Distribution and Frequency of *Fusarium* Species Associated with Soybean Roots in Iowa

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Abstract

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A 3-year survey was conducted in Iowa to characterize the distribution and frequency of species of *Fusarium* associated with soybean roots. Ten plants were collected from each of 40 to 57 fields each year at V2 to V5 and R3 to R4 soybean growth stages. *Fusarium* colonies were isolated from symptomatic and symptomless roots and identified to species based on cultural and morphological characteristics. Species identification was confirmed by amplification and sequencing of the translation elongation factor (EF1- α) gene. Fifteen species were identified; *Fusarium oxysporum* was isolated most frequently, accounting for more than 30% of all isolates. *F. acuminatum*, *F. graminearum*, and *F. solani* were also among the most frequent and widespread species.

Eleven other species were recovered from few fields, accounting for less than 10% of all isolates in a given year. No consistent trends were observed in geographic distribution of species. Variability in species frequency was found between soybean growth stages. *Fusarium oxysporum* was recovered at higher frequency during vegetative stages (40%) than reproductive stages (22%). Conversely, species such as *F. acuminatum*, *F. graminearum*, and *F. solani* were recovered more often from reproductive-stage plants. No significant differences in species composition were observed among fields differing in tillage practices and row spacing.

Fungi in the genus *Fusarium* cause several diseases of soybean (*Glycine max* (L.) Merr.), including Fusarium wilt, caused by *Fusarium oxysporum* Schltdl. (2); sudden death syndrome (SDS), caused in North America by *F. virguliforme* O'Donnell & T. Aoki (1); as well as seed and seedling diseases and root rot, caused by numerous species (7,28,33,37). Fusarium root rot is a common and widespread disease in the United States (40). At least 19 *Fusarium* spp. have been isolated from soybean roots in different soybean production areas (4,5,10,23,24,28,32). Previous studies have demonstrated that *F. oxysporum* and *F. solani* are generally the most common species in soybean tissues and the main causal agents of soybean root rot in the United States (9,13,24,28) and other countries (3,20,41). Affected plants may exhibit poor or slow emergence and root symptoms, including dark brown lesions, especially in the lower portion of the root system, or decay of the entire taproot (28).

Several studies on occurrence, distribution, pathogenicity, and cultivar resistance have been conducted for *F. oxysporum* and *F. solani* (13,14,22,29) but there is limited work on the other *Fusarium* spp. isolated from soybean roots (4,9,24). These reports as well as field observations indicate that colonization of soybean roots by several of these species is common; however, there is very limited information about their pathogenicity on soybean and their impacts on root health and yield.

Fusarium root rot was first reported as a problem in soybean in Iowa in 1953 by Dunleavy (30). The disease was initially attributed to *F. orthoceras* Appel & Wollenw. but later it was documented that *F. oxysporum* was the predominant fungus isolated from symptomatic soybean roots (14,30). Several other *Fusarium* spp. have

been consistently isolated from soybean plants in Iowa fields. Nyvall (30), who conducted a 3-year study, isolated *Fusarium* spp. from pods, stems, and roots from soybean plants collected in nine fields in central and north Iowa. In this study, *F. oxysporum* was the predominant colonizer of roots and soil, followed by *F. solani*. Another important soybean root rot pathogen in Iowa is *F. virguliforme*, the cause of SDS, and various studies have been done on the biology, epidemiology, and cultivar resistance for this disease (16,27).

Although Fusarium root rot has long been known as a common soybean disease, the importance of this disease to Iowa soybean production is not fully understood. In addition, there is a lack of information about the species involved and their abundance and geographical distribution around the state. Therefore, the objectives of the present study were to (i) characterize the diversity and frequency of *Fusarium* spp. associated with soybean roots in Iowa and (ii) determine the geographic distribution of *Fusarium* spp. in Iowa during vegetative and reproductive stages of soybean growth.

