Plant Health Survey

Survey of *Fusarium* Species Associated with Fusarium Head Blight of Spring Wheat (*Triticum aestivum*) in Southeastern Idaho

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Abstract

In Idaho, losses due to Fusarium head blight (FHB) of spring wheat (*Triticum aestivum*) have been infrequent and have historically been dominated by *Fusarium culmorum* (Wm. G. Sm.) Sacc. However, the incidence of FHB and deoxynivalenol-contaminated grain has increased in spring wheat in southeastern Idaho since 2009, indicating that other species of *Fusarium* may be contributing to disease. In 2011 and 2012, 17 spring wheat fields were scouted and sampled for FHB in southern Idaho. Contaminated grains were cultured, and putative *Fusarium* isolates were identified using species-specific polymerase chain reaction. In 2011, 87% of all recovered isolates were identified as *F. graminearum*, whereas only 13% were identified as *F. culmorum*. Of the isolates collected in 2012, 51% were identified as *F. graminearum* and 49% as *F. culmorum*. In both years, more *F. graminearum* isolates were recovered as compared to a survey conducted in 1984. Implementation of effective disease management practices will be necessary to minimize the establishment and spread of *F. graminearum*–responsible FHB in southeastern Idaho.

Fusarium head blight (FHB), caused by numerous *Fusarium* species, occurs in many wheat (*Triticum aestivum*) growing regions of the world (McMullen et al. 1997; Stack and McMullen 1995) and has become an emerging threat to grain production in southeastern Idaho. In the United States, FHB reemerged in the 1980s as a devastating disease in cereal crops in North America (Shaner 2003) and was responsible for $2.6 billion in economic losses in the Midwest from 1998 to 2000 (Nganje et al. 2004). FHB was first reported in the Pacific Northwest in the early 1980s in Washington (Inglis and Maloy 1983) and Idaho (Mihuta-Grimm and Forster 1989). Although occurring in similar geographical regions, the primary *Fusarium* species associated with infection differed. In Washington, *Fusarium graminearum* (Schwabe) was found to be the primary causal agent of FHB (Inglis and Maloy 1983), whereas *F. culmorum* (Wm. G. Sm.) Sacc. was the primary species contributing to *Fusarium* infection in Idaho (Mihuta-Grimm and Forster 1989). Disease surveys have not been published since these first reports; therefore, it has not been determined which species of *Fusarium* are currently responsible for the increasing level of disease and deoxynivalenol (DON) contamination in grain in southeastern Idaho. Up to 17 species of *Fusarium* have been reported as contributing to FHB (Obst et al. 1997). However, *F. graminearum* and *F. culmorum* are recognized as the most common species responsible for FHB.

Shifts in the dominant species of *Fusarium* responsible for FHB from *F. culmorum* to *F. graminearum* have been reported in many cereal-producing regions since the 1980s following increased maize cultivation (Clear and Patrick 2000; Isebaert et al. 2009; Miller 2008; Obst et al. 1997; Sutton 1982; Waalwijk et al. 2003). Surveys of FHB symptomatic heads conducted in several European countries (Audenaert et al. 2009; Isebaert et al. 2009; Jennings et al. 2004; Kosiak et al. 2003; Waalwijk et al. 2003) and Canada (Clear and Patrick 2000, 2010) all reported an increase in incidence of *F. graminearum* recovered from symptomatic heads, suggesting that *F. culmorum* is no longer the primary *Fusarium* species responsible for FHB in those regions. In Idaho, the potential for this shift has increased with changes occurring in maize cultivation.

Since the early 1990s, the acreage of maize planted in southern Idaho has more than tripled (NASS 2017) in response to its use in the enlarging dairy industry and an increase in grain for ethanol production. This expansion of maize acreage provides residues on which *F. graminearum* can readily proliferate (Sutton 1982). Shifting irrigation practices to center pivot systems that run constantly also increase humidity within the crop canopy, creating the ideal environment for the development of FHB. Added to that, environmental changes in the past decade resulted in conditions conducive for the development of increased FHB in wheat, which led to a significant amount of *Fusarium*-damaged kernels and DON-contaminated grain. Little is known about the current species of *Fusarium* contributing to FHB in Idaho. The objective of this study was to determine which of the primary FHB-contributing *Fusarium* species is responsible for infection in southeastern Idaho.

