

Comparison of the Distribution and Occurrence of *Fusarium graminearum* and Deoxynivalenol in Hard Red Winter Wheat for 1993–1996¹

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ABSTRACT

Cereal Chem. 75(6):841–846

Hard red winter wheat samples collected from different locations in Kansas from the 1993, 1994, 1995, and 1996 harvests were plated to determine *Fusarium graminearum* infection and analyzed for deoxynivalenol by thin-layer and gas chromatography. Rainfall, temperature, and cultivar were important factors affecting the severity of *F. graminearum* infection as well as deoxynivalenol production. The 1993 and 1995 growing seasons had high percentages of samples infected with *F. graminearum* and con-

taminated with deoxynivalenol. Averaged over the four years, cultivars 2163 and Karl had significantly higher levels of infection than did TAM 107. These widely grown cultivars were used in comparison. Northeastern Kansas had the highest levels of *F. graminearum* infection and deoxynivalenol contamination but also had the lowest acreage planted to hard red winter wheat.

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is the fungus that causes head blight or scab of wheat. In recent years, wheat scab has again caused devastating problems in many parts of the United States (McMullen et al 1997). Damage from head blight or scab includes shrunken and discolored (pink or chalky white “tombstone”) kernels, reductions in yield and seed quality, and toxin contamination. These factors also reduce test weight and lower market grade.

One of the most important toxins produced by *F. graminearum* is deoxynivalenol (DON or vomitoxin). It causes feed refusal, diarrhea, and vomiting in swine and other animals (Marasas and Nelson 1987). DON also is considered one of the most agriculturally important fungal toxins.

Wet weather conditions promote scab development and toxin production. Frequent and high levels of rainfall, especially during flowering, cause severe scab infections because rain aids in the liberation and spread of spores (Atanasoff 1920). Continued moist weather during the growing season favors development of the fungus (McMullen et al 1997). Susceptible wheat cultivars also contribute to outbreaks of scab. After a scab outbreak in 1982, Love and Seitz (1987) reported that cultivars commonly grown in the midwestern United States showed consistent differences in susceptibility to scab infection that generally were not related to maturity factors.

The occurrence of wheat scab during recent years also may be attributed to low-tillage farming practices, which allow the continuing presence of *F. graminearum* inoculum in the crop residue. Moreover, because *F. graminearum* also is associated with corn stalk and ear rot, the fungus persists and multiplies on infected crop residues of small grains and corn (McMullen et al 1997).

Beginning with the scab outbreak in 1993, our major objectives were to annually determine the distribution and occurrence of *F. graminearum* and DON in Kansas hard red winter wheat (HRW) wheat and to determine the relationship of rainfall and temperature to severity of *F. graminearum* infection and DON production.

MATERIALS AND METHODS

Wheat Samples

HRW wheat samples were obtained from yield and quality surveys of the 1993, 1994, 1995, and 1996 harvests. The numbers of samples collected were proportional to the acreage grown. The total numbers of samples were 276 (1993), 274 (1994), 271 (1995), and 305 (1996). The samples were stored at 5°C until they were analyzed.

Determination of Fungal Invasion

To identify species of fungi, HRW wheat samples were plated on agar according to the procedure described by Sauer et al (1982). Wheat kernels were shaken for 1 min in 2% (v/v) NaClO and rinsed in sterile water. Then 100 kernels were placed on malt-salt-Tergitol agar (MS6T) with 6% (w/v) NaCl and 200 ppm of Tergitol NPX (Sigma, St. Louis, MO). After incubation at 25°C for 7–10 days, fungi growing from the kernels were identified and counted with the aid of a dissecting microscope. The presence of *F. graminearum* was confirmed by using carnation leaf agar (Nelson et al 1983). All samples from the 1993, 1994, and 1995 harvests were plated, and 176 samples from 1996 were plated.

Mycotoxin Analyses

The procedure followed for toxin analyses has been described previously (Trigo-Stockli et al 1995). Ground samples were analyzed for DON with three-toxin quantitative test kits (Romer Labs, Union, MO) for thin-layer chromatography (TLC). Randomly selected samples infected with *F. graminearum* along with representative noninfected samples were confirmed by gas chromatography (GC). Total samples analyzed by TLC were 116 (1993), 102 (1994), 206 (1995), and 99 (1996).

