Crop Rotation and Management Effect on *Fusarium* spp. Populations

David A. Marburger,* Muthusubramanian Venkateshwaran, Shawn P. Conley, Paul D. Esker, Joseph G. Lauer, and Jean-Michel Ané

ABSTRACT

Fusarium spp. are common fungal pathogens that infect a number of field and vegetable crops. Crop rotation, genetic resistance, and fungicides are the primary methods used for managing these pathogens; however, there is a lack of information regarding the interactions between these management strategies and how they impact *Fusarium* spp. population dynamics. Therefore, the objective of this research was to quantify the effect of crop rotation and management (i.e., variety selection and fungicide use) on *F. graminearum, F. oxysporum*, and *F. virguliforme* populations in the soil using real-time quantitative polymerase chain reaction (qPCR). Soil samples were collected in 2011 and 2012 from a long-term corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.]–wheat (*Triticum aestivum* L.) rotation study near Arlington, WI, and populations for each species (spores g^{-1} of soil) were quantified from extracted soil DNA. *Fusarium oxysporum* was the most prevalent *Fusarium* sp. found. Crop rotation and management did not impact *F. oxysporum* populations nor *F. virguliforme* presence. A crop rotation by fungicide interaction was found for *F. graminearum* ($P < 0.001$), but this interaction was primarily affected by crop rotation. As expected, *F. graminearum* was found more often in plots with wheat as part of the rotation. This study found few interactions among crop rotation, variety selection, and fungicide use for controlling populations of three *Fusarium* spp. in the soil, and significant interactions or individual control methods were dependent on the species being examined.

D.A. Marburger, S.P. Conley, J.G. Lauer, and J.M. Ané, Dep. of Agronomy, Univ. of Wisconsin, 1575 Linden Drive, Madison, WI 53706; M. Venkateshwaran, School of Agriculture, Univ. of Wisconsin, 313 Pioneer Tower, 1 University Plaza, Platteville, WI 53818; P.D. Esker, CIP-ROC, Universidad de Costa Rica, San Jose, Costa Rica. This material is based on work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-088. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. Received 10 Mar. 2014. *Corresponding author (dmarburger@wisc.edu).

Abbreviations: CFU, colony forming unit; CSW, corn followed by soybean followed by wheat; C_sW_sS, corn followed by wheat followed by soybean rotation system used to mimic a livestock operation in which corn was harvested as silage and wheat straw was removed where appropriate; DON, deoxynivalenol; FHB, *Fusarium* head blight; Cq, quantification cycle; CWS, corn followed by wheat followed by soybean; PCR, polymerase chain reaction; qPCR, real-time quantitative polymerase chain reaction; *R*, relative resistance; S, relative susceptibility; SDS, sudden death syndrome; WW, continuous wheat.

Fusarium spp. are pathogenic fungi known to cause numerous diseases on a wide range of host plants (Leslie and Summerell, 2006; Summerell et al., 2003). These species are widely distributed throughout the world in soil, on aerial and subterranean plant parts and debris, and on other organic substrates (Booth, 1971; Burgess, 1981; Leslie et al., 1990; Nelson et al., 1983). Several species (*F. graminearum*, *F. oxysporum*, and *F. virguliforme*) are particularly important pathogens on vegetable and field crops.

Published in Crop Sci. 55:365–376 (2015).
doi: 10.2135/cropsci2014.03.0199

Fusarium graminearum is the most dominant of several *Fusarium* spp. that cause *Fusarium* head blight (FHB; also known as "head scab") on wheat and other cereals around the world (McMullen et al., 1997). Yield loss can range from 30 to 70% when disease is severe (Bai and Shaner, 1994). Grain quality can also be affected through the contamination with trichothecene mycotoxins such as deoxynivalenol (DON; commonly known as vomitoxin) (Goswami and Kistler, 2004; McMullen et al., 1997). The sexual stage of *F. graminearum*, *Gibberella zeae*, causes *Gibberella* ear and stalk rot on corn. Yield loss from *Gibberella* ear and stalk rot has been estimated at 5 to 15% annually, but actual loss can vary greatly between years and locations (Lipps et al., 2001). Additionally, this fungus has also been shown to be pathogenic to soybean, causing seed decay and damping-off (Broders et al., 2007; Ellis et al., 2011; Pioli et al., 2004).

Fusarium oxysporum is the most widely distributed *Fusarium* sp. and can be recovered from most soils (Leslie and Summerell, 2006). Many *F. oxysporum* isolates are host specific, and, as a result, more than 100 *formae specialis* and races have been described. Diseases caused by this species include vascular wilts, damping-off, and crown and root rots (Leslie and Summerell, 2006; Leslie et al., 1990). *Fusarium oxysporum* has been commonly recovered from corn and soybean fields in the United States (Leslie et al., 1990).

Sudden death syndrome (SDS) on soybean, caused by *Fusarium virguliforme*, was discovered in Arkansas in 1971 (Hirrel, 1983) and has spread to most of the soybean-growing areas in the Midwest (Bernstein et al., 2007; Roy et al., 1989, 997; Wrather and Koenning, 2009). Yield losses of up to 80% in individual fields have been attributed to SDS, but yield losses of 5 to 15% are more common. Yield loss is caused by a combination of reduced photosynthetic area, premature defoliation, flower and pod abortion, and reduced seed size (Hershman et al., 1990; Roy et al., 1997).

Single or multiple management strategies are often implemented for managing different *Fusarium* spp., including variety selection, crop rotation, and fungicide use. Choosing a resistant variety against specific diseases caused by *Fusarium* spp. can help reduce disease risk and increase the probability of maximizing yield (Krupinsky et al., 2002). Of the diseases caused by the three *Fusarium* spp. previously mentioned, extensive research has been conducted related to developing resistant varieties for diseases such as SDS and FHB (Bai and Shaner, 2004; Ellis et al., 2012; Hershman et al., 1990; Rupe et al., 1991; Sciumbato and Keeling, 1985; Vick et al., 2006).

Even with the use of resistant varieties, the use of a nonhost crop can also help reduce the risk of disease development (Krupinsky et al., 2002), but given the wide geographic and host ranges for many members of the *Fusarium* genus, crop rotation is not necessarily an effective control strategy. A survey conducted by Leslie et al. (1990) revealed the distribution of *Fusarium* spp. within corn, soybean, and sorghum [*Sorghum bicolor* (L.) Moench.] fields in the United States found in the north (Illinois, Indiana, Missouri, Ohio, and West Virginia) were similar to those found in the south (Alabama, Arkansas, Florida, Georgia, Mississippi, North Carolina, and South Carolina). Two species related to the current study (*F. graminearum* and *F. oxysporum*) were isolated from either tissue, debris, or soil from all three crops sampled. For FHB, it has been long recommended to rotate wheat and corn with nonhost crops such as soybean; however, recent research has shown that *F. graminearum* can be pathogenic on soybean (Broders et al., 2007; Pioli et al., 2004; Xue et al., 2007) and can survive on the stubble of other crops such as canola (*Brassica napus* L.), field pea [*Pisum sativum* subsp. *arvense* (L.) Asch.], and flax (*Linum usitatissimum* L.) (Chongo et al., 2001). Therefore, residues of these crops can serve as potential inoculum sources (Guo et al., 2010). In addition, it has been shown for several *Fusarium* spp. that the pathogens can survive for long periods of time, including up to 2 yr, thereby reducing the efficacy of crop rotation (Cotten and Munkvold, 1998; Inch and Gilbert, 2003; Manzo and Claflin, 1984).

Another tactic to manage the diseases caused by *Fusarium* spp. is the use of fungicides. However, the use of a seed treatment or foliar fungicide will depend on the *Fusarium* spp. Fungicides are commonly used on aboveground foliar diseases such as FHB. Many studies have assessed the efficacy of foliar fungicides for controlling FHB and reducing DON accumulation, but results have ranged from good to limited control (Cromey et al., 2001; Hollingsworth et al., 2006; Ioos et al., 2005; Jones, 2000; Mesterhazy et al., 2003; Milus and Parsons, 1994; Wegulo et al., 2011; Willyerd et al., 2010, 2012). Unfortunately, foliar fungicides will not control underground, root rotting diseases such as SDS (Groves and Smith, 2013). Using fungicide seed treatments has become common when dealing with these types of soilborne pathogens (Broders et al., 2007; Esker and Conley, 2012). Studies have demonstrated seed treatment efficacy on *Fusarium* spp., but the efficacy of different active ingredients varies with different *Fusarium* spp. (Broders et al., 2007; Ellis et al., 2011; Munkvold and O'Mara, 2002).

