

Occurrence of *Fusarium* Species in Scabby Wheat from Minnesota and their Pathogenicity to Wheat

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ABSTRACT

Wilcoxson, R. D., Kommedahl, T., Ozmon, E. A., and Windels, C. E. 1988. Occurrence of *Fusarium* species in scabby wheat from Minnesota and their pathogenicity to wheat. *Phytopathology* 78:586-589.

Fifteen *Fusarium* species were identified in 23,726 isolates obtained from scabby spring wheat. The wheat was collected in 1984, 1985, and 1986 from farm fields and agricultural experiment stations in 24 Minnesota counties located in the wheat growing areas of the state. *Fusarium graminearum* comprised 75% of the isolates, *F. poae* 17%, and the other species (*F. equiseti*, *F. sporotrichioides*, *F. acuminatum*, *F. oxysporum*, *F. semitectum*, *F. moniliforme*, *F. avenaceum*, *F. subglutinans*, *F.*

proliferatum, *F. sambucinum*, *F. tricinctum*, and *F. crookwellense*) 1 to 2%. Most of the *F. graminearum* isolates produced perithecia in culture. *F. graminearum* and *F. culmorum* infected spikelets of Era wheat in glasshouse tests and spread from the inoculated spikelets into more than half of the spikelets in the spikes. Infection by the other 11 *Fusarium* species tested was confined to inoculated spikelets.

Additional key words: *Gibberella zeae*, *Triticum aestivum*.

Head blight or scab of wheat (*Triticum aestivum* L.) is destructive in the humid and semihumid areas of the world where wheat is grown. In Minnesota, incidence of affected spikes within fields may range from a trace to 100% each year; at approximately 5-yr intervals, scab is widespread and epidemic, especially in southern and western regions of the state (*unpublished*). In 1983 and 1986, scab was common in the Red River Valley of northwest Minnesota, which caused concern because previously this major wheat producing area had been relatively free from the disease.

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch) is reported to be the most common and destructive cause of scab in the United States. There are, however, reports of other *Fusarium* species associated with wheat scab (9-11, 14, 15) or wheat kernels (2, 4). The purpose of our study was to identify *Fusarium* species associated with scabby wheat in Minnesota, report their distribution in the state, and evaluate their pathogenicity. A preliminary report has been made (6).

MATERIALS AND METHODS

Isolation of *Fusarium* species. During July and August of 1984, 1985, and 1986 spikes of spring wheat with symptoms of scab were collected from farm fields and experimental plots throughout Minnesota. At each collection site, 25-100 spikes were collected. Collections were at 14 sites in 11 counties in 1984, at 23 sites in 10 counties in 1985, and at 12 sites in 12 counties in 1986. Samples were collected from 24 Minnesota counties. All kernels from collected spikes were dipped in 95% ethanol, immersed in 0.5% NaOCl for 30 sec, rinsed in sterile distilled water, and assayed for fungi. From two sites in 1984, spikes were threshed in the field, the kernels bulked, and shriveled kernels were selected for isolation.

Surface-treated kernels were placed on pentachloronitrobenzene-peptone agar (PCNB) (7) supplemented with chlorotetracycline and streptomycin sulfate, and incubated at 24 C for 6 or 7 days. Petri dishes were rechecked after 14 days for slow-growing isolates. All isolates were transferred to potato-dextrose agar (PDA) made with fresh potatoes and incubated at 24 C at 5,300 lx for 14-21 days to stimulate formation of macroconidia and perithecia. Light sources were fluorescent and

near ultraviolet lamps. All *F. graminearum* cultures were kept on PDA until the medium began to dry and curl in the dish to favor formation of perithecia both on upper and lower surfaces of the dried agar. If no perithecia formed on the drying PDA, cultures were transferred to carnation leaf agar (CLA), which favors perithecia formation (13). *Fusarium* species were identified according to Nelson et al (8).

