

# Prevalence and Virulence of *Fusarium* spp. Associated with Stalk Rot of Corn in Colorado

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## ABSTRACT

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Stalk rot caused by *Fusarium* spp. was the most prevalent type of stalk-rot disease found in 1982 and 1983 in Colorado. *Fusarium* stalk rot, caused by *F. moniliforme* and *F. subglutinans*, and Gibberella stalk rot, caused by *F. graminearum* (teleomorph: *Gibberella zeae*), were detected in 1982, whereas *Fusarium* stalk rot and charcoal rot, caused by *Macrophomina phaseolina*, were detected in 1983. Virulence tests of stalk-rot *Fusarium* spp. from different sources (corn seed, stubble, stalks, soil, and air) and locations in Colorado in 1983 indicated that *F. graminearum* isolates were more virulent than *F. moniliforme* and *F. subglutinans* isolates, regardless of source. *F. moniliforme* and *F. subglutinans* were similar in ability to cause stalk rot; *F. subglutinans* is an important stalk-rot fungus in Colorado. Results from isolation studies revealed that corn stubble provides an overwintering site for pathogenic stalk rot and saprophytic *Fusarium* spp. in Colorado.

Stalk rot of corn is a disease of worldwide importance (3-5,17,18,20,22,24) that can cause serious losses in Colorado (8). *Fusarium* spp. cause stalk rot in most corn-growing regions of the United States (5,13,15,20,22,24). *F. moniliforme* Sheld. and *F. graminearum* Schwabe are the most frequent pathogens causing stalk rot (5,13,15), though the newly described species *F. subglutinans* (Wr. & Reink.) Nelson, Toussoun, & Marasas also incites stalk rot (3,5,15,17,18,20,22,24). Stalk rots are divided into two separate diseases: *Fusarium* stalk rot, caused by *F. moniliforme* and *F. subglutinans*, and Gibberella stalk rot, caused by *F. graminearum* (teleomorph: *Gibberella zeae* (Schw.) Petch. (5,13,20,22,24). Both diseases can occur in the same corn-growing regions, though one often predominates in any given region (3-5,13,15,20,22,24,28).

*F. moniliforme*, *F. subglutinans*, and

*F. graminearum* can occur in stalk-rotted corn from the same region, and it is difficult to determine which species is most important. The predominant species varies from year to year, depending on certain environmental conditions (4,5,13,15,20,22,24). Thus, *Fusarium* stalk rot predominates in warm, dry regions, whereas Gibberella stalk rot predominates in cool, moist regions (5,15,22,24).

There is little information on the identity and virulence of fungi causing stalk rot of corn in Colorado. Preliminary research indicated that *Fusarium* spp. were involved (8). Therefore, the objectives of this study were to isolate and identify fungi associated with stalk rot of corn in Colorado and to test the relative virulence of selected isolates.

## MATERIALS AND METHODS

**Prevalence of fungi.** Diseased cornstalks and ears collected in Colorado by county extension agents, other cooperators, and the authors were examined in 1982 and 1983. In 1982, cornstalks from nine fields from five northeastern counties and two southeastern counties and grain from three fields from three northeastern counties were examined for stalk and ear rot. In 1983, stalks from nine fields (different from fields sampled in 1982) from four northeastern counties, one southeastern county, and one western county and four grain samples from three southeastern counties and one northeastern county were examined for stalk and ear rot. Five to 10 stalks per field, and 50-125 kernels per grain sample were used for isolations. A grain cornfield with severe stalk rot in 1982 in Greeley, CO, was extensively sampled and is

designated the MF field.

Cornstalks were evaluated for external and internal stalk-rot symptoms. Isolations were made from pith and vascular tissue at the nodes. Tissue pieces (about  $0.5 \times 1 \text{ cm}^2$ ) were excised, surface-disinfested in 25 ml of 1% sodium hypochlorite plus 3 ml of polyethylene-sorbitan monolaurate surfactant for 2-5 min (depending on condition of sample), and plated on modified Komada's *Fusarium*-selective medium (KM) (14) and on a general medium of acidified potato-carrot agar (PCAL) (24). Corn kernels were visually examined for rot and randomly selected for direct plating on media or surface-disinfested in a 0.3% sodium hypochlorite solution for 5 min before plating. Macroconidia produced on PCAL were uniform and similar to those described on carnation leaf agar (CLA) (19).