Although these different management practices (e.g., variety selection, crop rotation, and fungicide use) have been studied extensively as individual practices, the impact of interactions among these practices on *Fusarium* spp. population dynamics is not yet understood. This lack of information may stem from difficulty in detecting and quantifying *Fusarium* to the species level when using traditional methods such as the colony forming unit (CFU). Newer technologies, such as qPCR, allow for the unique opportunity to detect and quantify different *Fusarium* spp. in a high-throughput manner. Using this technology has potential for helping better understand how to manage common diseases caused by *Fusarium* spp.

Table 1. Crop rotation sequences for the corn (C), soybean (S), and wheat (W) rotations from 2002 to 2012.

	Crop rotation sequences†													
	Continuous			CS rotation		CSW rotation			CWS rotation			C_sW_sS rotation		
Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2012	C	S	W	C	S	S	W	C	W	S	C	W_s	S	C_s
2011	C	S	W	S	C	C	S	W	C	W	S	C_s	W_s	S
2010	C	S	W	C	S	W	C	S	S	C	W	S	C_s	W_s
2009	C	S	W	S	C	S	W	C	W	S	C	W_s	S	C_s
2008	C	S	W	C	S	C	S	W	C	W	S	C_s	W_s	S
2007	C	S	W	S	C	W	C	S	S	C	W	S	C_s	W_s
2006	C	S	W	C	S	S	W	C	W	S	C	W_s	S	C_s
2005	C	S	W	S	C	C	S	W	C	W	S	C_s	W_s	S
2004	C	S	W	C	S	W	C	S	S	C	W	S	C_s	W_s
2003	C	S	W	S	C	S	W	C	W	S	C	W_s	S	C_s
2002	C	S	W	C	S	C	S	W	C	W	S	C_s	W_s	S

† CS, corn alternated annually with soybean; CSW, corn followed by soybean followed by wheat; CWS, corn followed by wheat followed by soybean; C_sW_sS, corn followed by wheat followed by soybean rotation system used to mimic a livestock operation in which corn was harvested as silage (C_s) and wheat straw was removed (W_s) where appropriate.

To examine interactions among multiple disease management practices, a comprehensive study was designed and implemented to meet two objectives: (i) identify possible combinations of practices which could reduce risk of disease development by decreasing *Fusarium* spp. populations (current study) and (ii) quantify the effect of combinations of these practices on yield (Marburger et al., 2014). This paper will focus on the first objective in which the effect of crop rotation and management (i.e., variety selection and fungicide use) on *F. graminearum*, *F. oxysporum*, and *F. virguliforme* populations in the soil was examined.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected in the spring of 2011 and 2012 and fall of 2012 from three replicates of 14 crop rotation sequences representing seven different crop rotations (Table 1) in a corn–soybean–wheat rotation study conducted at the University of Wisconsin-Madison Agricultural Research Station near Arlington, WI (Table 2). This study was established in 2002 on a Plano silt loam soil with a 2 to 6% slope, and no-tillage practices have been performed since establishment. The experimental design was a randomized complete block in a split-split-plot arrangement. The main plot factor consisted of the 14 crop rotation sequences, representing each phase of each crop rotation, and main plots were 18.3 m wide and 18.3 m long. The subplot and sub-subplot treatments were established in 2010 as part of a 3-yr cycle. The subplot treatments consisted of two varieties chosen on the basis of their relative resistance (*R*) or susceptibility (S) to important *Fusarium*-related pathogens regarding each crop and were arranged in four rotations: RRR, SSS, SSR, and RRS. Sub-subplots consisted of two fungicide treatments and use of a fungicide versus an untreated check. Fungicides used were different for each crop. Headline (pyraclostrobin) was applied to the corn at the V5 growth stage (Ritchie et al., 1992) at 439 mL ha^{-1}. Maxim seed treatment (fludioxonil [0.0076 mg per seed]) was used for the soybean and Prosaro 421 (propiconazole and tebuconazole) was applied to the wheat at the Feekes 10.5.1 growth stage (Large, 1954) at 600 mL ha^{-1}. Sub-subplots were 3 m wide and 9 m long. One soil sample was collected from each sub-subplot. A soil sample consisted of five soil cores to a depth of 20 cm with a diameter of 1.9 cm and were randomly taken throughout the middle 2 m of the sub-subplot. The five soil cores were combined and stored at −20°C until a subsample was used for pathogen quantification by qPCR. A sub-subplot will be referred to as 'plot' or 'sample' hereafter. Fertilizers and pesticides were applied according to University of Wisconsin-Madison best management recommendations.

Table 2. Soil fertility, varieties used, and dates of field operations for each crop during the 2010 to 2012 growing seasons at the Arlington Agricultural Research Station (43°18′ N, 89°20′ W; Plano silt loam; Fine-silty, mixed, mesic Typic Argiudoll).

	Year		
	2010	2011	2012
Soil fertility			
Phosphorus, mg kg^{-1}	19	19	19
Potassium, mg kg^{-1}	127	102	104
pH	6.9	6.2	6.9
Organic matter, g kg^{-1}	3.1	3.2	2.9
Varieties used			
Corn			
Susceptible	Pioneer 37N16	Pioneer 37N16	Pioneer 0392AMX-R
Resistant	Pioneer 37N68	Pioneer 37N68	Pioneer 9917AM1
Soybean			
Susceptible	Pioneer 92M33	Pioneer 92M33	Pioneer 92M33
Resistant	Pioneer 92Y30	Pioneer 92Y30	Pioneer 92Y30
Wheat			
Susceptible	Pioneer 25R47	Pioneer 25R47	Pioneer 25R47
Resistant	Excel 234	Excel 234	Excel 234
Field operations			
Corn			
Planting date	10 May	11 May	11 May
Harvest date			
Silage	2 Sept.	7 Sept.	4 Sept.
Grain	30 Sept.	12 Oct.	25 Sept.
Soybean			
Planting date	4 May	4 May	11 May
Harvest date	4 Oct.	6 Oct.	3 Oct.
Wheat			
Planting date	19 Oct. (2009)	10 Oct. (2010)	7 Oct. (2011)
Harvest date	19 July	26 July	2 July

Soil Sample DNA Extraction

Total DNA from 500 mg of soil from each plot was extracted using MoBio UltraClean-htp 96 Well Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA). The overall quality and quantity of

Table 3. Real-time quantitative polymerase chain reaction primers and probes used for each *Fusarium* species.

Species	Source†	Target gene	Sequence (5' ⇒ 3')‡	Annealing temperature	Length of amplicon
				°C	bp
F. graminearum	This study	EF1α	TGGTCTCATTTTCCTCGATCG (F)	54.5	130
			AAATTACGACAAAGCCGCAA (*R*)	53.4	
			56-FAM– CTCGATACG/ZEN/CGCCTGTTACCCC– 3IABkFQ (P)	61.9	
F. oxysporum	Mishra et al. (2003)	ITS region	ACATACCACTTGTTGCCTCG (F)	55.2	339
			CGCCAATCAATTTGAGGAACG (*R*)	54.9	
F. virguliforme	Mbofung et al. (2011)	FvTox1	GCAGGCCATGTTGGTTCTGTA (F)	57.8	244
			GCACGTAAAGTGAGTCGTCTCATC (*R*)	57.5	
			6-FAM- ACTCAGCGCCCAGGA- MGBNFQ (T)	–	

† Primer and probe sequences for *F. graminearum* were developed using the RealTime SciTool software from Integrated DNA Technologies (IDT).

