Effects of Location and Cultivar on Fusarium Head Blight (Scab) in Wheat from Kansas in 1982 and 1983[1]

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ABSTRACT

The occurrence of Fusarium head blight (scab) in wheat cultivars from test plots across Kansas in 1982 and 1983 was measured by assaying for deoxynivalenol (DON, a metabolite of *F. graminearum*), ergosterol (an index of total fungal invasion), and viable *F. graminearum* by seed plating. Infection levels were low in 1983 compared to 1982. In both years, locations in eastern, and to a lesser extent in north central, Kansas exhibited higher scab infection than areas to the west and south. Differences in infection levels among some locations were only partially explained by weather parameters, particularly in 1983. Cultivars commonly grown in the midwestern U.S. showed significant and consistent differences in susceptibility to scab infection that were generally not associated with maturity factors. Significantly positive correlations between DON, ergosterol, and viable *F. graminearum* indicated that *F. graminearum* was the fungus principally associated with scab and that it consistently produced DON. The ergosterol-to-DON ratio asymptotically approached 2.4 as degree of infection became severe.

In 1982, occurrence of the fungus *Fusarium graminearum* Schwabe and one of its metabolites, 4-deoxynivalenol (DON), was unusually high in parts of eastern and north central Kansas (Kansas Agricultural Experiment Station 1983). *F. graminearum* is generally considered to be the principal cause of Fusarium head blight (scab) in wheat in North America (Cook 1981, Sutton 1982). Because DON produces some toxicological effects in animals (Ueno 1983), there was concern regarding the possible use of contaminated wheat in food products and animal feed. Also, yields and grade quality of wheat were greatly reduced in some areas.

The scab disease is favored by continuously wet and warm weather during a critical stage of wheat maturation, usually from anthesis through soft dough stage of kernel development (Schroeder and Christensen 1963, Sutton 1982). Weather was favorable for scab infection during May and June of 1982 in some areas of Kansas, but actual relationships between weather conditions and stages of wheat maturation at specific locations across Kansas were not well documented.

In addition, Kansas State University specialists and others who visited many fields reported that certain cultivars were generally more infected than others. Differences in susceptibility to scab among cultivars have been observed in inoculated wheat (Atanasoff 1920, Christensen et al 1924, Takegami and Sasai 1967, Miller et al 1985), and some reports suggest that susceptibility varies with the stage of maturation at which inoculation occurs (Schroeder and Christensen 1963, Hart et al 1984).

To determine whether cultivars currently grown in the midwestern United States vary in susceptibility to scab, and to better assess effects of weather and location versus wheat maturity, we used chemical (ergosterol and DON) and microbiological (whole kernel plating) assays to measure the degree of scab infection in selected cultivars grown across Kansas in test plots where weather and wheat maturation records were kept. The results also provide information concerning the occurrence of DON in scabby wheat, as well as relationships among DON, ergosterol, and percentage of kernels with viable *F. graminearum*.

MATERIALS AND METHODS

Wheat Samples

Samples were combine-harvested from 12 performance test plots managed by the Kansas Agricultural Experiment Station (KAES) (Table I). Assays were performed on two cultivars (Agripro Hawk and Arkan) from all locations in 1982 and 1983, and on 18 cultivars from two locations (Powhattan and Ottawa) in 1982. One exception was that Agripro Hawk was not available from the Manhattan plot in 1982. Seed samples were stored at 4° C. Samples were ground (about 250 g) immediately before extractions for ergosterol and DON assays.

Chemical Assays

DON was determined by the method of Seitz and Bechtel (1985) and ergosterol by the method of Seitz et al (1979). Final extracts were analyzed with a Hewlett-Packard model 1084B high-performance liquid chromatograph (HPLC) equipped with a 1040A photodiode array detector. Chromatograms are formed by monitoring the difference between absorbances at sample and reference wavelengths. For all analyses the reference wavelength

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was centered at 550 nm with a 50-nm bandwidth. Sample wavelengths, each with 8-nm bandwidth, were centered at 224 for DON and 282 for ergosterol.

