Research

Eight Species of *Fusarium* Cause Root Rot of Corn (*Zea mays*) in South Dakota

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Abstract

Fusarium root rot of corn (*Zea mays* L.) is yield-limiting in the United States, but there is no information available on the disease in South Dakota. In 2015, corn seedlings with discolored roots were arbitrarily sampled from 50 South Dakota fields, and 198 isolates were recovered. Eight species (*F. acuminatum*, *F. boothii*, *F. equiseti-incarnatum* complex, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans*) were identified by morphology and translation elongation factor 1-α gene sequencing. *F. graminearum* (26.8%) was the most common fungus, and *F. boothii* (0.5%) was the least recovered. Fourteen isolates, representing the eight species, were evaluated for their pathogenicity on 2-week-old seedlings of inbred 'B73' using the inoculum layer method in the greenhouse. Fourteen days post-inoculation, root rot severity was evaluated on a 1-to-5 rating scale and expressed as relative treatment effects (RTEs). *F. proliferatum* isolate P2 caused significantly greater RTE (based on 95% confidence intervals) on seedlings than the other isolates and the noninoculated control, except *F. graminearum* isolate FG23. This study indicates that the eight species of *Fusarium* are aggressive root rot pathogens of corn in South Dakota, and this information will help evaluate strategies for producers to manage these pathogens in their fields.

Keywords: *Fusarium*, corn, root rot, maize

Diseases of corn (*Zea mays* L.) caused by species of *Fusarium* (e.g., Fusarium ear rot, root rot, and stalk rot) are yield limiting in the United States and Ontario, Canada (Mueller et al. 2016). In 2015, the total yield losses owing to *Fusarium*-associated diseases of corn in the United States and Ontario, Canada, were estimated at 6.3 million metric tons (Mueller et al. 2016). For all *Fusarium*-associated diseases, the causal pathogens are either soil-borne or seed-borne (Dodd and White 1999; Ocamb and Kommedahl 1994).

Among the *Fusarium*-associated diseases, root rot of corn may be understudied (Smit 1998). This may be because diagnosis of Fusarium root rot is complicated, because multiple organisms can be isolated from a single diseased corn plant, and these include species of *Fusarium* (Dodd and White 1999; Ocamb and Kommedahl 1994), *Rhizoctonia* (Sumner and Bell 1982), and *Pythium* (Matthiesen et al. 2016). Among the species of *Fusarium* reported to colonize corn roots, *F. acuminatum* Ellis and Everhart, *F. chlamydosporum* Wollenweber and Reinking, *F. culmorum* (Smith) Saccardo, *F. equiseti* (Corda) Saccardo (syn. *F. equiseti-incarnatum* complex), *F. graminearum* Schwabe, *F. oxysporum* Schlechtendal, *F. poae* (Peck) Wollenweber, *F. proliferatum* (Matsushima) Nirenberg, *F. redolens* Wollenweber, *F. semitectum* Berkeley and Ravenel, *F. solani* (Martius) Saccardo, *F. subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas, and *F. verticillioides* (Saccardo) Nirenberg (syn. *F. moniliforme* Sheldon), are important (Kuhnem et al. 2015; Leslie et al. 1990; Munkvold and Desjardins 1997; Munkvold and O'Mara 2002; Ocamb and Kommedahl 1994; Parikh et al. 2018; Ranzi et al. 2017; Soonthornpoct et al. 2000). In general, corn seedlings affected by Fusarium root rot have brown to dark discoloration and decayed roots (Gilbertson et al. 1985; Soonthornpoct et al. 2000; Wise et al. 2016).

In South Dakota, most producers rotate corn with soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), or sunflower (*Helianthus annuus* L.) and combine the rotation with no-tillage systems. Such cropping practices can favor the survival of species of *Fusarium* because a substantial amount of crop residue may be left on the soil surface, which can increase the amount of inoculum for the crop in the subsequent season. Despite that fungicides are used to treat corn seeds, seedling and root diseases of corn are becoming a concern in the United States. At this time, there is no information available on the pathogens causing root rot of corn in South Dakota. However, several species of *Fusarium* were recently isolated from diseased corn seedlings; therefore, the objectives of this study were to characterize species of *Fusarium* associated with root rot of corn and determine their aggressiveness in the greenhouse.

