Trichothecene-Producing *Fusarium* Species Isolated from Soybean Roots in Ethiopia and Ghana and their Pathogenicity on Soybean

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Abstract

Numerous pathogen surveys have reported that diverse *Fusarium* spp. threaten soybean production in North and South America. However, little research has been conducted to characterize *Fusarium* pathogens of soybean in sub-Saharan Africa. Our objectives were to (i) identify *Fusarium* spp. isolated from discolored root segments of soybean grown in Ethiopia and Ghana using DNA sequence data, (ii) determine whether isolates nested in the *Fusarium incarnatum-equiseti* and *F. sambucinum* species complexes (FIESC and FSAMSC, respectively) produced trichothecene mycotoxins in vitro, and (iii) test these isolates for pathogenicity on soybean. Molecular phylogenetic analyses revealed that the trichothecene mycotoxin-producing isolates comprised three undescribed species within the FIESC and FSAMSC. Mycotoxin type B trichothecene 4,

15-diacetylvalenol or T-2 toxin and related type A neosolanol trichothecenes were produced by 18 of the 21 isolates. Of the 12 isolates from Ethiopia and Ghana tested for their impact on seed germination, 5, comprising two undescribed phylospecies (i.e., *Fusarium* sp. number 3 and *Fusarium* sp. FIESC 2), completely inhibited germination, whereas 4 caused no reduction in germination. Root lesions induced by all 12 isolates were greater than the uninoculated negative control. Additional variation among the isolates was reflected in differences ($\alpha = 0.05$) in lesion lengths, which ranged from 34 to 67% of total root length. This is the first report characterizing FIESC and FSAMSC isolates from soybean roots in Ethiopia and Ghana.

Keywords: field crops, fungi, oilseeds and legumes, pathogen diversity

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important legumes owing to its high oil and protein content (<https://www.infonet-biovision.org/>). Currently, it is one of the top-traded commodities worldwide, accounting for approximately 6% of earth's arable acreage (Hartman et al. 2011). Diseases, pests, and abiotic factors threaten soybean production by reducing seed quality and yields (Hartman et al. 2015).

In the United States, there have been a number of studies that have identified important *Fusarium* spp. that infect soybean, causing root, collar, and pod rot, and wilt (Chitrampalam and Nelson 2016; Nelson 2015a,b). Although it is not clear that species are restricted to certain soybean plant parts, many are known to cause root rot, including *F. avenaceum*, *F. graminearum*, the *F. incarnatum-equiseti* species complex (FIESC), the *F. oxysporum* species complex (FOSC), *F. proliferatum*, *F. pseudograminearum*, *F. redolens*, *F. semitectum*, the *F. solani* species complex (FSSC), *F. sporotrichioides*, and the *F. tricinctum* species complex (FTSC) (Nelson 2015a,b). Of these, *F. graminearum* was shown to be

highly aggressive in causing soybean root rot followed by *F. proliferatum* and *F. virguliforme* (Díaz Arias et al. 2013). In the Red River Valley of North Dakota and Minnesota, FSSC predominated in causing soybean root rot (Chitrampalam and Nelson 2016). Recently, *F. cerealis* was reported to cause root rot on soybean in the province of Manitoba in northwestern Canada (Abdelmagid et al. 2018).

Several *Fusarium* spp. pathogenic to soybean are known to produce mycotoxins, including fumonisins by *F. verticillioides* (reported as *F. moniliforme*) (Liu et al. 2017) and trichothecenes by *F. graminearum* (Díaz Arias et al. 2013) and *F. cerealis* (Abdelmagid et al. 2018). Trichothecene mycotoxins comprise more than 200 compounds that are produced primarily by *Fusarium* spp. (Yoshinari et al. 2018). Virtually all of the *Fusarium* spp. that produce trichothecenes are nested in the *F. sambucinum* species complex (FSAMSC) and FIESC (O'Donnell et al. 2013). These species typically produce type A or B trichothecenes, which are distinguished by the absence or presence, respectively, of a keto group at the C-8 position of the trichothecene ring (McCormick et al. 2011). Type A trichothecenes HT-2 or T-2 toxin were detected in soybean from Argentina (Barros et al. 2011) and the Midwest United States (Jacobsen et al. 1995), and soy food in Germany (Schollenberger et al. 2007).

