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Impact of Crop Residue and Corn-soybean Rotation on the Survival of *Fusarium virguliforme* a Causal Agent of Sudden Death Syndrome of Soybean

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Abstract

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is an economically important soil-borne disease and is a major risk to many soybean [*Glycine max*, (L.) Merr.] production regions worldwide. Two-year studies were conducted in a greenhouse and in fields to examine survival of the fungus in corn-soybean residues. Corn kernels consistently showed significantly ($P<0.05$) higher *F. virguliforme* colony-forming units per gram of dry soil (CFU/g) in a greenhouse and in field micro-plots compared to no additional residue added treatment (control). None of the soil samples from commercial fields in Iowa showed significant ($P<0.05$) difference in *F. virguliforme* CFU/g within year of sampling, but between years there were numerical differences but not statistically different in samples if the previous crop had been corn, compared with winter wheat or soybean. In *Fusarium* spp. not causing SDS, CFU/g were significantly ($P<0.05$) higher in micro-plots amended with six different corn-soybean residue treatments compared with the control in 2008, while in 2009, only corn stock spread on soil showed significant ($P<0.05$) difference over the control. Our results suggest that a clean corn harvest could reduce SDS risk by reducing colonization of corn kernels that supports survival of *F. virguliforme*, while a considerable corn loss during harvest could increase SDS risk.

Keywords: *Fusarium virguliforme*; Crop rotation; Crop residue; Sudden death syndrome

Introduction

Sudden death syndrome of soybean [*Glycine max*, (L.) Merr.], caused by *Fusarium virguliforme* O'Donnell & T. Aoki, is an economically important soil-borne disease, and a threat to many soybean production areas worldwide. The disease was first observed by H.J. Walters in Arkansas in 1971 [1], but it was only in 1983 that the disease was named as sudden death syndrome (SDS) of unknown etiology [2]. Since then, the disease has been reported in 21 U.S. states where corn and soybean are grown, as well as in seven other countries (Shrishail S. Navi, Lan Jing and Xiao-Bing Yang, unpublished), the most recent of SDS has been in South Dakota [3] and South Africa [4]. After the disease was first noticed in Iowa in 1993 [5], an extensive investigation confirmed SDS in 28 counties by 1996 [6]. By 2010, the disease had spread to 96 of the 99 counties in Iowa. This spread of SDS suggested that *F. virguliforme* may have been present in most Iowa soils in previous years, but had not been detected in several counties because of low inoculum densities and/or unfavorable environmental conditions [7]. Currently, SDS is a major concern to soybean producers in the US. north-central region [8]. Nationally, yield losses due to SDS from 1988 to 2010 ranged from 3.4 million metric tons in 1988 ($24 million) to 114.3 million metric tons in 2010 ($875 million), and one of the highest yield losses of 125.7 million metric tons was reported in 2000 ($893 million) [9]. However, yield losses due to SDS vary widely depending on agronomic practices, like planting dates, row spacing, maturity groups and cultivar selection [10], as well as climatic and environmental trends in the growing season [7].

An SDS epidemic is highly correlated with soybean planting date and tends to be more severe in earlier planted soybeans [11,12]. Cool soil temperature (22°C to 24°C) increases root infection by *F. virguliforme* [13,14], conditions that are typical in early planted soybeans. The occurrence of *F. virguliforme* infection early in the season is likely to result in colonization of xylem tissue, a process essential for foliar symptom expression because xylem vessels are upward pathways in transporting water and nutrients [15]. Thus, delayed planting has been recommended as a management approach to reduce SDS risk in the north-central production region [16].

The effects of crop rotation on soybean SDS may be different than on other soil-borne diseases. Crop rotation with sorghum, fescue, and wheat significantly affected the soil population densities of *F. virguliforme* and lowest densities were observed in plots with wheat and sorghum [17]. However, the corn-soybean rotation system is highly vulnerable to the development of severe soil-borne disease complexes [18]. Due to a wide host range of *F. virguliforme*, Kolander et al. [19] disagree with crop rotation as a realistic method of controlling SDS. In addition, they argue that alternative hosts do not necessarily show foliar symptoms, but their root colonization can cause destructive effects on the plant, and can serve as a medium for the fungus to survive and increase the population of *F. virguliforme*. Other research suggests that annual corn-soybean rotations do not reduce the risk of SDS [12,17,18]. Research was conducted to quantify the impact of crop residue and a corn-soybean rotation system on the presence of *F. virguliforme* under greenhouse and field conditions.