Isolation and Identification of Species of *Fusarium*

In 2015, corn plants with discolored roots were arbitrarily sampled from a total of 50 commercial fields (five samples per field) across 24 counties in eastern South Dakota, where over 50% of corn production takes place. The corn plants were sampled early in the season (following rain) between V1 (first leaf) and V3 (third leaf)

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vegetative growth stages (Ritchie et al. 1992), along five transects (50 m) that covered an area of 2 ha of the field where excess moisture was observed in soils. The distance between the corn fields was approximately 2 km.

To isolate fungi, the corn roots were washed under running tap water for 2 to 5 min to remove soil particles and any debris from the field. The infected root tissues from each plant were cut into small pieces of approximately 15 mm long and surface disinfested in sodium hypochlorite (0.05%) and ethanol (70%) for 1 min each, rinsed with sterile distilled water, and blotted dry with sterile paper towels. Three root pieces (≈15 mm) were plated on potato dextrose agar (PDA) amended with streptomycin sulfate (0.02%) and incubated at 23 ± 2°C for 7 days.

From five plants sampled per field, one to four putative *Fusarium* isolates were recovered on PDA. In total, 198 isolates were collected and identified to species level by transferring hyphal tips of the colonies onto fresh PDA plates to obtain pure cultures. From the growing edge of the colony of the *Fusarium* isolates, one mycelial plug (≈3-mm square) was removed with a sterile scalpel and transferred to carnation leaf agar (CLA) to examine morphological characteristics (Leslie and Summerell 2006). The isolates were identified to eight species of *Fusarium* (*F. acuminatum*, *F. boothii* O'Donnell, Aoki, Kistler and Geiser, *F. equiseti-incarnatum* complex, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans*). Among the 198 isolates, *F. graminearum* (26.8%) was the most commonly recovered, followed by *F. oxysporum* (22.2%), *F. equiseti-incarnatum* complex (16.2%), *F. acuminatum* (13.6%), *F. proliferatum* (11.1%), *F. subglutinans* (5.6%), *F. solani* (4.0%), and *F. boothii* (0.5%).

Twenty-seven isolates identified as *F. acuminatum* produced red pigment, curved macroconidia with three to five septate (n = 100; 7.0 to 10.0 × 0.7 to 1.0 μm), and no microconidia but with chain formation of chlamydospores. One isolate was tentatively identified as *F. boothii* because it produced thick-walled macroconidia with five to seven septate (n = 100; 5.9 to 10.0 × 0.8 to 1.1 μm). Thirty-two isolates were tentatively identified as *F. equiseti-incarnatum* complex, given the macroconidia on CLA were relatively long and narrow (n = 100; 12.6 × 20.4 μm), with an average of five septa and whip-like bent apical cells. Chlamydospores and microconidia were not observed on CLA. Fifty-three isolates were tentatively identified as *F. graminearum* given the macroconidia were slender and slightly curved with five to six septate (n = 100; 4.5 to 11.5 × 0.8 to 1.1 μm). No microconidia were observed, but chlamydospores were present in singular form. Forty-four isolates were tentatively identified as *F. oxysporum* given the macroconidia were less abundant and were three septate (n = 100; 5.6 to 7.1 × 0.5 to 0.9 μm). The microconidia were in abundance and formed on false heads (n = 100; 0.6 to 2.0 × 0.3 to 0.6 μm). Twenty-two isolates were tentatively identified as *F. proliferatum* given the macroconidia were relatively straight with a curved apical cell. The septate were two to five in number (n = 100; 4.8 to 8.1 × 0.7 to 1.2 μm). The microconidia were produced in abundance, oval in shape, without septate. They were formed in chains on both monophialides and polyphialides (n = 100; 0.6 to 1.3 × 0.5 to 0.7 μm). Eight isolates were tentatively identified as *F. solani* because they produced green sporodochia. The macroconidia had five to seven septate (n = 100; 4.6 to 6.6 × 0.6 to 1.1 μm). The microconidia were oval shaped with one to two septate (n = 100; 1.2