The analytical column (3.9 × 300 mm) contained C_{18} packing of 5-μm particle size (two 15-cm "Resolve" columns from Waters Associates, Milford, MA, placed in series). A guard column (C_{18}, 5-μm, 4.6 × 30 mm; Brownlee Laboratories, Santa Clara, CA) was positioned between the analytical column and the injector. Column temperature was 50° C. Mobile phases were methanol-water mixtures, 19:81 (V:V) at 0.80 ml/min for DON and 95:5 (V:V) at 1.0 ml/min for ergosterol. Volume injected was 20 μl for both assays.

All samples were extracted in duplicate, and each final extract was analyzed in duplicate by HPLC.

Whole-Seed Plating

The assay for viable fungi in surface-sterilized, whole kernels was a modified method of Sauer et al (1984). Kernels were rinsed 10-15 sec in 100% ethanol, shaken for 1 min in 2% sodium hypochlorite (Clorox), and rinsed in sterile water. Then 100 seeds were placed on Difco malt agar containing 4% NaCl and 200 ppm of Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO). After 5–7 days at 25° C, fungi growing from the kernels were identified and counted with the aid of a dissecting microscope. Fusaria producing red pigmentation were categorized as *F. graminearum*. Fusaria that produce bright red pigments in malt agar with 4% NaCl are primarily *F. graminearum*, and their prevalence in wheat from the midwestern United States in most years rarely exceeds 1–2% kernel infection (D. B. Sauer, *personal communication*).

TABLE I
Deoxynivalenol (DON) and Ergosterol Concentrations (ppm) and Percentages of Kernels Infected with *F. graminearum* in Agripro Hawk and Arkan Cultivars Grown at 12 Kansas Agricultural Experiment Station Plots in 1982 and 1983

		1982			1983		
Location[a]	Cultivar	DON[b] (ppm)	Ergosterol[b] (ppm)	*F. gram.* (%)	DON[b] (ppm)	Ergosterol[b] (ppm)	*F. gram.* (%)
Powhattan (NE)	Hawk	10.56	25.10	74	1.29	3.20	5
	Arkan	1.46	7.40	52	0.12	1.97	0
Ottawa (EC)	Hawk	10.42	27.45	68	1.21	5.53	12
	Arkan	1.78	10.13	45	0.11	4.28	9
Parsons (SE)	Hawk	0.83	8.32	21	1.35	4.06	8
	Arkan	0	4.24	0	0.32	3.24	6
Manhattan (NE)	Hawk	NS[c]	NS	NS	0.02	3.42	8
	Arkan	0	4.74	8	0	4.06	2
Belleville (NC)	Hawk	2.26	11.76	32	0.23	2.03	2
	Arkan	0	4.13	7	0	1.33	0
Hesston (SC)	Hawk	0.47	5.72	17	0.02	3.32	1
	Arkan	0	4.09	3	0	2.66	4
Hutchinson (SC)	Hawk	0	3.37	4	0	4.89	0
	Arkan	0	4.07	4	0	5.08	5
St. John (SC)	Hawk	0	2.54	2	0	2.12	0
	Arkan	0	2.42	7	0	3.05	3
Hays (C)	Hawk	0	3.19	0	0	1.58	0
	Arkan	0	2.51	0	0	1.54	2
Colby (NW)	Hawk	0.14	3.29	4	0	1.15	1
	Arkan	0	4.23	3	0	1.38	0
Garden City (SW)	Hawk	0	3.48	2	0	2.47	0
	Arkan	0	4.12	0	0	2.06	0
Tribune (WC)	Hawk	0	2.63	0	0	0.84	0
	Arkan	0	3.24	0	0	1.12	0

[a] Plots were near the cities listed and within the following geographical divisions: northeast (NE), east central (EC), southeast (SW), north central (NC), south central (SC), central (C), northwest (NW), southwest (SW), west central (WC).
[b] Each value is a mean of duplicate assays. Coefficients of variation of duplicates averaged 10.4 and 3.6% for DON and ergosterol, respectively.
[c] NS = No sample was available.