‡ F, forward primer; R, reverse primer; T, TaqMan probe; P, IDT probe.

each DNA sample was checked with a NanoDrop 1000 spectrophotometer from Thermo Scientific (Waltham, MA). Absorbance at 260 nm was used as a measure of DNA quantity, and quality was checked using the 260:280 absorbance ratio.

qPCR Optimization

Pure cultures of each species, *F. graminearum* (isolate z-3639), *F. oxysporum* (isolate 11390), and *F. virguliforme* (isolate 20485), were obtained from Kansas State University (APHIS permit P526P-11–02917). These isolates were used because pure isolates of these species from Wisconsin were not available at the time of qPCR optimization. DNA from each isolate grown on potato dextrose agar for 7 d at 23 ± 2°C was extracted using the FastDNA Spin Kit from MP Biomedicals (Santa Ana, CA). The resulting DNA was used to optimize each primer–probe set. Primer and probe sequences for each species were selected from the literature on the basis of their specificity to each species (Table 3). For *F. oxysporum*, the primer set developed by Mishra et al. (2003) targeted all *formae specialis* and races. Primer sequences for *F. graminearum* were originally adapted from Nicolaisen et al. (2009). Initial testing of this primer set with soil DNA revealed non-*F. graminearum*-specific amplicons. The cDNA sequence (Accession JQ862471.1) from which the Nicolaisen et al. (2009) primer set was developed was obtained. Using the Basic Local Alignment Search Tool from the National Center for Biotechnology Information revealed a region approximately 250 bp upstream from the Nicolaisen et al. (2009) primer set with low query cover for other *Fusarium* spp. The sequence from this region was used in the RealTime SciTool software from Integrated DNA Technologies to develop the primer and probe sequences for *F. graminearum* (Table 3). Reaction volume, primer concentration, annealing temperature, primer efficiency, and primer specificity were tested. Optimization of each primer set resulted in a reaction protocol specific to each species:

Fusarium graminearum: Reaction volume of 25 μL; containing 1 μL of DNA template, 12.5 μL of PerfeCTa qPCR Supermix (Quanta Biosciences, Gaithersburg, MD), 0.5 μL of each primer (from 10 μM stock), 0.5 μL of probe (from 10 μM stock), and 10 μL of Ambion diethylpyrocarbonate-treated water (Life Technologies, Carlsbad, CA). Initial denaturation took place at 95°C for 2 min followed by 40 cycles of 95°C for 10 sec, 60°C for 20 sec, and 68°C for 30 sec. The threshold value of the qPCR machine was manually set at 25.

Fusarium oxysporum: Reaction volume of 20 μL; containing 1 μL of DNA template, 10 μL of SsoAdvanced SYBR Green (BioRad, Hercules, CA), 1.5 μL of each primer (from 10 μM stock), and 6 μL of autoclaved Milli-Q purified water. Initial denaturation took place at 95°C for 2 min followed by 30 cycles of 95°C for 10 sec, 65°C for 20 sec, and 72°C for 30 sec. The threshold value was automatically set by the qPCR machine.

Fusarium virguliforme: Reaction volume of 25 μL; containing 1 μL of DNA template, 12.5 μL of PerfeCTa qPCR Supermix (Quanta Biosciences, Gaithersburg, MD), 0.5 μL of each primer (from 10 μM stock), 0.5 μL of probe (from 10 μM stock), and 10 μL of Ambion diethylpyrocarbonate-treated water (Life Technologies, Carlsbad, CA). Initial denaturation took place at 95°C for 2 min followed by 40 cycles of 95°C for 10 sec, 60°C for 20 sec, and 68°C for 30 sec. The threshold value of the qPCR machine was manually set at 25.

qPCR Standard Curve Development

Spores from several 7-d-old plates of the same isolate for each species were harvested by flooding plates with autoclaved Milli-Q purified water. Spore concentrations (spores mL^{-1}) were determined using a compound microscope and Bright-Line hemocytometer (Hausser Scientific, Horsham, PA). Tenfold serial dilutions of the spores were prepared. A standard curve for quantification of spore concentration (spores g^{-1} of soil) for each species was developed according to the protocol outlined by (Mbofung et al., 2011). Soil was autoclaved for 60 min at 121°C for two consecutive days. Spore suspensions (*F. graminearum*: 2 mL each of 1.55×10^7, 1.55×10^6, 1.55×10^5, 1.55×10^4, 1.55×10^3, 155, and 15 spores mL^{-1}; *F. oxysporum*: 2 mL each of 6.56×10^7, 6.56×10^6, 6.56×10^5, 6.56×10^4, 6.56×10^3, 656, and 65 spores mL^{-1}; *F. virguliforme*: 2 mL each of 2.22×10^7, 2.22×10^6, 2.22×10^5, 2.22×10^4, 2.22×10^3, 222, and 22 spores mL^{-1}) were added to 2 g of autoclaved soil in 15 mL Falcon tubes and mixed. A control consisting of 2 mL of autoclaved Milli-Q water mixed with 2 g of autoclaved soil was included. The suspensions were lyophilized for 12 h. Three 250-mg samples from each suspension were weighed. Total soil DNA from each sample was isolated using the MoBio UltraClean Soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA). The resulting DNA was used for quantification using the optimized qPCR assay protocol for each species. A linear regression equation (Fig. 1) for each species was obtained from the average quantification of each dilution.

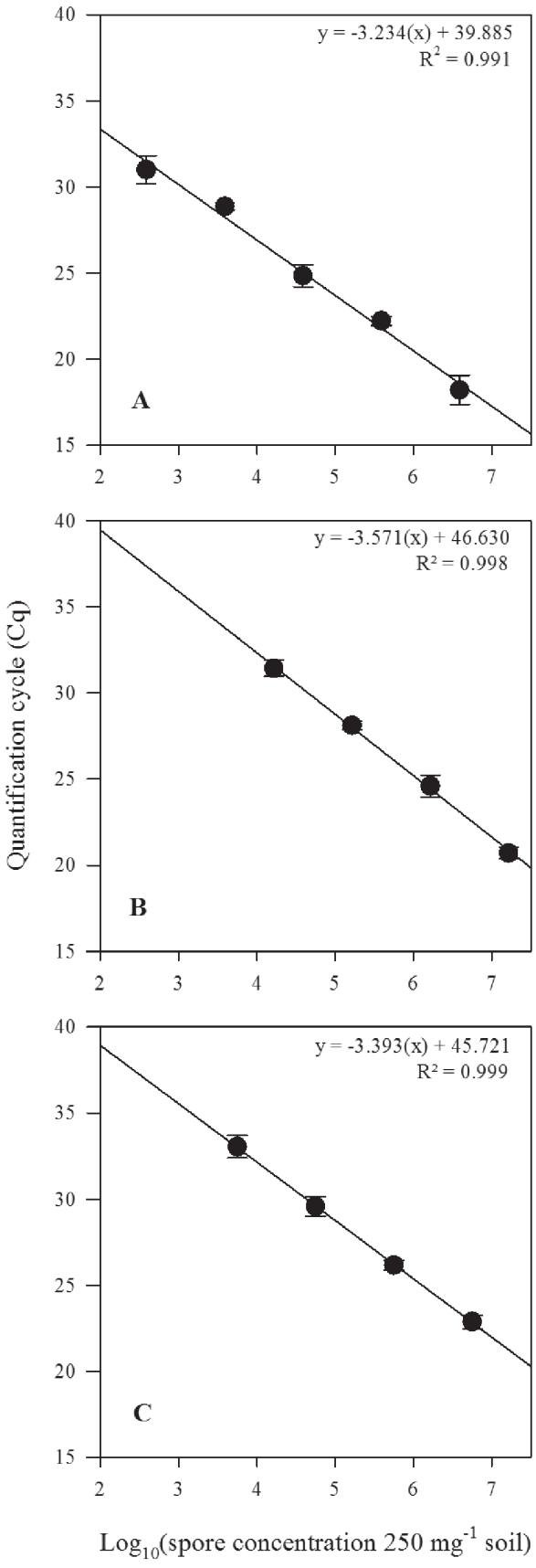

Figure 1. Standard curve quantification of (A) *Fusarium graminearum*, (B) *Fusarium oxysporum*, and (C) *Fusarium virguliforme* by method of real-time quantitative polymerase chain reaction. Error bars are ± one standard error of the mean (n = 9).

For *F. graminearum*, quantification occurred in all replicates (n = 9) at 1.55 × 10^3 spores g^{-1} of soil, whereas at 1.55 × 10^2 spores g^{-1} of soil, the fungus was quantified in zero out of nine replicates. Thus, the limit of quantification was defined as 1.55 × 10^3 spores g^{-1} of soil, corresponding to a quantification cycle (Cq) value (i.e., the number of polymerase chain reaction [PCR] cycles at which the target amplicon was quantified on the basis of the qPCR machine threshold) of 31.18, and the limit of detection was defined as Cq values between 31.18 and 33 for all subsequent reactions.