Materials and Methods

Sample collection. Soybean roots were collected during the 2007, 2008, and 2009 growing seasons across the nine Iowa crop reporting districts (Fig. 1). In collaboration with 12 Iowa State University extension specialists, 142 soybean fields from 98 Iowa counties were surveyed over the 3-year period (Fig. 1). All the fields sampled had corn as the previous crop, with the exception of three locations. Ten plants with as much of the root system as possible were collected arbitrarily from each field at both vegetative (V2 to V5) and reproductive (R2 to R4) growth stages (18); plants were dug and soil was gently shaken from roots before plants were placed in plastic bags. Samples were shipped overnight to the Iowa State University Plant & Insect Diagnostic Clinic or The Seed Science Center and stored at 4°C until processed. Some fields were sampled only at one growth stage and some counties were sampled in more than 1 year. In total, 101 samples were collected from 55 fields in 50 counties in 2007, 77 samples from 40 fields in 40 counties in 2008, and 84 samples from 47 fields in 40 counties in 2009 (Fig. 1). In each field, sampling date, location (geographic coordinates), and soybean growth stage were documented. Row spacing (19.1, 38.1, and 76.2 cm) and tillage type (conventional

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*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures and one supplementary table are available online.

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till, no till, and reduced tillage) were also recorded in order to determine possible differences in species frequency in relation to crop production practices. For each year, statewide average air temperature and precipitation from May to July were obtained from Iowa Environmental Mesonet, Department of Agronomy, Iowa State University (<http://mesonet.agron.iastate.edu/climodat/>).

Isolation and identification of *Fusarium* spp. Roots were rinsed under running tap water for 10 min to remove soil and debris from the field. Fungi were isolated from roots by arbitrarily excising two 1-cm-long root pieces from each plant, one from the taproot and one from the lateral root tissue. Root pieces were surface disinfected in 0.5% NaOCl for 2 min, rinsed three times in sterile distilled water, and blotted dry. Root pieces were embedded in antibiotic-amended Nash Snyder medium (per liter: 15 g of peptone, 1 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g of agar, 0.75 g of pentachloronitrobenzene (PCNB), streptomycin sulfate solution at 33 mg/ml, and neomycin sulfate solution at 40 mg/ml) and incubated at room temperature ($25 \pm 2^\circ\text{C}$) for 7 days under fluorescent light. Numbers of root pieces with putative *Fusarium* colonies were recorded for each sample. Colonies were transferred into 1.5-ml centrifuge tubes containing potato dextrose broth (PDB) by removing a small piece of mycelium from the colony margin, maintained on a rotary shaker at 200 rpm for 3 to 5 days to allow colony growth, and then stored at 4°C for future species identification.

From each set of 10 plants sampled per field, 1 to 20 putative *Fusarium* isolates were recovered. In total, 2,193 isolates (793 isolations in 2007, 588 in 2008, and 812 in 2009) were collected. For identification to species level, 1 to 15 isolates were selected arbitrarily from within each field sample, for a total of 1,640 isolates. Each isolate was transferred from PDB to carnation leaf agar (CLA) (25) amended with KCl (15 g of agar and 6 g of KCl per liter) and antibiotic-amended potato dextrose agar (PDA) (streptomycin sulfate solution at 33 mg/ml, neomycin sulfate solution at 40 mg/ml, and 39 g of PDA per liter), and cultures were incubated for 7 to 20 days at room temperature under fluorescent light. Each of the 1,640 isolates was examined microscopically and identified to species according to the system of Leslie and Summerell (25). Species were identified based on the most distinctive morphological characteristics, including macroconidia and microconidia, production of mesoconidia (e.g., *F. semitectum*), conidial arrangements (singly, false heads, and chains), nature of the conidiogenous cells (monophialides [e.g., *F. oxysporum*] or polyphialides [e.g., *F. proliferatum*]), conidiophore length (e.g. *F. solani* versus *F. oxysporum*), formation and arrangement of chlamydospores (e.g. *F.*

equiseti), production and color of sporodochia on CLA, and colony appearance and pigment formation on PDA (Supplementary Figure 1). Of the 1,640 isolates identified, 1,254 were species of *Fusarium* based on cultural and morphological characteristics.