Over the past decade, soybean production increased dramatically in several sub-Saharan Africa countries to meet the demand for food and feed (Murithi et al. 2016; Sinclair et al. 2014). Based on the first author's communications with scientists from Africa and his personal observations, there are a number of important soybean diseases in Africa (Hartman and Murithi 2017) but there are few published reports of *Fusarium* spp. isolated from symptomatic soybean plants in Africa. Tewoldemedhin et al. (2017)'s report of *F. virguliforme* and a novel *Fusarium* sp. causing sudden death syndrome (SDS) in South Africa represents the only molecular phylogenetic-based survey of phytopathogenic *Fusarium* spp. on soybean in the African continent. Therefore, the present study was conducted to (i) identify *Fusarium* spp. isolated from discolored root segments from symptomatic soybean grown in Ethiopia and Ghana using DNA sequence data, (ii) determine whether isolates nested in the FSAMSC and FIESC produce trichothecene

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Funding: We thank US AID for funding through the Soybean Innovation Lab.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is published online.

The author(s) declare no conflict of interest.

Accepted for publication 23 March 2019.

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mycotoxins in vitro, and (iii) test these isolates for pathogenicity on soybean.

Materials and Methods

Origin of isolates. Fusaria were isolated from soybean plants exhibiting root rot symptoms in Ghana ($n = 20$) and Ethiopia ($n = 8$); four isolates from Champaign County, IL, U.S.A., also isolated from soybean roots, were included for comparison. The samples collected from Ethiopia were from the Jimma Agricultural Research Center and those from Ghana were from northern Ghana near Tamale in November 2016. Each sample represented a plant that consisted of a portion of the root showing discoloration. Samples were sent by Animal and Plant Health Inspection Service permit to the United States Department of Agriculture–Agricultural Research Service Soybean Disease and Pest Laboratory for isolation. Root segments were surface disinfected in 0.5% NaOCl for 4 min, rinsed 2 min each in three exchanges of distilled water, then blotted dry on sterile towels prior to plating on water agar. Pure cultures were obtained by transferring hyphal tips to acidified potato dextrose agar (APDA). Isolates that produced fusiform, multiseptate macroconidia on APDA were tentatively identified as *Fusarium*. These colonies were single spored and stored in a -80°C freezer in 15% glycerol.

Molecular identification of isolates. To obtain total genomic DNA for the molecular identifications, isolates were cultured at 25°C in 25 ml of yeast-malt broth on a rotary shaker set at 200 rpm for 3 to 4 days, after which mycelium was harvested over a Büchner funnel and then freeze dried overnight. A hexadecyltrimethyl-ammonium bromide (cetyltrimethylammonium bromide; Sigma-Aldrich, St. Louis, MO, U.S.A.) protocol was used to extract total genomic DNA from 50 to 100 mg of pulverized mycelium, as previously described (Gardes and Bruns 1993). To identify isolates nested in the FSAMSC and FIESC (predicted to produce trichothecene mycotoxins) (O'Donnell et al. 2013), a portion of the phylogenetically informative locus, translation elongation factor 1- α (*TEF1*), was amplified by the polymerase chain reaction (PCR) and sequenced following a published protocol (O'Donnell et al. 2009). PCR amplifications were conducted with Platinum *Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, U.S.A.) followed by purification of the amplicons with Montage₉₆ filter plates (Millipore Corp., Billerica, MA, U.S.A.). After amplicons were sequenced with BigDye 3.1 Terminator reaction mix (Applied Biosystems, Foster City, CA, U.S.A.), they were purified with BigDye Xterminator before they were run on an ABI 3730 genetic analyzer. Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, MI, U.S.A.) was used to edit ABI sequence chromatograms, and then the sequences were exported as a NEXUS file. The partial *TEF1* sequences were used to conduct BLASTn queries of GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and *Fusarium* *MLST* (<http://www.westerdijkinstituut.nl/fusarium/>) (O'Donnell et al. 2015) to identify isolates nested in the FSAMSC and FIESC. To focus on *Fusarium* spp. that produced trichothecene toxins, other *Fusarium* spp. were not characterized further. The FIESC isolates were identified to species by conducting a maximum-parsimony analysis (Swofford 2002) of a more inclusive three-locus dataset for this complex (O'Donnell et al. 2009) that included portions of *TEF1*, DNA-directed RNA polymerase II second-largest (*RPB2*) subunit, and the nuclear ribosomal internal transcribed spacer (ITS) + large subunit ribosomal DNA (rDNA). The indel-containing *TEF1* and ITS rDNA partitions were aligned using MUSCLE (Edgar 2004); then, the alignments were improved manually using TextPad, version 5.1.0 for Windows (<https://www.textpad.com/>). The FIESC isolates were identified using a three-locus typing scheme that employed an ad hoc nomenclature in which Arabic numerals identified species and lowercase roman letters identified unique haplotypes within each species (O'Donnell et al. 2009). Multilocus haplotypes within the FIESC were identified using the software program COLLAPSE (softpedia.com, version 1.2). The FSAMSC isolates were identified to species by adding their partial *RPB2* subunit sequences to a more inclusive dataset for this species complex prior to analyzing them via maximum parsimony. Based on the results of this analysis, sequences