Materials and Methods

Culturing of *Fusarium virguliforme*, inoculum preparation and inoculum increase

A single-spore isolate Fsg-ISU 1 of *F. virguliforme* [20] obtained

from an Iowa field was subcultured on 9-cm-diameter disposable Petri dishes containing one-third strength PDA with 0.1 g streptomycin sulfate added per liter. The isolate was incubated at 22 ± 1°C under a continuous photoperiod of 0.42 w/m^2 visible light intensity (PMA2100, Solar Light Company, Inc., 100 East Glenside Ave, Glenside, PA) for one month. When the isolate was fully grown on the culture plate, 10-ml distilled sterile water (DSW) was transferred under aseptic conditions to release the fungal tissue, which was then stirred using a sterilized Cotman Series 666 (1.9-cm) brush (Winsor and Newton, England), followed by a second 10-ml DSW wash. The resultant conidial suspension was transferred to a 100-ml sterilized conical graduated flask. The conidial population density was adjusted to 1×10^5 conidia / ml using a hemocytometer. The suspension was then transferred to a 200-ml Nalgene aerosol spray bottle for inoculation in the crop residue treatments.

White sorghum grain was soaked in lukewarm tap water (32 ± 1°C) for 10–12 h, with about 5 cm of water above the grain surface. The grain was rinsed in running lukewarm tap water until the water ran clear. The excess water was decanted and completely drained using number 16 sieves (U.S. Standard Sieve Series, the W.S. Tyler Company, Mentor, OH, 44060). One scoop (710-ml aluminum ice scoop) of grain was transferred to each of the half-full Ball quart jars, which were sterilized at 121°C at 10.5 kg per m^3 for 60 min with loosely covered lids. Immediately after sterilization, the lids were tightened and the jars were allowed to cool down to the ambient temperature (22 ± 1°C). Under aseptic conditions, five or six agar plugs of full-grown isolate Fsg-ISU 1 were transferred into the jars and were incubated in the dark for three to four weeks. For uniform colonization of the grain during this period, the jars were shaken at least once a week. After the incubation period was complete, jars were opened in a pre-disinfected NuAire class II type B2 biological safety cabinet, and the colonized grain was transferred onto trays layered with brown paper using 25-cm forceps (Fisher brand F 10-316B). The grain was spread as uniformly as possible to air-dry in the cabinet for two to three days. The dried grain was sieved through an oblong commercial sieve (0.4 cm × 1.9 cm, Seedburo Equipment Company, Chicago, IL) to take out clumps and collect the grain in plastic bags. If inoculum was not used immediately, it was stored at <3°C.

Establishing crop residue treatments in the greenhouse

At physiological maturity, 50 healthy corn plants and 50 healthy soybean plants were removed from the Hinds Research Farm, Ames, IA, in September 2007 and dried on greenhouse benches. Plants were considered healthy if they showed no major visible disease symptoms at the time of sampling. Both corn and soybean plants were separated into roots, stems, leaves, husks, and grain. Roots, stems, and leaves were cut into small (5 to 8 cm long) pieces using a hand pruner and the crop residue was steam-sterilized (121°C at 10.5 kg per square meter). The unsterilized soil (organic matter of 2.8, pH 7.6, and buffer index 7.3) was sieved through a Linding KM 10 model soil shredder (Linding Manufacturing Co., Inc., St. Paul 13, MN), mixed with crop residue (Table 1), and inoculated with 100 µl of the *F. virguliforme* conidial suspension per g of soil. This resulted in an inoculum density of 1×10^5 macroconidia/g of soil of the Fsg-ISU 1 isolate. Each clay pot (25.4 cm) was filled with 1500 cc of the soil and residue mix (Table 1). The experiment was arranged in a randomized complete block design with nine treatments and three blocks (Table 1). Each block contained 12 replicate pots per treatment (total of 36 pots per treatment). Baseline *F. virguliforme* population in the soil prior to inoculation was not estimated. Pots were incubated on the greenhouse benches at 23 ±

2°C in 16 h light. Moisture was maintained as uniformly as possible, watering to the saturation point once a week.

Soil sampling in the greenhouse

Three months after treatments were established, soil from 12 pots/block of each of the nine treatments was collected to a depth of 10.2 cm using a Hoffer 91.4 cm soil sampler (Ben Meadows Company, Janesville, WI). The soil sampler removes approximately a 2.5-cm-diameter core of soil. Samples from the 12 pots were bulked in plastic bags as one bulked sample per block. The samples were transferred to Fisher hexagonal polystyrene weigh boats (10.1 cm top × 7.6 cm base) and were air-dried in a laminar flow at 22 ± 1°C for two days. All samples were individually ground using a pestle and mortar and were transferred back to the weigh boats. Care was taken to not contaminate samples collected while powdering. Soil sampling was repeated in 2009, 12 months after the first soil sampling in 2008.