Species identities were confirmed for a subset of 170 isolates by amplification and sequencing of the elongation factor 1- α (*EF1- α*) gene region. The isolates were selected to represent all the distinguishable and unknown morphological groups that were observed. Single-spore cultures were obtained for each of the 170 isolates used for molecular identification. All isolates were grown on PDA at room temperature for 7 days. The mycelia were harvested, 50 to 100 mg of mycelia was placed into a 1.5-ml centrifuge tube, and then each sample was lyophilized for 2 days and immediately crushed with a sterile pestle in the tube. Genomic DNA was extracted using the cetyltrimethylammonium bromide procedure (19). DNA was quantified using a spectrophotometer and then stored at -20°C until processed. Primers pair used for amplification and sequencing were *ef1/ef2* (5'-ATGGGTAAGGAGGACAAGAC-3'/5'-GGAAGTACCAGTGATCATGTT-3') and *ef1/ef22* (5'-AGG AACCCTTACCGAGCTC-3'), respectively (15,31). Polymerase chain reactions (PCRs) were conducted in 20- μl volumes. Each reaction tube contained approximately 1 μl of a 1-ng/ μl DNA template, 3.2 μl of 10 $\times$ Titanium *Taq* buffer (35 mM MgCl_2), 9.7 μl of sterile distilled water, 2 μl of 2 mM dNTPs, 2 μl each of forward and reverse primers (2.5 μM), and 0.1 μl of DNA *Taq* polymerase (Titanium *Taq*). The thermal cycling parameters were initial denaturation at 94°C for 1 min followed by 31 cycles consisting of denaturation at 94°C for 30 s, annealing at 53°C for 45 s, and extension at 72°C for 1 min. A final extension at 72°C for 5 min was done at the end of the amplification. PCR products were purified adding 2 μl of ExoSAP-IT reagent (Affymetrix Inc.) in 5 μl of PCR. PCR products were sequenced at the Iowa State University DNA and Sequencing Facility. DNA sequences were trimmed to 400 to 500 bp using CodonCode Aligner 4.1.1 (CodonCode Corp.). Sequences were submitted for BLAST searches for comparison to known DNA sequences in the National Center for Biotechnology Information (NCBI) (<http://ncbi.nlm.nih.gov>) and the FUSARIUM-ID/FUNCBS databases in Fusarium MLST (<http://www.cbs.knaw.nl/Fusarium>). Sequences were also compared with reference strains from The *Fusarium* Laboratory Manual (25) using BLASTn from NCBI (<http://ncbi.nlm.nih.gov>). The accession numbers for the reference strains and the percent identity of the isolates to those strains are listed in Supplementary Table 1.

Data analysis. For each *Fusarium* sp., relative frequency and incidence were calculated using the total number of *Fusarium* isolates and root pieces, respectively, from all fields sampled in a given year. The relative frequency (*F*) of *Fusarium* spp. was evaluated using the formula $F = 100 \times (n/N)$, in which *n* = the number of isolates of each species and *N* = the total number of isolates of all species (34). Incidence of each species was defined as the percentage of all root pieces which yielded that species (26), and was estimated as $I_s = 100 \times (r_s/r_i) \times r_a/R$, in which *r_s* = number of root pieces yielding a species, *r_i* = number of root pieces selected for identification, *r_a* = number of root pieces yielding any isolate, and *R* = total number of root pieces sampled. Prevalence of *Fusarium* spp. was calculated at the state level, using both field and county as sampling units, by dividing total number of fields or counties in which each *Fusarium* spp. was present by total number of fields or counties sampled around the state. In addition, *Fusarium* spp. prevalence was calculated at the district level as the total number of fields in which each *Fusarium* spp. was present ($\times 100$) divided by the total number of fields sampled per district.