Pathogenicity of *Fusarium* species. Tests were made in 1985 and 1986 with four isolates of each *Fusarium* species, except that one isolate was used for *F. sambucinum* and *F. tricinctum*. The isolates were from wheat kernels during 1984 and 1985. Pathogenicity of *F. proliferatum* and *F. crookwellense* was not studied because these species were found only in 1986 after the pathogenicity test was completed. Each year the tests were made on Era wheat in a glasshouse during March and April at about 21 C, but temperatures were not controlled for short periods.

Fusarium species were grown in 9-cm dishes on PDA for 14 days, after which two cultures of each isolate were comminuted in a blender containing 100 ml of sterile water. About 0.2 ml of the suspension of hyphae and conidia was injected into each of two spikelets located near the middle of the spike using a hypodermic syringe fitted with a 21-gauge needle. Spikelets were inoculated when spikes were at development stages ranging from emergence to kernels 3/4 filled. Control plants were inoculated with a suspension made from sterile agar. After inoculation, plants were placed in a mist chamber for 48 hr. The number of spikes and spikelets inoculated with each *Fusarium* species is shown in Table 1. The number of necrotic spikelets was recorded 3 wk after inoculation.

The kernels from inoculated spikes were surfaced-treated with ethanol and NaOCl and placed on PCNB agar as outlined above. *Fusarium* species that grew from the kernels were transferred to PDA and identified.

RESULTS

***Fusarium* species isolated.** Fifteen species of *Fusarium* were isolated during 1984, 1985, and 1986 from scabby wheat spikes collected in Minnesota. The wheat cultivars are not reported, but Era, Marshall, and Wheaton were widely grown in Minnesota during the study. In decreasing order of frequency of isolation from the spikes, the species were: *F. graminearum* Schwabe, *F.*

poae (Peck) Wollenw., *F. equiseti* (Corda) Sacc. sensu Gordon, *F. sporotrichioides* Sherb., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. culmorum* (W. G. Smith) Sacc., *F. oxysporum* Schlecht. emend. Syd. & Hans., *F. semitectum* Berk. & Rav., *F. moniliforme* Sheldon, *F. avenaceum* (Fr.) Sacc., *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas, *F. proliferatum* (Matsushima) Nirenberg, *F. sambucinum* Fuckel, *F. tricinatum* (Corda) Sacc., and *F. crookwellense* Burgess, Nelson & Toussoun.

Prevalence of *Fusarium* species. During the 3-yr study, 23,726 isolates of *Fusarium* species were identified (Table 2). *F. graminearum* constituted 75% of the isolates, *F. poae* 17%, and each of the other 13 species not more than 2%. Such low frequencies indicate that their role in scab development probably was negligible.

During 1985 and 1986, when precise records were kept on both numbers of spikes and kernels, *F. graminearum* was most prevalent, being isolated from 67% of spikes and 70% of kernels. *F. poae* was second most prevalent, occurring in 54% of spikes and 24% of kernels. The other *Fusarium* species occurred in fewer than 10% of spikes and fewer than 4% of kernels (Table 3).

Of 17,796 cultures of *F. graminearum* studied, 99.8% produced perithecia on PDA or CLA in the laboratory. Most of the perithecia checked at random were mature and contained asci and ascospores.

Distribution of species by county. *F. graminearum* occurred in each of the 24 Minnesota counties sampled and *F. poae* in 22 of them. These two species were followed in frequency of occurrence by *F. equiseti*, *F. sporotrichioides*, *F. acuminatum*, and *F. semitectum* in 12–15 of the counties. The remaining species were in not more than eight of the counties. *F. culmorum* occurred in five of the nine counties in northwestern Minnesota and Ramsey County in east central Minnesota. The occurrence in Ramsey County probably was not natural, because the fungus was found only in 1986 near some plants that had been inoculated. *F. moniliforme* occurred primarily in the east central and southern areas although trace amounts were found in west central and northwestern areas. *F. moniliforme* occurred most frequently in kernels that had been air-blast inoculated with *Ustilago tritici* (Pers.) Rostrup in Ramsey County.