TABLE II
Temperature, Rainfall, and Relative Humidity (rh) at Kansas Agricultural Experiment Station Locations in 1982 and 1983 During a 25-Day Period Following the Heading Date for Agripro Hawk Cultivar

	1982								1983							
		Temperature (° C)			Rainfall[a]		rh[b]			Temperature (° C)			Rainfall[a]		rh[b]	
Location	Heading Date	Av. Max	Range	Av. at rh ≥90%	No. Days	Amt (cm)	Av. (%)	Hr at ≥90%	Heading Date	Av. Max	Range	Av. at rh ≥90%	No. Days	Amt (cm)	Av. (%)	Hr at ≥90%
Powhattan[c]	May 24	24	6–31	15.0	13	18.9	79	156	May 30	27	6–34	17.2	10	9.6	70	108
Ottawa	May 17	24	6–32	15.0	17	22.4	79	156	May 25	27	6–31	17.2	10	6.2	70	108
Parsons	May 10	24	8–32	···	16	17.6	···	···	May 16	23	5–29	···	12	5.3	···	···
Manhattan	May 17	24	5–32	···	18	15.8	···	···	May 22	NA	NA	···	NA	NA	···	···
Belleville	May 20	23	4–33	15.8	11	16.5	78	156	June 4	28	7–34	17.4	9	11.7	76	171
Hesston[c]	May 17	26	6–33	13.9	14	12.1	71	30	May 19	25	6–31	14.0	11	10.6	75	135
Hutchinson	May 10	24	7–29	13.9	21	17.2	71	30	May 19	22	4–31	14.0	18	11.8	75	135
St. John[c]	May 13	24	7–32	···	21	7.5	···	···	May 18	26	3–34	···	18	16.9	···	···
Hays	May 17	22	7–31	···	15	9.0	···	···	May 25	26	5–31	···	9	3.2	···	···
Colby	May 21	21	5–29	12.1	14	11.3	72	99	May 29	25	3–36	12.4	13	5.0	66	51
Garden City	May 14	23	7–32	14.3	13	4.4	69	18	May 27	25	7–36	14.0	13	13.6	65	36
Tribune	May 17	25	6–31	···	8	4.6	···	···	May 30	27	5–35	···	15	13.8	···	···

[a] Trace rainfall was estimated to be 0.01 cm.
[b] Relative humidity data were from weather reporting stations at Topeka (for Powhattan and Ottawa), Concordia (for Belleville), Wichita (for Hesston and Hutchinson), Goodland (for Colby), and Dodge City (for Garden City).
[c] Weather reporting stations for Powhattan, Hesston, and St. John were at Horton, Newton, and Larned, respectively.

Assessment of Weather Versus Scab Infection

To assess effects of weather on the geographical distribution of scab infection, we compiled weather data for a 25-day period following anthesis of Agripro Hawk wheat. This coincided with the period from anthesis to soft dough stage of kernel development, when wheat is most receptive to *F. graminearum* infection (Schroeder and Christensen 1963, Sutton 1982). Also, information from structural studies of mature wheat kernels suggested that the fungus does much of its damage between the second and third week after anthesis (Bechtel et al 1985). The date when heads emerged (heading date) was used as the start of the compilation period, because it was recorded by KAES and preceded anthesis by only one to two days.

Temperature and rainfall data were available from recording stations at or near the plot locations (U.S. Department of Commerce 1982a, 1983a). Other weather data were obtained from regional recording stations at Concordia, Dodge City, Goodland, Topeka, and Wichita (U.S. Department of Commerce 1982b, 1983b). Relative humidity was recorded at 3-hr intervals.

RESULTS AND DISCUSSION

Weather and Location Effects

Results from DON, ergosterol, and whole-seed plating assays of wheat samples from test plots across Kansas showed that scab was most severe in eastern Kansas for 1982 and 1983, and that infection levels were considerably lower in 1983 than in 1982 (Table I). This geographical distribution of scab infection is in general agreement with that reported previously for 1982 (KAES 1983). Because the cultivar Agripro Hawk was susceptible to scab infection by *F. graminearum* (cultivar susceptibility is discussed below), it was more sensitive than most of the other cultivars for indicating the potential for scab to occur at a location. In 1982, DON content in Hawk ranged from about 10 ppm at Powhattan and Ottawa in the east to undetectable levels in the west, whereas DON in Arkan reached only about 1.5 ppm and was detectable at only Powhattan and Ottawa. In 1983 wheat from locations where scab was observed, Hawk was again more infected than Arkan (Table I). At the southeast Kansas plot near Parsons, infection was more severe in 1983 than in 1982. Lack of infection at Manhattan in 1983 was surprising given its proximity to infected areas to the west and east. Unfortunately, no sample was available for Manhattan in 1982.