Establishing crop residue treatments in the field micro-plots

Field micro-plots with an area of 1.5 m × 1.5 m, and with a 0.9 m alley between plots, were established in a randomized complete block design at Hinds Research Farm, Iowa State University, Ames, IA, in April 2008 (Figure 1). Cropping history of this field was continuous soybean from 2004. The experimental area of 7.62 m wide × 26.82 m long was tilled twice using a 71.1-cm Troy-Bilt pony tiller (Garden Way Manufacturing Company, Garden Way Inc., Troy, NY). Any residue appearing on the surface from previous trials was raked from the plot area. Steam-sterilized crop residue was manually spread as per the treatments detailed in Table 1. Immediately after, steam-sterilized white sorghum grain colonized by Fsg-ISU 1 isolate was sprinkled manually on all the plots, as uniformly as possible, at a rate of 25 g/plot. After spreading the residue and the inoculum, the plots were carefully tilled to a depth of 7.6 cm. Care was taken to minimize the movement or spill of the residue and the inoculum into adjacent plots and or beyond the alleys between plots. The *F. virguliforme* population was not assessed in the micro-plots prior to spreading the residue and inoculum. Micro-plots of two treatments, with corn stalks only and a mixture of corn stalks, coarse-ground corn kernels, and inoculum, were spread on the surface and remained untilled. Soon after, the plots were watered manually at 2 L/plot using 7.57-L watering cans. The plots were not irrigated or watered after this initial watering to maintain soil moisture in the micro-plots. Around the experimental area (7.62 m × 26.82 m),

Crop residue treatments	Quantity of residue (g)	
	Clay pot[1]	Micro-plot[2]
Coarse-ground corn kernels (CK)	13	100
Corn roots	10	10,000
Corn stalks (CS)	7	10,000
No residue added	0	0
Coarse-ground soybean kernels (SK)	10.5	100
Soybean stalks (SS)	10	5000
Soybean roots	10	5000
CS spread on the soil surface[3]	7	5000
CK and CS spread on the soil surface[3]	13 and 7	100 and 5000

Table 1: Crop residue treatments for the greenhouse and micro-plots.
[1]Crop residues were mixed with soil and inoculated with spore suspension of *Fusarium virguliforme*, a single-spore Iowa isolate Fsg-ISU 1 (1×10^5 conidia per gram of soil) in greenhouse studies.
[2]Crop residue was spread on micro-plots and inoculated with 25 g steam-sterilized white sorghum grain colonized by Fsg-ISU 1 and tilled.
[3]Crop residue was spread on micro-plots and inoculated with 25 g steam-sterilized white sorghum grain colonized by Fsg-ISU 1 and micro-plots remained untilled.
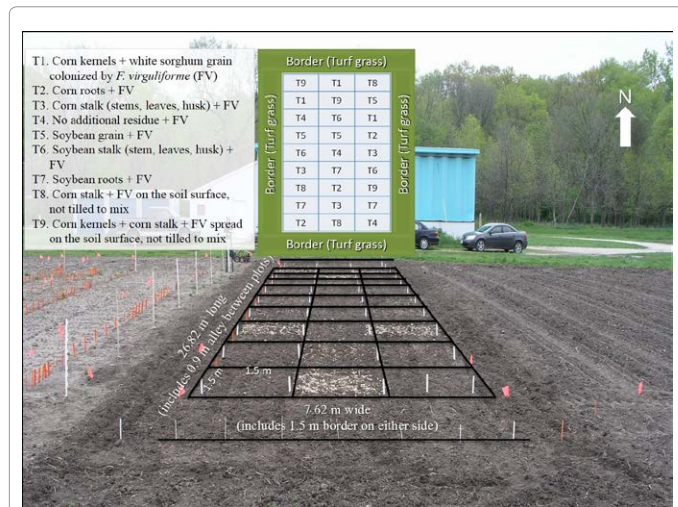
Figure 1: Layout of corn-soybean residue treatments in field micro-plots along with treatment details, Hinds Research Farm, Iowa State University, Ames, IA.

1.5 m borders on all four sides were seeded with turf grass. Weed-free plots were maintained by manual weeding.

Soil sampling in micro-plots

Three months after treatments were established, the soil was sampled from a depth of 8 to 10 cm in all plots using a Hoffer soil sampler. The samples from five arbitrarily selected spots in each plot was bulked and collected in plastic bags. Subsequently, the samples were air-dried and powdered similar to the greenhouse samples. Samples that were not processed were stored in a walk-in cold room at $3 \pm 2°C$. Soil sampling was repeated in 2009, 12 months after the first soil sampling in 2008.

Establishing treatments in commercial fields based on crop rotation history

Five farmers' fields with diverse crop rotation backgrounds through 2007 were selected to assess *F. virguliforme* in a commercial setting. Fields were selected through consultations and established through a memorandum of understanding between Iowa State University and the farmers in 2008. The crop rotation history of selected commercial fields in Black Hawk, Delaware, and Boone counties in Iowa, from which soil samples were collected to assess the *F. virguliforme* CFU/g of dry soil, is provided in Table 2.

Soil sampling on commercial farms

Five soil samples from a farm in Black Hawk County, two samples from two farms in Delaware County, and five samples from two farms in Boone County were collected using a soil sampler during 2008 and 2009. Each sample collected in a plastic bag was a bulk of five arbitrarily selected spots over approximately 0.5 ha. Samples were collected from between crop rows when the crops (either soybean or corn) were in reproductive growth stages. Samples were collected as previously described in the micro plot study. While collecting soil samples, SDS incidence and severities based on foliar symptoms were recorded in fields planted to soybean. Soil sampling was repeated in 2009, 12 months after the first soil sampling in 2008.