Extraction. Ground wheat (25 g) and 100 mL of acetonitrile and water (84:16, v/v) were blended for 3 min at high speed in a Waring blender. Filtered extract (≈5 mL) was placed in a 15- × 85-mm culture tube, and 10 μL of concentrated acetic acid was added. After the tube was shaken for a few seconds, at least 2 mL of the filtrate was passed through a multifunctional cleanup column (Mycosep no. 224, Romer Labs). A 2-mL aliquot of purified extract was transferred to a 10-mL culture tube and evaporated to dryness by using a hot water bath at 60°C and vacuum.

TLC. Toluene (100 μL) and acetonitrile (97:3, v/v) was added to the residue in the culture tube. The tube was stoppered and placed on a vortex mixer for ≈30 sec. Samples (20 μL each) and 5, 10, and 20 μL of DON and zearalenone standards were spotted on a silica gel TLC plate 10-cm high. The plate was developed in a tank containing 50 mL of toluene and acetone (1:1, v/v) until the solvent traveled to ≈1.0 cm from the top of the plate. The plate was allowed to dry and was then dipped into 20% aluminum chloride in methanol. The plate was allowed to air-dry and was then observed

¹ Contribution 98-28-J. Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS. Presented in part at the AACCC 80th Annual Meeting, San Antonio, TX, November 1995.

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under long-wave UV light for a light blue fluorescent spot at 0.7 R_f value, which indicates zearalenone. The TLC plate was placed on a hot plate (140–150°C) and viewed under long-wave UV light while being heated. The plate was removed from the hot plate when DON standard spots appeared at 0.3 R_f . The levels of DON and zearalenone were estimated visually by comparison with DON standards of 100, 200, and 400 ng. The amount (ng) of toxin in the sample spot was divided by 0.1-g sample equivalent and by 1,000 to obtain parts per million of toxin in the initial sample. The lower detection limit for TLC was 500 ng.

Confirmation of DON

The GC procedure, in which an electron capture detector is used, was based on the process described by Trigo-Stockli et al (1996). Evaporated extracts in culture tubes, obtained as described above, were dissolved in 1 mL of toluene and acetonitrile (95:5, v/v) and then mixed for 5 sec on a vortex mixer. Heptafluorobutyrylimidazole (50 μ L) was added, and then the mixture was agitated for 5 sec on

a vortex mixer and heated at 60°C in a sand bath for 1 hr. After the mixture was cooled to room temperature, 1 mL of 3% (w/v) sodium bicarbonate was added and the mixture was agitated vigorously on a vortex mixer for 30 sec to form a fine emulsion. The phases were allowed to separate. A syringe was used to transfer 50 μ L of the upper phase into a 1.8-mL crimped vial along with 950 μ L of hexane. The DON standard (100–125 μ g/ μ L) in acetonitrile was evaporated and derivatized similarly.

A gas chromatograph (model 3600, Varian, Palo Alto, CA) with a ^{63}Ni electron capture detector, a Megabore (0.53 mm) DB-5 capillary column 15 m long, and 1.5- μ m film thickness was used. The carrier and make-up gas, carrier-grade nitrogen, were set at 6.5 and 20 mL/min, respectively. Column temperature was programmed from 150 to 210°C at 5°C/min. The initial column hold time was 1 min, and the final column hold time was 2 min. The injector and detector temperatures were 220 and 300°C, respectively. The retention time for the DON-heptafluorobutyryl derivative at this setting was \approx 10.5 min.

The amount of DON present in the sample was calculated by the official method of the Association of Official Analytical Chemists (AOAC 1997). Sample extract (2 μ L) was injected into the GC apparatus under the same conditions used for preparing a standard curve. The amount of DON in the sample was calculated by comparing the peak area of the sample with the peak area of the derivatized DON standard with the formula:

$$\text{DON, ng/g} = (C'/C) \times (V'/V) \times (PA/PA')$$

in which C' = concentration of DON standard (ng/ μ L); V' = volume of DON standard injected (μ L); PA = peak area of sample; PA' = peak area of standard; C = concentration of sample (0.0002 g/ μ L using a 25-g sample); and V = volume of sample extract injected (μ L). A standard curve was prepared before the sample extracts were injected into the GC apparatus. Derivatized DON (1–5 μ L) was injected into the column to obtain a peak response. A standard curve was constructed by plotting the amount of derivatized DON versus detector response for a range of 100–500 μ g.