Frequencies of isolation were compared for the overall data set to test for differences among species, and between crop growth stages, tillage practices, row spacing, and root type (lateral versus taproot). Analyses of variance were conducted using the general linear model procedure (PROC GLIMMIX) of SAS (version 9.2, SAS Institute) using year as replication (random factor). Differences among means were compared using the Tukey-Kramer test and considered significantly different if $P \leq 0.05$. Incidence and

Fig. 1. Location of 142 soybean fields surveyed in 98 Iowa counties during the 2007, 2008, and 2009 growing seasons across the nine Iowa crop reporting districts: Northwest (NW), North Central (NC), Northeast (NE), West Central (WC), Central (C), East Central (EC), Southwest (SW), South Central (SC), and Southeast (SE).

prevalence data were analyzed for the overall data set, as well as for tillage and row spacing, using the same model as above.

Results

Fifteen species were identified, including *F. acuminatum*, *F. armeniacum*, *F. avenaceum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, *F. subglutinans*, *F. tricinctum*, *F. verticillioides*, and *F. virguliforme* (Fig. 2). Morphological characteristics such as mycelium appearance, growth rate, pigment, and sporodochia formation varied among isolates within a species, particularly for *F. armeniacum*, *F. semitectum*, *F. oxysporum*, and *F. solani*. Strains belonging to the *F. oxysporum* complex showed the widest variation in culture morphology on PDA.

Frequency of isolation of *Fusarium* spp. Mean frequency of isolation averaged over years (data not shown) varied among *Fusarium* spp. ($P < 0.0001$). Frequency of isolation of *F. oxysporum*, *F. acuminatum*, and *F. solani* was greater ($P < 0.0001$) than all the other species. *F. oxysporum* showed significantly higher frequency (32%) than all other species, except *F. acuminatum* (23.9%). Frequency of *F. solani* was 21.5% and did not differ from *F. acuminatum* but did differ from all other species which were isolated with a frequency of 8% or lower. There were no differences in frequency among the remaining species.

The main trends observed in the data plotted per year (Fig. 2) were similar to those observed for data combined over years. *F. oxysporum* was the most frequently recovered species, followed by *F. acuminatum* and *F. solani*, each ranging from 12 to 37% of the isolates (Fig. 2). Other species such as *F. graminearum*, *F. proliferatum*, and *F. sporotrichioides* represented 1 to 12% of the isolates. Remaining species such as *F. armeniacum*, *F. avenaceum*, *F. equi-*

seti, *F. semitectum*, *F. subglutinans*, *F. poae*, *F. tricinctum*, *F. verticillioides*, and *F. virguliforme* were less frequent (<1 to 3% of isolates) (Fig. 2). For example, in 2007 and 2008, *F. oxysporum* was the most frequently isolated (28 and 30%, respectively), followed by *F. acuminatum* (26 and 28%, respectively) whereas, in 2009, the most frequently isolated species were *F. oxysporum* (37%) and *F. solani* (27%). Frequency of isolation of *F. graminearum* was similar in 2007 (12%) and 2009 (8%); however, in 2008, a low percentage (4%) of isolates was recovered for this species (Fig. 2).

Soybean growth stage did not have a significant effect on frequency of isolation of *Fusarium* spp., in data combined over years, and there was no significant interaction between growth stage and species. When analyzed for each species separately, growth stage had a significant effect on *F. acuminatum*, with a higher ($P = 0.027$) frequency observed at reproductive stages (29.6%) compared with vegetative stages (19.3%) (Fig. 3). No significant differences in relative frequency were found between growth stages for any of the other species (Fig. 3). Although *F. oxysporum* frequency did not differ significantly between growth stages, the trend for higher frequency at vegetative growth stages compared with reproductive growth stages was present each year (30 versus 26% in 2007, 40 versus 17% in 2008, and 54 versus 27% in 2009 for vegetative and reproductive stages, respectively). The species *F. avenaceum* and *F. poae* were only recovered in reproductive stages, whereas *F. tricinctum* and *F. virguliforme* were only recovered at vegetative stages.

