Research

Cross Pathogenicity Studies Show South Dakota Isolates of *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* from Either Soybean or Corn are Pathogenic to Both Crops

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Abstract

In South Dakota, despite that integrated pest management options are available, *Fusarium* root rot is an emerging disease on soybean (*Glycine max* L.) and corn (*Zea mays* L.). Surveys were conducted across South Dakota on soybean and corn fields in 2014 and 2015, respectively, to assess the prevalence of species of *Fusarium* causing root rot. *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* were identified common to soybean and corn. A total of 21 isolates, representing these seven species, were evaluated for their pathogenicity on soybean ('Williams 82') and corn ('B73') using the inoculum layer inoculation method in the greenhouse. At

14 days postinoculation, the seedlings were evaluated for root rot severity (1-to-5 rating scale), and relative treatment effects (RTEs) were estimated. A significant effect of the treatments was observed on RTE for soybean ($P = 1.1 \times 10^{-7}$) and corn ($P = 3.0 \times 10^{-14}$). Two *F. proliferatum* isolates and one *F. graminearum* isolate from corn caused significantly greater RTE than the other treatments (including the noninoculated control) on soybean and corn. Results indicate that soybean and corn can serve as inoculum sources of the seven species of *Fusarium* that are pathogenic to both crops.

Keywords: *Fusarium*, pathogenicity, corn, soybean, root rot

Root rots and seedling diseases, together, are among the 10 most destructive diseases that limit corn (*Zea mays* L.) (Mueller et al. 2016) and soybean (*Glycine max* L.) (Allen et al. 2017) production in the United States and Canada (Ontario). For example, on corn, the total yield losses owing to root rots and seedling diseases were estimated to be 1.5 million metric tons in 2015 (Mueller et al. 2016). On soybean, the total yield losses owing to seedling diseases were estimated to be 6.6 million metric tons in 2014 (Allen et al. 2017). Among the pathogens that cause root rot and seedling diseases of soybean and corn, species of *Fusarium* are found in all corn and soybean growing areas of the United States. Currently, there are no commercial cultivars of the two crops available that have complete resistance to *Fusarium* root rot. In South Dakota, most producers plant corn and soybean early into cool and wet soils. They also use a corn/soybean rotation in combination with tillage practices that leave more than 30% crop residue cover on the soil surface. These cropping practices can increase the risk of root rot and seedling diseases for the two crops in the production areas.

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In 2014 and 2015, root rots and seedling diseases were observed in commercial corn and soybean fields in South Dakota with symptoms such as yellowing, loss of stand, wilting, and stunting of plants (F. Mathew, *unpublished*). Root rot surveys were conducted across South Dakota on soybean and corn fields, respectively, to assess the presence of pathogens causing root rot. Species of *Fusarium* were identified to be most prevalent among the fungal pathogens causing root rot of soybean and corn. Of the total 11 and eight species of *Fusarium* identified on soybean and corn (P. Okello and F. Mathew, *unpublished*; Okello et al. 2019), seven species of *Fusarium*—*F. acuminatum* Ellis and Everhart, *F. equiseti* (Corda) Saccardo (syn. *F. equiseti-incarnatum* complex), *F. graminearum* Schwabe, *F. oxysporum* Schlecht, *F. proliferatum* Matsushima, *F. solani* (Martius) Saccardo, and *F. subglutinans* Wollenweber and Reinking—were common to the two crops. Although *F. acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, and *F. subglutinans* were demonstrated to be pathogenic on both corn and soybean in cross-pathogenicity studies (Broders et al. 2007; Parikh et al. 2018), there is no information available on the cross-pathogenicity of *F. proliferatum* and *F. solani* causing root rot of the two crops. For this study, a total of 21 isolates representing *F. acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* were selected from the isolates recovered from corn and soybean surveys in South Dakota based on their aggressiveness levels on a susceptible variety of corn (Adhikari et al. 2016) or soybean (Okello et al. 2016). The objective of this study was to evaluate the cross-pathogenicity of the 21 isolates from soybean and corn on the two crops in the greenhouse.

