EVALUATION OF AGGRESSIVENESS OF *FUSARIUM VIRGULIFORME* ISOLATES THAT CAUSE SOYBEAN SUDDEN DEATH SYNDROME

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SUMMARY

Fusarium virguliforme (Akoi, O'Donnell, Homma & Lattanzi), formerly named *F. solani* (Mart.) Sacc. f.sp. *glycines*, is the cause of soybean sudden death syndrome (SDS). Over the last 6 years, an international collection of *F. virguliforme* isolates has been established and maintained at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign. Using part of the collection, aggressiveness of *F. virguliforme* isolates to a susceptible soybean cultivar, Great Lakes 3202, was evaluated under controlled conditions in the greenhouse. After an initial evaluation of 123 isolates on soybean, 30 isolates from different geographic origins with different levels of foliar severity were selected to further evaluate both foliar and root severities. Variability of aggressiveness based on measurements of SDS foliar severity, shoot, root, and root lesion lengths, shoot and root dry weights, and total dry weights was found among isolates ($P \leq 0.01$). Isolate FSG1(Mont-1), a reference isolate that has been widely used by the soybean community for basic and applied research, caused the greatest reduction in shoot weight and shoot length compared to the non-inoculated control plants, but six isolates caused higher foliar severity and 15 isolates caused longer root lesion length than the isolate FSG1. Isolate FSG5 caused the greatest reduction in root weight among isolates. Knowledge about the variability of the pathogen is important for selection of isolates for testing for broad-based SDS resistant soybean lines.

Key words: Fusarium virguliforme isolates, soybean, sudden death syndrome.

INTRODUCTION

Fusarium virguliforme (Akoi, O'Donnell, Homma & Lattanzi), a soilborne fungus and formerly named *F. solani* (Mart.) Sacc. f.sp. *glycines*, is the causal agent of sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.) (Roy, 1997; Roy *et al*., 1989; Rupe, 1989). The pathogen colonizes soybean roots causing root rot and vascular discoloration of roots and stems. Although the pathogen infects roots, the most conspicuous symptoms occur on leaves beginning with chlorotic mottling and proceeding to interveinal chlorosis, necrosis and defoliation (Hartman *et al*., 1995; Rupe and Hartman, 1999). The fungus has been isolated from soybean roots and occasionally from lower stems (Roy, 1997; Roy *et al*., 1989; Rupe, 1989), but has not been found in leaves. Foliar symptoms were proposed to be caused by fungal toxins produced on or in colonized roots and translocated to the leaves (Hartman *et al*., 2004; Jin *et al*., 1996; Li *et al*., 1999). It was reported that light was essential for degradation of Rubisco large subunit and accumulation of free radicals and initiated programmed cell death leading to foliar SDS symptoms in soybean (Ji *et al*., 2006).

SDS has become a widespread and recurring problem causing significant yield losses in many soybean-growing areas (Hartman *et al*., 1995; Rupe and Hartman, 1999). From 1996 to 2005, SDS was listed as one of the most important diseases in the United States (Wrather *et al*., 2001, 2003, 2006). This disease has been reported not only in the United States, but also in Argentina, Brazil, and Canada (Anderson and Tenuta, 1998; Nakajima *et al*., 1993; Ploper, 1993). A feature article (Roy *et al*., 1997) provided a review of the history and symptomatology of the disease. Characterization of the fungal cultural morphology (Li *et al*., 1998; Roy, 1997; Roy *et al*., 1989) and chlamydospore formation, production, and nuclear status of the causal agent (Li *et al*., 1998) have been reported. The biochemical response of soybean roots to *F. virguliforme* infection was studied (Lozovaya *et al*., 2004, 2006).The nuclear rRNA genes and ITS regions (O'Donnell and Gray, 1995), and the mitochondrial small subunit ribosomal rRNA gene (Li *et al*., 2000) of *F. virguliforme* were sequenced, and the mitochondrial DNA restriction fragment length polymorphisms (Rupe *et al*., 2001) were used to compare other *Fusarium* non-SDS causing isolates in order to differentiate and analyze their phylogenetic relationship and genetic differences. PCR-based detection

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methods have been used for the specific detection of *F. virguliforme* DNA from field-grown soybean roots and field soil (Li and Hartman, 2003) and to quantify fungal DNA from soybean roots (Gao *et al*., 2004; Li *et al*., 2008a). *F. virguliforme* isolates are not known to develop perithecia, whereas *F. tucumaniae*, a related species from South America, does form perithecia (Covert *et al*., 2007).