Pathogenicity of *Fusarium* species. All spikes of Era inoculated

TABLE 1. Pathogenicity of 13 *Fusarium* species collected from scabby wheat inoculated on *Triticum aestivum* 'Era' in the glasshouse

<i>Fusarium</i> species ^a	Spikes inoculated (no.)	Spikelets ^b in spikes (no.)	Spikelets inoculated (no.)	Spikelets necrotic (%) ^c	Range necrotic spikelets/spike (no.)
<i>F. graminearum</i>	105	1,785	210	51	12–100
<i>F. poae</i>	103	1,751	206	12	6–24
<i>F. equiseti</i>	103	1,751	206	10	0–18
<i>F. sporotrichioides</i>	104	1,768	208	14	6–29
<i>F. acuminatum</i>	114	1,938	228	12	6–24
<i>F. semitectum</i>	98	1,666	196	12	6–24
<i>F. oxysporum</i>	106	1,802	212	1	0–24
<i>F. culmorum</i>	114	1,938	228	78	12–100
<i>F. moniliforme</i>	103	1,751	206	11	6–18
<i>F. avenaceum</i>	108	1,836	216	10	0–24
<i>F. subglutinans</i>	115	1,955	230	11	0–18
<i>F. sambucinum</i>	76	1,292	152	14	6–41
<i>F. tricinatum</i>	35	595	70	10	0–12
Control ^d	38	646	76	1	0–6

^a Four isolates of each *Fusarium* species tested except for one isolate each of *F. sambucinum* and *F. tricinatum*.

^b Calculated as mean of 17 spikelets per spike.

^c Calculated from spikelets in spikes.

^d Plants inoculated with culture medium only.

TABLE 2. Percentage of *Fusarium* isolates in different species from scabby wheat in Minnesota and percentage and range for each species in four areas of the state in 1984, 1985, and 1986

<i>Fusarium</i> species	(%)	Northwest ^b		West Central ^c		East Central ^d		Southern ^e	
		(%)	Range	(%)	Range	(%)	Range	(%)	Range
<i>F. graminearum</i>	75	85	0–100	82	50–100	46	21–87	88	1–96
<i>F. poae</i>	17	9	0–51	15	0–49	38	>–43	9	1–96
<i>F. equiseti</i>	2	<1	0–14	<1	0–1	6	0–9	1	0–17
<i>F. sporotrichioides</i>	2	1	0–17	2	0–4	3	0–8	<1	0–2
<i>F. acuminatum</i>	2	2	0–40	<1	0–<1	3	0–23	<1	0–4
<i>F. moniliforme</i>	<1	<1	0–<1	<1	0–<1	3	0–26	<1	0–<1
<i>F. oxysporum</i>	<1	<1	0–5	0	...	<1	0–6	<1	0–1
<i>F. avenaceum</i>	<1	<1	0–4	<1	0–<1	<1	0–1	0	...
<i>F. subglutinans</i>	<1	<1	0–<1	0	...	<1	0–<1	<1	0–<1
<i>F. semitectum</i>	<1	<1	0–<1	<1	0–<1	<1	0–10	<1	0–<1
<i>F. tricinatum</i>	<1	0	...	0	...	<1	0–<1	0	...
<i>F. proliferatum</i>	<1	0	...	0	...	<1	0–<1	0	...
<i>F. sambucinum</i>	<1	<1	0–<1	0	...	0	...	<1	0–<1
<i>F. culmorum</i>	<1	1	0–30	0	...	<1	0–<1	0	...
<i>F. crookwellense</i>	<1	0	...	0	...	<1	0–<1	0	...

^a Based on 23,726 isolates, 9,392 collected in 1984, 7,682 in 1985, and 6,652 in 1986.