TABLE I
Fusarium graminearum Infection of Kansas Hard Red Winter Wheat During Four Years^a

District	1993	1994	1995	1996
Northeast	33.1a (88)	1.2bc (33)	36.1a (100)	13.0a (70)
East central	16.2b (100)	2.3b (86)	11.0bc (100)	5.1b (100)
Southeast	15.2b (100)	5.9a (72)	11.0bc (93)	2.2bc (54)
North central	7.8c (79)	1.0bc (44)	18.0b (89)	3.4b (77)
Central	1.2d (42)	0.4bc (26)	5.1cd (87)	2.0bc (24)
South central	1.0d (33)	0.4bc (27)	3.0de (83)	0.3c (10)
Northwest	0.1d (7)	1.0bc (33)	1.5de (62)	0.3c (9)
West central	0.0d (0)	0.3bc (21)	1.3de (38)	0.2c (8)
Southwest	0.1d (7)	0.1c (9)	0.8e (39)	0.2c (8)
Mean	8.3b	1.4c	9.8a	3.0c

^a Percentage of kernels invaded. Percentage of samples infected is in parentheses. Data are means of several counties within a district. Means within a year followed by common letters are not significantly different at $P \leq 0.05$. The 1993 data are from Trigo-Stockli et al (1995).

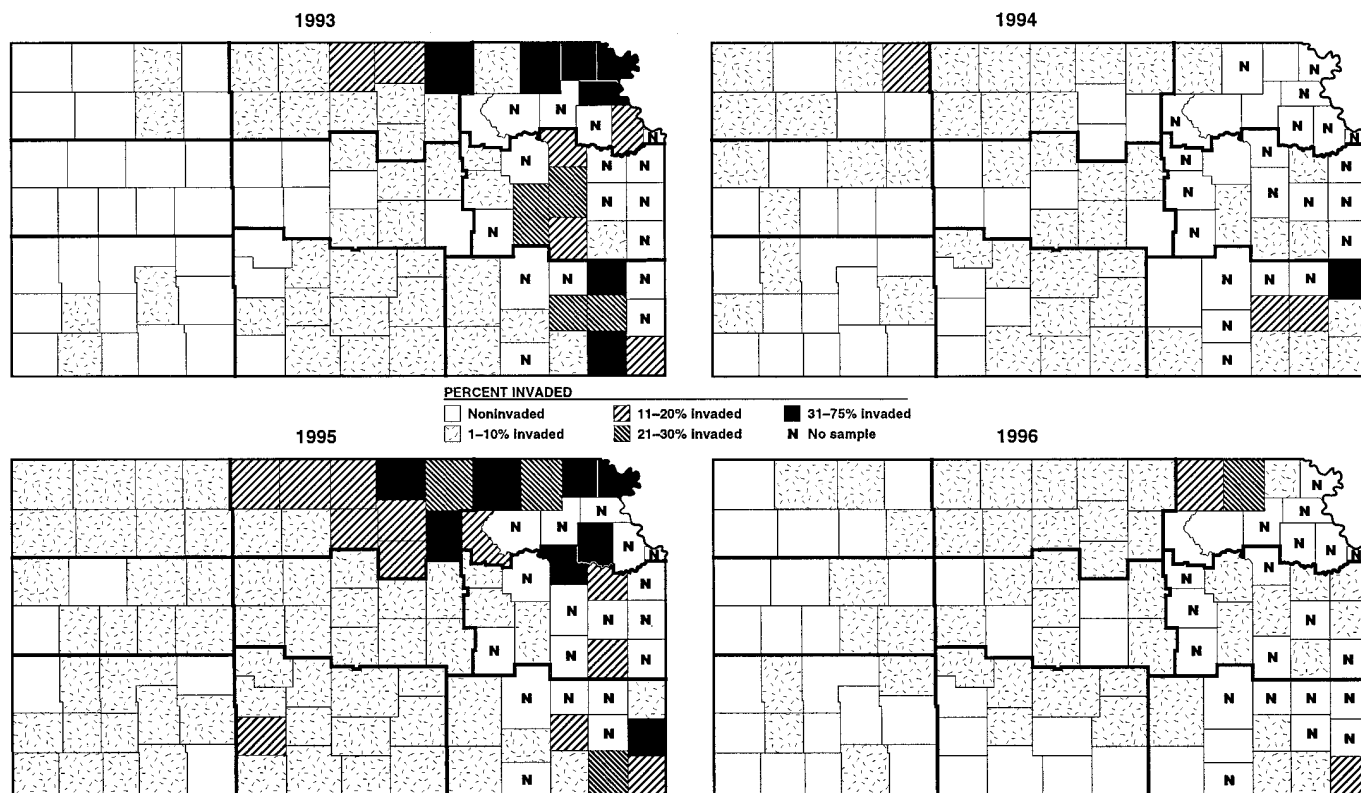


Fig. 1. Distribution of *Fusarium graminearum* in Kansas hard red winter wheat, 1993–1996.