Little is known about the variability of aggressiveness on soybean among *F. virguliforme* isolates from different geographic origins. More detailed knowledge about the variability of the pathogen is essential for understanding the population structure. This information will be essential for selecting isolates in studies for developing broad-based SDS-resistant soybean lines.

The objectives of this study were to evaluate and compare the aggressiveness of *F. virguliforme* isolates from the National Soybean Pathogen Collection Center at the University of Illinois on soybean.

MATERIALS AND METHODS

Fungal cultures. A total of 123 *F. virguliforme* isolates from the National Soybean Pathogen Collection Center (NSPCC) at the University of Illinois was used in the initial pathogenicity tests on causing foliar severity in the greenhouse. These fungal isolates were isolated or received from 10 states in the United States, as well as Argentina, Brazil, and Canada (Table 1). Isolate FSG1 (Mont-1) was used as a reference isolate because it has been widely used by the soybean community for basic and applied research (Hartman *et al*., 1997; 2004; Achenbach *et al*., 1996; Li and Hartman, 2003; Mueller *et al*., 2003; Iqbal *et al*., 2005; Lozovaya *et al*., 2006; Farias *et al*., 2006). This isolate was originally isolated from Monticello, Illinois in 1991 and has been re-isolated from infected soybean and maintained at the NSPCC and the USDA-ARS Crop Genetics and Production Research Unit in Mississippi. After initial evaluation, a subset of 30 isolates with different levels of aggressiveness and from different geographic origins was selected for further tests (Table 2). Fungal cultures were either maintained in 2% water agar plates at 4ºC, stored in 15% glycerol at –80ºC or stored in a cryogenic freezer with liquid nitrogen.

Inoculum preparation. Sorghum grain (80 cm^3) was soaked in tap water in a 250-ml Erlenmeyer flask overnight. Floating sorghum seed and debris were removed. After soaking, the grain was washed with tap water three to five times. The excess water was drained, and the grain was autoclaved on 2 consecutive days for 40 min at 121ºC. Each of the flasks containing sterilized sorghum grain was infested with an individual isolate by transferring five 4-mm-diameter plugs from the edge of a

Table 1. Geographic origins of 123 *Fusarium virguliforme* isolates used in this study including the year isolated or acquired, the source, and the mean foliar severity in the initial screening under greenhouse conditions.

Geographic origin	No. of isolates	Year isolated or acquired	Isolator/ Contributor	Mean foliar severity (%) [a]	Foliar severity range
Argentina	10	2000	J.C. Rupe	41.5	35 – 65
Arkansas, USA	4	1996	J.C. Rupe	57.5	35 – 80
Brazil	1	2002	K. O'Donnell	72.5	65 – 90
Canada	1	2002	S. Li, T. Anderson	57.5	50 – 65
Illinois, USA	75	1991-2003	S. Li, G.L. Hartman, L. Gray	40.3	5 – 90
Indiana, USA	6	1996, 2003	T.S. Abney, A. Westphal	35.8	35 – 50
Iowa, USA	4	1996, 1999	X.B. Yang	32.5	15 – 65
Minnesota, USA	1	2002	J.E. Kurle	35.0	35 – 50
Missouri, USA	17	2002	T.L. Niblack	24.3	5 – 80
Wisconsin, USA	4	1998	L.A. Achenbach, C.R. Grau	27.5	15 – 50
Total isolates	123				

[a] Means of foliar severity ratings of 10 plants from two trials for each isolate were based on a 1 to 5 scale, where 1 = no foliar symptoms; 2 = light symptom development with mottling and mosaic (1 to 20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21 to 50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51 to 80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81 to 100% foliage affected). Data were converted to percentages with the midpoint value (Campbell and Madden, 1990; Hartman *et al*., 1997; Mueller *et al*., 2003) based on the range within each severity rating scale.

Table 2. A list of 30 *Fusarium virguliforme* isolates selected for both foliar and root severity evaluations under greenhouse conditions.

