox) and rinsed in sterile distilled water. Then 100 kernels were plated on malt agar (MS6T) containing 6% NaCl and 200 ppm of Tergitol NPX (Sigma Chemical Co., St Louis, MO). The plates were incubated at room temperature (25–27°C) for five to seven days. The number of kernels yielding fungi was counted and species were identified using a dissecting microscope. Identification of *F. graminearum* species was confirmed by growing cultures on carnation leaf agar (Nelson et al 1983).

Mycotoxin Analyses

A total of 116 samples was analyzed for DON, zearalenone, and aflatoxin B_1 using three toxin quantitative test kits (Mycotest, Romer Labs, Inc.) for thin-layer chromatography. All samples in which *F. graminearum* was detected were tested, along with a representative number of noninvaded samples. Wheat (50–200 g) was ground for analysis.

Extraction. Ground wheat (50 g) and 100 ml of 9:1 acetonitrile (CH_3CN) and water were blended for 2 min at high speed. Filtered extract (≈5 ml) was placed in a 15- × 85-mm culture tube. Then 10 µl of acetic acid was added using a microliter capillary pipet. After mixing, ≈2 ml of the filtrate was pushed through a Mycosep 224 multifunctional clean-up (MFC) column; 2 ml of purified extract was then transferred to a 10-ml culture tube and evaporated to dryness using a hot water bath at 60°C and vacuum.

Thin-layer chromatography. A 500-µl syringe was used to add 200 µl of toluene and CH_3CN (97:3) to the residue in the culture tube. The tube was stoppered and vortexed for ≈30 sec. Capillary pipets were used for spotting 20 µl of each sample, along with 10, 20, and 50 µl of aflatoxin B_1, DON, and zearalenone standards on a silica-gel thin-layer chromatography plate (10-cm high), which was then placed into a developing tank containing 50 ml of 1:1 toluene and acetone until the solvent traveled to ≈1.0 cm from the top of the plate. The plate was blow-dried and then observed under long-wave UV light for aflatoxin B_1 (blue fluorescing spots half-way up the plate). The plate was sprayed with 20% aluminum chloride ($AlCl_3$) in methanol and then observed under long-wave UV light for zearalenone (light blue fluorescent spots about two-thirds of the way up the plate). The plate was then heated (140–150°C) and viewed under long-wave UV light. It was removed when DON standard spots appeared about one-third of the way up the plate. The levels of DON, zearalenone, and aflatoxin B_1 were estimated visually by comparison to standard spots. The nanograms of toxin in the sample spot were divided by 0.1-g sample equivalent to obtain parts per billion of toxin in the initial sample. The levels of DON and zearalenone obtained are presented in parts per million.

Aflatoxin B_1 analysis. The level of aflatoxin B_1 in the samples was also verified using a test based on an enzyme-linked immunosorbent assay (VICAM Aflatest). Ground wheat (50 g), NaCl (5 g), and 80% high-pressure liquid chromatography (HPLC) grade methanol (100 ml) were blended at high speed for 1 min. The extract (50–100 ml) was filtered, and 5 ml was diluted with 10 ml of distilled water and mixed. The dilute extract was filtered using a microfiber filter. Clean extract (1 ml) was passed through the Aflatest-P affinity column. The column was washed twice with 1 ml of distilled water. Aflatoxin B_1 was eluted with 1 ml of HPLC grade methanol and collected in a cuvette. The eluate was mixed with 1 ml of freshly prepared developer containing 0.002% bromine. The level of aflatoxin B_1 (ppb) was measured after 1 min with a Torbex Series-3 fluorometer.

Data Analysis

The data for *F. graminearum* invasion (%) were categorized as low, medium, or high. The frequency was analyzed statistically. To determine whether differences existed among wheat cultivars grown in different locations, comparisons were made on four of the 42 cultivars collected: Karl, 2163, TAM 107, and Victory. These cultivars were grown at the greatest number of locations. Analyses also were made by combining data in the eastern, central, and western districts, as well as northern, central, and southern districts to determine whether differences existed among geographical regions. Correlation analysis determined whether the percentage of *F. graminearum* invasion and the level of DON observed were correlated significantly (SAS 1989a,b).