Isolation and Identification of *Fusarium* Species

For soybean, 10 plants with diseased roots (e.g., reduced tap root, no secondary roots, brown discoloration) were arbitrarily sampled along five transects (50 m) and covering an area of 0.5 ha from a total of 200 commercial fields in 22 counties (nine fields per county) in 2014. The soybean plants were sampled between R1 (beginning flowering) and R5 (beginning seed) growth stages (Fehr et al. 1971), where excess moisture was observed in soils following rain and the previous crop was corn. For corn, five plants were arbitrarily sampled along two transects (50 m) and covering an area of 0.5 ha from a total of 50 commercial fields in 24 counties (two fields per county) in 2015. The corn plants were sampled between V1 (first leaf) and V3 (third leaf) growth stages (Ritchie et al. 1992), where moisture was observed in soils from rain and the previous crop was soybean. The commercial fields were randomly selected for the two surveys and located in eastern South Dakota, where over 50% of soybean and corn production takes place in the state. Distance between the commercial fields ranged from approximately 1 to 2 km.

For fungal isolation, the main root of all plant samples was first rinsed under running tap water for 2 to 5 min and then cut into small pieces (≈ 15 mm long). The root pieces were surface disinfested in 0.05% sodium hypochlorite and 70% ethanol for 1 min each, washed with sterile distilled water, and then dried on autoclaved filter papers. Three root pieces of each plant were placed on Petri dishes containing potato dextrose agar (PDA; prepared as per the protocol by Leslie and Summerell [2006]) amended with 0.02% streptomycin sulfate and incubated at $23 \pm 2^\circ\text{C}$ for 7 days under 16-h fluorescent light/dark conditions. Seven days after incubation, cultures were examined for the presence of *Fusarium* using the Olympus CX31 Binocular Microscope (Olympus Corporation, Center Valley, PA). Putative *Fusarium*

colonies were transferred to fresh PDA plates by removing a mycelial plug (≈ 3 -mm square) from the edge of a growing colony with a sterile scalpel. A total of 1,130 and 198 isolates (= 1,328) were recovered from soybean and corn, respectively, which were grouped into a total of 12 species (11 species from soybean and eight from corn) based on morphology on PDA (colony appearance) and carnation leaf agar (sporodochia production and color) (Leslie and Summerell 2006). Of the 12 species of *Fusarium*, seven species (*F. acuminatum*, 27.6%; *F. equiseti*, 2.7%; *F. graminearum*, 47.4%; *F. oxysporum*, 10.1%; *F. proliferatum*, 3.4%; *F. solani*, 1.6%; and *F. subglutinans*, 0.9%) were identified as common to corn and soybean. The other five species of *Fusarium* were identified only to soybean (*F. armeniacum*, 2.9%; *F. commune*, 0.1%; *F. sporotrichioides*, 2.0%; and *F. virguliforme*, 1.4%) or to corn (*F. boothii*, 0.1%).

From the 1,328 isolates, 84 isolates (57 isolates from soybean and 27 from corn) were selected from different South Dakota counties to represent the 12 species, and single-conidial isolates were established. DNA was extracted from the single-conidial cultures of the 84 isolates using the FastDNA Spin Kit (MP Biomedicals, Solon, OH), and the translation elongation factor 1- α (EF1- α) gene region was sequenced using the primers EF1F/EF1R (EF1F, ATGGGTAAGGARGACAAGAC; and EF1R, GGARGTACCAGTSATCATGTT) (Geiser et al. 2004). The polymerase chain reaction (PCR) amplifications were performed in a 25- μl mixture containing 2.0 μl of fungal DNA (10 ng/ μl), 0.75 μl of forward primer (10.0 μM), 0.75 μl of reverse primer (10.0 μM), 12.5 μl of 2 $\times$ Taq PCR Master Mix containing Taq DNA Polymerase (Qiagen, Valencia, CA), and 9.0 μl of sterile nuclease-free water. The PCR parameters included an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and

TABLE 1
Information on the 21 *Fusarium* isolates evaluated on soybean and corn in the greenhouse