Isolate[a]	Alternate collection No.[b]	Geographic origin	Year isolated or acquired	Source
FSG1	i300	Illinois	1991	P. A. Stephens and L. E. Gray[c]
FSG2	i518	Canada	2002	S. Li[d]
FSG3	i525	Brazil	2002	K. O'Donnell
FSG4	i522	Illinois	2002	S. Li
FSG5	i524	Illinois	2003	S. Li
FSG6	i307	Argentina	2000	J. C. Rupe
FSG7	i502	Missouri	2002	T. L. Niblack,
FSG8	i503	Missouri	2002	T. L. Niblack,
FSG9	i504	Missouri	2002	T. L. Niblack,
FSG10	i506	Missouri	2002	T. L. Niblack,
FSG11	i9	Arkansas	1996	J. C. Rupe
FSG12	i12	Arkansas	1996	J. C. Rupe
FSG13	i45	Illinois	1994	G. L. Hartman
FSG14	i50	Iowa	1996	X. B. Yang
FSG15	i52	Illinois	1996	G. L. Hartman
FSG16	i169	Illinois	1999	S. Li
FSG17	i171	Illinois	1999	S. Li
FSG18	i173	Illinois	1999	S. Li
FSG19	i446	Illinois	1999	S. Li
FSG20	i447	Illinois	1999	S. Li
FSG21	i501	Illinois	2001	S. Li
FSG22	i521	Minnesota	2002	J. E. Kurle
FSG23	i528	Illinois	2002	S. Li
FSG24	i530	Indiana	2003	T. S. Abney
FSG25	i56	Arkansas	1996	J. C. Rupe
FSG26	i94	Illinois	1998	S. Li
FSG27	i96	Illinois	1998	S. Li
FSG28	i98	Wisconsin	1998	L. A. Achenbach and C. R. Grau
FSG29	i101	Wisconsin	1998	L. A. Achenbach and C. R. Grau
FSG30	i65	Illinois	1993	G. L. Hartman

[a] Isolate codes were designated at the National Soybean Pathogen Collection Center, University of Illinois at Urbana-Champaign.
[b] Alternate culture collection number was designated before establishing the culture database at the National Soybean Pathogen Collection Center, University of Illinois.
[c] Isolated from Monticello-1 isolate (obtained from Dr. Lynn Gray)-inoculated soybean (Great Lakes 3202) roots and confirmed with a specific PCR assay (Li and Hartman, 2003) by S. Li.
[d] Plant sample was provided by T. Anderson.

2-week-old *F. virguliforme* culture on water agar. Flasks were incubated at 23ºC in the dark and shaken by hand every other day to promote uniform fungal growth. After a 2-weeks incubation, inocula for both greenhouse and the colony-forming units (CFU) assay were stored at 4ºC. CFU assays were performed before the greenhouse experiment but adjusted the inoculum dose right before the inoculation in greenhouse. Inoculum for each isolate was prepared in the same way, most of the inocula had similar amount of the spores.

The CFU assays of the infested sorghum grain inoculum for 30 selected isolates were conducted as previously reported (Li *et al*., 2008b). Briefly, 1 gram of sorghum inoculum from each isolate was soaked in a 250-ml Erlenmeyer flask containing 100 ml of sterile distilled water. The flasks were shaken at 150 rpm in a Lab-Line Orbit Shaker (Lab-Line Instruments, USA) for 30 min, and then diluted 10 fold with sterile distilled water. From this dilution, 100 µl of inoculum dilution from each isolate was spread on an agar plate (100 x 15 mm) containing *F. virguliforme* semi-selective medium (Huang and Hartman, 1996). Six plates were used for each inoculum dilution. The plates were incubated at 23ºC in the dark for 10 days. Colonies of *F. virguliforme* were identified and counted on each plate to determine the CFU values per gram of sorghum inoculum for each isolate. The inoculum was adjusted to be 10^4 spores/gram of inoculum by diluting infested sorghum grain with non-infested sorghum grain (w/w). Three grams of infested sorghum grain were used for each cone inoculation.

Isolate comparison. The experiments consisted of two greenhouse trials. For the initial evaluation of foliar severity on 123 isolates, experiments were run from March through August in 2003. For the tests of 30 selected isolates, the first trial started in June 2004 and ended in August 2004, and the second trial started in October 2004 and ended in December 2004. Soybean seeds of a susceptible entry, Great Lakes 3202, were sown in Cone-Tainers (Ray Leach Cone-Tainers, Stuewe & Sons, USA) and inoculated with three cm^3 of infested sorghum grains placed 2 to 3 cm below a soybean seed in each Cone-Tainer. Non-infested sorghum grain was used as a control. The Cone-Tainers were placed in racks on a greenhouse bench under a 14- h photoperiod with a light intensity of 434 $\mu Em^{-2}\ s^{-1}$ at 25 ± 2ºC and watered daily. For each of the isolates, five plants inoculated per isolate served as the five replications (one cone-tainer/replication) in a randomized complete block design. The soil, soil:sand mix (1:1 vol/vol) at pH 7, was pasteurized and autoclaved before filling the Cone-Tainer.