Data Analyses

A general linear models procedure for unbalanced data (Milliken and Johnson 1992) was used to analyze *F. graminearum* infection (percentage of infected kernels) and DON levels. Comparisons among locations (districts) were made for each year. Arcsine (square root) transformation was performed to normalize percent *F. graminearum* infection data.

Correlation analysis was used to determine whether the level of *F. graminearum* infection was related to rainfall, temperature, or DON level. Monthly rainfall (mm) and temperature (°C, departures from 30-year normal) data were obtained from the Weather Data Library, Kansas State University. Averages of rainfall and temperature from April through July of each year in each district were used in correlation analysis. For correlation analysis of DON level and *F. graminearum* infection, all observations for each year were used.

For comparison of cultivars, 2163, Karl, and TAM 107 were used because they were widely grown during 1993–1996. Cultivars were compared yearly across districts. However, because certain cultivars were grown only in certain regions, *F. graminearum* infection also was compared among eastern (northeastern, east central, and southeastern districts), central (north central, central, and south central districts), and western (northwestern, west central, and southwestern districts) regions (Trigo-Stockli et al 1995).

RESULTS

Occurrence and Distribution of *F. graminearum*

F. graminearum infection of Kansas HRW wheat was observed during 1993–1996, but the distribution and severity of infection varied, depending on location and year of harvest. Samples from northeastern Kansas had the highest average percentages of ker-

nels infected in the 1993, 1995, and 1996 harvests. In 1994, when *F. graminearum* infection was low statewide, the southeastern district had the highest level of kernels infected at 5.9%. Generally, the western districts (northwestern, west central, and southwestern) had low levels of infection (Table I and Fig. 1).

The mean percentage of kernels infected was highest in 1995 (9.8%) and lowest in 1994 (1.4%) and 1996 (3.0%) (Table I). Moreover, in 1995, *F. graminearum* infection was more widespread to the west (Fig. 1). Levels of 1–10% infected kernels were more common in most counties in the central, south central, and western districts in 1995 than in the other three years. The 1995 harvest also had the highest number of samples (73%) with detectable *F. graminearum* compared with the 1993 (38%), 1994 (31%), and 1996 (25%) harvests.

However, the 1993 and 1995 harvests had the highest levels of *F. graminearum* infected kernels; individual HRW wheat samples had levels as high as 75 and 71%, respectively (data not shown).

TABLE II
Correlation Coefficients for Amount of Rainfall and Temperature Conditions^a vs. *Fusarium graminearum* Infection for 1993–1996

Year of Harvest	Amount of Rainfall	Temperature
1993	0.77***	0.35
1994	0.97***	-0.53
1995	0.57	0.76**
1996	0.88***	-0.04
Overall	0.72***	-0.19

^a Rainfall (mm) and temperature (°C, departures from 30-year normal) are averages of April through July, 1993–1996.

^b **, $P < 0.01$; ***, $P < 0.001$.