Isolate ID ^a	Pathogen	Year of isolation	Host of origin	Location in South Dakota	GenBank accession number
14-OP-FUS-041	<i>Fusarium acuminatum</i>	2014	Soybean	Kingsbury	MH822059
14-OP-FUS-045	<i>F. acuminatum</i>	2014	Soybean	Miner	MH822068
14-OP-FUS-052	<i>F. graminearum</i>	2014	Soybean	McCook	MH822072
14-OP-FUS-055	<i>F. graminearum</i>	2014	Soybean	Grant	MH822048
14-OP-FUS-027	<i>F. oxysporum</i>	2014	Soybean	Moody	MH822081
14-OP-FUS-026	<i>F. proliferatum</i>	2014	Soybean	McCook	MH822083
14-OP-FUS-025	<i>F. proliferatum</i>	2014	Soybean	Clay	MH822084
14-OP-FUS-030	<i>F. equiseti</i>	2014	Soybean	McCook	MH822040
14-OP-FUS-028	<i>F. solani</i>	2014	Soybean	Clay	MH822037
14-OP-FUS-035	<i>F. subglutinans</i>	2014	Soybean	Sanborn	MH822031
F8	<i>F. acuminatum</i>	2015	Corn	Charles Mix	MH595499
F6	<i>F. acuminatum</i>	2015	Corn	Browns	MH595497
F13	<i>F. graminearum</i>	2015	Corn	Codington	MH595507
F12	<i>F. graminearum</i>	2015	Corn	Codington	MH595505
F4	<i>F. oxysporum</i>	2015	Corn	Brookings	MH595513
F3	<i>F. oxysporum</i>	2015	Corn	Brookings	MH595510
F14FP-PB	<i>F. proliferatum</i>	2015	Corn	Codington	MH595517
F1	<i>F. proliferatum</i>	2015	Corn	Brookings	MH595516
F16	<i>F. equiseti</i>	2015	Corn	Days	MH595503
F11	<i>F. solani</i>	2015	Corn	Clay	MH595519
P5	<i>F. subglutinans</i>	2015	Corn	Browns	MH595522

^a Species identity of the 21 isolates (10 from soybean and 11 from corn) was established by morphology and sequencing the EF1- α gene region using the primers EF1F/EF1R (Geiser et al. 2004). The EF1- α sequences of the 10 soybean and 11 corn isolates are deposited in the GenBank (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/>).

elongation at 72°C for 1 min, and a final elongation at 72°C for 10 min (O'Donnell et al. 1998). To confirm amplification, a 5-μl aliquot of both PCR products was run on an agarose gel (2%). The PCR products were sequenced by GenScript USA, Piscataway, NJ. The isolates were identified to 12 species of *Fusarium* using the Basic Local Alignment Search Tool Nucleotide (BLASTN) searches at the *Fusarium*-ID database (<http://isolate.fusariumdb.org/index.php>). For this study, the EF1-α sequences of the 10 soybean isolates were deposited in GenBank (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/>) under the accession numbers MH822031, MH822037, MH822040, MH822048, MH822059, MH822068, MH822072, MH822081, MH822083, and MH822084, and those of the 11 corn isolates are under the accession numbers MH595503, MH595499, MH595503, MH595505, MH595507, MH595510, MH595513, MH595516, MH595517, MH595519, and MH595522 (Table 1).

Pathogenicity of *Fusarium* Isolates from Soybean and Corn

For the cross-pathogenicity experiment, a total of 21 isolates from soybean and corn were used to determine their pathogenicity on 'Williams 82' and 'B73' in the greenhouse in two experiments. These isolates were selected based on the level of aggressiveness

of 57 soybean isolates on a soybean susceptible variety (Okello et al. 2016) or 27 corn isolates on a corn susceptible hybrid (Adhikari et al. 2016) in greenhouse experiments. It was determined in the studies by Okello et al. (2016) and Adhikari et al. (2016) that although individual isolates varied significantly in their aggressiveness on soybean or corn, respectively, significant differences in aggressiveness were not observed among isolates within a particular species. In the total 21 isolates used for this study, there were four isolates each of *F. acuminatum*, *F. graminearum*, and *F. proliferatum*, three isolates of *F. oxysporum*, and two isolates each of *F. equiseti*, *F. solani*, and *F. subglutinans* (Table 1). To prepare inoculum, each isolate was initially grown on PDA, and five mycelial plugs (≈15-mm square) were transferred into a 250-ml Erlenmeyer flask containing a previously autoclaved sand/corn meal mixture (54 g of play sand, 6 g of cornmeal, and 10 ml of distilled water) based on the protocol of Bilgi et al. (2008). The inoculum for each of the 21 isolates was incubated for 14 days at 22 ± 2°C and mixed every other day by manually shaking the flask.