of six closely related species within the FSAMSC were added to the *RPB2* dataset as a reference, then analyzed phylogenetically (Fig. 1). DNA sequences generated in this study were accessioned in GenBank as MH845414 to MH845434 and the NEXUS and tree file were deposited in TreeBASE (<https://www.treebase.org/treebase-web/>) as S23281 and Tr113977, respectively.

Mycotoxin analyses. Isolates were screened for mycotoxin production in two liquid media and on a solid rice culture. Each isolate was initially grown on V8 juice agar plates (20% V8 juice, 0.3% CaCO_3 , and 2% agar) (Stevens 1974). For rice cultures, two 0.5-cm² plugs from the V8 plates were used to inoculate 4.4 g of rice that had been autoclaved with 1.8 ml of water in 6-dram vials. After 7 days, each rice culture was extracted with 10 ml of ethyl acetate, the extracts were dried under a nitrogen stream, and the residues were resuspended in 1 ml of ethyl acetate for gas chromatography–mass spectrometry (GC-MS) analysis.

For liquid cultures, 20 ml of agmatine or yeast extract-peptone-dextrose (YEPD) media were inoculated with two 0.5-cm² plugs cut from V8 plates and grown at 28°C on a rotary shaker at 200 rpm in the dark. Agmatine media was prepared with 30 g of sucrose, 1.14 g of agmatine, 1 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g of KCl, 10 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 200 μl of a trace element solution (5 g of citric acid, 5 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 50 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 50 mg of H_3BO_3 , and 50 mg of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ per 100 ml) per liter of distilled water (Gardiner et al. 2009). YEPD medium was prepared with 50 g of dextrose, 1 g of peptone, and 1 g of yeast extract per liter of distilled water. After 7 days, the cultures were extracted with 8 ml of ethyl acetate and dried under a nitrogen stream, and the residue was resuspended in 1 ml ethyl acetate for GC-MS analysis.

GC-MS analysis was performed with an Agilent 6890 gas chromatograph fitted with an Agilent HP-5MS column (30 m, 0.25 mm, and 0.25 μm) and an Agilent 5973 mass detector (Agilent Technologies, Wilmington, DE, U.S.A.). Helium was used as the carrier gas with a 20:1 split ratio and a 20 ml/min split flow. The column was held at 150°C for 1 min following injection, heated to 280°C at $30^{\circ}\text{C}/\text{min}$, and held for 7.7 min. Compound identifications were based on comparisons of GC-MS data with published data (Blackwell and Savard 1994) and results for purified standards (Fig. 2). The limit of detection was toxin at 0.25 $\mu\text{g}/\text{ml}$ of culture.

Seed germination and root lesion assays. Each isolate was transferred to PDA in 9-cm Petri plates and incubated in the dark at 24°C for 5 days. A 1.5-cm² agar plug was cut from the colony margin of each isolate and placed individually in an empty sterile Petri dish. Seed of cultivar Williams were surface disinfected in a 1% NaOCl solution for 1 min and rinsed for 2 min each in three exchanges of distilled water. A single seed was placed on each colonized agar plug. There were eight blocked replications, with each block represented by a 9-cm Petri plate. An uninoculated 1.5-cm² agar plug was placed in each Petri dish as a negative control. The cultures were incubated in the dark for 5 days at 24°C ; the experiment was repeated once. Seed germination was evaluated based on percentage of seed that germinated, as defined by the radicle being as long as the imbibed seed.

For the root lesion assay, seed of Williams were surface disinfected in a 1% NaOCl solution for 1 min, then rinsed for 1 min each in three exchanges of distilled water. Surface-disinfected seed were placed inside a seed germinator on moist paper towels set at 25°C for 3 days. Seedlings with roots 2 to 4 cm long were surface disinfected as above and seven seedlings were placed inside each clear plastic container (23 by 14 by 3 cm [length by width by height]) on paper towels saturated with deionized water. A 1.5-cm² agar plug was cut from the colony margin of each isolate and placed individually on the midsection of a root. Two containers made up a replication in a randomized complete block design, with two replications in the first trial and three replications in the second trial. Root lesions and root and shoot lengths were recorded 5 days after inoculation.