Disease ratings. Foliar symptoms were recorded 21 days after planting. Plant assessments were made using a visual disease rating based on a 1 to 5 disease severity scale (Hartman *et al*., 1997), where 1 = no foliar symptoms; 2 = light symptom development with mottling and mosaic (1 to 20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21 to 50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51 to 80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81 to 100% foliage affected). At 21 days after sowing seed, all cones were soaked in tap water for 15 to 20 min, and whole plants were uprooted and washed to remove adhering soil. Plants were blotted with a paper towel to remove excess moisture. Shoot length (from soil line to shoot tip), root length (from soil line to root tip) and discolored lesion lengths on the taproots were measured. After measuring, all plants were cut at the soil line. Both shoot and root parts were dried for 48 h at 50ºC and weighed.

Data analysis. Data from the two trials were tested for homogeneity of error variances, and experiment by treatment interactions. To analyze foliar disease severity ratings, the scales were converted to percentages using the midpoint value (Campbell and Madden, 1990; Hartman *et al*., 1997; Mueller *et al*., 2003), where 1 = 0%; 2 = 10%; 3 = 35%; 4 = 65%; 5 = 90%. Shoot and root lengths, and shoot and root dry weights were calculated as percentage of the non-inoculated control plants. Root lesion lengths were calculated as a percentage of the root length. For isolate studies, data were subjected to analysis of variance using a general linear mixed model procedure (PROC MIXED) of SAS (version 9.1, SAS Institute, USA). In this analysis, isolate was treated as fixed effect while trial, replications within trial, and trial x isolates were treated as random effects. Replication x isolates within trial was the residual error component. Means were compared by Fisher's protected least significant difference (LSD) at $P \leq 0.05$. The PROC CORR procedure of SAS was used to compute Pearson's correlation coefficients between the variables of SDS foliar severity, shoot and root lengths and weights, total plant weights, and root lesion length.

RESULTS

In the initial screening of 123 isolates, variation of aggressiveness based on SDS foliar severity was found among isolates ($P \leq 0.01$) on susceptible soybean entry Great Lakes 3202. Thirty isolates with different levels of aggressiveness and from different geographic origins were selected to evaluate for foliar severity and root infection (Table 2). The F-test for isolate as a source of variation indicated there were significant ($P < 0.01$) differences among isolates. For the random effects, the values of variance components were 5.98, 0.33, 15.46, and 141.87 for "Trial", "Replication within Trial", "Trial x isolate" and "Residual error", respectively. Since the random effect of "Trial x isolate" was not significant ($P \geq 0.05$), data from two trials were pooled and analyzed together. The means of sudden death syndrome severity ratings of soybean cv. Great Lakes 3202 inoculated with 30 isolates in two replicated trials in the greenhouse was presented in Tables 3 and 4.

The three isolates with the highest foliar severity

Table 3. Means of sudden death syndrome foliar severity ratings, shoot length, and shoot weights of soybean line Great Lakes 3202 after inoculation with 30 *Fusarium virguliforme* isolates for 21 days in two replicated trials under greenhouse conditions.

Isolate	Foliar Severity[a]		Shoot length[b]		Shoot weight[b]	
FSG1	52	abcde	57	n	35	m
FSG2	52	abcde	68	ghijk	44	ghijkl
FSG3	62	a	64	jklm	41	jklm
FSG4	42	efghi	74	bcde	55	abc
FSG5	53	abcd	67	hijkl	38	lm
FSG6	59	ab	62	lmn	46	efghij
FSG7	52	abcde	63	klm	45	fghijk
FSG8	55	abcd	68	ghijk	41	hijklm
FSG9	48	cdefg	76	bc	52	bcd
FSG10	38	ghi	78	b	59	a
FSG11	50	bcde	66	ijkl	45	efghij
FSG12	56	abc	64	jklm	38	klm
FSG13	43	efghi	68	fghijk	43	ghijkl
FSG14	33	i	69	fghijk	53	abcd
FSG15	33	i	70	defghi	52	bcde
FSG16	52	abcde	73	bcdefg	38	lm
FSG17	34	i	76	bc	51	bcdef
FSG18	38	fghi	75	bcd	56	ab
FSG19	48	cdefg	70	defghi	47	defghi
FSG20	45	defgh	69	efghij	51	bcdef
FSG21	57	abc	59	mn	40	jklm
FSG22	42	efghi	72	bcdefg	51	bcdef
FSG23	49	cdef	60	mn	41	ijklm
FSG24	47	cdefg	70	efghi	49	cdefg
FSG25	55	abcd	62	lmn	41	hijklm
FSG26	38	ghi	105	a	45	efghij
FSG27	35	hi	74	bcdef	52	bcd
FSG28	51	bcde	73	bcdefg	47	defgh
FSG29	47	cdefg	78	b	52	bcde
FSG30	46	cdefg	74	bcdef	45	fghijk
Mean	47		70		46	