Plating

Three 5-g subsamples were drawn from each of the original

powdered soil samples. Each subsample was transferred to a 250-ml conical flask containing 100-ml de-ionized water, and the flasks were sealed using corks. Each time eight flasks were placed on a wrist action shaker (Burrell Scientific, Pittsburg, PA) and were agitated for 30 minutes. Two 1-ml aliquots were pipetted out from each flask and transferred into a 50-ml conical flask containing 9-ml de-ionized water for 10-fold dilutions and another 50-ml flask containing 19-ml de-ionized water for 20-fold dilutions. From each dilution, 100-µl were pipetted out and transferred onto a 9-cm Petri dish containing modified Nash and Snyder's medium [21]. Fifteen MNSM Petri dishes per dilution and per treatment were used. The suspension on the MNSM plates was spread as uniformly as possible using a sterile disposable 10-µl inoculation loop (Midsci, 280 Vance Rd., St. Louis, MO). Petri dishes were incubated in florescent light for seven days at $23 \pm 1°C$.

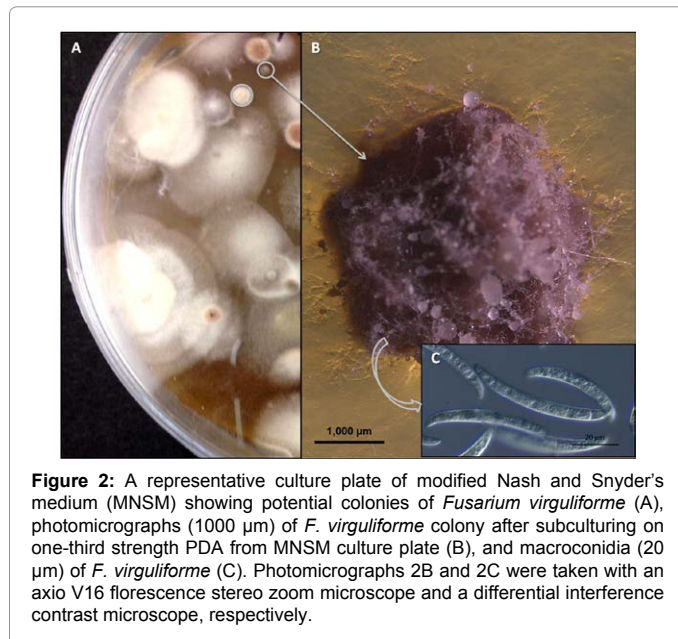## Assessment of *F. virguliforme* colonies

Soil samples from the greenhouse and commercial farms were assessed for colonies of *F. virguliforme*, and that of field micro-plots were assessed for *F. virguliforme*, *Fusarium* spp. (*F. verticillioides*, *F. proliferatum*, and *F. solani*), and other soil microorganisms (species of *Aspergillus*, *Candida*, *Chrysosporium*, *Cladosporium*, *Colletotrichum*, *Cryptococcus*, *Macrophomina*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizoctonia*, and *Trichoderma*) as colony-forming units per gram of dry soil (CFU/g of dry soil). Of the various fungal colonies observed, major focus was on identifying and assessing the CFU/g dry soil of *F. virguliforme*. The difficulty of assessing the growth of *F. virguliforme* within seven days of incubation in a Petri dish, wherein colonies of other soil microorganisms were likely to interfere, was anticipated. Therefore, Putative colonies of *Fusarium* spp. including *F. virguliforme* were observed under a stereo microscope (Stemi 200-C Zeiss) and subsequently, subcultured on one-third strength PDA under aseptic conditions. Identity of *F. verticillioides*, *F. proliferatum*, and *F. solani* were confirmed following Leslie and Summerell [22]. Putative colonies of *F. virguliforme* (Figure 2A) subcultured on one-third strength PDA were characterized for morphological characters like colony pigmentation, sporodochia production, colony growth (Figure 2B), spore morphology, and macroconidia (Figure 2C). To ensure that colonies counted were that of *F. virguliforme*, the identity confirmation steps mentioned above (Figures 2A to 2C) were followed when required. However, identity confirmation of *F. virguliforme* colonies was not subjected to molecular methods due to a very high number of samples and number of colonies observed. While processing the colonies for identity confirmation, plates that were under evaluation were stored in a refrigerator at $2 \pm 1°C$. Identity of other soil microorganisms mentioned above were confirmed following Baron [23] and Barnett and Hunter [24]. CFU of *F. virguliforme* were recorded from 15 plates of the 10- and of 20-fold dilutions of soil sampled from greenhouse

Selected commercial field locations in Iowa*	Crop rotation sequence		
	2007	**2008**	**2009**
Waterloo, Black Hawk County	Soybean, followed by winter wheat	Soybean	Corn
Manchester, Delaware County (field 1)	Soybean	Corn	Soybean
Manchester, Delaware County (field 2)	Corn	Soybean	Corn
Boone, Boone County (field 1)	Soybean	Corn	Soybean
Boone, Boone County (field 2)	Corn	Soybean	Corn

Table 2: Corp rotation sequence in selected commercial fields in Iowa from where soil samples were collected to assess the colony forming units per gram of dry soil of *Fusarium virguliforme*.
*Fields were selected by consultations and a memorandum of understanding between Iowa State University and grower(s).