Statistical analyses were performed with JMP Pro 13 (SAS Institute, Cary, NC, U.S.A.). Prior to conducting the analyses, a “1”

was added to the seed germination percentage, then transformed by arcsine to correct for heterogeneity of variances. Data were detransformed prior to presentation. The O'Brien test was performed on the transformed data using the JMP Fit Y-by-X platform to test for

homogeneity of variance between trials. An analysis of variance (ANOVA) for both experiments was fitted using the JMP Fit Model platform. ANOVA was performed with the transformed seed germination and root lesion lengths, with isolates as a fixed effect. Means

Fig. 1. One of two most-parsimonious trees (MPTs) inferred via maximum-parsimony analysis of a partial RNA polymerase II second-largest (*RPB2*) dataset comprising 14 isolates from Africa and 1 isolate from Illinois, all isolated from soybean roots symptomatic for root rot (highlighted in gray). In all, 10 of the isolates from soybean are nested within the *Fusarium incarnatum-equiseti* species complex (FIESC), including 1 from Illinois, and the other 5 are members of the *F. sambucinum* species complex (FSAMSC). Sequences of six NRRL isolates from the FSAMSC are included as a reference. The *Fusarium* spp. from soybean comprise three unnamed species within the FIESC, which are distinguished using an informal ad hoc nomenclature (i.e., FIESC 2, 29, and 37), and three unnamed species within the FSAMSC (*Fusarium* sp. 1, 3, and 4). An Arabic number followed by a lowercase roman letter distinguishes species-haplotypes within the FIESC. Numbers above nodes represent maximum parsimony bootstrap support $\geq 70\%$ based on 2,000 pseudoreplicates of the data implemented in PAUP*, version 4.0b10 (Swofford 2002). T-2 toxin or 4,15-diacetylinalenol (4,15-diANIV) mycotoxins were detected in 18 of the 21 isolates analyzed by gas chromatography–mass spectrometry. CI = consistency index, PIC = parsimony informative character, RI = retention index, and — = none detected.

separation was performed using Fisher's least significant difference test at $\alpha = 0.05$ for the two experiments.

Results

Molecular identification of isolates and mycotoxin production in vitro. From the 32 total isolates (28 from Ethiopia and Ghana and 4 from Illinois) sequenced, BLASTn searches of GenBank and *Fusarium* MLST, using the partial *TEF1* sequences as the queries, placed 10 isolates (2 from Ethiopia, 7 from Ghana, and 1 from Illinois) in the FIESC, 5 isolates (1 from Ethiopia and 4 from Ghana) in the FSAMSC, and 13 isolates (5 from Ethiopia, 6 from Ghana, and 2 from Illinois) in the FOSC ($n = 13$, *F. oxysporum*) and FSSC ($n = 4$, *F. falciforme*). Because members of the latter two species complexes do not produce trichothecene mycotoxins, they were not characterized further. However, given the expectation that the FIESC and FSAMSC isolates might produce trichothecene mycotoxins, multilocus DNA sequence data were used to identify them to species prior to assessing them for the production of mycotoxins in vitro. The FIESC isolates were identified as three undescribed phylopecies (i.e., FIESC 2, 29, and 37) using a three-locus typing scheme (O'Donnell et al. 2009). Results of the COLLAPSE version 1.1 analyses revealed that the eight FIESC 2 isolates comprised six unique three-locus haplotypes (2-d to 2-i), KOD1718 from Illinois was identified as the undescribed *Fusarium* sp. FIESC 29-c and KOD1721

from Ethiopia represented a newly discovered phylospecies designated FIESC 37-a.