Row spacing did not have a significant effect on relative frequency averaged over all species or when analyzed for each species separately. For example, mean frequency of isolation of *F. acuminatum* was 14, 15, and 21% for fields with row spacing less than 19, 38, and 76 cm, respectively, and frequency of *F. oxysporum* varied from 22 and 26% between row spacing treatments. There were also no significant differences between tillage practices in the frequency of overall *Fusarium* spp. or each species individually. For example, frequency of *F. acuminatum* was 14.4, 21.9, and 20.5% for no-till, reduced till, and conventional till, respectively, and varied from 18 to 25% for *F. oxysporum* and 11 to 18% for *F. solani*. The type of root piece (tap or lateral) from which isolations were made had no significant effect on frequency of *Fusarium* spp.

***Fusarium* spp. incidence and prevalence.** Significant differences among species were found for mean incidence ($P < 0.0001$), prevalence at the county level ($P < 0.0001$), and prevalence at the field level ($P = 0.003$) when averaged over years. *F. oxysporum*, *F.*

Fig. 2. Frequency of isolation (%) of *Fusarium* spp. from soybean roots in Iowa in 2007, 2008, and 2009.

Fig. 3. Frequency (%) of isolation of *Fusarium* spp. recovered from soybean roots at two plant stages, V2 to V5 and R2 to R4, from 2007 to 2009. *Fusarium oxysporum* (FO), *F. acuminatum* (FA), *F. solani* (FS), *F. graminearum* (FG), *F. proliferatum* (FP), *F. sporotrichioides* (FSP), *F. semitectum* (FSE), *F. equiseti* (FE), *F. subglutinans* (FSUB), *F. verticillioides* (FVER), *F. virguliforme* (FVIR), *F. armeniacum* (FAR), *F. avenaceum* (FAVE), *F. poae* (FPO), and *F. tricinctum* (FTRI).

solani, *F. acuminatum*, and *F. graminearum* were the species with the highest prevalence (recovered from the highest percentage of fields or counties) and the highest incidence (recovered from the greatest percentage of root pieces) in 2007 to 2009. Other species, such as *F. armeniacum*, *F. avenaceum*, *F. equiseti*, *F. poae*, *F. semitectum*, *F. subglutinans*, *F. tricinatum*, and *F. virguliforme*, showed the lowest prevalence and incidence (Table 1). Mean incidence of *F. oxysporum* (14.9%) did not differ significantly from *F. acuminatum* (12.3%) and *F. solani* (10.2%) but was greater than all other species. The same results were obtained for prevalence at the county level, with *F. oxysporum* (84.5%), *F. acuminatum* (73.3%), and *F. solani* (73.0%) showing the higher prevalence compared with all other species. Due to greater variation among fields, differences in prevalence at the field level were not as significant ($P = 0.003$).

For the most frequent species identified in this study, such as *F. oxysporum*, *F. solani*, and *F. acuminatum*, prevalence was similar among years (Supplementary Figure 2); these species were recovered statewide in each year. For some species, such as *F. graminearum*, *F. semitectum*, and *F. verticillioide*s, consistent patterns were evident in prevalence among year and geographically. For example, *F. graminearum* was present in all districts in 2007 but the fungus was not present in fields sampled from the East-Central, Southeast, and Northwest districts in 2008 or from the Southeast district in 2009. In contrast, prevalence patterns for *F. semitectum* and *F. verticillioide*s were highly variable (Supplementary Figure 2). Species such as *F. avenaceum*, *F. poae*, and *F. tricinatum*, were found in only one field within one district. No significant differences in incidence were observed among different tillage practices or row spacing averaged over all *Fusarium* spp. or when analyzed for each species separately for (data not shown).

Discussion

This study provides the first documentation regarding frequency, prevalence, and geographic distribution of *Fusarium* spp. associated with soybean roots among the nine Iowa crop districts. Overall, *F. acuminatum*, *F. graminearum*, *F. oxysporum*, and *F. solani* were omnipresent and abundant in all nine Iowa crop districts, whereas *F. armeniacum*, *F. avenaceum*, *F. equiseti*, *F. sporotrichioides*, *F. poae*, *F. proliferatum*, *F. semitectum*, *F. subglutinans*, *F. tricinatum*, *F. verticillioide*s, and *F. virguliforme* were less frequently found. These observations would indicate that diversity of species associated with soybean roots in Iowa is higher than previously recognized by Nyvall (30), who found four species of *Fusarium* (*F. oxysporum*, *F. solani*, *F. tricinatum*, and *F. roseum*) colonizing soybean roots, stems, and pods.