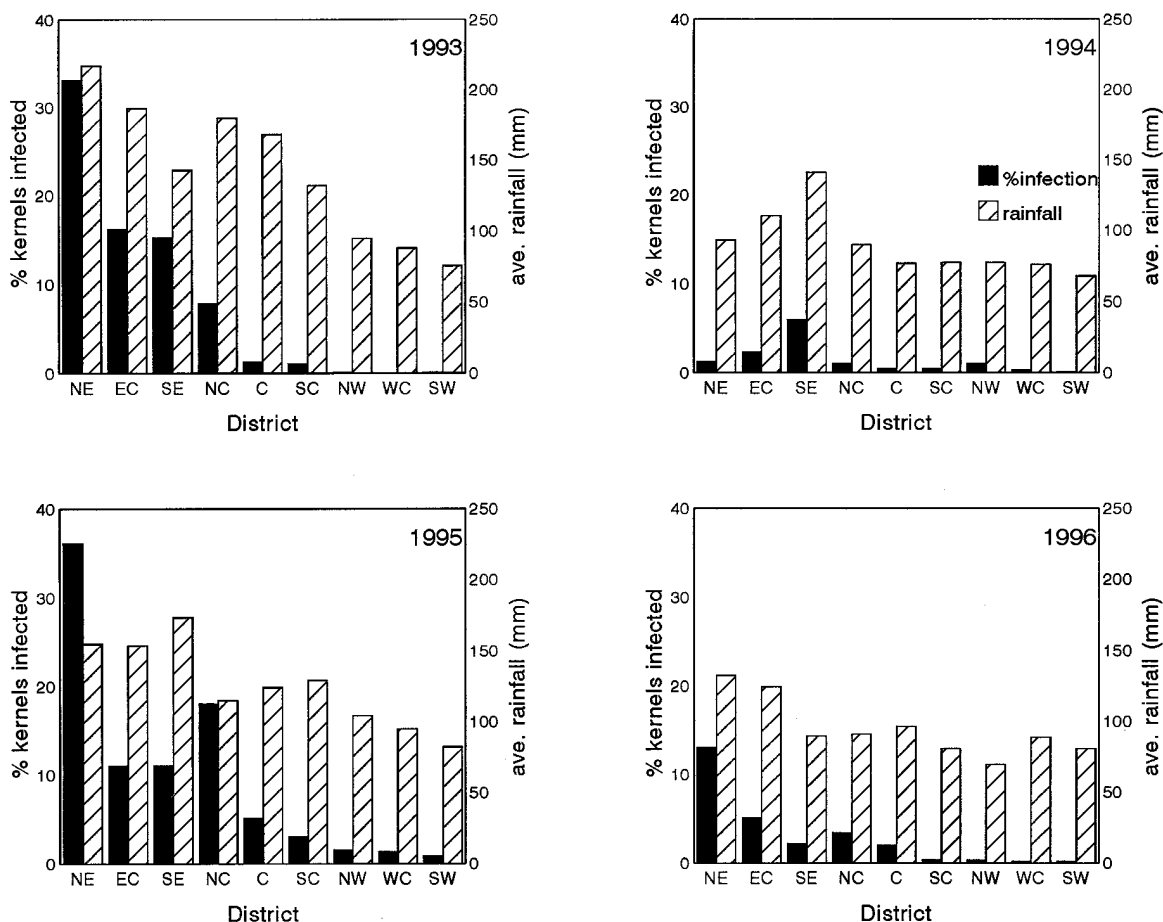


Fig. 2. Kernel infection (%) and average monthly rainfall (mm), April–July 1993–1996, in nine Kansas districts. N = north; E = east; C = central; S = south; and W = west.

The 1994 and 1996 harvests had lower levels of *F. graminearum*-infected kernels; the highest were 36 and 30%, respectively.

Effect of Rainfall and Temperature on *F. graminearum* Infection

Rainfall from April to July correlated positively with the level of *F. graminearum* infected kernels for the 1993, 1994, and 1996 harvests. No significant correlation was observed in the 1995 harvest (Table II). The districts that had high rainfall levels generally had high levels of infection (Fig. 2).

In 1993 and 1995, when rainfall levels were high in the eastern and north central districts, infection levels were also high. In 1994, when *F. graminearum* infection was low statewide, the southeastern district had the highest average percentage of kernels infected (5.9%) and also had the most rainfall (150 mm).

Temperature also affected the levels of *F. graminearum* infection. A significant positive correlation was observed between temperature and infection levels in 1995 (Table II). Temperatures in April through July of 1993 (although not significantly correlated) and 1995 were generally lower (Fig. 3), and infection levels were higher than in 1994 and 1996.

In addition to rainfall and temperature, cultivar differences affected the levels of kernels infected by *F. graminearum*. Of the three cultivars compared, 2163 and Karl had higher levels of kernels infected (average across years) than TAM 107 (Table III).

Previously (Trigo-Stockli et al 1995), we reported that the levels of *F. graminearum* infection of Karl differed among regions ($\chi^2 = 45$, $df = 4$, $P < 0.001$). A similar analysis of the levels of infection of Karl in relation to regions in this study indicated that

infection levels differed significantly for the 1994 harvest ($\chi^2 = 30$, $df = 4$, $P < 0.001$). Differences were less significant in 1995 ($\chi^2 = 8$, $df = 4$, $P = 0.079$), and no statistics were computed for 1996 because of the small sample size. This lack of sample size may have been because more farmers planted Karl 92 in 1996 than in previous years.