TABLE 1
Information on the representative *Fusarium* isolates used for molecular identification and greenhouse aggressiveness study

Isolate[a]	Fungal names	County in South Dakota	Growth stage at time of collection	Previous crop	GenBank accession number
F7	*Fusarium acuminatum*	Sanborn	V2	Soybean	MH595496
F25	*F. acuminatum*	Clark	V2–V3	Wheat	MH595498
F6	*F. acuminatum*	Miner	V2	Corn	MH595499
F8	*F. acuminatum*	Spink	V1	Corn	MH595497
F9	*F. boothii*	Clark	V2–V3	Wheat	MH595500
F16	*F. equiseti-incarnatum*	Minnehaha	V3	Corn	MH595503
F27	*F. equiseti-incarnatum*	Hamlin	V3	Soybean	MH595501
F28	*F. equiseti-incarnatum*	Hamlin	V3	Soybean	MH595502
F10	*F. graminearum*	Codington	V1	Corn	MH595504
E22	*F. graminearum*	Hutchinson	V1–V2	Corn	MH595506
F12	*F. graminearum*	Clark	V2–V3	Wheat	MH595505
F13	*F. graminearum*	Lincoln	V2	Soybean	MH595507
F23	*F. graminearum*	Douglas	V2	Corn	MH595508
F2	*F. oxysporum*	Brookings	V1	Corn	MH595509
F3	*F. oxysporum*	Brookings	V1	Corn	MH595510
E15	*F. oxysporum*	Davison	V1–V2	Corn	MH595511
P1	*F. oxysporum*	Brown	V2–V3	Corn	MH595514
P4	*F. oxysporum*	Lake	V2	Corn	MH595515
P7	*F. oxysporum*	Minnehaha	V3	Soybean	MH595512
F1	*F. proliferatum*	Brookings	V1	Corn	MH595516
F14-PB	*F. proliferatum*	Lincoln	V3	Soybean	MH595517
P2	*F. proliferatum*	Brookings	V1	Corn	MH595518
F11	*F. solani*	Clay	V2	Corn	MH595519
P6	*F. solani*	Browns	V1–V2	Wheat	MH595520
P5	*F. subglutinans*	Miner	V2	Corn	MH595522

[a] These isolates were recovered from diseased corn roots sampled from 50 commercial fields (across 24 counties) in South Dakota during the 2015 growing season.

to 2.5 × 0.4 to 1.0 µm). Eleven isolates were tentatively identified as *F. subglutinans*, given the macroconidia were abundant, slender, thin-walled, and with curved apical cell (n = 100; 5.2 to 7.3 × 0.4 to 1.1 µm). The microconidia were produced in abundance, oval shaped, and nonseptate (n = 100; 1.2 to 2.6 × 0.4 to 0.8 µm).

For molecular identification, a total of 25 isolates were selected by South Dakota county to represent the eight species (Table 1). DNA was extracted from the isolates, and the translation elongation factor 1-α (TEF1-α) gene region was sequenced using the primers EF1F and EF1R (Geiser et al. 2004). The TEF1-α sequences were used for performing maximum parsimony phylogenetic analysis in Molecular Evolutionary Genetics Analysis (MEGA) software (version 7; Kumar et al. 2016). Out of 761 aligned characters, 343 were parsimony-informative characters, which was included in the maximum parsimony analyses and resulted in seven most parsimonious trees. The consistency index was 0.72, the retention index was 0.93, and the composite index was 0.67 (parsimony-informative sites = 0.66) for all sites. The