For *F. oxysporum*, quantification occurred in all replicates (n = 9) at 6.56 × 10^3 spores g^{-1} of soil, but the mean of this dilution (Cq = 31.76) was similar to the mean at 6.56 × 10^4 spores g^{-1} of soil (Cq = 31.44). Thus, the limit of quantification and detection was defined as 6.56 × 10^4 spores g^{-1} of soil. However, when testing soil DNA from the field, amplification of PCR products nonspecific to *F. oxysporum* were observed at Cq values similar or larger than the quantification and detection limit but not at Cq values <30 (data not shown). Therefore, the limit of quantification and detection was redefined to a Cq value of 30 and reactions were not carried past 30 cycles to eliminate detection and quantification of *F. oxysporum* nonspecific products.

For *F. virguliforme*, detection occurred in all of the replicates (n = 9) at 2.22 × 10^4 spores g^{-1} of soil, whereas at 2.22 × 10^3 spores g^{-1} of soil, it was only detected in six out of nine replicates. Thus, the limit of quantification was defined as 2.22 × 10^4 spores g^{-1} of soil, corresponding to a Cq value of 33.17, and the limit of detection was defined as Cq values between 33.17 and 35 for all subsequent reactions.

qPCR Analysis of Soil Samples

The qPCR reactions were performed in 96-well plates with a BioRad CFX96 Touch Real-Time PCR System. Analysis of each soil sample included three replicates using the procedure for each species outlined above. An average of the Cq values obtained from the three replicates was used to estimate the population:

Fusarium graminearum: The average Cq value for samples ≤31.18 was inserted into the standard curve equation:

$$\text{Spores g}^{-1}\text{ of soil} = \{10^{[(\text{Cq value} - 39.885)/-3.234]}\}2.$$

Cq values ranging from 31.19 to 33 were considered 'Present' but not quantifiable. Samples with Cq values more than 33 were considered absent of the pathogen (Fig. 1).

Fusarium oxysporum: The average Cq value for samples ≤30 was inserted into the standard curve equation:

$$\text{Spores g}^{-1}\text{ of soil} = \{10^{[(\text{Cq value} - 46.630)/-3.571]}\}2.$$

Cq values ≤30 were considered 'Present' and quantifiable (Fig. 1).

Fusarium virguliforme: The average Cq value for samples ≤33.17 was inserted into the standard curve equation:

$$\text{Spores g}^{-1}\text{ of soil} = \{10^{[(\text{Cq value} - 45.721)/-3.393]}\}2.$$

Cq values ranging from 33.18 to 35 were considered 'Present' but not quantifiable. Samples with Cq values more than 35 were considered absent of the pathogen (Fig. 1).

Disease Assessments

The incidence and severity of FHB occurring on the wheat were visually determined at the Feekes 11.1 to 11.2 growth stage (Large, 1954). Two samples, each measuring 0.3 m of row, from each plot were rated and combined to give an average percent incidence and severity for each plot. At harvest, approximately 500 g of wheat grain from each plot was sent to the University of Minnesota-Twin Cities for analysis of DON mycotoxin content.

The incidence and severity of SDS on the soybeans were visually determined at R6 (Fehr and Caviness, 1977) on each plot using the methods outlined by de Farias Neto et al. (2007).

Visual disease assessments were not performed on the corn. Corn grain and silage samples from each plot were sent to North Dakota State University for DON mycotoxin analysis.

Yield

At plant maturity for each crop, excluding the corn silage, each plot was harvested with a plot combine (Table 2). Grain weight and moisture were recorded and yield was converted to Mg ha^{-1} and adjusted to moisture content of 155 g kg^{-1} (corn) and 130 g kg^{-1} (soybean and wheat). Corn silage was harvested at approximately 50 to 60% grain moisture and yield was converted to Mg ha^{-1} and adjusted to 650 g kg^{-1} moisture content. Wheat yield in the continuous wheat rotation was not taken in 2010 and 2011 due to extremely low yields. In addition, relative mean yield (%) for each plot was calculated, where (%) = (plot yield/average crop yield within year)100. Relative mean yield was used for combining the yields from all three crops.

Statistical Analyses

The population of each *Fusarium* spp. from each plot was considered the response variable for statistical analysis. Mixed-model analysis of variance was conducted using the PROC GLIMMIX procedure in SAS Version 9.3 (SAS Institute Inc., Cary, NC). In addition to the population variable, a second variable (Presence) was created, which was considered to be a binomial variable for each pathogen, where a positive detection and/or quantification of each pathogen was coded as 1 and all other events as 0. Models were constructed individually for each species. For *F. oxysporum*, populations were natural log transformed to meet model assumptions. Degrees of freedom were calculated using the Kenward-Rogers method (Littell et al., 2006). Due to the low number samples with a population estimate for *F. graminearum* and *F. virguliforme*, analysis of variance was conducted only on the 'Presence' variable using a binomial distribution along with the Laplace approximation within PROC GLIMMIX (Bolker et al., 2008). The 'ilink' function was used to back transform the model estimate to the estimated incidence (i.e., the total number of plots where the pathogen was present divided by the total plots sampled) to better understand significant main effects and/or interactions.

Because the variety rotations, RRS and SSR, for the wheat were not made in the fall 2011, two separate analyses were conducted to examine the effect of variety and variety rotation. The first analysis included data from all three crops to examine the effect of variety (i.e., either a resistant or susceptible variety used). Crop rotation, variety, fungicide use, and all two-way and three-way interactions were considered fixed effects. Sampling period, replication(sampling period), replication × crop rotation(sampling period), replication × crop rotation × variety(sampling period), and the overall error term were considered random effects. In the second analysis, the wheat data was removed to investigate the effect of variety rotation (e.g., SSR). For this analysis, crop rotation, variety rotation, fungicide use, and all two-way and three-way interactions were considered fixed effects. Sampling period, replication(sampling period), replication × crop rotation(sampling period), replication × crop rotation × variety rotation(sampling period), and the overall error term were considered random effects. For both analyses, fixed effects were tested for significance at $\alpha = 0.05$. Final models were a function of the model fit statistics (AIC, BIC, −2 Res Log Likelihood) as well as biological interpretation.

Mixed-model analysis of variance was also conducted on the FHB assessments (incidence, severity, and DON concentration) by year using PROC GLIMMIX. The fixed and random effects used were similar to the first analysis mentioned above. Fixed effects were tested for significance at $\alpha = 0.05$, and means comparisons were calculated on the basis of Fisher's protected LSD.

All correlations were done using the Kendall tau rank correlation coefficient (PROC CORR Kendall) in SAS, with the exception of using the Spearman rank correlation coefficient to measure the association between *F. oxysporum* populations and relative yield (PROC CORR Spearman).

RESULTS AND DISCUSSION

Environment

Air temperatures in April to June and September were lower in 2011 than in 2010 and 2012 (Table 4). Air temperatures were similar to the 30-yr average for all other months except for higher temperatures experienced in July 2012. Above-average rainfall occurred in 2010 and was higher than amounts in 2011 and 2012. Rainfall in 2011 was lower than normal in May to August. In 2012, total precipitation was below normal, except in July when all precipitation was received after July 15th. Drought conditions were experienced from June through mid-July in 2012 (U.S. Drought Monitor, 2012).

Fusarium graminearum

Fusarium graminearum was present and quantified in 13.3 and 2.7% of the plots ($n = 1008$), respectively. Presence varied among sampling periods. In spring 2011, *F. graminearum* was present in 6.0% of plots, increased to 20.8% in spring 2012, and decreased to 13.0% in fall 2012. Quantification followed a similar trend as presence with 0.9% of plots in spring 2011 followed by 3.9% in spring 2012 and 3.6% in fall 2012. Populations ranged from 0 to 23,710 spores g^{-1} of soil, and for plots with a population greater than zero, population averaged 3692 spores g^{-1} of soil ($n = 27$).

In the first analysis, crop rotation was the only significant main effect observed ($P < 0.001$) (Table 5). In addition, there was a significant crop rotation × fungicide interaction ($P < 0.001$) (Table 5). However, this interaction was driven

Table 4. Mean monthly air temperature and total monthly precipitation during the 2010 to 2012 growing seasons and during the past 30 yr.