Levels of DON

DON levels were highest in the northeastern district in 1993, 1995, and 1996 and the southeastern district in 1994. They correlated significantly ($r = 0.84$ in 1993, 0.89 in 1994, 0.93 in 1995, and 0.84 in 1996; $P < 0.001$) with the level of *F. graminearum* infection. DON was generally not detected in the western districts except in one county in the northwest in 1996 (Fig. 4).

TABLE III
Fusarium graminearum Infection (% kernels) of Three Cultivars During Four Years^a

Year of Harvest	2163	Karl	TAM 107
1993	7.3 a	6.1 a	0.0 b
1994	0.7 b	2.0 a	0.1 b
1995	11.9 a	9.8 a	1.9 b
1996	1.3 b	4.4 a	0.3 b
Mean	5.3 a	5.6 a	0.6 b

^a Means in a row followed by the same letter are not significantly different at $P \leq 0.05$. The 1993 data are from Trigo-Stockli et al (1995).

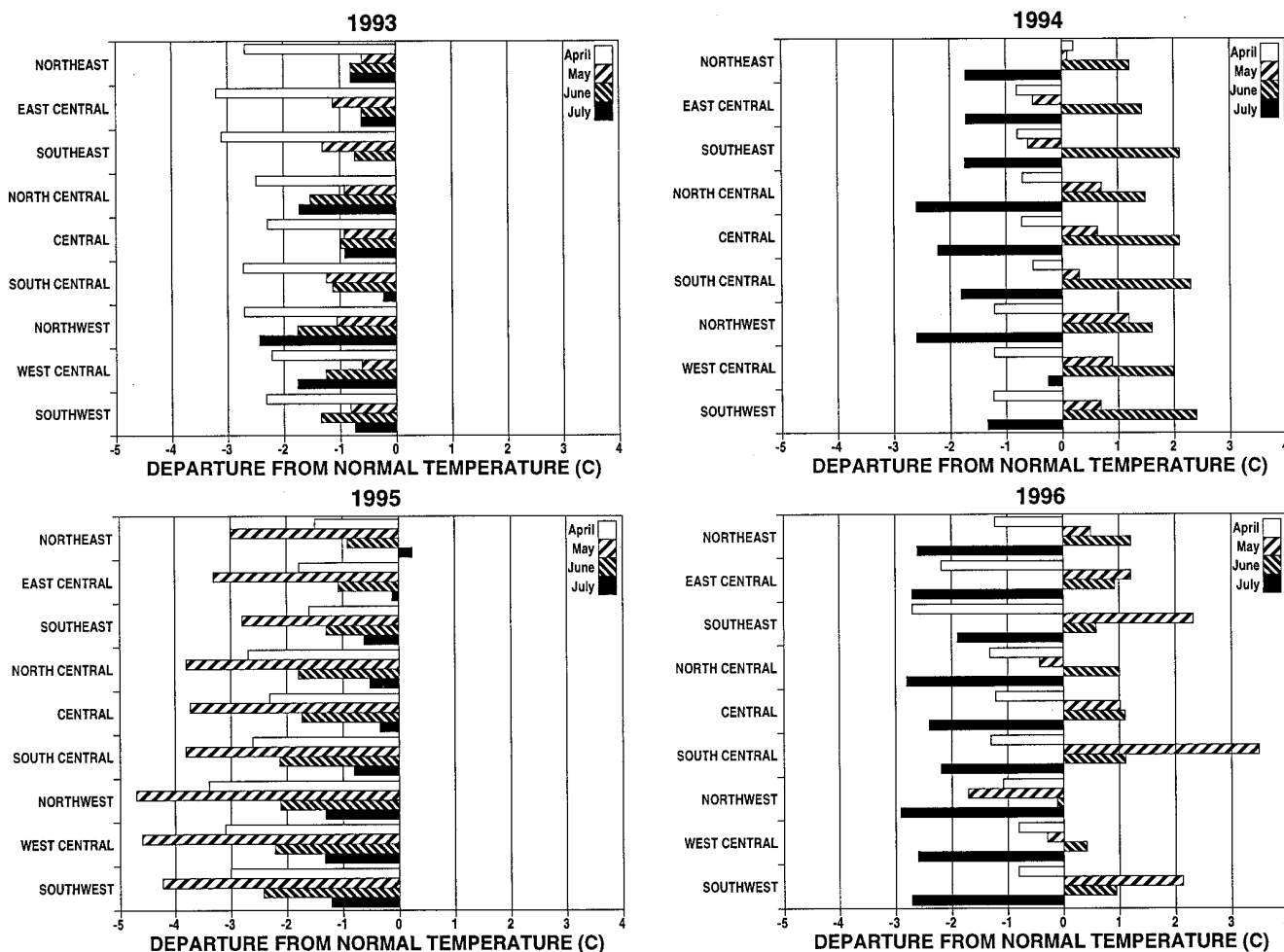


Fig. 3. Kansas mean temperature (°C) departures from the 30-year normal by district, April–July, 1993–1996.

DON contamination was more widespread in 1995 when *F. graminearum* infection was also widespread (Figs. 1 and 4). Overall, DON levels were higher in 1995 than in 1993, 1994, and 1996. On the basis of a production-weighted average, the statewide average DON levels were 0.10, 0.03, 0.21, and 0.16 ppm for 1993, 1994, 1995, and 1996, respectively. The northeastern district, which usually had >2 ppm of DON, also had the lowest wheat production acreage in Kansas (Kansas Agricultural Statistics 1996).

DISCUSSION

The levels of *F. Graminearum* infected kernels in Kansas HRW wheat during the 1993–1996 harvests varied. The differences in the levels of infection were influenced by the amount of rainfall (which is related to location), temperature from flowering until harvest (April–July), and cultivars. The levels of infection were higher in eastern than in western Kansas and higher in 1993 and 1995 (when rainfall levels were higher) than in 1994 and 1996. Love and Seitz (1987) reported a similar observation for the 1982 and 1983 Kansas HRW wheat crops.

Rainfall appeared to be an important factor affecting the severity of the disease. Severe scab infections occurred in 1993 and 1995 when the amount of rainfall was higher than that in 1994 and 1996. Most districts in Kansas had above-normal precipitation in July of 1993. In 1995, above-normal precipitation occurred in April, which coincided with the flowering stage of wheat. Cook (1981) reported that moisture during flowering has a major effect on the incidence of *Fusarium* head blight. Moreover, Andersen (1948) stated that after flowering, most wheat cultivars continue to be receptive to scab infection as long as the required moisture and temperature conditions are present.