RESULTS

Kernels Invaded with *F. graminearum* and Other Fungi

Surface-disinfected wheat kernels yielded *Alternaria* sp., *Cladosporium* sp., *Epicoccum* sp., *F. graminearum* and other *Fusarium* species, *Helminthosporium* sp., *Stemphyllium* sp., *Penicillium* sp., and *Aspergillus* sp. The *Aspergillus* group species observed included: *A. candidus*, *A. glaucus*, *A. flavus*, *A. niger*, and *A. ochraceus*.

F. graminearum was observed in most of the counties included in the study, except in west central Kansas. The level of invasion ranged from 1 to 75%. All of the samples collected from the east central and southeast districts were invaded with *F. graminearum.* In the northeast and north central districts, respectively, 88 and 79% of the samples collected were invaded with *F. graminearum* (Table I). Higher percentages of kernels invaded with *F. graminearum* were observed on samples collected from the three eastern districts. The highest percentage of invasion (75%) was observed in a sample from Doniphan County in northeast Kansas. Low percentages of invaded kernels were observed on samples from the central, south central, and western districts (Fig. 1). The average invasion for the state was 3.6%.

The levels of invasion by *F. graminearum* differed depending on the wheat cultivar and the location where the wheat was grown. Based on statistical analysis of data for Karl, 2163, TAM 107, and Victory, differences in *F. graminearum* infection levels were affected significantly by cultivars and location (x^2 = 131, df = 24, $P < 0.001$).

Because only Karl was grown in most districts, a comparison was made of *F. graminearum* infection levels on Karl among eastern (northeast, east central, and southeast); central (north central, central, and south central); and western (northwest, west central, and southwest) regions. Highly significant differences (x^2 = 45, df = 4, $P < 0.001$) existed from east to west. The differences from north to south were less significant (x^2 = 8, df = 4, P = 0.062).

Comparison of the four cultivars was restricted to the central (north central, central, and south central) region, where they were commonly grown. As shown in Table II, Victory had the highest percentage of samples (83.3%) invaded with *F. graminearum,* followed by 2163 (52.4%), Karl (41.6%), and TAM 107 (10%).

Distribution and Levels of Mycotoxins

DON. Approximately 20% of the samples showed levels of DON ranging from 0 to >5 ppm, based on 1, 2, and 5 ppm standards. The samples that had high levels of *F. graminearum* infection also had high levels of DON (Table I, Figs. 1 and 2). The presence of DON on random samples was confirmed using gas chromatography with an electron capture detector, based on the procedure described by Bennett et al (1983). DON was not detected in samples collected from counties in central, northwest, west central, and southwest districts (Table I, Fig. 2). In the south central district, only 1 of 25 samples tested had DON (<1 ppm). The average statewide level of DON, weighted by 1993 wheat production (Table I), was 0.1 ppm.

Zearalenone. Of the 116 samples analyzed, zearalenone was detected in only one sample. A sample of cultivar 2163 collected from Doniphan county in northeast Kansas had 5 ppm of zearalenone. This sample also had the greatest level of invasion by *F. graminearum* (75%) and the highest level of DON (>5 ppm). Zearalenone was confirmed using the Target zearalenone test column from Terratek, Inc.

Aflatoxin B_1. Seven of 116 samples (6%) analyzed showed aflatoxin B_1 levels of ≈20 ppb using the Romer Mycotest and the

TABLE I
Fusarium graminearum **Invasion and Deoxynivalenol (DON) Levels in 1993 Kansas Hard Red Winter Wheat by District**

District	Number of Samples		Kernels Invaded (%)		DON Level (ppm)[a]		Wheat Production[b]
	Collected	Invaded	Mean	(Range)	Mean	(Range)	(bu)
Northeast	8	7	33.1	(0–75)	2.2	(0.0–5.5)	4,731
East central	8	8	16.2	(3–29)	0.3	(0.0–2.0)	3,977
Southeast	14	14	15.2	(1–52)	0.4	(0.0–2.0)	15,183
North central	34	27	7.8	(0–54)	0.2	(0.0–0.7)	35,807
Central	42	19	1.2	(0–10)	0.0	(0.0)	48,854
South central	73	24	1.0	(0–16)	0.0	(0.0–0.1)	118,770
Northwest	27	2	0.1	(0–1)	0.0	(0.0)	46,122
West central	27	0	0.0	(0)	0.0	(0.0)	55,725
Southwest	43	3	0.1	(0–2)	0.0	(0.0)	83,588

[a] 0.0 = not detected.
[b] Data from Kansas Agricultural Statistics (1994).