Phylogenetic analysis of the partial *RPB2* sequence data and the *TEF1* BLASTn queries of GenBank and *Fusarium* MLST indicated that the five FSAMSC isolates represented three undescribed phylospecies reported here as *Fusarium* sp. numbers 1, 3, and 4 (Fig. 1). The three isolates of *Fusarium* sp. 1 from Ghana appeared to be conspecific with NRRL 28725 ex wheat in Argentina. KOD1545 *Fusarium* sp. 4 from Ethiopia and reference strain NRRL 3299 *F. sporotrichioides* ex corn from France were resolved as a strongly supported subclade (99% bootstrap) in the FSAMSC phylogeny. These two fusaria were unique in that they were the only ones tested that produced the type A trichothecene T-2 toxin and related neosolaniol mycotoxins on the solid rice culture (Fig. 2). Five of the seven phylospecies in the FSAMSC, including *Fusarium* spp. 1 and 3 from Ghana, produced the type B trichothecene 4,15-diacetylnevalenol on solid rice cultures, as did seven of eight isolates of phylospecies FIESC 2 (Figs. 1 and 2). Trichothecene mycotoxins, however, were not detected in broth or rice cultures of KOD1712 FIESC 2-e, KOD1718 FIESC 29-c, and KOD1721 FIESC 37-a.

Seed germination and root lesion assays. Data for trials within the two experiments were combined based on homogeneity of variance between trials. Seed failed to germinate when inoculated individually with five of the isolates (Table 1), whereas 80 to 100% of

Fig. 2. Reconstructed ion chromatograms of extracts of **A**, KOD1545 *Fusarium* sp. 4 from Ethiopia and **B**, KOD1716 *Fusarium incarnatum-equiseti* species complex 2-f from Ghana. DAS = 4,15-diacetoxyscirpenol; and 4,15-diANIV = 4,15-diacetylnevalenol. Trichothecene components were identified by comparison of mass spectral fragmentation pattern with libraries of standard trichothecenes.

Table 1. Soybean seed germination (experiment 1) and root lesion length and root lesion length per root (experiment 2) on soybean cultivar Williams inoculated with three *Fusarium* isolates from Ethiopia, nine from Ghana, and one from Illinois

Species ^a	Isolate code	Origin	Germination (%) ^b	Lesion length	
				Root (mm) ^c	Per total (%) ^{c,d}
<i>Fusarium</i> sp. FSAMSC 1	KOD1715	Ghana	100*	24	59
<i>Fusarium</i> sp. FSAMSC 1	KOD1723	Ghana	60	21	45
<i>Fusarium</i> sp. FSAMSC 3	KOD1722	Ghana	0	23	56
<i>Fusarium</i> sp. FSAMSC 4	KOD1545	Ethiopia	60	26	51
<i>Fusarium</i> sp. FIESC 2-d	KOD1719	Ghana	60	19	34
<i>Fusarium</i> sp. FIESC 2-e	KOD1712	Ghana	80*	34	61
<i>Fusarium</i> sp. FIESC 2-f	KOD1716	Ghana	0	21	58
<i>Fusarium</i> sp. FIESC 2-f	KOD1724	Ethiopia	60	36	62
<i>Fusarium</i> sp. FIESC 2-g	KOD1714	Ghana	0	20	43
<i>Fusarium</i> sp. FIESC 2-h	KOD1713	Ghana	20	26	48
<i>Fusarium</i> sp. FIESC 2-i	KOD1717	Ghana	0	28	54
<i>Fusarium</i> sp. FIESC 29-c	KOD1718	Illinois	0	22	42
<i>Fusarium</i> sp. FIESC 37-a	KOD1721	Ethiopia	80*	26	67

^a FSAMSC = *F. sambucinum* species complex and FIESC = *F. incarnatum-equiseti* species complex.

^b Seed germination. Numbers followed by an asterisk did not differ (Fischer's least significant difference [LSD] test at $\alpha = 0.05$) from the uninoculated control based on an analysis of "1" added to the data before arcsine transformation.

^c All data differed (Fischer's LSD test at $\alpha = 0.05$) from the uninoculated control (2 mm for lesion length and 4% for lesion length percentage).

^d Lesion length per total root length.

the seed germinated when inoculated with three of the isolates. Root lesion lengths were all greater ($\alpha = 0.05$) than the control (2 mm), ranging from 19 to 36 mm in length (Table 1). The length of lesions induced by five isolates did not differ from the longest (36 mm) root lesion caused by KOD1724 *Fusarium* sp. FIESC 2-f from Ethiopia. Percent lesion lengths per total root length were all greater ($\alpha = 0.05$) than the control (4%), ranging from 34 to 67% (Table 1).