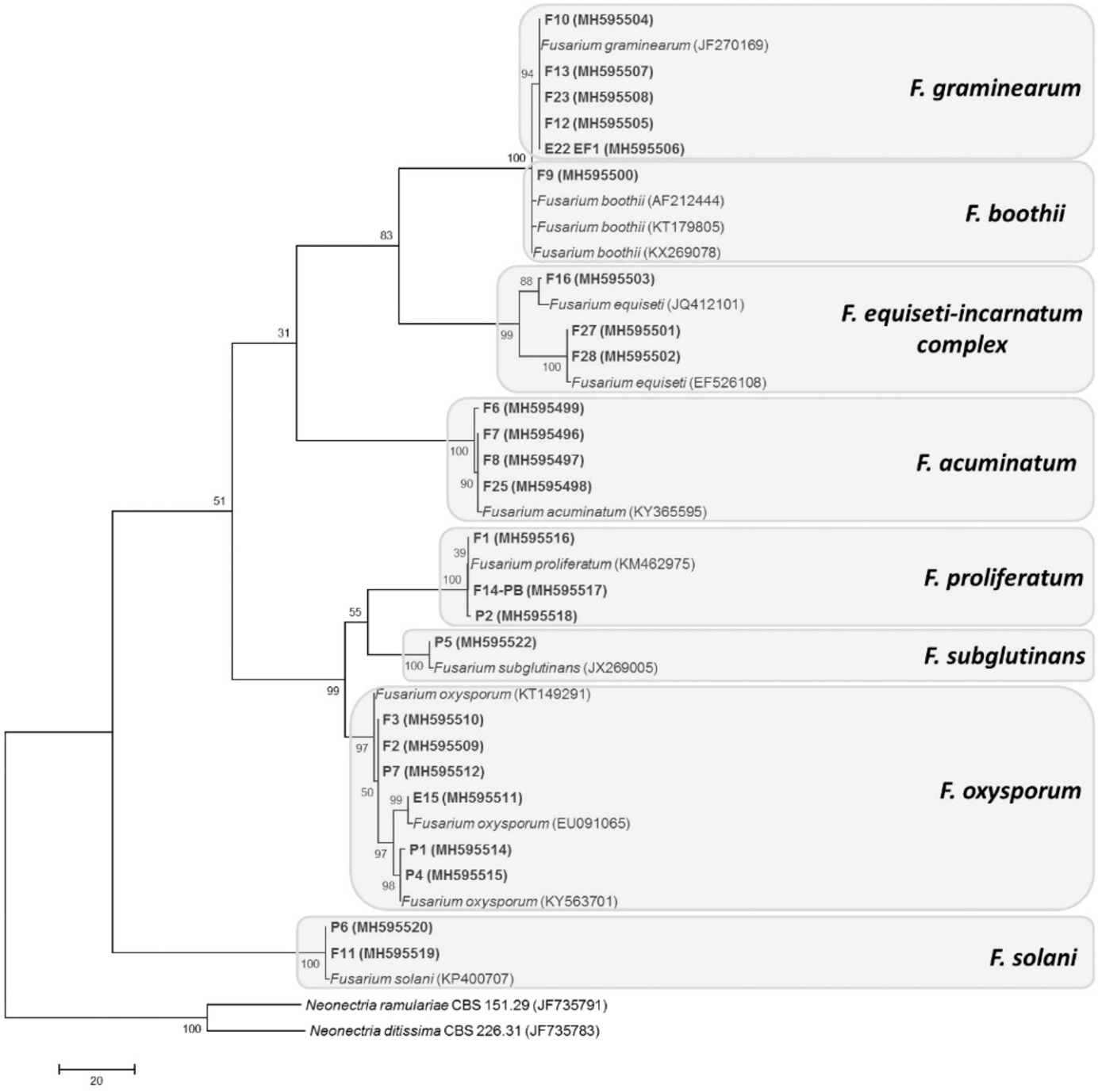

FIGURE 1

The evolutionary history was inferred using the maximum parsimony method in Molecular Evolutionary Genetics Analysis software (MEGA version 7; Kumar et al. 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The analysis involved 40 nucleotide sequences and included the outgroups *Neonectria ramulariae* and *N. ditissima*. The newly generated sequences are in bold.

TEF1-α based phylogeny grouped the isolates in eight well-supported clades (bootstrap value from 94 to 100%) that included type sequences of the eight species identified by BLASTN searches in the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) (Fig. 1).