Figure 2: A representative culture plate of modified Nash and Snyder's medium (MNSM) showing potential colonies of *Fusarium virguliforme* (A), photomicrographs (1000 μm) of *F. virguliforme* colony after subculturing on one-third strength PDA from MNSM culture plate (B), and macroconidia (20 μm) of *F. virguliforme* (C). Photomicrographs 2B and 2C were taken with an axio V16 florescence stereo zoom microscope and a differential interference contrast microscope, respectively.

experiments, field micro-plots, and commercial fields, both in 2008 and in 2009. Colony counts of *F. verticillioides*, *F. proliferatum*, and *F. solani* and other soil microorganisms were recorded only in soil samples of field micro-plots. However, we could not collect soil samples and assess the CFU of *F. virguliforme* in 2010 due to severe flooding in Iowa fields.

Data analysis

Mean CFU of (i) *F. virguliforme* isolated from soil sampled from pots in the greenhouse, field micro-plots, and commercial farms, (ii) non-SDS causing *Fusarium* spp. (*F. verticillioides*, *F. proliferatum*, and *F. solani*), and other microorganisms (species of *Aspergillus*, *Candida*, *Chrysosporium*, *Cladosporium*, *Colletotrichum*, *Cryptococcus*, *Macrophomina*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizoctonia* and *Trichoderma*) isolated from soil sampled in micro-plots assessed in 2008 and 2009 was analyzed for each year using PROC ANOVA in SAS 9.4. (SAS, LLC, Cray, NY). Fisher's least significant difference was used to detect the significant differences among the means ($P=0.05$). Although data were collected separately for 10- and 20- fold dilutions, the analysis was done across dilutions in each year.

Results

Fusarium virguliforme in crop residue treatments in a greenhouse

In soil samples collected from nine crop residue treatments in 2008 across dilutions, the coarse-ground corn kernel treatment showed significantly ($P<0.05$) higher *F. virguliforme* CFU/g of dry soil compared to all other treatments, except in soil amended with corn roots (Figure 3A). In 2009, highest *F. virguliforme* CFU/g of dry soil was observed in pots amended with corn roots compared with eight other residue treatments (Figure 3A).
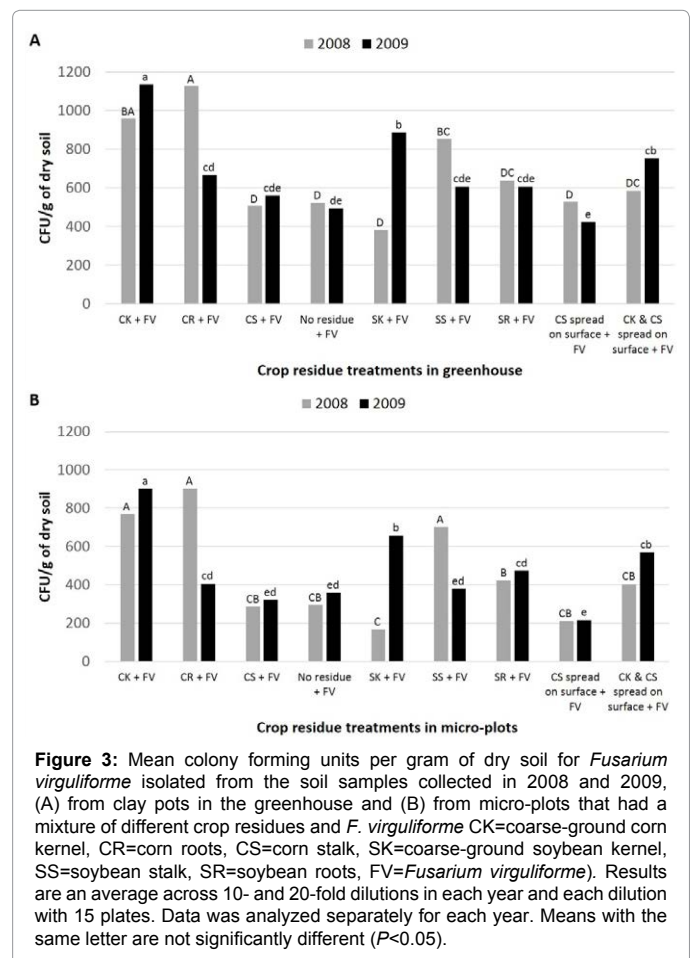
Fusarium virguliforme, non-SDS causing *Fusarium* spp., and other microorganisms in crop residue treatments on micro-plots
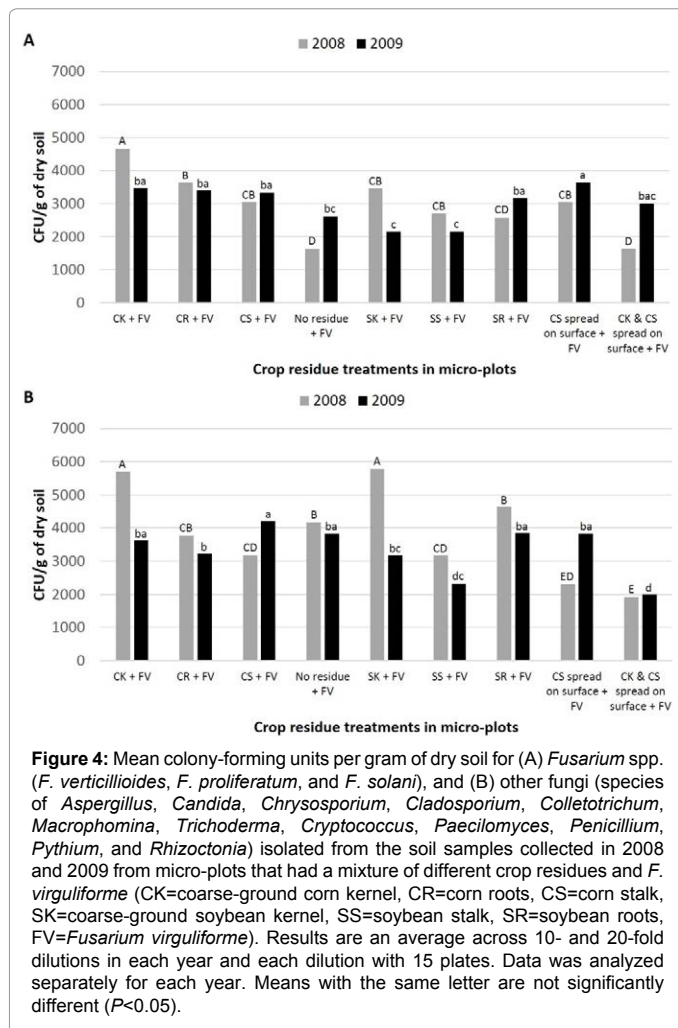
In 2008, plots amended with coarse-ground corn kernels, corn roots, and soybean stalks did not differ significantly ($P<0.05$) for *F. virguliforme* CFU/g. In 2009, the CFU of *F. virguliforme* were