Discussion

This is the first report characterizing isolates of FIESC and FSAMSC from soybean roots in Ethiopia and Ghana. These isolates in vitro produced trichothecenes and some caused 0% seed germination when seed were inoculated, and all isolates produced root lesions on inoculated roots. It is not known whether these isolates are restricted to roots or whether, under field conditions, they infect aboveground plant parts. Most *Fusarium* spp. that infect soybean can be recovered on multiple plant parts. For example, of the 11 reported seedborne *Fusarium* spp., most have also been recovered from soybean roots and other plant parts (Nelson 2015a,b). We do not know if the isolates in our study can be recovered from aboveground plant parts such as seed; thus, it remains an important question to know whether these *Fusarium* spp. that we isolated affect feed and food-grade quality of soybean in Ethiopia and Ghana.

To our knowledge, the Tewoldemedhin et al. (2017) survey of soybean SDS pathogens in South Africa represents the only published study of *Fusarium* pathogens of soybean in sub-Saharan Africa. They discovered that *F. virguliforme* and a closely related novel *Fusarium* sp. within clade 2 of the FSSC were the etiological agents of soybean SDS. However, Tewoldemedhin et al. (2017) did not test their isolates for trichothecene production in vitro because there are no authentic reports that *Fusarium* spp. causing SDS or other members of the FSSC produce trichothecenes (Marasas et al. 1984; Munkvold 2017) because they lack the gene cluster essential for producing these toxins (Coleman et al. 2009; Kim et al. 2017; Srivastava et al. 2014).

Isolates of *F. falciforme*, a cosmopolitan soilborne member of the FSSC (Zhang et al. 2006), and *F. oxysporum* were recovered from symptomatic soybean in Ethiopia and Ghana, and identified by using partial *TEF1* sequences to conduct BLASTn queries of GenBank and *Fusarium* MLST. These isolates were not characterized further because they are not known to produce trichothecene mycotoxins. However, isolates in the FSAMSC and FIESC were tested for trichothecene mycotoxin production in agmatine broth, which is known to induce trichothecene biosynthesis (Gardiner et al. 2009), and on solid rice cultures because these two lineages are known to comprise virtually all of the *Fusarium* spp. that produce these toxins (Kim et al. 2017; O'Donnell et al. 2013). As predicted by their phylogenetic placement in these two species complexes, all 11 FSAMSC and 7 of 10 FIESC isolates tested for toxin production produced the type B trichothecene 4,15-diacetylivalenol or type A T-2 toxin and related neosolaniol trichothecenes in vitro. Our discovery that the FSAMSC and FIESC isolates from Ethiopia and Ghana represented five novel, undescribed phylopecies highlights the importance of conducting more extensive pathogen surveys on the African continent to expand our knowledge base about which *Fusarium* spp. predominate on soybean in Africa.

Of the numerous assays for testing pathogenicity and aggressiveness of *Fusarium* spp. on soybean, we used those that measured inhibition of seed germination and length of lesions on seedling roots. The ability of the isolates to inhibit seed germination and induce lesions varied considerably among and within the species tested, and similar variability in disease severity has been reported for other *Fusarium* pathogens of soybean (Díaz Arias et al. 2013; Ivic 2014). Although 6 of the 13 isolates tested completely inhibited seed germination, all 13 isolates were able to induce root lesions 19 to 36 mm in length. Many trichothecenes are phytotoxic, including T-2 toxin (Desjardins et al. 2007), and some such as 4,15-diacetoxyscirpenol have been shown to inhibit seed germination (Muhitch et al. 2000). However, it is unclear whether trichothecenes were involved in inhibiting soybean seed germination and inducing lesions in the

present study. Future studies are needed to determine whether toxins are produced in these assays, and if so, to correlate their expression with disease severity. Also, because results of the pathogenicity assays may not match those obtained from experiments conducted in the field or containment facilities, additional research is needed to better predict what threat these pathogens pose to soybean production in Africa, especially as it relates to plant stands and yield, and to soybean feed and food products.

Acknowledgments

We thank G. Zhang, G. Doehring, and N. Orwig for skilled technical assistance in various aspects of this study; A. Tesfay for sending samples from Ethiopia; and N. Denwar and C. Miranda for assisting with samples from Ghana.