^b Based on 5,720 isolates collected in Becker, Clay, Clearwater, Kittson, Mahanomen, Marshall, Norman, Polk, and Roseau counties.

^c Based on 2,623 isolates collected in Chippewa, Douglas, Stearns, Stevens, and Swift counties.

^d Based on 6,566 isolates collected in Carver, Dakota, Ramsey, and Wright counties.

^e Based on 8,817 isolates collected in Blue Earth, Brown, Nicollet, Redwood, Renville, and Waseca counties.

DISCUSSION

with *F. graminearum* and *F. culmorum* became necrotic; the necrosis began in the inoculated spikelets and spread into more than half of the other spikelets of the spikes. Most of the spikelets inoculated with the other 11 *Fusarium* species tested became necrotic, but the necrosis usually did not spread into adjacent spikelets. Only 1% of the spikelets inoculated with PDA became necrotic (Table 1).

Kernels from spikes with necrosis were placed on PCNB agar to reisolate the *Fusarium* species used as inoculum. The same *Fusarium* species were recovered from the kernels originally inoculated (Table 4). *F. graminearum* and *F. culmorum* were recovered from all spikes and from 28 and 42% of the kernels, respectively. The other *Fusarium* species were recovered less frequently. From a few of the kernels, *F. poae* and the *F. moniliforme* / *oxysporum* group were isolated along with the *Fusarium* species originally used as inoculum. The *F. moniliforme* / *oxysporum* group was not identified further. Control kernels inoculated with only the culture medium were mostly free from *Fusarium* species, but 1% were infected with the *F. moniliforme* / *oxysporum* group (Table 4).

TABLE 3. *Fusarium* species isolated from scabby wheat kernels collected from 18 wheat-growing counties in Minnesota in 1985 and 1986

<i>Fusarium</i> species	Counties ^a with <i>Fusarium</i> spp. (no.)	Spikes infected		Kernels infected	
	(no.)	(no.)	%	(no.)	(%) ^c
<i>F. graminearum</i>	18	1,445	67	9,941	70
<i>F. poae</i>	17	1,152	54	3,377	24
<i>F. equiseti</i>	10	189	9	385	3
<i>F. sporotrichioides</i>	11	173	8	243	2
<i>F. acuminatum</i>	10	110	5	159	1
<i>F. semitectum</i>	8	24	1	28	0.2
<i>F. oxysporum</i>	6	39	2	45	0.3
<i>F. culmorum</i>	6	12	0.6	99	0.7
<i>F. moniliforme</i>	4	23	1	25	0.2
<i>F. avenaceum</i>	4	12	0.6	13	<0.1
<i>F. subglutinans</i>	4	8	0.4	8	<0.1
<i>F. proliferatum</i>	1	5	0.2	7	<0.1
<i>F. sambucinum</i>	2	3	0.1	2	<0.1
<i>F. tricinctum</i>	1	1	0.05	1	<0.1
<i>F. crookwellense</i>	1	1	0.05	1	<0.1

^a 18 counties sampled, 10 in 1985 and 12 in 1986.

^b Based on 2,153 infected spikes, 1,436 collected in 1985 and 717 in 1986.

^c Based on 14,159 infected kernels, 7,632 in 1985 and 6,527 in 1986.

Fusarium species are well recognized in causing scab (head blight) of wheat, and *F. graminearum* has been generally identified as the principal pathogen. Our results are similar to those reported by others (1,10,11,15). In addition, most of our *F. graminearum* isolates belonged to Group II (3), as virtually all produced perithecia. The occurrence of Group II isolates is also consistent with the findings of Windels and Kommedahl (16,17) who reported Group II isolates from cornstalks (*Zea mays* L.) in about the same areas of Minnesota where both corn and wheat are grown.