VICAM Aflatest methods. However, high-pressure liquid chromatography tests on the same samples did not confirm the presence of aflatoxin B_1 (David Wilson, *personal communication*). These samples were collected from north central and central Kansas.

DISCUSSION

Our data show that *F. graminearum* was prevalent in the three eastern districts, which account for only 7.1% of the wheat production in Kansas. The highest level of invasion was observed in northeast Kansas. These areas had unusually high precipitation during flowering in April and May, and during harvest in June and July (Fig. 3).

Cook (1981) stated that moisture during anthesis is the most important factor affecting incidence of *Fusarium* head blight. After anthesis, most wheat cultivars continue to be receptive to infection as long as the required moisture and temperature conditions are present (Andersen 1948).

The weather obviously was an important factor affecting infection levels by *F. graminearum* in 1993 Kansas wheat. Samples obtained from areas where precipitation was lowest (e.g., south central and western districts) had very low levels of *F. graminearum* infection. In northeast Kansas, where precipitation was highest from flowering to harvest (May–July), the level of infection was high. Roozeboom (1993) reported that scab infection was associated with severe reduction in yield in several varieties in Brown County. He also stated that the rate of infection was more dependent on environmental conditions at flowering than on varietal response to the disease.

Love and Seitz (1987) reported that weather parameters as well as cultivars affected infection levels by *F. graminearum* for some locations in Kansas. They reported that some cultivars in the midwestern United States show significant and consistent differences in susceptibility to scab infection. In this study, the level of invasion by *F. graminearum* was also affected by cultivar and weather conditions. Our data indicate that among the four commonly grown cultivars, Victory was the most susceptible cultivar, followed by 2163, Karl, and TAM 107. In addition, the level of invasion by *F. graminearum* was higher in the eastern districts than in the west.

Little information is available about field occurrence of aflatoxins on wheat. However, under laboratory conditions, wheat is a suitable substrate for aflatoxin formation (Hesseltine et al 1966, Stubblefield et al 1967). Shotwell et al (1969) reported higher aflatoxin levels on low-grade samples (U.S. grades 4 and 5, and sample grade) collected from the market channels. Hagler et al (1984) reported simultaneous occurrence of DON, zearalenone, and aflatoxin in 1982 scabby wheat, collected from Kansas and Nebraska.

Although DON was not detected in all samples invaded with *F. graminearum*, levels were highly correlated with the level of *F. graminearum* invasion ($r = 0.839$, $P < 0.001$). This is consistent with the observations of McMullen et al (1993) that DON levels were positively correlated with percent of visible scab infection on spring wheat.

Based on the weighted mean level of DON by district, samples from the northeast, east central, and southeast districts had 2.2 0.3, 0.4 ppm of DON, respectively (Table I). The mean level of DON by district decreased from north to south, which is similar to the observation reported by Shotwell et al (1985). The decreasing DON levels from north to south may be attributed to weather conditions. As shown in Figure 3, the northeast had higher pre-

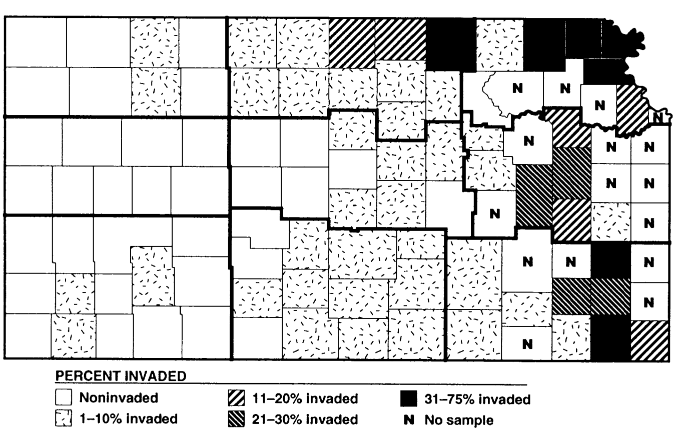

Fig. 1. Distribution of *Fusarium graminearum* in 1993 Kansas hard red winter wheat.