In the spring of 1983, corn stubble was collected from the MF field before planting the 1983 crop. Twenty 20-cm pieces of stubble were collected from each of four plots in the MF field. Two plots had been chisel-plowed and two were not tilled. Five pieces (about  $0.5 \times 1 \text{ cm}^2$ ) of each stubble section were excised, surface-disinfested in 0.3% sodium hypochlorite plus surfactant for 5 min, and plated on KM.

All plates were incubated at 23 C for 4-7 days. Colonies were identified directly or subcultured on PCAL for identification. *Fusarium* identifications were based on the descriptions of Nelson et al (19).

**Virulence testing.** A total of 42 *Fusarium* isolates were obtained from corn seed, corn stubble, cornstalks, soil, and air. Isolates were obtained from Sterling, Akron, Windsor, Fort Collins, and Fruita, CO. All isolates were single-spored, grown in pure culture on PCAL, and maintained in moist, autoclaved soil containing 10% (v/v) barley straw. In late July, isolates were grown on sterile toothpicks washed with potato-dextrose broth (PDB) (5,30). A plot of field corn (Northrup King cultivar PX 15) was planted in Fort Collins, CO, on 9 May 1983; standard agronomic practices were followed (224 kg of nitrogen and  $6.2 \times 10^4$  plants per hectare). In mid-August, when plants were near maturity (ears almost fully developed), toothpicks were

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inserted in the second node of randomly selected corn plants. A completely randomized design was used with four replicates (plants) per isolate. Noncolonized toothpicks washed with PDB were inserted in the second node of control plants. Stalks were harvested 17 days later, longitudinally sectioned, and the amount of rot in the internode above the inoculation point was measured. Rot constituted discolored and degraded tissue. Disease estimates in this internode were based on a scale of 0–4, where 0 = no rot, 1 = less than 25% rot, 2 = 26–50% rot, 3 = 51–75% rot, and 4 = greater than 76% rot. Ratings of stalks inoculated with each isolate were averaged and expressed as a mean disease rating (MDR). After evaluation, isolations were made from selected stalks to determine the presence of the *Fusarium* sp. originally inoculated.

In fields where *F. graminearum* was consistently isolated from stalks, corn stubble and debris were examined for the presence of perithecia to determine if group I or group II *F. graminearum* predominated (26,27). Additionally, 20 *F. graminearum* isolates were grown on wheat straw and CLA in an attempt to induce perithecia formation (19).

## RESULTS

**Prevalence of fungi.** Stalk-rotted plants examined in 1982 and 1983

expressed symptoms of stalk rot caused by *Fusarium* spp., including pith breakdown and red-pink discoloration of vascular strands. *Fusarium* spp. were the predominant fungi isolated from diseased stalks, with *F. graminearum*, *F. moniliforme*, and *F. subglutinans* most frequently isolated. In 1982, *F. graminearum* was isolated on KM as frequently (35%) as *F. moniliforme* and *F. subglutinans* combined (36%) and more frequently on PCAL (55 vs. 22%) (Table 1). Stalks obtained from three fields from northeastern Colorado consistently yielded *F. graminearum*, whereas stalks from six other fields consistently yielded *F. moniliforme* and *F. subglutinans*. In 1983, *F. graminearum* was infrequently isolated (1–3%) regardless of medium, whereas *F. moniliforme* (54%) and *F. subglutinans* (10%) were isolated from stalks from all nine fields sampled. Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid., was found in three fields in the Arkansas Valley in southeastern Colorado. Although all three stalk-rot *Fusarium* spp. were isolated from stalks from individual fields, either *F. graminearum* or *F. moniliforme* and *F. subglutinans* predominated. Stalks affected by charcoal rot frequently yielded *F. moniliforme*.

Three lots of corn kernels from three

fields in eastern Colorado were examined in 1982 (Table 2). Kernels from sample 1 showed rot and red-brown discoloration, whereas kernels from samples 2 and 3 were symptomless. *F. graminearum* was isolated from discolored kernels on PCAL, whereas *F. graminearum*, *F. moniliforme*, and *F. subglutinans* were isolated on KM. Symptomless kernels yielded *F. moniliforme*, *F. subglutinans*, and *Penicillium* spp. Surface-disinfection failed to eliminate kernel contamination. In 1983, four kernel samples, sample 1 from northeastern Colorado and samples 2–4 from three fields in southeastern Colorado, were examined (Table 2). Kernels from sample 1 showed red-brown discoloration and yielded *F. graminearum*, *F. subglutinans*, and *Penicillium* spp. on KM and *F. graminearum* and *Penicillium* spp. on PCAL. Surface-disinfection failed to eliminate kernel contamination. Kernels from samples 2–4 showed some brown discoloration or no symptoms, and all kernels yielded *F. moniliforme*.