The nonsignificant correlation between the amount of rainfall and infection level in 1995 indicates that other factors may be involved. Timing (Miller 1995) and duration (Hart et al 1984) of precipitation also affect invasion and toxin formation by *F. graminearum*. In controlled studies, Hart et al (1984) reported that longer exposure

of the wheat heads to wetness increased the severity of infection as well as DON production by *F. graminearum*.

Other factors such as relative humidity and amount of inoculum also affect *F. graminearum* infection. Although they were not measured in this study, Love and Seitz (1987) stated that *F. graminearum* grows ideally at or above 92–94% rh. Moreover, they reported that both average relative humidity and total number of hours of $\geq 90\%$ rh appear to be related to severity of scab.

The high number of HRW wheat samples invaded by *F. graminearum* and the widespread occurrence of scab infection in the western region in 1995 compared with 1993 also suggests that other factors such as duration and timing of rainfall affect *F. graminearum* infection. An example is the high percentage (80%) of TAM 107 samples that were infected in 1995, the highest in four years. TAM 107 was grown mostly in western and central Kansas, where levels of infection were usually low.

Cultivar differences also affected the level of infection by *F. graminearum*. From 1993 to 1996, 2163 and Karl had levels of infection higher than those of TAM 107. Because weather conditions are beyond control, information on cultivar differences is valuable. Cultivar resistance is an effective way to control *F. graminearum* infection. Other reports on the effect of cultivar differences on *F. graminearum* infection were published by Schroeder and Christensen (1963), Love and Seitz (1987), and Bai and Shaner (1996).

Our observation on the correlation of DON and the level of *F. graminearum* infection is similar to those previously reported (McMullen et al 1993, Trigo-Stockli et al 1995, Sinha and Savard 1997). Thus, in cropping years when rainfall levels are high from flowering to harvest, *F. graminearum* infection and DON level will be high.