	Year			
	2010	2011	2012	30 yr.
Air temperature, °C				
Apr.	9.4	5.0	6.7	7.8
May	13.9	12.2	15.0	13.9
June	18.9	18.3	20.0	19.4
July	21.7	22.8	24.4	21.7
Aug.	21.1	20.0	19.4	20.6
Sept.	14.4	13.9	14.4	16.1
Average	16.6	15.4	16.7	16.6
Precipitation, mm				
Apr.	94.0	88.9	78.7	88.9
May	106.7	40.6	73.7	94.0
June	193.0	104.1	7.6	116.8
July	236.2	63.5	109.2	104.1
Aug.	119.4	38.1	73.7	96.5
Sept.	114.3	99.1	25.4	91.4
Total	863.6	434.3	368.3	591.7

Table 5. Results from the ANOVA for Analysis 1 and Analysis 2 for fixed main effects and interactions for *Fusarium graminearum*, *F. oxysporum*, and *F. virguliforme*.

Source of variation	*F. graminearum*†	*F. oxysporum*‡	*F. virguliforme*†
Analysis 1			
Crop rotation (CR)	***	NS§	NS
Variety selection (VS)	NS	NS	NS
CR × VS	NS	NS	–
Fungicide use (FUNG)	NS	NS	NS
CR × FUNG	***	NS	–
VS × FUNG	NS	NS	–
CR × VS × FUNG	NS	NS	–
Analysis 2			
CR	NS	NS	NS
VR	NS	NS	NS
CR × VR	–	NS	–
FUNG	***	NS	NS
CR × FUNG	–	NS	–
VR × FUNG	–	NS	–
CR × VR × FUNG	–	NS	–

*** Significant at the 0.001 probability level.

† Modeling performed on the 'Presence' variable because of the low number of plots with a population estimate; – indicates modeling could not be performed due to the low number of plots where the pathogen was present.

‡ Modeling performed on the 'Population' variable.

§ NS, no significant differences at $P \leq 0.05$.

primarily by crop rotation, particularly where wheat was part of the rotation (Table 6). Presence in the continuous wheat (WW) rotation occurred in 44% of plots, compared with 13% in the rotations corn followed by soybean followed by wheat (CSW), corn followed by wheat followed by soybean (CWS), and corn followed by wheat followed by soybean in which corn was harvested as silage and wheat straw was removed where appropriate (CsWsS). Presence in the continuous corn (CC), continuous soybean (SS), and corn alternated annually with soybean (CS) rotations occurred in 10, 8, and 4% of plots, respectively. Presence was lower in fungicide-treated plots within all rotations except for the CWS and WW rotations (data not shown).

For the second analysis, modeling could only be performed with main effects due to the low number of plots where *F. graminearum* was present. A significant fungicide-use main effect was observed ($P < 0.001$) (Table 5). Presence was lower in the fungicide-treated plots (5.2%) versus the untreated plots (7.2%).

Presence of *F. graminearum* in the soil and relative yield across all plots ($n = 984$) were weakly negatively correlated ($\tau = -0.07$, $P = 0.01$). Population and relative yield were not correlated ($\tau = -0.02$, $P = 0.36$).

Deoxynivalenol was found in 6.3% of corn grain and silage samples ($n = 240$), with a range of 0 to 2.2 mg kg^{-1} (data not shown). However, no correlation was found between presence of *F. graminearum* in the soil and DON concentration in the corn grain, corn silage, or corn grain and silage samples combined.

For the FHB disease assessments, higher amounts of FHB were observed in 2010 and 2011 compared with 2012 (Table 7). Infection by *F. graminearum* favors high humidity and moderately warm temperatures (Sutton, 1982), both of which were present during the time of flowering in 2010 and 2011. Hot, dry weather present during the summer of 2012 led to a low intensity of FHB. The impact of crop rotation and variety selection on FHB development varied by year (Table 7). Fungicide use showed more consistency with decreased FHB incidence in 2 out of 3 yr and decreased FHB severity for all 3 yr. For the correlations between *F. graminearum* and the FHB disease assessments, presence of *F. graminearum* in the soil was weakly negatively correlated with incidence ($\tau = -0.19$, $P < 0.001$), severity ($\tau = -0.17$, $P = 0.001$), and DON concentration ($\tau = -0.28$, $P < 0.001$). When examining 2011 and 2012 separately, presence in the soil was positively correlated with incidence and severity for spring 2011, but no correlation was found for incidence, severity, and DON in spring or fall 2012. The low amounts of FHB from the hot, dry weather during the summer of 2012 may explain the lack of association for spring and fall 2012. Presence in the soil and DON concentration across all wheat and corn samples ($n = 622$) were weakly negatively correlated ($\tau = -0.11$, $P = 0.003$). These results suggest the likelihood of FHB symptoms in wheat or DON accumulation in corn grain and silage are a function of several factors, and the ability to quantify *F. graminearum* in the soil may not serve as a good predictor. While it has been shown that *F. graminearum* can survive on buried crop residues, inoculum for the development of FHB probably comes from aboveground crop residues

Table 6. Combined summary of number of plots screened, number of plots where each pathogen was present, and spore population densities by main effects for *Fusarium graminearum*, *F. oxysporum*, and *F. virguliforme*.

Main effect	Plots	F. graminearum		F. oxysporum		F. virguliforme	
		Present	Population†	Present	Population† (×10⁶)	Present	Population†
	No.	No.		No.		No.	
Crop rotation‡							
CC	72	7	P–8,034	72	0.47–16.59	1	P
SS	72	6	P	72	0.47–8.52	8	P–12,945
WW	72	32	P–3,117	72	0.21–12.73	3	P
CS	144	6	P–1,019	144	0.33–24.79	11	P–19,851
CSW	216	28	P–23,710	216	0.32–9.46	10	P–10,489
CWS	216	27	P–8,627	216	0.24–15.75	16	P–25,345
C_sW_sS	216	28	P–7,324	216	0.50–7.16	13	P–66,887
Total	1,008	134	P–23,710	1,008	0.21–24.79	62	P–66,887
Variety selection							
Resistant	504	63	P–23,710	504	0.21–16.34	30	P–54,198
Susceptible	504	71	P–8,034	504	0.22–24.79	32	P–66,887
Total	1,008	134	P–23,710	1,008	0.21–24.79	62	P–66,887
Fungicide use							
Untreated	504	65	P–10,530	504	0.22–16.34	32	P–66,887
Treated	504	69	P–23,710	504	0.21–24.79	30	P–54,198
Total	1,008	134	P–23,710	1,008	0.21–24.79	62	P–66,887

† Estimated number of spores g^{-1} of soil; P, pathogen present but not quantifiable.

‡ CC, continuous corn; SS, continuous soybean; WW, continuous wheat; CS, corn alternated annually with soybean; CSW, corn followed by soybean followed by wheat; CWS, corn followed by wheat followed by soybean, C_sW_sS, corn followed by wheat followed by soybean rotation system used to mimic a livestock operation in which corn was harvested as silage (C_s) and wheat straw was removed (W_s) where appropriate.

or from other sources (e.g., windblown from neighboring fields) (Cotten and Munkvold, 1998).

Nonetheless, although our results suggested that presence of *F. graminearum* in the soil was not a good predictor of observed FHB, we did observe that the trend of the average percent of *F. graminearum* presence for a sampling period followed the trend for the average FHB percent incidence and severity before each sampling period. This suggested FHB was contributing to *F. graminearum* presence in the soil, making it a potential inoculum source. This trend was probably driven by the increased presence of *F. graminearum* within rotations containing wheat, particularly from the WW rotation, where percent incidence of this rotation (44%) was more than three times higher than percent incidence of all other rotations containing wheat (13%). From our analyses, presence of *F. graminearum* could be lowered by removing wheat from the crop rotation or using a fungicide. If wheat remained in the rotation, using a fungicide lowered presence of *F. graminearum* in the CSW and C_sW_sS rotations but not in the WW and CWS rotations. If wheat was removed from the rotation, using a fungicide lowered the presence of *F. graminearum* in the soil.