Of 15 species of *Fusarium* isolated, *F. graminearum* was dominant, occurring in all 24 counties of Minnesota examined, with *F. poae* the next most prevalent. Although *F. equiseti*, *F. acuminatum*, *F. sporotrichioides*, and *F. semitectum* occurred over a wide area, they were not frequently isolated. Stack and McMullen (10) inoculated wheat spikelets with many of the species tested in our study and also found that only *F. graminearum* and *F. culmorum* spread from the inoculated spikelet. Each of their other *Fusarium* species caused necrosis only in the inoculated spikelets. We obtained similar results with *F. graminearum* and *F. culmorum*. With seven of our *Fusarium* species, only inoculated spikelets became necrotic, but with *F. poae*, *F. sambucinum*, *F. semitectum*, and *F. sporotrichioides* a few spikelets became necrotic other than those that were inoculated. Atanasoff (1) reported that isolates infecting only single spikelets could kill rachis tissue and thereby shut off the water and nutrient supply to distal portions of the spike. This means that species much less pathogenic than *F. graminearum* and *F. culmorum* could cause damage to kernels distal to the infected spikelet. This was also pointed out by Sutton (12), especially when there are numerous infections.

The inoculation method used for this study facilitated infection and spread of the fungi within the spike. Though the method did not simulate natural conditions, it did direct inoculum into selected spikelets from where the progress of the pathogen into other spikelets could be easily observed. While the injection of inoculum may favor infection, the method did not cause the fungi to appear to be more important than indicated from isolations made from naturally infected kernels.

Most of the *Fusarium* species that we found on wheat kernels were also isolated from corn (16) in nearly the same areas of Minnesota. This suggests that inoculum of *Fusarium* species is common to these two crops throughout the state. Because both corn and wheat contribute to this inoculum pool, wheat scab and corn stalk rot cannot be reduced by cultural practices unless the residue of both crops is destroyed. However, the destruction of

TABLE 4. Isolation of 13 *Fusarium* species from *Triticum aestivum* 'Era' kernels that had been inoculated with these species in the glasshouse

<i>Fusarium</i> species	Spikes tested (no.)	Kernels tested (no.)	Range kernels tested/spike (no.)	Spikes with <i>Fusarium</i> (%)	Kernels with <i>Fusarium</i> (%)	Range infected kernels/spike (%)
<i>F. graminearum</i>	21	512	7-36	100	28	4-60
<i>F. poae</i>	23	642	11-43	70	4 ^a	0-9
<i>F. equiseti</i>	20	501	6-47	55	7 ^b	0-25
<i>F. sporotrichioides</i>	18	471	1-44	83	13 ^a	0-34
<i>F. acuminatum</i>	18	528	9-41	78	7 ^a	0-22
<i>F. semitectum</i>	18	446	3-40	83	17	0-81
<i>F. oxysporum</i>	18	479	5-45	83	11	0-28
<i>F. culmorum</i>	18	437	5-40	100	42	20-100
<i>F. moniliforme</i>	19	557	9-43	80	14	0-43
<i>F. avenaceum</i>	20	355	1-35	80	7 ^a	0-100
<i>F. subglutinans</i>	18	538	17-39	100	23 ^a	8-57
<i>F. sambucinum</i>	12	343	18-42	83	20 ^b	0-58
<i>F. tricinctum</i>	4	151	20-45	100	25 ^c	0-31
Control	14	407	12-39	14	1 ^a	0-17

^a *F. moniliforme*/oxysporum group also present.

^b *F. moniliforme*/oxysporum group and *F. poae* also present.

^c *F. poae* also present.

corn residue is hampered by widespread usage of conservation tillage, and this tillage system is rapidly spreading to wheat. This information leads us to believe that scab will remain an important wheat disease in Minnesota unless resistant cultivars are developed.

The culture of corn is moving northward into the Red River Valley, an area where scab has not been common in the past. This poses a threat to wheat in this region unless cultural practices are initiated to destroy corn and wheat residues (5) or resistant cultivars are developed.

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