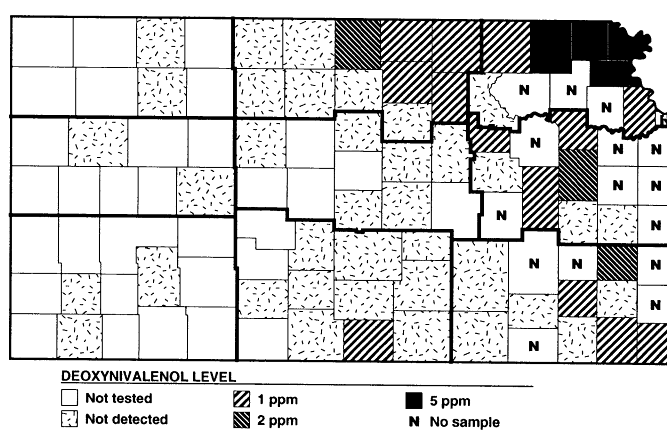

Fig. 2. Occurrence of deoxynivalenol in 1993 Kansas hard red winter wheat.

TABLE II
***Fusarium graminearum* Invasion in Four Kansas 1993 Hard Red Winter Wheat Cultivars**

	Number of Samples Per Cultivar							
	2163		Karl		TAM 107		Victory	
District	Collected	Invaded	Collected	Invaded	Collected	Invaded	Collected	Invaded
Northeast	2	2	4	3	0	0	0	0
East central	1	1	5	5	0	0	0	0
Southeast	1	1	12	12	0	0	0	0
North central	6	5	9	6	2	1	8	7
Central	3	1	13	8	6	0	5	5
South cntral	12	5	26	6	2	0	5	3
Northwest	0	0	4	0	10	1	0	0
West central	0	0	0	0	16	0	1	0
Southwest	1	0	1	0	16	1	0	0
Total	26	15	74	40	52	3	19	15
Infection (Range)	1–75%		1–57%		1–8%		1–41%	

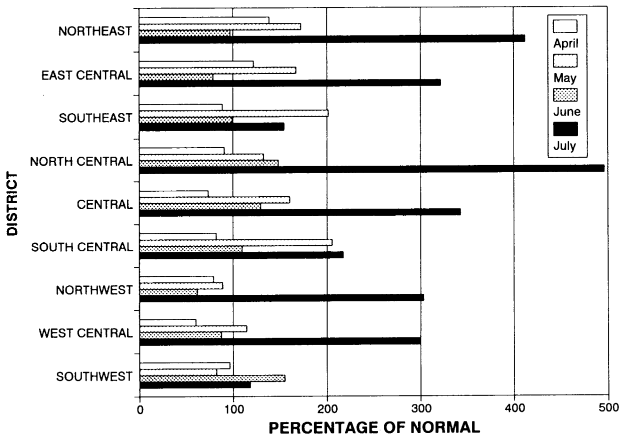

Fig. 3. Kansas precipitation as a percent of normal, by district, April–July, 1993.

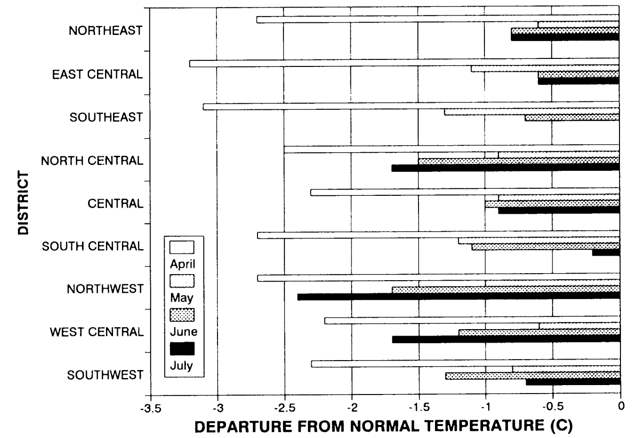

Fig. 4. Kansas mean temperature (°C) deviation from normal, by district, April–July, 1993.