significantly ($P<0.05$) higher in plots amended with coarse-ground corn kernels compared with all other treatments (Figure 3B). Apart from coarse-ground corn kernels and corn roots, *F. virguliforme* CFU/g of dry soil were significantly ($P<0.05$) higher in treatment with soybean stalks tilled into the soil compared with no residue treatment in 2008. A treatment with a mixture of coarse-ground corn kernels and corn stalks spread on the soil surface was also significantly ($P<0.05$) higher than the no residue treatment in 2009 (Figure 3B).

The means of CFU/g of dry soil for *Fusarium* spp. not causing SDS (*F. verticillioides*, *F. proliferatum*, and *F. solani*) were significantly ($P<0.05$) higher in plots amended with coarse-ground corn kernels compared with seven other corn-soybean residue treatments and no residue added treatment in 2008 (Figure 4A). In 2009, CFU of *Fusarium* spp. were significantly ($P<0.05$) higher in plots with corn stalks spread on the soil surface compared with soybean stalk, soybean kernel, and no additional residue added treatments (Figure 4A). Similar to *Fusarium* spp., mean CFU of other fungi (species of *Aspergillus*, *Candida*, *Chrysosporium*, *Cladosporium*, *Colletotrichum*, *Macrophomina*, *Trichoderma*, *Cryptococcus*, *Paecilomyces*, *Penicillium*, *Pythium*, and *Rhizoctonia*) isolated from micro-plots showed significantly ($P<0.05$) higher in treatments amended with coarse-ground corn kernels and coarse-ground soybean kernels compared with the no residue treatment in 2008, while no significant differences were observed between these treatments in 2009 (Figure 4B).

Soil samples from a farm in Black Hawk County showed no significant ($P<0.05$) difference for CFU of *F. virguliforme* both in 2008