Literature Cited

- Abdelmagid, A., Hafez, M., Lawley, Y., Adam, L., and Daayf, F. 2018. First report of *Fusarium cerealis* causing root rot on soybean. Plant Dis. 102:2638.
- Barros, G., García, D., Oviedo, M. S., Ramirez, M. L., Torres, A., Lattanzio, V., Pascale, M., and Chulze, S. 2011. Survey of T-2 and HT-2 toxins in soybean and soy meal from Argentina using immunoaffinity clean-up and high performance liquid chromatography. World Mycotoxin J. 4:189-197.
- Blackwell, B. A., and Savard, M. E. 1994. Spectral characteristics of secondary metabolites from *Fusarium* fungi. Pages 59-257 in: Mycotoxins in grain. Compounds other than aflatoxin. J. D. Miller and H. L. Trenholm, eds. Eagon Press, St. Paul, MN, U.S.A.
- Chitrapalam, P., and Nelson, B., Jr. 2016. Multilocus phylogeny reveals an association of the agriculturally important *Fusarium solani* species complex (FSSC) 11, and clinically important FSSC 5 and FSSC 3+4 with soybean roots in the north central United States. Antonie Leeuwenhoek 109:335-347.
- Coleman, J. J., Rounsley, S. D., Rodriguez-Carres, M., Kuo, A., Wasmann, C. C., Grimwood, J., Schumtz, J., Taga, M., White, G. J., Zhou, S., Schwartz, D. C., Freitag, M., Ma, L.-J., Danchin, E. G. J., Henrissat, B., Coutinho, P. M., Nelson, D. R., Straney, D., Napoli, C. A., Barker, B. M., Gribskov, M., Rep, M., Kroken, S., Molnár, I., Rensing, C., Kennell, J. C., Zamora, J., Farman, M. L., Selker, E. U., Salamov, A., Shapiro, H., Pangilinan, J., Lindquist, E., Lamers, C., Grigoriev, I. V., Geiser, D. M., Covert, S. F., Temporini, E., and VanEtten, H. D. 2009. The genome of *Nectria haematococca*: Contribution of supernumerary chromosomes to gene expansion. PLoS Genet. 5:e1000618.
- Desjardins, A. E., McCormick, S. P., and Appell, M. 2007. Structure-activity relationships of trichothecene toxins in an *Arabidopsis thaliana* leaf assay. J. Agric. Food Chem. 55:6487-6492.
- Díaz Arias, M., Leandro, L., and Munkvold, G. 2013. Aggressiveness of *Fusarium* species and impact of root infection on growth and yield of soybeans. Phytopathology 103:822-832.
- Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.
- Gardes, M., and Bruns, T. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113-118.
- Gardiner, D. M., Kazan, K., and Manners, J. M. 2009. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. Fungal Genet. Biol. 46:604-613.
- Hartman, G. L., and Murithi, H. M. 2017. Field Guide to African Soybean Diseases and Pests. <http://soybeaninnovationlab.illinois.edu/soybean-disease-diagnostic-guide>
- Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., David, J. A., and Steffey, K. L. 2015. Compendium of Soybean Diseases and Pests, 5th ed. American Phytopathological Society, St. Paul, MN, U.S.A.
- Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the world 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. Food Secur. 3:5-17.
- Ivic, D. 2014. Pathogenicity and potential toxicity of seed-borne *Fusarium* species on soybean and pea. J. Plant Pathol. 96:541-551.
- Jacobsen, B. J., Harlin, K. S., Swanson, S. P., Lambert, R. J., Beasley, V. R., Sinclair, J. B., and Wei, L. S. 1995. Occurrence of fungi and mycotoxins associated with field mold damaged soybeans in the Midwest. Plant Dis. 79: 86-88.
- Kim, H.-S., Proctor, R. H., and Brown, D. W. 2017. Comparative genomic analysis of secondary metabolite gene clusters in 207 isolates of *Fusarium*. Abstr. 297F in: 29th Fungal Genet. Conf.
- Liu, J., Deng, J., Yang, C., Huang, N., Chang, X.-L., Zang, J., Yang, F., Liu, W., Wang, X., Yong, T., Du, J., Shu, K., and Yang, W.-y. 2017. Fungal diversity in field mold-damaged soybean fruits and pathogenicity identification based on high-throughput rDNA sequencing. Front. Microbiol. 8:779.
- Marasas, W. F. O., Nelson, P. E., and Toussoun, T. A. 1984. Toxicogenic *Fusarium* species: Identity and Mycotoxicology. The Pennsylvania State University Press, University Park, PA, U.S.A.
- McCormick, S. P., Stanley, A. M., Stover, N. A., and Alexander, N. J. 2011. Trichothecenes: From simple to complex mycotoxins. Toxins (Basel) 3: 802-814.