cipitation than the southeast in April and July 1993. Additionally, the crop year in Kansas for 1993 was generally cooler than normal (Fig. 4). Low temperatures with concomitant high rainfall and humidity favors *F. graminearum* infection and toxin production (Marasas et al 1984). Moreover, Hart et al (1984) reported that the level of DON produced is dependent on the duration of moist periods.

Only ≈15% of the total wheat harvested in Kansas in 1993 had some DON. A similar percentage was observed during the 1982 outbreak of wheat scab in Kansas (Seitz 1982). The northeast district, which had DON levels of >2 to >5 ppm, comprised only 1.3% of the total state wheat production, and most of the fields were abandoned and planted to soybeans (Kansas State Board of Agriculture 1993). The level of DON is further reduced by cleaning procedures implemented by the millers before milling (Tkachuk et al 1991), and the separation of flour and feed fractions during milling (Seitz et al 1986). Thus, the likelihood of a finished food product containing a biologically significant amount of toxin is small.

The statewide weighted mean level of 0.1 ppm of DON was much lower than the advisory levels, which range from 1 ppm for finished wheat products, such as flour, bran, and germ destined for human consumption, to 5–10 ppm on grains and grain byproducts for animal feeds (FDA 1993). Therefore, even in a year conducive to scab and head blight development, the 1993 wheat produced in Kansas, as a whole, had a relatively low level of mycotoxin.

ACKNOWLEDGMENTS

We thank Glenn Bennett at the National Center for Agricultural Utilization Research, Peoria, IL, for the gas chromatography analysis of deoxynivalenol in our samples; David Wilson, Dept. of Plant Pathology, Coastal Plain Experiment Station, Tifton, GA, for testing samples for aflatoxins; Dallas Johnson and Carl Reed for discussions on statistical analyses and for critiquing the article; Robert Bequette, for suggestions on the manuscript; and Mary Knapp for the weather data. This research was conducted in conjunction with Regional Project NC-129, Occurrence of Mycotoxins and the Implications to Animal and Human Health.

LITERATURE CITED

ANDERSEN, A. L. 1948. The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:599-611.

BENNETT, G. A., and SHOTWELL, O. L. 1979. Zearalenone in cereal grains. J. Am. Oil Chem. Soc. 56:812-819.

BENNETT, G. A., STUBBLEFIELD, R. D., SHANNON, G. M., and SHOTWELL, O. L. 1983. Gas chromatographic determination of deoxynivalenol in wheat. J. Assoc. Off. Anal. Chem. 66:1478-1480.

CAST. 1989. Mycotoxins: Economic and Health Risks. Report 116. Council for Agricultural Science & Technology: Ames, IA.

COOK, R. J. 1981. *Fusarium* diseases of wheat and other small grains in North America. Pages 39-52 in: *Fusarium* Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press: University Park.

FDA. 1993. News release. Sept. 16, 1993. U. S. Dept. of Health and Human Services, Food and Drug Administration: Washington, DC.

HAGLER, W. M., TYCZKOWSKA, K., and HAMILTON, P. B. 1984. Simultaneous occurrence of deoxynivalenol, zearalenone, and aflatoxin in 1982 scabby wheat from the midwestern United States. Appl. Environ. Microbiol. 47:151-154.

HART, L. P., PESTKA, J. J., and LIU, M. T. 1984. Effect of kernel development and wet periods on production of deoxynivalenol in wheat infected with *Gibberella zeae*. Phytopathology 74:1415-1418.

HESSELTINE, C. W., SHOTWELL, O. L., ELLIS, J. J., and STUBBLEFIELD, R. D. 1966. Aflatoxin formation by *Aspergillus flavus*. Bacteriol. Rev. 30:795-805.

KANSAS AGRICULTURAL STATISTICS. 1993. Wheat Quality 1993. Kansas State Board of Agriculture: Topeka.