Figure 3: Mean colony forming units per gram of dry soil for *Fusarium virguliforme* isolated from the soil samples collected in 2008 and 2009, (A) from clay pots in the greenhouse and (B) from micro-plots that had a mixture of different crop residues and *F. virguliforme* CK=coarse-ground corn kernel, CR=corn roots, CS=corn stalk, SK=coarse-ground soybean kernel, SS=soybean stalk, SR=soybean roots, FV=*Fusarium virguliforme*). Results are an average across 10- and 20-fold dilutions in each year and each dilution with 15 plates. Data was analyzed separately for each year. Means with the same letter are not significantly different ($P<0.05$).
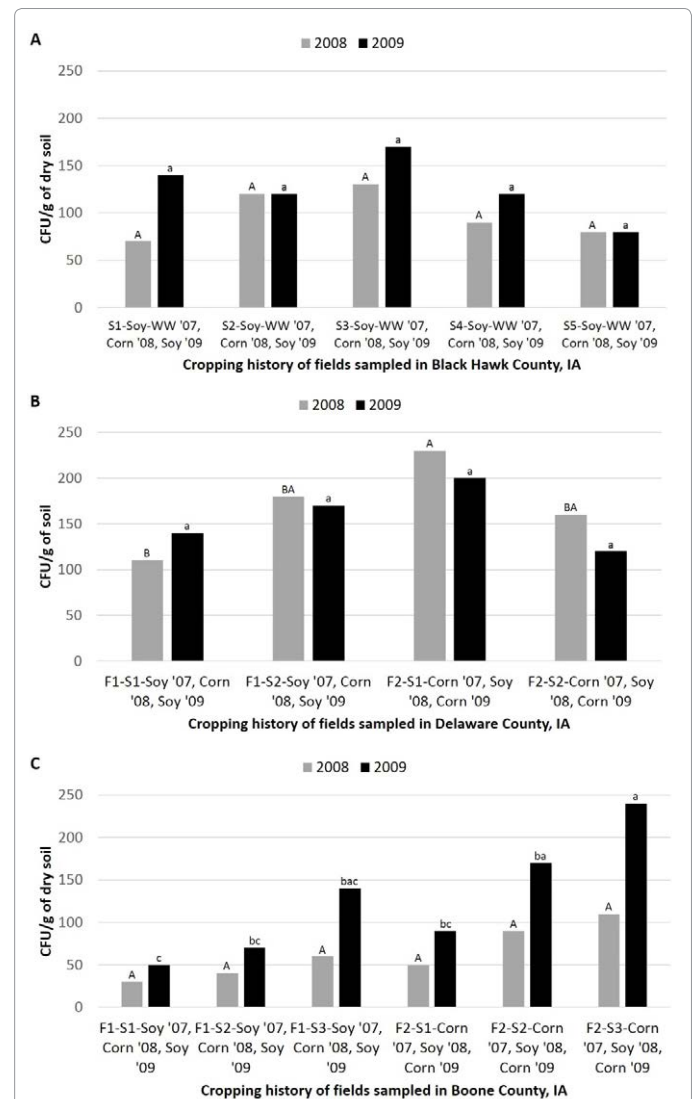
Figure 4: Mean colony-forming units per gram of dry soil for (A) *Fusarium* spp. (*F. verticillioides*, *F. proliferatum*, and *F. solani*), and (B) other fungi (species of *Aspergillus*, *Candida*, *Chrysosporium*, *Cladosporium*, *Colletotrichum*, *Macrophomina*, *Trichoderma*, *Cryptococcus*, *Paecilomyces*, *Penicillium*, *Pythium*, and *Rhizoctonia*) isolated from the soil samples collected in 2008 and 2009 from micro-plots that had a mixture of different crop residues and *F. virguliforme* (CK=coarse-ground corn kernel, CR=corn roots, CS=corn stalk, SK=coarse-ground soybean kernel, SS=soybean stalk, SR=soybean roots, FV=*Fusarium virguliforme*). Results are an average across 10- and 20-fold dilutions in each year and each dilution with 15 plates. Data was analyzed separately for each year. Means with the same letter are not significantly different (*P*<0.05).

Researchers have been puzzled by the fact that SDS has now become more prevalent in the Corn Belt compared to when the disease was first reported in Arkansas. Our results from the greenhouse (Figure 3A) and micro-plots (Figure 3B) showed that coarse-ground corn kernels affect survival of *F. virguliforme*. Sampling from the 2009 greenhouse experiment showed significantly (*P*<0.05) higher *F. virguliforme* CFU/g of dry soil in pots amended with coarse-ground soybean kernel residue compared with no residue (Figure 3A), although these treatment differences were not observed in 2008. These results indicate that *F. virguliforme* survival is increased on coarse-ground soybean

Figure 5: Mean colony-forming units per gram of dry soil for *Fusarium virguliforme* isolated from soil samples collected in 2008 and 2009 (A) from Waterloo, Black Hawk County, IA, wherein the field was planted with soybean in 2007 followed by winter wheat, corn in 2008, and soybean in 2009; (B) from two fields in Manchester, Delaware County, IA, wherein field 1 had soybean in 2007, corn in 2008, and soybean in 2009 and in field 2, corn in 2007, soybean in 2008, and corn in 2009; and (C) from two fields in Boone, Boone County, IA, wherein field 1 was planted with soybean in 2007, corn in 2008 and soybean in 2009; whereas, field 2 was planted with corn in 2007, soybean in 2008 and corn in 2009. Values are the mean of 30 plates per treatment (15 each from 10-fold and 20-fold dilutions). Data presented here are an average across 10- and 20-fold dilutions. Data was analyzed separately for each year. Means with the same letter are not significantly different (*P*<0.05). (F1 and F2=field numbers, S1 to S3=soil sample numbers 1 to 3, Soy=soybean, and WW=winter wheat).

and 2009 (Figure 5A). Similar to Black Hawk County, two samples from each of two farms in Delaware County with different cropping patterns showed no significant difference in both years (Figure 5B). Mean CFU/g of dry soil of *F. virguliforme* in samples collected in Boone County showed no significant (*P*<0.05) difference in 2008 across two crop rotations in two fields (Figure 5C).