During a crop year conducive to scab development (e.g., 1995), $\approx 7\%$ of the HRW wheat samples had DON levels of >1 ppm. This information is important, because the Food and Drug Administration has set an advisory level of 1 ppm for finished wheat products intended for human consumption. For grains and grain by-products intended for feed, however, the advisory levels are 5 ppm for swine

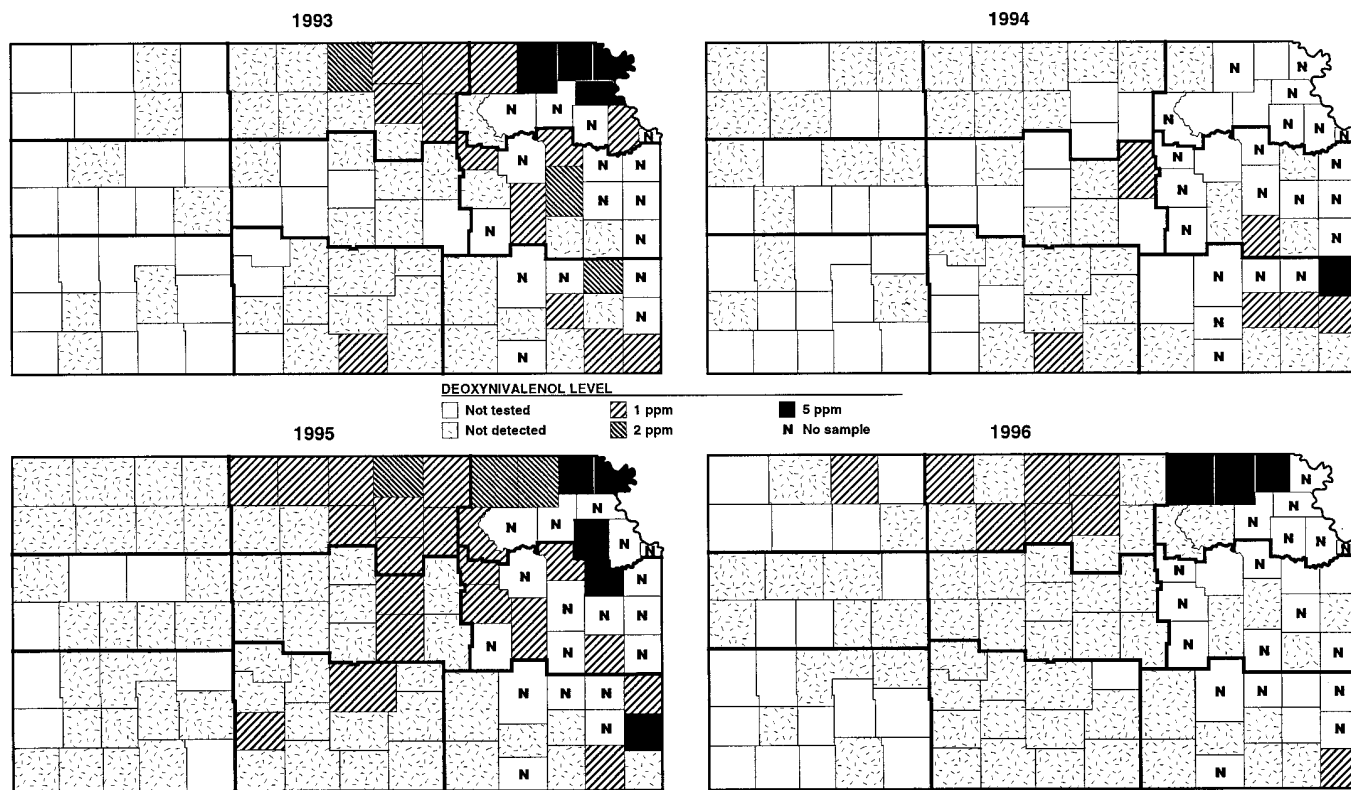


Fig. 4. Occurrence of deoxynivalenol in Kansas hard red winter wheat, 1993–1996.

and other animals and 10 ppm for cattle and chickens, depending on the ration (FDA 1993).

ACKNOWLEDGMENTS

We thank F. Anderson and C. M. Tan for preparing the figures, M. Knapp for providing the weather data, and C. Reed and G. Milliken for help in statistical analysis. This research was conducted in conjunction with Regional Project NC-129, Occurrence of Mycotoxins and the Implications to Animal and Human Health.

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[Received August 8, 1997. Accepted August 28, 1998.]