F. oxysporum was the most common species and also the species showing the most morphological variability in this study. The high frequency of isolation of *F. oxysporum* found is consistent with work by Nyvall (30) and French and Kennedy (14), who both found that *F. oxysporum* was the most common *Fusarium* sp. isolated from soybean roots in Iowa and Minnesota, respectively. *F. oxysporum* and *F. solani* are well known as major causal agents of soybean root rot (13,14,22,28,30) but there is limited information about pathogenicity of the other *Fusarium* spp. isolated from soybean roots. For most species, there is also scarce information about their epidemiology and impact of soybean yield. A recent study (9) compared the aggressiveness and yield impact of nine of the *Fusarium* spp. isolated from soybean roots in the Iowa survey reported here. In that study, at least some isolates of each of the nine species tested were found to be pathogenic on soybean. Further work is needed to better understand the role of *Fusarium* spp. as causal agents of seedling and root rot, as well as their epidemiology and yield impact in other soybean-producing regions.

It is generally accepted that *F. oxysporum* and *F. solani* (25) are species complexes, showing a high level of variation within each group. In the study by Diaz et al. (9), variation in aggressiveness was found among and within *Fusarium* spp., particularly within *F. solani* and *F. oxysporum*. Additional work is needed to understand the genotypic and phenotypic diversity of these fungi on soybean and other crops. Some efforts are underway, and this has led to the recognition of *F. commune* K. Skovg., O'Donnell & Nirenberg as a pathogenic component of the *F. oxysporum* complex on Iowa soybean (11). In the current study, however, *F. commune* is included in the *F. oxysporum* data. This work also led to new records of two other *Fusarium* spp. causing soybean root rot, *F. armeniacum* (12) (formerly described as *F. acuminatum* subsp. *armeniaceum*) and *F. proliferatum* (10).

Although most of the species identified in our survey have been reported on soybean previously, often as seedling pathogens (7,9,12,17,18,22,30,33), some species have not been associated with soybean roots at both vegetative (V2 to V5) and reproductive plant stages (R1 to R3), including *F. avenaceum*, *F. poae*, *F. semitectum*, *F. sporotrichioides*, *F. subglutinans*, and *F. tricinatum*. In this study, *F. acuminatum*, *F. graminearum*, *F. oxysporum*, and *F. solani* differed in frequency of isolation depending on plant stage. For example, *F. oxysporum* was more frequently isolated in early stages of the crop, supporting studies that have associated *F. oxysporum* with seedling diseases in soybean (13,14,17,21,22). In contrast, *F. solani* was more frequently observed at reproductive stages than at vegetative stages, suggesting that *F. solani* colonization of roots may increase with plant growth. Other species did not differ

Table 1. Incidence (%) and prevalence (%) by field and county of *Fusarium* species recovered from soybean roots in 2007, 2008, and 2009

<i>Fusarium</i> spp.	2007			2008			2009		
	P ^a			P			P		
	I ^b	County	Field	I	County	Field	I	County	Field
<i>F. acuminatum</i>	19.1	84.0	17.9	12.9	70.0	70.0	4.8	65.0	51.2
<i>F. armeniacum</i>	0.0	0.0	0.0	1.5	12.5	12.5	0.3	7.5	7.0
<i>F. avenaceum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	2.5	2.3
<i>F. equiseti</i>	1.0	14.0	12.5	1.0	12.5	12.5	0.1	2.5	2.3
<i>F. graminearum</i>	8.6	62.0	50.0	2.1	22.5	22.5	1.9	37.5	35.0
<i>F. oxysporum</i>	20.3	86.0	12.5	14.1	80.0	80.0	10.3	87.5	81.4
<i>F. poae</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	2.5	2.3
<i>F. proliferatum</i>	2.4	34.0	32.1	2.1	32.5	32.5	0.2	2.5	2.3
<i>F. semitectum</i>	1.2	18.0	19.6	0.6	10.0	10.0	0.2	5.0	4.7
<i>F. solani</i>	15.5	80.0	19.6	8.0	62.5	62.5	7.0	77.5	74.4
<i>F. sporotrichioides</i>	2.2	28.0	26.8	2.3	20.0	20.0	0.6	15.0	14.0
<i>F. subglutinans</i>	1.0	18.0	14.3	0.3	5.0	5.0	0.3	7.5	7.0
<i>F. tricinatum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	2.5	2.3
<i>F. verticillioide</i> s	0.6	10.0	8.9	0.0	0.0	0.0	0.6	10.0	9.3
<i>F. virguliforme</i>	0.4	4.0	3.6	0.0	0.0	0.0	0.2	5.0	4.7