Greenhouse Experiment to Evaluate Aggressiveness of *Fusarium* Isolates

For the aggressiveness study, 14 isolates representing the eight species of *Fusarium* were selected arbitrarily based on morphological grouping from the 25 isolates and evaluated on the corn inbred 'B73' (PI 550473) in the greenhouse. To prepare the inoculum, each of the 14 isolates was initially grown on PDA, and then five mycelial plugs (≈15-mm square) were transferred into a 250-ml Erlenmeyer flask containing autoclaved sand/corn meal mixture (54 g of play sand, 6 g of cornmeal, and 10 ml of distilled water). The flasks containing the inoculum were mixed every other day by manually shaking the flask to ensure uniform colonization of the sand/corn meal mixture. For the noninoculated control, sand/cornmeal mixture without any fungus was used for inoculum. These were incubated at 23 ± 2°C for 14 days.

At planting, the inoculum layer method from Bilgi et al. (2008) was used. The plastic cups (473 ml) were first filled with 40 g of coarse vermiculite, followed by 20 g of inoculum and then 20 g of coarse vermiculite before planting the pregerminated seeds of B73. The seeds were spread evenly on a wet filter paper in Petri plates and germinated for 3 days at 23 ± 2°C. After the seeds sprouted roots, they were transplanted into plastic cups. In each cup, two sprouting seeds were planted and covered with an additional 20 g of coarse vermiculite. The experiment was set up as a completely randomized design with five replications (cups) and performed twice. The temperature in the greenhouse was maintained at 22 ± 2°C under a 16-h photoperiod. The plants were watered once daily, and no fertilizer was added during the experiment.

The experiment was terminated at 14 days after inoculation, and root rot severity caused by the isolates on corn seedlings was evaluated on 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 root rot severity data were not normally distributed and, therefore, were analyzed using nonparametric statistics (Shah and Madden 2004). The Fligner-Killen test for homogeneity of variance between the two experimental repeats was tested and satisfied (P = 0.09) prior to data analysis. The nparLD package (Noguchi et al. 2012) in R version 2.1 (R Core Team 2013) was used to determine the analysis of variance type test statistics (ATS) of ranked data, which indicated the overall effect of the treatments.