- Muhitch, M. J., McCormick, S. P., Alexander, N. J., and Hohn, T. M. 2000. Transgenic expression of the *Tri101* or *PDR5* gene increases resistance to tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol. *Plant Sci.* 157:201-207.
- Munkvold, G. P. 2017. *Fusarium* species and their associated mycotoxins. Pages 51-106 in: *Mycotoxigenic Fungi: Methods and Protocols*. A. Moretti and A. Susca, eds. Humana Press, New York, NY, U.S.A.
- Murithi, H. M., Beed, F., Tukamuhabwa, P., Thomma, B. P. H. J., and Joosten, M. H. A. J. 2016. Soybean production in eastern and southern Africa and threat of yield loss due to soybean rust caused by *Phakopsora pachyrhizi*. *Plant Pathol.* 65:176-188.
- Nelson, B. D. 2015a. *Fusarium* seed rot and *Fusarium* species associated with seed. Pages 108-109 in: *Compendium of Soybean Diseases and Pests*. G. L. Hartman, J. C. Rupe, E. F. Sikora, L. L. Domier, J. A. Davis, and K. L. Steffey, eds. American Phytopathological Society, St. Paul, MN, U.S.A.
- Nelson, B. D. 2015b. *Fusarium* blight or wilt, root rot, and pod and collar rot. Pages 69-73 in: *Compendium of Soybean Diseases and Pests*. G. L. Hartman, J. C. Rupe, E. F. Sikora, L. L. Domier, J. A. Davis, and K. L. Steffey, eds. American Phytopathological Society, St. Paul, MN, U.S.A.
- O'Donnell, K., McCormick, S. P., Busman, M., Proctor, R. H., Ward, T. J., Doehring, G., Geiser, D. M., Alberts, J. F. and Rheeder, J. P. 2013. Marasas et al. 1984. "Toxigenic *Fusarium* Species: Identity and mycotoxicology" revisited. *Mycologia* 110:1058-1080.
- O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., Ward, T. J., Frandsen, R. J. N., Lysøe, E., Rehner, S. A., Aoki, T., Robert, V. A. R. G., Crous, P. W., Groenewald, J. Z., Kang, S., and Geiser, D. M. 2013. Phylogenetic analyses of *RPB1* and *RPB2* support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genet. Biol.* 52:20-31.
- O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., and Geiser, D. M. 2009. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *J. Clin. Microbiol.* 47:3851-3861.
- O'Donnell, K., Ward, T. J., Robert, V. A. R. G., Crous, P. W., Geiser, D. M., and Kang, S. 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. *Phytoparasitica* 43:583-595.
- Schollenberger, M., Müller, H.-M., Rühle, M., Terry-Jara, H., Suchy, S., Plank, S., and Drochner, W. 2007. Natural occurrence of *Fusarium* toxins in soy food marketed in Germany. *Int. J. Food Microbiol.* 113:142-146.
- Sinclair, T. R., Marrou, H., Soltani, A., Vadez, V., and Chandolu, K. C. 2014. Soybean production potential in Africa. *Glob. Food Secur.* 3:31-40.
- Srivastava, S. K., Huang, X., Brar, H. K., Fakhoury, A., Bluhm, B. H., and Bhattacharyya, M. K. 2014. The genome sequence of the fungal pathogen *Fusarium virguliforme* that causes sudden death syndrome in soybean. *PLoS One* 9:e81832.
- Stevens, R. B. 1974. *Mycology Guidebook*. Mycology Guidebook. University of Washington Press, Seattle, WA, U.S.A.
- Swofford, D. L. 2002. PAUP*. *Phylogenetic Analysis Using Parsimony* (and other methods), version 4.0b10. Sinauer Associates, Sunderland, MA, U.S.A.
- Tewoldemedhin, Y. T., Lamprecht, S. C., Vaughan, M., Doehring, G., and O'Donnell, K. 2017. Soybean SDS in South Africa is caused by *Fusarium brasiliense* and novel undescribed *Fusarium* sp. *Plant Dis.* 101:150-157.
- Yoshinari, T., Takeda, N., Watanabe, M., and Sugita-Konishi, Y. 2018. Development of an analytical method for simultaneous determination of the modified forms of 4,15-diacetoxyscirpenol and their occurrence in Japanese retail food. *Toxins (Basel)* 10:178.
- Zhang, N., O'Donnell, K., Sutton, D. A., Nalim, F. A., Summerbell, R. C., Padhye, A. A., and Geiser, D. M. 2006. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *J. Clin. Microbiol.* 44:2186-2190.