^a P = Prevalence (%): $100 \times (\text{total number of fields or counties in which each } Fusarium \text{ species was present} / \text{total number of fields or counties sampled})$.

^b I = Incidence (%): $100 \times [(\text{number of root pieces yielding a species} / \text{number of root pieces selected for identification}) \times (\text{number of root pieces yielding any isolate})] / \text{total number of root pieces assessed}$.

in frequency of isolation depending of plant growth stage. These results could lead to new research to describe trends in soybean root colonization in relation to plant development or growth stages.

In our study, the frequency of isolation of species of *Fusarium* varied substantially geographically and among years. Possible reasons for these patterns include differences in climate and topography, sampling error, crop management practices, and environmental conditions possibly related to soil types. In addition, the complexity of the soil microbial community may have played a role in the results obtained. In this survey, 23% of the total isolates obtained from soybean roots plated into semiselective medium were not *Fusarium* spp. Although we did not identify these other isolates, this illustrates the potential for diverse communities of root-associated microorganisms to influence colonization by *Fusarium* spp., thereby affecting our ability to detect tillage or geographical differences.

Several studies on distribution of *Fusarium* spp. around the world suggest that climate is a major factor determining the distribution of these fungi in soil (6,35,36). Although the precise combinations of environmental conditions favoring *Fusarium* root rot on soybean are not known, it has been reported that the disease is more severe under cool, wet weather conditions (28,30). During our study, average state temperatures from May to July 2007 were 18.3 to 23.3°C and precipitation was 78.2 to 131.1 mm. For the 2008 season, temperature and precipitation were 14.4 to 23.3°C and 139.7 to 211.3 mm, respectively. In contrast, average state temperatures for 2009 from May to August were 15.6 to 21.1°C and precipitation was 89.7 to 133.8 mm. The cool and wet weather pattern in 2008 might have favored development of damping-off by *Fusarium* spp. early in the season that could cause plant mortality in Iowa soybean fields. Also, the high precipitation and flooding conditions in summer 2008 may have favored disease development and increased incidence and prevalence of the most frequent species, such as *F. acuminatum*, *F. graminearum*, *F. oxysporum*, and *F. solani* (Table 1). Similarly, cool temperatures during summer 2009 may have influenced the abundance and frequency of isolation of some *Fusarium* spp.

In our study, no differences were found in *Fusarium* sp. composition and frequency of isolation among tillage practices and among different row spacing. Reports on the effects of tillage practices on *Fusarium* populations are contradictory; tillage has been reported to increase (8,38), decrease, or have no effect on *Fusarium* populations (39). Further research might be needed to determine whether there is an effect of tillage practices and row spacing on *Fusarium* root rot species on soybean and to better understand if root rot disease in soybean could increase under these management practices in Iowa fields.

Using morphological characterization in combination with molecular analysis, we documented 15 *Fusarium* spp. associated with soybean roots at two soybean growth stages. Our results confirm that, in Iowa soils, the *Fusarium* root rot complex is diverse, as previously recognized (9,28,30). The new information generated in this study on the diversity and frequency of *Fusarium* spp. associated with soybean roots suggests the need to understand the importance of root-infecting *Fusarium* spp. on soybean productivity in Iowa and test the effectiveness of some management tools, such as seed treatments, to control this pathogen complex.

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