Identification of *Fusarium* Species

Fungal isolation. Wheat samples were collected from fields throughout southeastern Idaho in 2011 and 2012. Wheat heads exhibiting typical FHB symptoms or the presence of pink-, orange-, or salmon-colored sporodochia on the glume tissue were collected over a 2-year period. Over the course of the two seasons, isolates were

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obtained from 17 arbitrarily selected fields: Aberdeen (two sites), Burley (one site), Idaho Falls (three sites), Minidoka (two sites), Pingree (two sites), Rockford (three sites), and Rupert (four sites). Collections began the day visual signs and symptoms of infection were first observed and continued over the course of each growing season. From each field, at least 10 heads exhibiting signs and symptoms of infection were randomly selected, placed in paper bags, and stored at room temperature until they could be processed. Disease incidence (percent of symptomatic heads per 50 randomly selected heads) and total the number of recovered isolates at each location in each year are listed in Table 1.

Each wheat head had two kernels removed from symptomatic florets, which were then surface sterilized for 3 min in a 10% commercial bleach solution (0.06% NaOCl). Seeds were rinsed in sterile deionized water, as modified from the protocol set forth by Gale et al. (2002), and excess water was allowed to drip off the kernel before it was plated on half-strength potato dextrose agar (PDA, Difco, Detroit, MI) in 24-well cell culture plates (Benton, Dickinson, and Company, Bedford, MA). Cultures were incubated for 1 week at room temperature (25°C), after which pure cultures obtained by hyphal tipping were grown on half-strength PDA and incubated at room temperature for 1 week. Putative *Fusarium* cultures were prepared for DNA extraction with DNeasy Plant Mini kits (Qiagen, Germantown, MD) according to the manufacturer's instructions.

Species identification. For this analysis, genomic DNA was amplified using species-specific primers for *F. graminearum* and *F. culmorum*. Primer sequences were obtained from Nicholson et al. (1998) and are listed in Table 2. Each fungal isolate was tested using both primers. Polymerase chain reaction (PCR) reactions were performed in a 20-µl mixture containing 10 µl of DreamTaq Master Mix 2X (Thermo Scientific, Waltham, MA), 1 µl each of 0.5 µM forward and reverse primers paired by species, and 8 µl of template DNA. PCR amplification was performed using the following cycle parameters: initial denaturation at 95°C for 120 s; 30 cycles of denaturation at 95°C for 45 s, annealing at 65°C for 60 s, and extension at 72°C for 120 s; and a final extension at 72°C for 5 min. After amplification, 4-µl aliquots of PCR products were visualized on a 1% agarose gel using Amresco EZvision dye (Amresco, Solon, OH).

Fusarium graminearum Is Recovered More Frequently

Overall, the incidence of FHB in many fields was low in both years. Few fields showed high incidence of FHB, which was not related to the species recovered. Total disease incidence varied from field to field, as did the species of recovered isolates. *F. graminearum* was recovered more frequently, overall, than *F. culmorum.*

A total of 848 *Fusarium* isolates were collected from all fields in 2011 and 2012 (Table 1). Of the 848 isolates collected, 624 isolates (73.6%) were positively identified as *F. graminearum* and 224 isolates (26.4%) were identified as *F. culmorum*. Fewer isolates were recovered in 2012 than in 2011, and the percentage of each species recovered differed by year. In 2011, a large percentage of isolates collected (87.0%) were identified as *F. graminearum*, whereas only 13.0% were identified as *F. culmorum.* Of the isolates recovered in 2012, there was an almost even split, with 51.1% identified as *F. graminearum* and 48.9% identified as *F. culmorum.* When broken down by year, the percent of all isolates attributed to *F. culmorum* was much higher in 2012 than in 2011.

By location, the percent of isolates recovered from each species was not consistent from year to year. In 2011, *F. graminearum* was recovered more frequently than *F. culmorum* at five of the six locations. In contrast, only three of the six locations in 2012 had 50% or more of the isolates identified as *F. graminearum*. Several of these sites were in similar locales from year to year, making the changes in isolate frequency of interest. The most dramatic example of this change was at the Aberdeen location, where in 2011 96.3%

TABLE 2
Primer sequences and fragment length for each primer pair[a]

Primer name	Species	Sequence (5′-3′)	Size
FC01F	*Fusarium culmorum*	ATGGTGAACTCGTCGTGGC	570 bp
FC01R		CCCTTCTTACGCCAATCTCG	
Fg16F	*Fusarium graminearum*	CTCCGGATATGTTGCGTCAA	450 bp
Fg16R		GGTAGGTATCCGACATGGCAA	

[a] Source: Nicholson et al. (1998).