Other *Fusarium* spp. were isolated from cornstalks and kernels. Stalks yielded more *Fusarium* spp. than kernels, and some stalk sections yielded three or four species. *F. equiseti* (Cda.) Sacc. sensu Gordon was most frequently isolated from stalks and kernels. *F. oxysporum* Schlecht. emend. Snyder & Hans., *F.*

**Table 1.** Fungi isolated from stalk-rotted corn from Colorado in 1982 and 1983

Year	Medium <sup>a</sup>	Fields sampled	No. of stalks	No. of isolations	Number and species isolated <sup>b</sup>			
					<i>Fusarium moniliforme</i>	<i>F. subglutinans</i>	<i>F. graminearum</i>	<i>Macrophomina phaseolina</i>
1982	PCAL	8	52	89	11	9	49	... <sup>c</sup>
	KM	9	62	253	51	41	88	...
1983	PCAL	9	65	65	29	6	2	6
	KM	9	65	187	107	20	2	20

<sup>a</sup>PCAL = potato-carrot agar acidified to pH 4.0 with lactic acid; KM = modified Komada's (14) *Fusarium*-selective medium.

<sup>b</sup>Five to 10 stalks per field were sectioned longitudinally, and excised nodal tissue pieces were surface-disinfested and plated on KM and PCAL. Isolates were directly identified from PCAL plates, whereas isolates on KM were transferred to PCAL and identified.

<sup>c</sup>Indicated species not isolated.

**Table 2.** Fungi isolated from corn kernels<sup>a</sup> from Colorado in 1982 and 1983

Year	Field	Medium <sup>b</sup>	Treatment <sup>c</sup>	No. of kernels	Number and species isolated			
					<i>Fusarium moniliforme</i>	<i>F. subglutinans</i>	<i>F. graminearum</i>	<i>Penicillium</i> spp.
1982	1	KM	NT	50	9	4	... <sup>d</sup>	35
		KM	NT	75	12	37	45	...
	3	PCAL	NT	50	...	...	34	...
		KM	NT	20	4	7	...	3
		PCAL	NT	20	3	3	...	15
		PCAL	SD	20	2	3	...	5
1983	1	KM	NT	15	...	5	2	2
		KM	SD	25	...	6	6	...
		PCAL	NT	6	...	...	2	2
		PCAL	SD	6	...	...	2	...
	2	KM	NT	50	50	...	...	...
		KM	NT	50	50	...	...	...
	4	KM	NT	50	50	...	...	...

<sup>a</sup>Kernels randomly selected from samples.

<sup>b</sup>PCAL = potato-carrot agar acidified to pH 4.0 with lactic acid; KM = modified Komada's (14) *Fusarium*-selective medium.

<sup>c</sup>NT = no disinfection treatment; SD = surface-disinfested with sodium hypochlorite.

<sup>d</sup>Indicated species not isolated.

*solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. acuminatum* Ell. & Ev. sensu Gordon, and *F. sporotrichioides* Sherb. were less frequently isolated.

Stubble collected from the MF field yielded *F. graminearum*, *F. moniliforme*, *F. subglutinans*, *F. equiseti*, and *F. oxysporum*. Ninety percent of stubble sections from the two chiseled plots yielded *F. graminearum*, whereas 70% from the no-till plots yielded the fungus. All stubble sections from both chiseled plots yielded *F. moniliforme* and/or *F. subglutinans*, whereas 90% of stubble sections from the no-till plots yielded these fungi. *F. graminearum*, *F. subglutinans*, and *F. moniliforme* were frequently isolated from the same stubble piece.

**Virulence testing.** *F. graminearum* isolates were significantly ( $P < 0.01$ ) more virulent on cornstalks than *F. subglutinans* or *F. moniliforme* isolates regardless of isolate source (Table 3). *F. graminearum* isolates associated with corn had a combined MDR of 3.6. *F. graminearum* colonized corn stubble extensively from the MF field, and isolates were highly virulent (MDR = 3.5).

*F. subglutinans* isolates from corn plant parts, soil, and air had a combined MDR of 2.5, and virulence was not associated with isolate source. *F. subglutinans* isolates from three cornfield soils from different areas of Colorado (western, eastern, and northeastern) showed similar virulence.