Discussion

For soybean producers, current management options to reduce SDS include but are not limited to planting resistant varieties [14], delayed planting [19], fall tillage [16], planting with corn or other non-soybean crops [5,16,25], modifying agronomic practices like row spacing and seeding rate, [4], and fungicide seed treatments [26,27]. In addition, our results suggest that with clean harvest, the level of *F. virguliforme* spores should be lower in the absence of either coarse-ground corn kernels and corn roots or coarse-ground soybean kernels. The first two practices (resistant varieties and adjusted planting dates) have not been fully adopted by growers; indeed delayed planting is in disagreement with agronomic recommendations for early planting to promote maximum yield potential [28]. Delayed planting in northern Iowa could result in severe yield penalties, but early planting could lead to higher levels of SDS [9,18,28]. The effects of crop rotation, tillage practices, and row spacing on SDS are inconclusive. In fact, there are contradictions (advantages in some soils and disadvantages in other soils) about tillage practices to reduce SDS [3,12].

kernel residue and the fungus could not increase without the support of residue.

Field micro-plots amended with coarse-ground corn kernels, corn roots, and soybean stalks showed significantly higher *F. virguliforme* CFU/g of dry soil over the no residue control but there was no significant difference among those three residue treatments in 2008 (Figure 3B). However, in 2009, higher *F. virguliforme* CFU/g of dry soil was observed in coarse-ground corn kernel residue compared with other eight residue treatments (Figure 3B). This indicates that survival and reproduction of *F. virguliforme* were higher on coarse-ground corn kernels within a year after the first sampling than on corn roots and soybean stalk, and that corn kernels were the most preferred residue by *F. virguliforme* in either tilled (mixed) or untilled micro-plots (Figure 3B). Our result suggest that a clean harvest, as well as incorporation of corn residue into the soil through tillage, would help reduce *F. virguliforme*.

Studies by Almeida et al. [29] showed that soybean debris support increased CFU of *F. solani*, *F. verticillioides*, *F. equiseti*, *F. dimerum* and *F. oxysporum*, while studies of Cotten and Munkvold [30], showed that corn stalks are good residue for *F. moniliforme*, *F. proliferatum*, and *F. subglutinans*, and Kommedahl et al. [31] showed corn roots support *F. oxysporum, F. solani,* and *F. roseum* and corn stalks support *F. moniliforme*. Recently, Marburger et al. [32] observed no significant effect of crop rotation sequences and management (variety selection and fungicide use) on *F. oxysporum* and *F. virguliforme*. However, in their study they observed that the *F. virguliforme* population was not quantifiable from fields with wheat in rotation. Results of our micro-plot studies with diverse crop residue amendment are in agreement with findings of Almeida et al. [29], Cotten and Munkvold [30] and Kommedahl et al. [31] but differ from Marburger et al. [32] for *F. virguliforme*.

Based on our results, it appears that corn residue, particularly coarse-ground corn kernels, harbor *F. virguliforme* significantly in the absence of soybeans. In fact, we consistently observed the highest CFU of *F. virguliforme* in treatment amended with coarse-ground corn kernels both in the greenhouse and field micro-plots. Similarly, studies conducted by Abdelsamad et al. [33] with a 2-year rotation of corn and soybean showed greater SDS incidence and severity, and lower yield, compared to the 3-year corn-soybean-oat/red clover, and 4-year corn-soybean-oat/alfalfa-alfalfa rotations with an inconclusive mechanism of reduction in SDS population with 3- and 4-year rotations. Therefore, further studies on crop rotation with winter wheat or other cereals may indicate whether or not planting such crops helps to reduce *F. virguliforme* populations and would be a long-term SDS management option. Additional options are seed treatments such as fluopyram (ILeVO; Bayer Crop Science) and our own recommendation of a clean harvest [34].

Some of our results agree and disagree with those reported in the literature, and our current study had some limitations, including the source of soil used in the greenhouse experiments, lack of information about the baseline population of *F. virguliforme* prior to amending residues and inoculum into pots or field micro-plots, and identity confirmation of colonies of *F. virguliforme* observed in soil samples processed from greenhouse, field micro-plots, and commercial farms by molecular techniques [35]. Since information about the source of greenhouse soil was not available, we conducted a soil profile analysis that included organic matter content and pH. In spite of some of these limitations, our results have shown a fairly clear understanding of the effects of different crop residue on the survival of *F. virguliforme* from soils sampled in a greenhouse, field micro-plots, and commercial farms.

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