TABLE 1
Percent disease incidence of Fusarium head blight and number and percentage of *Fusarium* isolates recovered from each location in southeastern Idaho in 2011 and 2012[a]

Location	Year	Number of sites	Disease incidence (%)	*Fusarium graminearum*		*Fusarium culmorum*		Total number of isolates
				Number of isolates	Percentage	Number of isolates	Percentage	
Aberdeen	2011	1	5	183	96.3	7	3.7	190
	2012	1	<1	1	2.2	45	97.8	46
Burley	2011	1	<1	31	93.9	2	6.1	33
Idaho Falls	2011	1	5	20	60.6	13	39.4	33
	2012	2	40	44	95.7	2	4.3	46
Minidoka	2012	2	5	25	49.0	26	51.0	51
Pingree	2012	2	30	51	50.0	51	50.0	102
Rockford	2011	1	5	25	46.3	29	53.7	54
	2012	2	<1	0	0.0	21	100.0	21
Rupert	2011	2	20	203	91.9	18	8.1	221
	2012	2	10	41	80.4	10	19.6	51
Totals	2011	6		462	87.0	69	13.0	531
	2012	11		162	51.1	155	48.9	317
	Both	17		624	73.6	224	26.4	848

[a] Percent disease incidence was rated at Feekes growth stage 11.

of isolates were attributed to *F. graminearum*, but in 2012 97.8% were attributed to *F. culmorum*. It could be speculated that the shift in species prevalence from year to year was related to environmental differences among locations and/or the availability of inoculum.

A Shift from *F. culmorum* to *F. graminearum* Means Changes in Management

The purpose of this study was to determine the species of *Fusarium* responsible for FHB in southern and eastern Idaho over a 2-year period. In 1984, Mihuta-Grimm and Forster (1989) determined *F. culmorum* to be the most frequently isolated *Fusarium* species in Idaho wheat fields. Since that time, an increased incidence in wheat of FHB and DON contamination caused by a previously undetermined species of *Fusarium* has been reported in the region. Because *F. graminearum* was not yet endemic to Idaho wheat fields, knowledge of the species contributing to FHB will be useful in developing effective management measures (i.e., crop rotation or the development of species-specific host resistance) to minimize risk.

Over the 2 years of the study, the incidence of FHB varied by field. The frequent recovery of *F. graminearum* in addition to the already endemic *F. culmorum* in most fields could be attributed to many factors. One such factor is that maize residues are generally not linked to the presence of *F. culmorum* but are known to increase the presence of *F. graminearum* (Dill-Macky and Jones 2000). Maize acreage has increased in a direct linear relationship with the number of dairy cows in the state (NASS 2017), and it has become a common practice to rotate spring wheat directly after maize. These rotations increase FHB in wheat crops (Dill-Macky and Jones 2000; Wilcoxson 1993), because maize residues at the soil surface allow for the development of perithecia of *F. graminearum*. Previously low levels of *F. graminearum* could be the result of lack of appropriate substrates for its proliferation, or the absence of aggressive populations. The inoculum of *F. graminearum* on maize residues at the soil surface, coupled with optimal temperatures for fungal development and high relative humidity in the crop canopy resulting from continuous irrigation, may be contributing to the increasing presence of this pathogen in Idaho.

Shifts from *F. culmorum* to *F. graminearum* have been observed in several European countries and in Canada in the past 20 years (Audenaert et al. 2009; Clear and Patrick 2010; Isebaert et al. 2009; Jennings et al. 2004; Kosiak et al. 2003; Waalwijk et al. 2003) and could provide insight into the changing dominant species in Idaho. It has been suggested that climate changes, in addition to changes in cropping practices, may favor the growth of *F. graminearum* over *F. culmorum* owing to the former having a higher optimum temperature for ideal growth (Madgwick et al. 2011; Waalwijk et al. 2003). With the acreage of maize in southern Idaho steadily increasing since the early 1990s, further investigation is warranted to study its impacts on the presence of *F. graminearum*.

In 2017, the state of Idaho ranked sixth in the United States for total wheat production and was number one in barley production (NASS 2017). Because the presence of FHB and *F. graminearum* has already been detected and is increasing in southern Idaho, the implementation of proper disease management practices is necessary to prevent the development of further epidemics. Integrating fungicide applications and the planting of less susceptible cultivars are means by which disease can be reduced but not eliminated in the region. Wheat fields in the region are now vulnerable to infection and to the production of mycotoxins that threaten food safety. The suppression of FHB is necessary to ensure continued production of high-quality grain in areas of Idaho where FHB previously had rarely occurred.

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