Temperatures during the compilation period were fairly uniform across Kansas in 1982 and 1983 (Table II). The averages of the daily maximum and minimum temperatures at all locations in 1982 and 1983 were 25 ± 2 and 12 ± 2° C, respectively. The daily maximum temperatures were generally within the 21–29° C range favorable for fungal growth (Booth 1971, Cook 1981), but the uniformity of temperature lessened its effect on the geographical pattern of scab infection. The only weather factor to indicate a possible correlation with scab infection in 1983 was the average temperature when relative humidity was ≥ 90% (Table III).

The frequency of rainfall was not related to the geographical pattern of scab infection, because rains were as frequent in western as in eastern Kansas (Table II). However, the amount of rainfall received at each location for this period appeared to be related to severity of scab in 1982 (Table III). The amount of rainfall was considerably greater in 1982 than in 1983, and for both years it was generally greater in eastern than in western Kansas. Neither frequency nor amount of rainfall apparently had an effect on scab in 1983 (Table III).

Because the fungus grows ideally at or above 92–94% relative humidity (rh), rh was expected to be a factor in infection. Unfortunately, rh data were available only from regional weather reporting stations as identified in Table II. Both average rh and total number of hours when rh was ≥ 90% appeared to be related to severity of scab in 1982 but not in 1983 (Table III). The rh at most locations was lower in 1983 than in 1982, which is in accord with the generally lower levels of scab infection in 1983 compared to 1982. Poor correlations in 1983 for rh versus scab were probably caused by the low infection levels and by rh being higher in noninfected north central and southcentral areas than in the northeast area where scab was definitely present (Tables I and II).

TABLE III
Correlation Coefficients for Weather Conditions[a] Versus Deoxynivalenol Content of Agripro Hawk Cultivar from all Kansas Agricultural Experiment Station Locations

Weather Condition	1982	1983
Days of rainfall	−0.03	−0.41
Amount of Rainfall	0.70*[b]	−0.40
Av. relative humidity (rh)	0.85***	−0.08
Hours at rh ≥90%	0.76**	0.12
Av. temp. when rh ≥90%	0.54	0.77**

[a] During a 25-day period following the heading date of Agripro Hawk cultivar.
[b] Significance levels: * = 5%, ** = 1%, *** = 0.1%.

TABLE IV
Deoxynivalenol (DON) and Ergosterol (ERG) Concentrations (ppm) and Percentages of Kernels with Fungi in 18 Wheat Cultivars from Two Eastern Kansas Locations in 1982[a]