Fusarium oxysporum

Fusarium oxysporum was present and quantified in 100% of the evaluated plots (n = 1008), making it the most prevalent of the three species tested. Other previous *Fusarium* spp. surveys have also shown *F. oxysporum* as the most prevalent species (Díaz Arias et al., 2013a; Leslie et al., 1990). Populations ranged from $0.21 \times 10^6 - 24.79 \times 10^6$ spores g^{-1} of soil with an average population of 2.27×10^6 spores g^{-1} of soil. Population averages for each sampling period increased with time (1.76×10^6, 1.98×10^6, and 3.08×10^6 spores g^{-1} of soil).

In the first analysis, no significant main effects or interactions were observed ($P > 0.05$) (Table 5). Similar to the first analysis, no significant main effects or interactions were observed in the second analysis ($P > 0.05$) (Table 5).

Population and relative yield across all plots (n = 984) was negatively correlated ($\rho = -0.11$, $P < 0.001$). Using a simple linear regression, a 1.6% relative yield loss was observed for every 1×10^6 increase in population. While qPCR has been demonstrated to predict yield loss in cereals resulting from *F. culmorum* and *F. pseudograminearum* (Hollaway et al., 2013), the large amount of variability ($R^2 = 0.02$) within our regression would not make this a useful tool for predicting yield loss from *F. oxysporum*. Yield loss attributed to *F. oxysporum* has been briefly studied in soybean but results have been mixed. Leath and Carroll (1985) found 48 and 56% yield loss in two soybean cultivars susceptible to *Fusarium* root rot after inoculation with *F. oxysporum*, but Díaz Arias et al. (2013b) found no difference in yield from inoculated field microplots. Although our results suggested the potential for small yield loss from increasing populations of *F. oxysporum*, none of the management strategies employed in this study significantly reduced *F. oxysporum* populations in a way that would potentially mitigate the loss. This lack of management effect on *F. oxysporum* could

Table 7. Results from the ANOVA for *Fusarium* head blight percent incidence (% Inc.), percent severity (% Sev.), and vomitoxin (DON) concentrations for the wheat rotations for the 2010 to 2012 growing seasons.

	2010†			2011†			2012		
	% Inc.	% Sev.	DON	% Inc.	% Sev.	DON	% Inc.	% Sev.	DON
			ppm			ppm			ppm
Crop rotation (CR)‡									
WW	–	–	–	20.0	49.0	–	0.01	1.4	0.01
CSW	6.6	8.3	1.1	9.3	27.3	0.5	0.01	7.0	0.01
CWS	12.8	12.8	2.2	7.4	26.1	1.2	0.01	6.3	0.02
C_sW_sS	11.2	11.4	1.4	9.9	24.7	1.0	0.01	13.9	0.03
LSD (0.05)	NS§	NS	0.5	4.4	14.5	0.3	NS	NS	NS
Variety selection (VS)									
Resistant	5.5	8.5	0.9	11.9	33.7	0.6	0.01	5.8	0.01
Susceptible	14.9	13.1	2.3	11.4	29.9	1.3	0.01	8.5	0.02
LSD (0.05)	4.2	NS	0.3	NS	NS	0.2	NS	NS	NS
Fungicide use (FUNG)									
Untreated	15.1	13.4	1.9	14.5	37.2	1.0	0.01	10.5	0.03
Treated	5.3	8.2	1.3	8.8	26.4	0.9	0.01	3.8	0.01
LSD (0.05)	3.2	4.4	0.3	2.5	5.3	NS	NS	5.3	0.01
ANOVA									
CR	NS	NS	**	**	*	**	NS	NS	NS
VS	**	NS	***	NS	NS	***	NS	NS	NS
CR × VS	NS	NS	*	NS	NS	*	NS	NS	NS
FUNG	***	*	***	***	***	NS	NS	*	***
CR × FUNG	NS	NS	NS	NS	NS	NS	NS	NS	NS
VS × FUNG	**	NS	NS	NS	NS	NS	NS	NS	*
CR × VS × FUNG	NS	NS	NS	NS	NS	**	NS	NS	NS

* Significant at the $P = 0.05$ probability level.

** Significant at the $P = 0.01$ probability level.

*** Significant at the $P = 0.001$ probability level.

† – indicates data not available.

‡ WW, continuous wheat; CSW, corn followed by soybean followed by wheat; CWS, corn followed by wheat followed by soybean, C_sW_sS, corn followed by wheat followed by soybean rotation system used to mimic a livestock operation in which corn was harvested as silage (C_s) and wheat straw was removed (W_s) where appropriate.

§ NS, no significant differences at $P \leq 0.05$.

be due to it being ubiquitous in most soils and having wide host range, with more than 100 *formae specialis* and races classified (Leslie and Summerell, 2006). In addition, the primer set developed by Mishra et al. (2003) targets *F. oxysporum* as a whole. Therefore, the method applied in this study quantified *F. oxysporum* including all *formae specialis* and races if present. Although we did not observe any significant effects from the management strategies employed, cereals are generally not affected by *F. oxysporum* (Leslie and Summerell, 2006), even though plants may be infected with the pathogen. This may be due to *formae specialis* and races of *F. oxysporum* present which are saprophytes and only colonize when dead or decaying plant tissue is present (Leslie and Summerell, 2006).

Fusarium virguliforme

Fusarium virguliforme was present and quantified in 6.2 and 1.4% of plots (n = 1008), respectively. Presence varied among sampling periods and decreased over time, from 7.4% in spring 2011 to 6.8% in spring 2012 and 0.3% by fall 2012. Quantification followed a similar pattern with 3.3% in spring 2011 to 0.6% in spring 2012 and 0.3% by fall 2012. Populations ranged from 0 to 66,887 spores g^{-1} of soil, and for plots with a population greater than zero, population averaged 25,095 spores g^{-1} of soil (n = 14).

For both analyses, modeling could only be performed with main effects due to the low number of plots where *F. virguliforme* was present. For each analysis, no significant effects were observed at $\alpha = 0.05$ (Table 5). In addition, presence of *F. virguliforme* did not relate to relative yield ($\tau = -0.03$, $P = 0.18$), across all plots respectively (n = 984).

Although crop rotation and management did not influence presence of *F. virguliforme* in the soil, we observed that the trend of average percent of presence followed the trend of total precipitation before each sampling period (Table 4). Increased soil moisture has been shown to increase SDS foliar symptoms (Scherm and Yang, 1996; Scherm et al., 1998; Vick et al., 2003), and increased soil moisture is often observed early in the growing season around the time of planting. For this experiment, above-average rainfall was observed during planting in May 2010, while below average rainfall was observed during May 2011 and 2012 (Table 4).

Unfortunately, no SDS symptoms were found during the course of this experiment, and historically, SDS symptoms have not been confirmed within the long-term crop rotation study. However, our results suggested *F. virguliforme* was present in some cases at populations capable of causing foliar symptoms. Populations of 10^2 to 10^3 CFU g^{-1} of soil isolated from dilution plating methods have been reported from symptomatic soybean plants (Mbofung et al., 2011; Rupe et al., 1997, 1999; Scherm et al., 1998), with Scherm et al. (1998) reporting a strong positive association between *F. virguliforme* soil populations and SDS severity. Furthermore, our study found *F. virguliforme* in four samples within rotations of continuous corn or continuous wheat (Table 6). Although no SDS symptoms were observed in the current study, studies have isolated *F. virguliforme* from asymptomatic soybean plants (Gao et al., 2004; Rupe, 1989), and in the greenhouse, it has been found that *F. virguliforme* can colonize and/or infect crops other than soybean, including corn and wheat as asymptomatic hosts (Kolander et al., 2012). These results suggest the role of individual or multiple factors directly or indirectly influencing the presence and reproduction of *F. virguliforme* in the field (Roy, 1997). Environmental conditions (e.g., soil moisture and temperature) coupled with different management practices (e.g., planting date, crop rotation, variety selection, tillage) certainly contribute to these mixed results (Kolander et al., 2012). While we were not able to test the interactions among the management tactics used in this study, additional research examining these practices under artificially inoculated fields or fields with a history of high SDS presence could reveal combinations of management strategies that would help lower populations of *F. virguliforme* in the soil and reduce the risk of SDS symptom development.