*F. moniliforme* isolates had a combined MDR of 2.2, and virulence was not associated with isolate source. *F. moniliforme* isolates recovered from a bean field soil in 1982, having been planted to corn in 1981, were slightly more virulent than any other isolate tested.

Control plants showed no stalk rot (MDR = 0.1), and isolations from inoculated cornstalks consistently yielded the *Fusarium* sp. placed in stalks on the toothpicks.

## DISCUSSION

**Prevalence of fungi.** Three stalk-rot diseases were detected in Colorado in 1982 and 1983: Gibberella stalk rot, caused by *F. graminearum*; Fusarium stalk rot, caused by *F. moniliforme* and *F. subglutinans*; and charcoal rot, caused by *M. phaseolina*. *F. moniliforme* and *F. subglutinans* were most frequently isolated from stalk-rotted corn in 1983; *F. graminearum* was isolated in 1982 but was rare in 1983. The low incidence of *F. graminearum* in 1983 may relate to climate or the different fields examined in 1982 versus 1983. Annual occurrence of *F. graminearum* has been shown to fluctuate in other corn-growing regions (5,13,15).

The prevalence of Gibberella stalk rot

in Colorado in 1982 was unexpected, because adjacent states have reported Fusarium stalk rot to be most prevalent (5-7,23,25). Gibberella stalk rot generally is the more damaging of the stalk rots caused by *Fusarium* spp. (4,5,13,29), and this may be the situation in Colorado. Fields with Gibberella stalk rot (e.g., MF field) were not harvestable, or they yielded small ears. *F. graminearum* was consistently isolated from stalks from the MF field.

Fusarium stalk rot generally is prevalent in drier environments, such as in Colorado (3,5,6,20,25). *F. subglutinans* was frequently isolated from stalk-rotted plants, and we feel *F. subglutinans* is an important component of the Fusarium stalk-rot complex in Colorado. Most investigators have not distinguished *F. subglutinans* from *F. moniliforme* (15,17,18); however, with the elevation of *F. moniliforme* var. *subglutinans* to species status (*F. subglutinans*) (19), it seems appropriate to distinguish between these fungi. *F. subglutinans* may be more common and therefore a more important stalk-rot organism than previously recognized.

Although stalk-rotted plants were infected by more than one stalk-rot *Fusarium* sp., trends were evident where either *F. graminearum* or *F. moniliforme* and *F. subglutinans* dominated. Isolation of other *Fusarium* spp. from stalks was expected, and other researchers have isolated *F. equiseti* (1,15,16), *F. oxysporum* (1,15,16,26), *F. solani* (26), and *F. tricinctum* (1,16,25) from corn plants. *F. equiseti* was the dominant species of these other *Fusarium* spp. encountered in Colorado and sometimes was isolated late in the season as frequently as stalk-rot *Fusarium* spp. The

fungus is a ubiquitous saprophyte common in cultivated Colorado soils (9) but does not cause stalk rot. If stalk rot is a disease complex involving a number of fungi (29), *F. equiseti* may be involved, and its pathogenicity needs to be tested.

Stalk-rot *Fusarium* spp. were isolated from kernels in 1982 and 1983. Severely diseased kernels consistently yielded *F. graminearum*. *F. graminearum* is only associated with damaged, discolored kernels (15,20,22,25), and sound kernels never yielded the fungus in our study. Kernel infection by *F. graminearum* was detected in overhead-irrigated fields where conditions may have been favorable for disease development. Kernels yielding *F. moniliforme* and *F. subglutinans* were symptomless, except for one sample in 1983, which showed brown kernel discoloration and rot. Most of the discolored kernels were associated with insect damage, but it was evident that *F. moniliforme* can cause severe kernel rot under appropriate conditions. *F. moniliforme* and *F. subglutinans* were commonly isolated from kernels, and *F. moniliforme* predominates on kernels in many states, including Iowa (7), Nebraska (23), Kansas (6), and others (10-12).

The occurrence of charcoal rot in southern Colorado was expected because of the hot, dry climate of the area (21). The importance of charcoal rot in Colorado is unknown, and plants examined having charcoal rot also had Fusarium stalk rot. With increasing soybean production in Colorado, charcoal rot may become more prevalent.