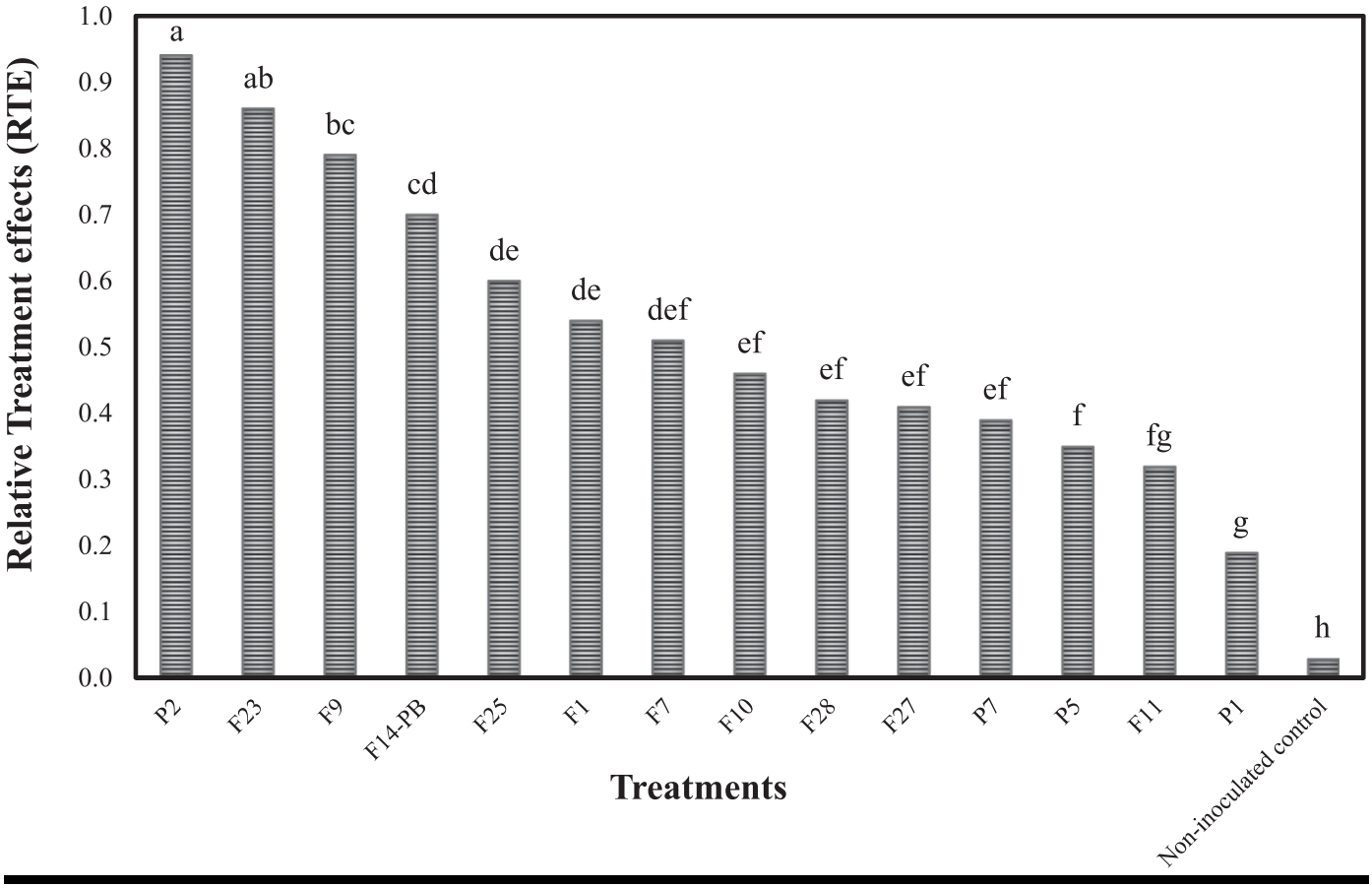

FIGURE 2

Root rot severity (expressed as relative treatment effects [RTEs]) caused by 14 isolates on the seedlings of the corn inbred 'B73' in the greenhouse. RTEs with the same letter are not significantly different among treatments based on 95% confidence intervals. Abbreviations: P2 = *F. oxysporum*; F23 = *F. graminearum*; F9 = *F. boothii*; F14-PB= *F. proliferatum*; F25 = *F. acuminatum*; F1 = *F. proliferatum*; F7 = *F. acuminatum*; F10 = *F. graminearum*; F28 = *F. equiseti-incarnatum*; F27 = *F. equiseti-incarnatum*; P7 = *F. oxysporum*; P5 = *F. subglutinans*; F11 = *F. solani*; and P1 = *F. oxysporum*.

The nparLD package was used to calculate the rank of each isolate (treatment) according to equation 1 (Akritas 1991):

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

where $\bar{R}_i$ is the mean rank for the ith treatment and R_{ik} is the rank of X_{ik} among all N observations (Shah and Madden 2004). The root rot severity 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 were compared at 95% confidence intervals using the nparLD package in R (Noguchi et al. 2012).

A significant effect of RTEs (ATS = 30.11; df = 4.94; $P = 2.20 \times 10^{-30}$) caused by the treatments was observed on corn seedlings at 14 days after inoculation. All 14 isolates caused discoloration on the roots of the corn seedlings. No discoloration was observed on the roots of the control seedlings. Among the treatments, *F. proliferatum* isolate P2 (median disease rating = 4.0) caused significantly higher RTE (based on 95% confidence intervals) than the other treatments except *F. graminearum* isolate F23 (median disease rating = 3.5). The RTE caused by *F. oxysporum* isolate P1 (median disease rating = 1.5) was significantly lower compared with that of all the other isolates (Fig. 2).