CONCLUSIONS

This study was aimed at identifying interactions among crop rotation, variety selection, and fungicide use that would reduce population levels of the three species examined and reduce the potential risk of disease development. To test these interactions, we developed and optimized a high-throughput approach for quantifying the populations of *F. graminearum*, *F. oxysporum*, and *F. virguliforme* from the soil using qPCR technology. No significant interactions or individual control tactics were found to reduce *F. oxysporum* populations or *F. virguliforme* presence. A crop rotation × fungicide interaction influenced *F. graminearum* presence. Introducing wheat into the rotation increased presence; however, fungicide use only lowered the presence of *F. graminearum* in certain crop rotations. The results from this study found few effective interactions among crop rotation, variety selection, and fungicide use for controlling the populations or presence of the three *Fusarium* spp. studied, with significant interactions or individual control methods being dependent on the species being examined.

Acknowledgments

The authors wish to thank the U.S. Wheat and Barley Scab Initiative, National Institute of Food and Agriculture, Wisconsin Institute for Sustainable Agriculture, and the University of Wisconsin-Madison College of Agriculture and Life Sciences for funding this research. We especially thank John Gaska, Adam Roth, Kent Kohn, Thierno Diallo, and all the members of the Conley, Lauer, and Ané programs for their technical support.

References

Bai, G., and G. Shaner. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760–766. doi:10.1094/PD-78-0760

Bai, G., and G. Shaner. 2004. Management and resistance in wheat and barley to *Fusarium* head blight. Annu. Rev. Phytopathol. 42:135–161. doi:10.1146/annurev.phyto.42.040803.140340

Bernstein, E.R., Z.K. Atallah, N.C. Koval, B.D. Hudelson, and C.R. Grau. 2007. First report of sudden death syndrome of soybean in Wisconsin. Plant Dis. 91:1201–1201. doi:10.1094/PDIS-91-9-1201B

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2008. Generalized linear mixed models: A practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135. doi:10.1016/j.tree.2008.10.008

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom

Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155–1160. doi:10.1094/PDIS-91-9-1155

Burgess, L.W. 1981. General ecology of the *Fusaria*. In: P.A. Nelson, T.A. Toussoun, and R.J. Cook, editors, *Fusarium*: Diseases, biology, and taxonomy. The Pennsylvania State Univ. Press, University Park. p. 225–235.

Chongo, G., B.D. Gossen, H.R. Kutcher, J. Gilbert, T.K. Turkington, M.R. Fernandez, and D. McLaren. 2001. Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. 23:132–137. doi:10.1080/07060660109506920

Cotten, T.K., and G.P. Munkvold. 1998. Survival of *Fusarium moniliforme, F. proliferatum*, and *F. subglutinans* in maize stalk residue. Phytopathology 88:550–555. doi:10.1094/PHYTO.1998.88.6.550

Cromey, M.G., D.R. Lauren, R.A. Parkes, K.I. Sinclair, S.C. Shorter, and A.R. Wallace. 2001. Control of *Fusarium* head blight of wheat with fungicides. Austral. Plant Pathol. 30:301–308.

de Farias Neto, A.L., R. Hashmi, M. Schmidt, S.R. Carlson, G.L. Hartman, S. Li, R.L. Nelson, and B.W. Diers. 2007. Mapping and confirmation of a new sudden death syndrome resistance QTL on linkage group D2 from the soybean genotypes PI 567374 and 'Ripley'. J. Mol. Breeding 20:53–62. doi:10.1007/s11032-006-9072-8

Díaz Arias, M.M., L.F. Leandro, and G.P. Munkvold. 2013b. Aggressiveness of *Fusarium* species and impact of root infection on growth and yield of soybeans. Phytopathology 103:822–832. doi:10.1094/PHYTO-08-12-0207-R

Díaz Arias, M.M., G.P. Munkvold, M.L. Ellis, and L.F.S. Leandro. 2013a. Distribution and frequency of *Fusarium* species associated with soybean roots in Iowa. Plant Dis. 97:1557–1562. doi:10.1094/PDIS-11-12-1059-RE

Ellis, M.L., K.D. Broders, P.A. Paul, and A.E. Dorrance. 2011. Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. Plant Dis. 95:401–407. doi:10.1094/PDIS-05-10-0317

Ellis, M.L., H.H. Wang, P.A. Paul, S.K. St. Martin, L.K. McHale, and A.E. Dorrance. 2012. Identification of soybean genotypes resistant to *Fusarium graminearum* and genetic mapping of resistance quantitative trait loci in the cultivar Conrad. Crop Sci. 52:2224–2233. doi:10.2135/cropsci2011.11.0624

Esker, P.D., and S.P. Conley. 2012. Probability of yield response and breaking even for soybean seed treatments. Crop Sci. 52:351–359. doi:10.2135/cropsci2011.06.0311

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Iowa Cooperative Extension Service Special Report 80. Iowa State University, Ames.

Gao, X., T.A. Jackson, K.N. Lambert, S. Li, G.L. Hartman, and T.L. Niblack. 2004. Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. Plant Dis. 88:1372–1380. doi:10.1094/PDIS.2004.88.12.1372

Goswami, R.S., and H.C. Kistler. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515–525. doi:10.1111/j.1364-3703.2004.00252.x

Groves, C., and D. Smith. 2013. Sudden death syndrome of soybean. University of Wisconsin Farm Facts. University of Wisconsin, Madison.

Guo, X.W., W.G.D. Fernando, P. Bullock, and H. Sapirstein. 2010. Quantifying cropping practices in relation to inoculum levels of *Fusarium graminearum* on crop stubble. Plant Pathol. 59:1107–1113. doi:10.1111/j.1365-3059.2010.02353.x

Hershman, D.E., J.W. Hendrix, R.E. Stuckey, P.R. Bachi, and G. Henson. 1990. Influence of planting date and cultivar on soybean sudden death syndrome in Kentucky. Plant Dis. 74:761–766. doi:10.1094/PD-74-0761

Hirrel, M.C. 1983. Sudden death syndrome of soybean: A disease of unknown etiology (Abstr.). Phytopathol. 73:501–502.

Hollaway, G.J., M.L. Evans, H. Wallwork, C.B. Dyson, and A.C. McKay. 2013. Yield loss in cereals, caused by *Fusarium culmorum* and *F. pseudograminearum*, is related to fungal DNA in soil prior to planting, rainfall, and cereal type. Plant Dis. 97:977–982. doi:10.1094/PDIS-09-12-0867-RE

Hollingsworth, C.R., C.D. Motteberg, and W.G. Thompson. 2006. Assessing fungicide efficacies for the management of *Fusarium* head blight on spring wheat and barley. Online. Plant Health Prog. 10.1094/PHP-2006–0906–01-RS.

Inch, S.A., and J. Gilbert. 2003. Survival of *Gibberella zeae* in *Fusarium*-damaged wheat kernels. Plant Dis. 87:282–287. doi:10.1094/PDIS.2003.87.3.282

Ioos, R., A. Belhadj, M. Menez, and A. Faure. 2005. The effects of fungicides on *Fusarium spp.* and *Microdochium nivale* and their associated trichothecene mycotoxins in French naturally-infected cereal grains. Crop Prot. 24:894–902. doi:10.1016/j.cropro.2005.01.014

Jones, R.K. 2000. Assessments of *Fusarium* head blight of wheat and barley in response to fungicide treatment. Plant Dis. 84:1021–1030. doi:10.1094/PDIS.2000.84.9.1021

Kolander, T.M., J.C. Bienapfl, J.E. Kurle, and D.K. Malvick. 2012. Symptomatic and asymptomatic host range of *Fusarium virguliforme*, the causal agent of soybean sudden death syndrome. Plant Dis. 96:1148–1153. doi:10.1094/PDIS-08-11-0685-RE

Krupinsky, J.M., K.L. Bailey, M.P. McMullen, B.D. Gossen, and T.K. Turkington. 2002. Managing plant disease risk in diversified cropping systems. Agron. J. 94:198–209. doi:10.2134/agronj2002.0198

Large, E.C. 1954. Growth stages in cereals illustration of the Feekes scale. Plant Pathol. 3:128–129. doi:10.1111/j.1365-3059.1954.tb00716.x

Leath, S., and R.B. Carroll. 1985. Use of ridge regression to predict yield reduction by *Fusarium* spp. *In* selected soybean cultivars. Can. J. Plant Pathol. 7:58–66. doi:10.1080/07060668509501516

Leslie, J.F., C.A. Pearsons, P.E. Nelson, and T.A. Toussoun. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathol. 80:343–350. doi:10.1094/Phyto-80-343

Leslie, J.F., and B.A. Summerell. 2006. The *Fusarium* laboratory manual. 1st ed. Blackwell Publishing, Ames, IA.