For the two experiments, each isolate was used to inoculate soybean or corn seedlings using the inoculum layer method modified from Bilgi et al. (2008). Briefly, plastic cups (473 ml)

FIGURE 1

Root rot severity (expressed as relative treatment effects [RTEs]) caused by *Fusarium* isolates from corn (black) and soybean (gray) on the soybean cultivar Williams 82 at 14 days postinoculation in the greenhouse. RTEs with the same letter are not significantly different based on 95% confidence intervals. *Fusarium* isolates: *F. acuminatum* = F6, F8, 14-OP-FUS-041, and 14-OP-FUS-045; *F. equiseti* = F16 and 14-OP-FUS-030; *F. graminearum* = F12, F13, 14-OP-FUS-052, and 14-OP-FUS-055; *F. oxysporum* = F3, F4, and 14-OP-FUS-027; *F. proliferatum* = F1, F14FP-PB, 14-OP-FUS-025, and 14-OP-FUS-026; *F. solani* = F11 and 14-OP-FUS-028; and *F. subglutinans* = P5 and 14-OP-FUS-035.

were first filled with 40 g of coarse vermiculite, followed by 20 g of fungal inoculum and then 20 g of coarse vermiculite to avoid direct contact of the seedlings with the inoculum. Two pre-germinated seeds of either Williams 82 or B73 were planted into the plastic cups containing vermiculite/inoculum mixture, and then 20 g of coarse vermiculite was used to cover the seedlings. To germinate the soybean or corn seeds, the seeds were spread on a moist filter paper placed in Petri plates and germinated at $23 \pm 2^\circ\text{C}$ for 3 days. After the seeds sprouted roots, these were planted into plastic cups containing vermiculite/inoculum mixture. For the noninoculated control treatments, previously autoclaved sand/cornmeal mixture containing no fungus was used as inoculum for the two experiments.

Following the experimental setup, soybean or corn plants were placed in a completely randomized design with five replications (each replication is a cup containing two plants) on a greenhouse bench at $22 \pm 2^\circ\text{C}$ with a 16-h photoperiod. The plants were watered once daily, and no fertilizer was added during the experiment. At 14 days after inoculation, root rot severity caused by the isolates on soybean and corn plants was evaluated using a 1-to-5 rating scale (Acharya et al. 2015), in which 1 = germination and healthy seedlings with no visible root colonization, 2 =

germination and 1 to 19% of the root having lesions, 3 = germination and 20 to 74% of the root having lesions, 4 = germination and 75% or more of the root having lesions, and 5 = no germination and complete colonization of seed. The experiments were performed twice.

Each plant in the cup was rated for root rot severity, and the mean root rot severity was calculated for the two plants in a cup. The root rot severity data are ordinal; hence, the data were analyzed using nonparametric statistics (Shah and Madden 2004). Prior to performing the nonparametric analyses, the Fligner-Killen test for homogeneity of variance was satisfied for the two experimental runs for soybean ($P = 0.85$) and corn ($P = 0.08$). Therefore, the data from the two experimental runs were combined, and the analysis of variance type test statistics (ATS) of ranked data were determined using the nparLD package (Noguchi et al. 2012) in R version 2.1 (R Core Team 2013). For either soybean or corn, the rank of each isolate was calculated according to equation 1 (Akritas 1991):

$$\bar{R}_i = \frac{1}{n_i} \sum_{k=1}^{n_i} R_{ik} \quad (1)$$

FIGURE 2

Root rot severity (expressed as relative treatment effects [RTEs]) caused by *Fusarium* isolates from corn (black) and soybean (gray) on the corn inbred line B73 at 14 days postinoculation in the greenhouse. RTEs with the same letter are not significantly different based on 95% confidence intervals. *Fusarium* isolates: *F. acuminatum* = F6, F8, 14-OP-FUS-041, and 14-OP-FUS-045; *F. equiseti* = F16 and 14-OP-FUS-030; *F. graminearum* = F12, F13, 14-OP-FUS-052, and 14-OP-FUS-055; *F. oxysporum* = F3, F4, and 14-OP-FUS-027; *F. proliferatum* = F1, F14FP-PB, 14-OP-FUS-025, and 14-OP-FUS-026; *F. solani* = F11 and 14-OP-FUS-028; and *F. subglutinans* = P5 and 14-OP-FUS-035.

where $\bar{R}_i$ is the mean rank for the i th treatment and R_{ik} is the rank of X_{ik} among all N observations (Shah and Madden 2004). The root rot severity caused by each isolate was expressed as relative treatment effects (RTEs) and was calculated from mean ranks according to equation 2:

$$\hat{p}_i = \frac{1}{N} \left(\bar{R}_i - \frac{1}{2} \right) \quad (2)$$

where $\bar{R}_i$ is the mean rank and N is the total number of observations (Shah and Madden 2004). RTEs of the treatments were compared at 95% confidence intervals using the nparLD package in R.