Significant differences in RTE were observed among isolates within *F. graminearum*, *F. oxysporum*, and *F. proliferatum*. The RTE caused by *F. graminearum* isolate F23 (median disease rating = 3.5) was significantly higher than that caused by F10 (median disease rating = 2.0). The RTE caused by *F. oxysporum* isolate P7 (median disease rating = 2.0) was observed to be significantly higher than that caused by PI (median disease rating = 1.5), and the RTE caused by *F. proliferatum* isolate P2 (median disease rating = 4.0) was significantly higher compared with that of either F14-PB (median disease rating = 3.0) or F1 (median disease rating = 3.0) (Fig. 2).

To fulfill Koch's postulates, roots of inoculated seedlings were randomly selected, and the fungi were reisolated by plating diseased root pieces on PDA as previously described. Colonies of the isolates were transferred to CLA for morphology-based identification. From the roots of the noninoculated corn seedlings, species of *Fusarium* were not recovered.

Summary and Importance

Our study confirms that eight species of *Fusarium* are capable of causing root rot of corn in South Dakota. Among the eight species, *F. graminearum* was the most commonly recovered from the roots of the diseased corn seedlings, followed by *F. oxysporum* and *F. equiseti-incarnatum* complex. In the greenhouse, isolates of *F. acuminatum*, *F. boothii*, *F. equiseti-incarnatum* complex, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* were determined to be aggressive on B73. In addition, significant differences in aggressiveness were observed among isolates within *F. graminearum*, *F. oxysporum*, and *F. proliferatum*.

In this study, *F. oxysporum* caused little discoloration from the two isolates on the corn roots when compared with the noninoculated plant roots. This is possibly because our experiment was performed at a greenhouse temperature of 22°C and the roots were not wounded. Based on the study by Warren and Kommedahl (1973), *F. oxysporum* can cause root rot of corn only under high temperatures (e.g., 29°C), in the presence of another species of *Fusarium* or other fungi, and if the roots are wounded. In contrast to *F. oxysporum*, isolates of *F. acuminatum*, *F. boothii*, *F. graminearum*, and *F. proliferatum* caused severe discoloration of corn roots. The severity of root rot observed in seedlings inoculated with *F. graminearum* isolates was consistent with that of previous research (Broders et al. 2007). However, in the case of *F. acuminatum* and *F. proliferatum* isolates, our study was not consistent with the previous studies that these two fungi contributed little or nothing to root rot development on corn (Mao et al. 1998; Ocamb and Kommedahl 1994; C. M. Ocamb and T. Kommedahl, *unpublished data*). In our study, isolates of *F. acuminatum* and *F. proliferatum* caused necrotic lesions on the root of corn plants, and we suspect that lower temperature in the greenhouse played a role in disease development. As for *F. boothii*, the fungus was reportedly caused Gibberella ear rot in China (Duan et al. 2016), Mexico (Cerón-Bustamante et al. 2018), and South Africa (Boutigny et al. 2011). *F. boothii* has been reported on corn in the United States, but the pathogenicity of the fungus was not examined (Aoki et al. 2012). Although one isolate of *F. boothii* was used for this study, the root rot severity caused by the fungus was not significantly different from that caused by *F. graminearum* (F23) and *F. proliferatum* (F14-PB) isolates. This suggests that *F. boothii* is pathogenic on corn, and to our knowledge, this is the first report of the fungus causing Fusarium root rot of corn.

This study suggests that species diversity of *Fusarium* associated with corn roots may have changed since the research by Warren and Kommedahl (1973), who identified six species of *Fusarium* (*F. episphaeria* [Tode] Snyder and Hansen, *F. moniliforme* Sheldon, *F. oxysporum*, *F. roseum* Link, *F. solani*, and *F. tricinctum* [Corda] Saccardo) colonizing corn roots, rhizosphere, residues, and soil. However, additional research is required to study the environmental factors affecting Fusarium root rot development of corn (e.g., temperature) and to evaluate integrated pest management tools (e.g., genetic resistance, fungicide seed treatments) to manage the disease in South Dakota corn fields.

Acknowledgments

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