Lipps, P., A.E. Dorrance, and D.R. Mills. 2001. *Gibberella* stalk rot of corn, AC-0033–01. Ohio State University Extension, Columbus.

Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models. 2nd ed. SAS Institute, Cary, NC.

Manzo, S.K., and L.E. Claflin. 1984. Survival of *Fusarium moniliforme* hyphae and conidia in grain sorghum stalks. Plant Dis. 68:866–867. doi:10.1094/PD-69-866

Marburger, D.A., S.P. Conley, P.D. Esker, J.G. Lauer, and J.M. Ané. 2014. Yield response to crop/genotype rotations and fungicide use to manage *Fusarium*-related diseases. Crop Sci. doi:10.2135/cropsci2014.03.0201 (in press).

Mbofung, G.C.Y., A. Fessehaie, M.K. Bhattacharyya, and L.F.S. Leandro. 2011. A new TaqMan real-time polymerase chain reaction assay for quantification of *Fusarium virguliforme* in soil. Plant Dis. 95:1420–1426. doi:10.1094/PDIS-02-11-0120

McMullen, M., R. Jones, and D. Gallenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340–1348. doi:10.1094/PDIS.1997.81.12.1340

Mesterhazy, A., T. Bartok, and C. Lamper. 2003. Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of *Fusarium* head blight. Plant Dis. 87:1107–1115. doi:10.1094/PDIS.2003.87.9.1107

Milus, E.A., and C.E. Parsons. 1994. Evaluation of foliar fungicides for controlling *Fusarium* head blight of wheat. Plant Dis. 78:697–699. doi:10.1094/PD-78-0697

Mishra, P.K., R.T.V. Fox, and A. Culham. 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic *Fusaria*. FEMS Microbiol. Lett. 218:329–332. doi:10.1111/j.1574-6968.2003.tb11537.x

Munkvold, G.P., and J.K. O'Mara. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. Plant Dis. 86:143–150. doi:10.1094/PDIS.2002.86.2.143

Nelson, P.A., T.A. Toussoun, and W.F.O. Marasas. 1983. *Fusarium* species: An illustrated manual for identification. The Pennsylvania State Univ. Press, University Park.

Nicolaisen, M., S. Suproniene, L.K. Nielsen, I. Lazzaro, N.H. Spliid, and A.F. Justesen. 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. J. Microbiol. Methods 76:234–240. doi:10.1016/j.mimet.2008.10.016

Pioli, R.N., L. Mozzoni, and E.N. Morandi. 2004. First report of pathogenic association between *Fusarium graminearum* and soybean. Plant Dis. 88:220. doi:10.1094/PDIS.2004.88.2.220A

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1992. How a corn plant develops. Iowa State University Extension Special Report 48. Iowa State University, Ames.

Roy, K.W. 1997. Sporulation of *Fusarium solani* f. sp. *glycines*, causal agent of sudden death syndrome, on soybeans in the mid-western and southern United States. Plant Dis. 81:566–569. doi:10.1094/PDIS.1997.81.6.566

Roy, K.W., G.W. Lawrence, H.H. Hodges, K.S. McLean, and J.F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation to *Heterodera glycines* to disease severity. Phytopathol. 79:191–197. doi:10.1094/Phyto-79-191

Roy, K.W., J.C. Rupe, D.E. Hershman, and T.S. Abney. 1997. Sudden death syndrome of soybean. Plant Dis. 81:1100–1111. doi:10.1094/PDIS.1997.81.10.1100

Rupe, J.C. 1989. Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome. Plant Dis. 73:581–584. doi:10.1094/PD-73-0581

Rupe, J.C., E.E. Gbur, and D.M. Marx. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Dis. 75:47–50. doi:10.1094/PD-75-0047

Rupe, J.C., R.T. Robbins, C.M. Becton, W.A. Sabbe, and E.E. Gbur. 1999. Vertical and temporal distribution of *Fusarium solani* and *Heterodera glycines* in fields with sudden death syndrome of soybean. Soil Biol. Biochem. 31:245–251. doi:10.1016/S0038-0717(98)00108-4

Rupe, J.C., R.T. Robbins, and E.E. Gbur. 1997. Effect of crop rotation on soil population densities of *Fusarium solani* and *Heterodera glycines* and on the development of sudden death syndrome of soybean. Crop Prot. 16:575–580. doi:10.1016/S0261-2194(97)00031-8

Scherm, H., and X.B. Yang. 1996. Development of sudden death syndrome of soybean in relation to soil temperature and soil water matric potential. Phytopathol. 86:642–649. doi:10.1094/Phyto-86-642

Scherm, H., X.B. Yang, and P. Lundeen. 1998. Soil variables associated with sudden death syndrome in soybean fields in Iowa. Plant Dis. 82:1152–1157. doi:10.1094/PDIS.1998.82.10.1152

Sciumbato, G.L., and B.L. Keeling. 1985. Sudden death syndrome (SDS) of soybeans in Mississippi in 1984 (Abstr.). Proceedings of the 12th Annual Meeting of the Southern Soybean Disease Workers, Birmingham, AL. 26–28 Mar. Southern Soybean Disease Workers, Birmingham, AL.

Summerell, B.A., B. Salleh, and J.F. Leslie. 2003. A utilitarian approach to *Fusarium* identification. Plant Dis. 87:117–128. doi:10.1094/PDIS.2003.87.2.117

Sutton, J. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4:195–209.

U.S. Drought Monitor. 2012. Drought monitor archives. The National Drought Mitigation Center. http://droughtmonitor.unl.edu/archive.html (accessed 1 Oct. 2013).

Vick, C.M., J.P. Bond, S.K. Chong, and J.S. Russin. 2006. Response of soybean sudden death syndrome to tillage and cultivar. Can. J. Plant Pathol. 28:77–83. doi:10.1080/07060660609507273

Vick, C.M., S.K. Chong, J.P. Bond, and J.S. Russin. 2003. Response of soybean sudden death syndrome to subsoil tillage. Plant Dis. 87:629–632. doi:10.1094/PDIS.2003.87.6.629

Wegulo, S.N., W.W. Bockus, J.H. Nopsa, E.D. De Wolf, K.M. Eskridge, K.H.S. Peiris, and F.E. Dowell. 2011. Effects of integrating cultivar resistance and fungicide application on *Fusarium* head blight and deoxynivalenol in winter wheat. Plant Dis. 95:554–560. doi:10.1094/PDIS-07-10-0495

Willyerd, K.T., C. Bradley, A. Grybauskas, D. Hershman, L. Madden, M. McMullen, L. Osborne, L. Sweets, and P. Paul. 2010. Multi-state evaluation of integrated management strategies for *Fusarium* head blight and deoxynivalenol in small grain. (Abstr.). Phytopathol. 100:S137.

Willyerd, K.T., C. Li, L.V. Madden, C.A. Bradley, G.C. Bergstrom, L.E. Sweets, M. McMullen, J.K. Ransom, A. Grybauskas, L. Osborne, S.N. Wegulo, D.E. Hershman, K. Wise, W.W. Bockus, D. Groth, R. Dill-Macky, E. Milus, P.D. Esker, K.D. Waxman, E.A. Adee, S.E. Ebelhar, B.G. Young, and P.A. Paul. 2012. Efficacy and stability of integrating fungicide and cultivar resistance to manage *Fusarium* head blight and deoxynivalenol in wheat. Plant Dis. 96:957–967. doi:10.1094/PDIS-09-11-0763

Wrather, J.A., and S.R. Koenning. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Prog. 10.1094/PHP-2009–0401–01-RS.

Xue, A.G., E. Cober, H.D. Voldeng, C. Babcock, and R.M. Clear. 2007. Evaluation of the pathogenicity of *Fusarium graminearum* and *Fusarium pseudograminearum* on soybean seedlings under controlled conditions. Can. J. Plant Pathol. 29:35–40. doi:10.1080/07060660709507435