At 14 days after inoculation, a significant effect of treatments was observed on root rot severity for soybean (ATS = 7.2, df = 6.0, $P = 1.1 \times 10^{-7}$) and corn (ATS = 11.5; df = 6.6, $P = 3.0 \times 10^{-14}$). All 21 isolates caused root rot on both soybean and corn. No damping-off was observed on any of the plants. On the noninoculated control plants, no root discoloration (disease rating = 1) was observed on either soybean or corn plants.

On soybean, significant differences in RTE were observed among individual isolates and the noninoculated control. *F. proliferatum* isolates C1FP and C14FP from corn caused the greatest RTEs, which were not significantly different from that of the *F. graminearum* isolate C12FG. Significant differences in RTE were observed among soybean and corn isolates within *F. graminearum* and *F. proliferatum* (Fig. 1).

On corn, significant differences in root rot severity were observed among individual isolates and the noninoculated control. *F. proliferatum* isolate C1FP from corn caused the greatest RTE, which was not significantly different from that of the *F. proliferatum* isolate C14FP. Significant differences in RTE were observed among soybean and corn isolates within *F. graminearum*, *F. subglutinans*, and *F. proliferatum* (Fig. 2).

To fulfill Koch's postulates, isolates from soybean and corn were reisolated from the roots of inoculated soybean and corn plants. The diseased plants were randomly selected, and discolored root pieces were plated on PDA as described previously. The identity of the isolates was confirmed by sequencing the EF1- α gene. No species of *Fusarium* was recovered from the roots of the noninoculated soybean and corn plants.

Summary

In this study, the severity of root rot on soybean and corn plants was observed to differ among the 21 isolates, with two *F. proliferatum* isolates (F1 and F14FP-PB) and one *F. graminearum* isolate (F12) from corn causing significantly greater root rot severity on both the hosts. Our findings of *F. graminearum* isolates from corn and soybean being virulent on the two hosts are consistent with previous cross-pathogenicity studies (Broders et al. 2007; Parikh et al. 2018). However, in contrast to the study by Broders et al. (2007), who observed that isolates of *F. acuminatum*, *F. equiseti*, *F. oxysporum*, and *F. subglutinans* caused no lesion on the roots of soybean or corn (a mean pathogenicity score of <1.0), we observed that isolates of these fungi caused brown to black discoloration of the primary root (20 to 74% of the root had lesions) of the seedlings in this study. Between Broders et al. (2007) and our study, we suspect differences in the soybean or corn cultivars used, inoculation method, type of inoculum, virulence of isolates, and greenhouse conditions may have contributed to the differences in disease development by *F. acuminatum*, *F. equiseti*, *F. oxysporum*, and *F. subglutinans* on the two hosts. As for *F. proliferatum* and *F. solani*, we have not

found published work on the cross-pathogenicity of isolates of these fungi on soybean and corn despite that these pathogens are known to cause root rot of soybean and corn. Our study was able to demonstrate that the isolates of *F. proliferatum* and *F. solani* from soybean and corn are virulent (at least 10% of the root had lesions) on the two hosts.

Overall, our cross-pathogenicity experiments showed that South Dakota isolates of *F. acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* from either soybean or corn are pathogenic to both crops, despite that only a small number of isolates was used for the study. This suggests that rotating soybean and corn in South Dakota will not help reduce inoculum of species of *Fusarium* that are pathogenic to the two crops. Unfortunately, these pathogens can cause root rot on other crops grown in South Dakota (e.g., wheat [*Triticum aestivum* L.], Kaur 2016), which suggests exposure of soybean and corn to virulent isolates of *Fusarium* can be expected even if crop rotations are diversified. Thus, it is critical to adopt an integrated approach to manage *Fusarium* root rot in South Dakota, which includes combining tillage practices, weed management, use of fungicide seed treatments, adjusting planting dates, and using resistant cultivars/hybrids. Additionally, future studies are needed to explain how much corn and soybean can individually contribute to maintaining inocula of these fungi in fields with a known rotation of the two hosts.

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