**Virulence testing.** The greater pathogenicity of *F. graminearum* vs. *F. subglutinans* and *F. moniliforme* is consistent with reports from other corn-

Table 3. Virulence<sup>v</sup> of stalk-rot *Fusarium* spp. isolated<sup>w</sup> from different sources in Colorado

Species	Source <sup>x</sup>	No. of isolates	Mean disease rating <sup>y</sup>	Species mean disease rating <sup>z</sup>
<i>Fusarium graminearum</i>	MF corn stubble	4	3.5	
	Corn soil	3	3.9	
	Corn seed	3	3.3	3.6 a
<i>F. subglutinans</i>	MF corn stubble	2	2.5	
	Corn soil (W)	5	2.5	
	Corn soil (A)	6	2.5	
	Corn soil (F)	1	2.5	
	Cornstalk	1	2.5	
<i>F. moniliforme</i>	Air	1	1.8	2.4 b
	Corn soil (A)	5	2.2	
	Corn soil (W)	4	1.9	
	Corn rhizosphere	4	2.3	
	Cornstalk	1	2.0	
	Bean soil	2	2.6	2.2 b

<sup>v</sup> Inoculations made by toothpick inoculation technique; four plants inoculated per isolate.

<sup>w</sup> Fungi isolated in 1982 and 1983 were stored in soil culture and tested in 1983.

<sup>x</sup> W = Windsor, A = Akron, and F = Fruita, CO.

<sup>y</sup> Rating system: 0 = no rot, 1 = less than 25% rot, 2 = 26-50% rot, 3 = 51-95% rot, and 4 = 76-100% rot. Analyses of variance of isolate source for each species showed no significant differences ( $P = 0.01$ ).

<sup>z</sup> Means followed by the same letter are not significantly different (analysis of variance, *t* test,  $P = 0.01$ ).

growing regions; this indicates *F. graminearum* is the most aggressive stalk-rot *Fusarium* sp. (5,14,29). Our results show that *F. graminearum* caused the losses in the MF field in 1982.

Pathogenic *F. graminearum* isolates overwintered in corn stubble in the MF field in chisel-tilled and no-till plots. Infested stubble may be the primary inoculum source for corn planted the next spring. *F. graminearum* overwintered in cornstalks in Minnesota (29), and host debris is the principal inoculum source for this fungus in Australia (28).

Ecological knowledge of *F. graminearum* populations in a given region can relate to management of Gibberella stalk rot. According to initial observations, the dry Colorado climate does not appear to be conducive to perithecia formation by *F. graminearum*. No perithecia were found in the field, and 15 of 20 *F. graminearum* isolates failed to form perithecia on CLA. This indicates that the *F. graminearum* population in Colorado is partly group I, which acts as a soilborne fungus surviving in organic debris and infecting belowground plant parts (2,3,27). Group I populations normally occur in dry, hot climates (2), such as in Colorado, and infect corn organs in contact with infested debris in the soil (20). Turning under infested stubble could enhance subsequent infection by group I *F. graminearum*, whereas turning under stubble may reduce infection by group II *F. graminearum*, which form perithecia and rely on aboveground infection by ascospores (3,28). More research is needed to determine the composition of the *F. graminearum* population in Colorado.

*F. subglutinans* and *F. moniliforme* isolates had MDRs of 2.4 and 2.2, respectively, and were not as virulent as *F. graminearum*. These data indicate that *F. subglutinans* and *F. moniliforme* are similar in ability to cause stalk rot in Colorado. Pathogenic *F. subglutinans* and *F. moniliforme* isolates overwintered in stubble, and pathogenic *F. moniliforme* isolates overwintered in bean field soil. Near the end of our study, *F. proliferatum* was differentiated from some *F. moniliforme* isolates according to a new taxonomic system (19). Therefore, *F. proliferatum* causes stalk rot of corn similar to that caused by *F. moniliforme* (5). It would be of interest to further investigate the distribution and virulence of *F. proliferatum* in other corn-growing regions.

Because Gibberella and Fusarium stalk rots cannot be distinguished by symptoms alone (13), growers are being encouraged to have *Fusarium* spp. associated with stalk rot isolated and identified. The potential differences in virulence and ecology among stalk-rot *Fusarium* spp. may influence the selection of management practices for reducing stalk rot, and management techniques for controlling Gibberella stalk rot may differ from those aimed at Fusarium stalk rot. When all three stalk-rot *Fusarium* spp. are prevalent, control practices may be selected for *F. graminearum* because of its greater virulence, unless population densities are very low. Further information is needed on survival of stalk-rot fungi and the effect of tillage on survival to develop the best stalk-rot management approach.

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