	Ottawa						Powhattan					
				Percent Kernels with						Percent Kernels with		
Cultivar[b]	Heading Date (May)	DON[c] (ppm)	ERG[c] (ppm)	*F. graminearum*	*Alternaria*	No Fungi	Heading Date (May)	DON[c] (ppm)	ERG[c] (ppm)	*F. graminearum*	*Alternaria*	No Fungi
Agripro Hawk	17	10.4	27.4	68	38	2	24	10.6	25.1	74	33	0
Agripro Rocky (x)	22	13.1	29.6	50	40	9	24	9.8	24.5	68	29	0
Centurk 78 (x)	20	7.5	20.8	36	43	11	24	6.4	20.7	51	40	4
Bennett	23	7.1	18.5	22	42	15	24	2.2	10.0	32	45	9
Parker 76	17	6.5	15.3	45	54	1	24	3.5	8.5	33	64	1
TAM 105	16	4.8	14.7	26	56	9	23	3.6	11.1	28	54	3
Newton (y)	19	4.6	21.8	34	52	7	23	2.0	10.8	42	49	4
KS75210 EXP (y)	19	3.9	22.4	45	45	6	25	2.5	11.2	48	43	1
Migro Archer	18	3.4	19.5	41	38	14	24	3.5	16.7	42	38	4
Buckskin	21	3.4	14.1	25	57	7	26	2.7	9.8	28	51	4
Pike	17	3.1	15.0	37	54	8	23	2.7	9.3	37	51	3
Vona (z)	15	3.2	17.0	32	45	22	22	3.4	11.1	51	43	0
Larned	19	2.8	15.3	25	43	15	23	2.6	8.6	29	56	4
Scout 66	20	2.7	12.3	17	56	13	23	1.9	10.3	37	47	0
Hart	18	1.8	10.9	20	54	9	24	1.7	8.5	36	45	8
Arkan	17	1.8	10.1	45	69	1	21	1.5	7.4	52	60	0
Agripro Wings (z)	14	1.8	10.7	38	53	12	23	1.6	8.0	46	44	2
Triumph 64	12	1.2	8.0	17	46	10	20	0.9	6.2	22	46	5

[a] Also present were other species of *Fusarium, Cladosporium, Epicoccum, Curvularia, Helminthosporium, Nigrospora*, and *Phoma*.
[b] Cultivars followed by like letters (x, y, or z) have the same genetic parentage.
[c] Each value is a mean of duplicate assays. Coefficient of variation of duplicates averaged 5.1 and 3.6% for DON and ergosterol, respectively.

TABLE V
Correlation Coefficients Between Deoxynivalenol (DON), Ergosterol, and Percent of Seeds with *F. graminearum*[a]

Correlation	Agripro Hawk at All Locations		18 Cultivars in Eastern Kansas	
	1982	1983	Powhattan	Ottawa
DON vs. *F. graminearum*	0.97***[b]	0.76**	0.77***[b]	0.59**
DON vs. ergosterol	0.98***	0.55	0.95***	0.86***
Ergosterol vs. *F. graminearum*	0.99***	0.66*	0.78***	0.66**

[a] Data for Agripro Hawk cultivar from all locations in 1982 and 1983, and for 18 cultivars from two eastern Kansas locations in 1982.
[b] Significance levels: * = 5%, ** = 1%, *** = 0.1%.

Comparison of weather conditions with levels of scab infection suggests that some factor(s) in addition to weather may regulate degree of scab infection at a location. Eastern locations, particularly Ottawa and Powhattan, consistently had more scab than locations to the west, even though some of the latter had similar or worse weather conditions. This was exemplified by Belleville compared to Ottawa and Powhattan in 1982 and 1983, and by Hutchinson and Hesston compared to Ottawa in 1983 (Tables I and II). In 1982, the relatively low number of hours with rh $\geq$ 90% might have suppressed infection at Hutchinson and Hesston. Nevertheless, the potential for infection in the south central area around Hutchinson appears to be low compared to areas to the east and north. The amount of *F. graminearum* inoculum available to infect wheat heads is probably an important factor in the differences among locations. For whatever reasons (i.e., generally more rainfall, consistently higher rh, favorable substrates, or cropping practices) inoculum levels may be considerably higher in eastern and northeastern Kansas than in other areas of the state. Unfortunately, data concerning the availability of fungal inoculum were not monitored at any of the locations.

Cultivar Effect

Degree of scab infection varied widely among 18 cultivars grown at Ottawa and Powhattan in 1982 (Table IV), and none of the cultivars was free of scab at either location. Evidently, the variation was a representation of genetic susceptibilities to *F. graminearum* because 1) DON concentration in cultivars from Ottawa was correlated with that of cultivars from Powhattan ($r = 0.90$, $P < 0.001$), 2) cultivars with the same genetic parentage (Table IV) exhibited similar susceptibility to infection, and 3) at each location cultivars with identical or similar heading dates had widely different degrees of scab infection (Table IV), i.e., consider Arkan versus Hawk and Buckskin versus Rocky at Ottawa and the narrow range in heading dates at Powhattan. Precipitation records indicate that even the earliest maturing cultivar, Triumph 64, did not escape wet conditions before and after heading.