KANSAS AGRICULTURAL STATISTICS. 1994. 1993 Wheat County Estimates. Kansas State Board of Agriculture: Topeka.

KANSAS STATE BOARD OF AGRICULTURE. 1993. Plant Disease Survey Report, Vol. 19, report 13. Plant protection and weed control. Kansas State Board of Agriculture: Topeka.

KURATA, H. 1990. Mycotoxins and mycotoxicoses: Overview. Pages 249-259 in: Microbial Toxins in Foods and Feeds. A. E. Pohland, V. R. Dowell, and J. L. Richard, eds. Plenum Press: New York.

LOVE, G. R., and SEITZ, L. M. 1987. Effects of location and cultivar on *Fusarium* head blight (scab) in wheat from Kansas in 1982 and 1983. Cereal Chem. 64:124-128.

MARASAS, W. F. O., and NELSON, P. E. 1987. Mycotoxicology. The Pennsylvania State University Press: University Park.

MARASAS, W. F. O., NELSON, P. E., and TOUSSOUN, T. A. 1984. Toxigenic *Fusarium* Species, Identity and Mycotoxicology. The Pennsylvania State University Press: University Park.

McMULLEN, M. P., CASPER, H., and STACK, R. W. 1993. Correlation of vomitoxin levels and visible scab in 1991 spring wheat. (Abstr). Phytopathology 83:885.

NELSON, P. E., TOUSSOUN, T. A., and MARASAS, W. F. O. 1983. *Fusarium* species, an illustrated manual for identification. The Pennsylvania State University Press: University Park.

ROOZEBOOM, K. 1993. 1993 Kansas Performance Tests with Winter Wheat Varieties. Report of Progress 691. Agricultural Experiment Station. Kansas State University: Manhattan.

SAS. 1989a. User's Guide: Basics, 5th ed. SAS Institute: Cary, NC.

SAS. 1989b. User's Guide: Statistics, 5th ed. SAS Institute: Cary, NC.

SEITZ, L. M. 1982. Deoxynivalenol analyses of Kansas wheat quality profile samples. Pages 10-12 in: Scab and Deoxynivalenol in the 1982 Wheat Crop. Report of Progress 440. Agricultural Experiment Station, Kansas State University: Manhattan.

SEITZ, L. M., EUSTACE, W. D., MOHR, H. E., SHOGREN, M. D., and YAMAZAKI, W. T. 1986. Cleaning, milling, and baking tests with hard red winter wheat containing deoxynivalenol. Cereal Chem. 63:146-150.

SHOTWELL, O. L., BENNETT, G. A., STUBBLEFIELD, R. D.,

SHANNON, G. M., KWOLEK, W. F., and PLATTNER, R. D. 1985. Deoxynivalenol in hard red winter wheat: Relationship between toxin levels and factors that could be used in grading. J. Assoc. Off. Anal. Chem. 68:954-957.
SHOTWELL, O. L., HESSELTINE, C. W., BURMEISTER, H. R., KWOLEK, W. F., SHANNON, G. M., and HALL, H. H. 1969. Survey of cereal grains and soybeans for the presence of aflatoxin: I. Wheat, grain sorghum, and oats. Cereal Chem. 46:446-454.
STUBBLEFIELD, R. D., SHOTWELL, O. L., HESSELTINE, C. W., SMITH, M. L., and HALL, H. H. 1967. Production of aflatoxin on wheat and oats. Measurement with a recording densitometer. Appl. Microbiol. 15:186-190.
TKACHUK, R., DEXTER, J. E., TIPPLES, K. H., and NOWICKI, T. W. 1991. Removal by specific gravity table of tombstone kernels and associated trichothecenes from wheat infected with *Fusarium* head blight. Cereal Chem. 68:428-431.
WILLIS, W. G. 1984. Wheat Diseases. Cooperative Extension Service. Kansas State University: Manhattan.
WOOD, G. E. 1992. Mycotoxins in foods and feeds in the United States. J. Anim. Sci. 70:3941-3949.

[Received June 30, 1994. Accepted May 5, 1995.]