For many years, differences in susceptibility to scab among wheat cultivars have been noted (Atanasoff 1920, Christensen et al 1924, Takegami and Sasai 1967, Miller et al 1985, Yu 1982), and other factors affecting susceptibility have been examined (Schroeder and Christensen 1963, Pearce et al 1976, Cook 1981, Sutton 1982, Miller et al 1985). Many of these observations were based on experimental inoculations or controlled environments. Results from our analyses of naturally produced grains indicated that cultivars currently grown in the midwestern United States differ in susceptibility to *F. graminearum*. Therefore, the planting of susceptible cultivars should be discouraged in areas with relatively high potential for scab infection. We do not know why the cultivars are different. Observations of susceptible and resistant cultivars growing in a KAES plot near Manhattan in 1984 did not reveal morphological differences in heads or spikelets (i.e., openness of the head or extent of anther extrusion) that might influence susceptibility.

Relationships Between DON, Ergosterol, and Viable Fungi

Results from DON, ergosterol, and viable *F. graminearum* assays used to assess the degree of scab infection were strongly correlated (Table V). Reduced correlations between the parameters in Hawk from all locations in 1983 were probably caused by considerably lower degrees of scab infection in 1983 than in 1982. High positive correlation of DON with viable *F. graminearum* shows that DON was produced consistently by *F. graminearum* and recommends DON as an indicator of scab infection. Strong positive correlations of DON and viable *F. graminearum* with ergosterol suggests that *F. graminearum* was the primary fungus causing the elevated ergosterol concentrations in wheat with moderate to severe scab infection.

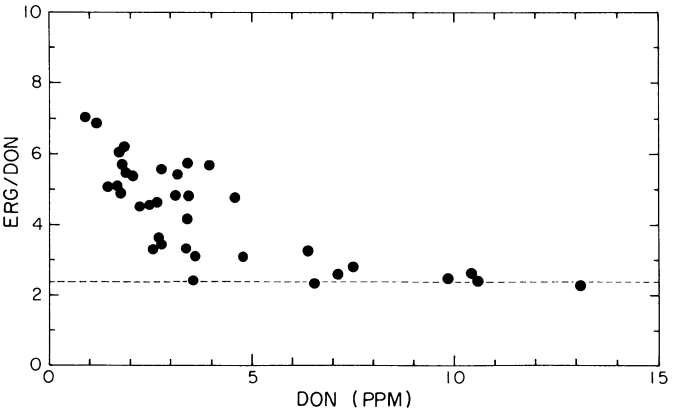

Fig. 1. Ergosterol-to-deoxynivalenol (**ERG/DON**) concentration ratios vs. deoxynivalenol (**DON**) concentration for 18 cultivars from Powhattan and Ottawa locations in 1982.

The ergosterol-to-DON ratio in the naturally infected winter wheats we studied asymptotically approached 2.4 as degree of scab infection became severe, i.e., DON concentration > 5 ppm (Fig. 1). This same ratio was observed by Miller and co-workers (1985) in susceptible, experimentally inoculated cultivars of spring wheats grown in Canada. They suggested that the ratio could be an index of susceptibility to DON synthesis in a wheat cultivar, i.e., a high ratio indicating a resistant cultivar and vice versa. Such use of the ratio would be appropriate only if *F. graminearum* were the only, or at least the predominant, fungus growing in the grain. Because other fungi are present in naturally inoculated wheat (Table IV), the ergosterol-to-DON ratio would depend on the growth of *F. graminearum* compared to other fungi. Therefore, a low degree of *F. graminearum* infection, as indicated by a low DON concentration, should give a high ergosterol-to-DON ratio, and this is generally what was observed in the 18 cultivars from Ottawa and Powhattan (Fig. 1). Also, the ergosterol-to-DON ratio in Hawk cultivar in 1982 was strongly influenced by the degree of scab infection (DON concentration) at a location, i.e., the ratio was 2:4, 2:7, 5:2, 10:0, 12:2, and 23:5 at Powhattan, Ottawa, Belleville, Parsons, Hesston, and Colby, respectively.

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