[a] Means of foliar severity ratings of 10 plants for each isolate were based on a 1 to 5 scale, where 1 = no foliar symptoms; 2 = light symptom development with mottling and mosaic (1 to 20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21 to 50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51 to 80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81 to 100% foliage affected). Data were converted to percentages using the midpoint value (Campbell and Madden, 1990; Hartman *et al.,* 1997; Mueller *et al*., 2003) based on the range within each severity rating scale. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).
[b] Means of shoot length and shoot dry weight of 10 plants for each isolate were calculated as percentage of the non-inoculated control.

were FSG3, FSG6, and FSG21 with values of 62, 59, and 57, respectively, while isolate FSG1 had a value of 52 (Table 3). Six of 30 isolates tested had a higher foliar severity value than isolate FSG1, and three isolates had the same foliar severity value as FSG1. Isolate FSG21, with the third highest severity, was isolated from Illinois, as was FSG15 that had the lowest foliar severity value of 33. The mean value of the shoot length as percentage of the control without inoculation was 70%. Isolates FSG1 and FSG21 caused the lowest shoot length with values of 57% and 59% of the non-inoculated control, respectively, while isolate FSG26 did not

Table 4. Means of root length, root lesion length, root weights, and total plant weights of soybean line Great Lakes 3202 after inoculation with 30 *Fusarium virguliforme* isolates for 21 days in two replicated trials under greenhouse conditions.

Isolate	Root length[a]		Lesion length[b]		Root weight[a]		Total weight[c]	
FSG1	71	jk	39	efgh	43	klm	78	k
FSG2	80	efghi	35	ghi	50	ghijkl	94	fghij
FSG3	72	jk	52	a	43	lm	84	jk
FSG4	82	defgh	42	bcdef	65	abc	120	ab
FSG5	67	k	51	a	40	m	78	k
FSG6	77	ghij	40	cdefg	55	efghi	101	efg
FSG7	71	jk	46	abcd	52	fghijk	97	efghij
FSG8	83	cdefg	31	ij	45	jklm	86	hijk
FSG9	94	a	39	efgh	55	defghi	107	bcde
FSG10	90	abc	39	efgh	69	a	128	a
FSG11	78	fghij	42	bcdefg	56	cdefghi	101	defg
FSG12	84	cdefg	37	efghi	44	klm	82	jk
FSG13	87	bcde	37	efghi	60	bcdef	103	cdef
FSG14	89	abcd	31	ij	66	ab	119	ab
FSG15	85	cdef	35	ghi	64	abcd	116	abc
FSG16	80	efgh	38	efghi	48	ijklm	86	hijk
FSG17	86	cde	27	j	58	bcdefgh	109	bcde
FSG18	84	cdefg	37	efghi	63	abcde	119	ab
FSG19	82	defgh	39	defg	59	bcdefg	106	bcdef
FSG20	81	efgh	40	cdefg	58	bcdefgh	109	bcde
FSG21	73	ijk	50	a	49	hijkl	89	ghijk
FSG22	84	cdef	36	fghi	63	abcde	114	bcd
FSG23	81	efgh	42	bcdefg	45	jklm	86	hijk
FSG24	75	hij	46	abc	50	ghijkl	99	efgh
FSG25	80	efgh	48	ab	44	klm	85	ijk
FSG26	94	ab	33	hij	59	bcdefg	104	cdef
FSG27	95	a	26	j	67	ab	119	ab
FSG28	89	abcd	36	ghi	55	defghi	102	cdefg
FSG29	86	cde	39	efgh	58	bcdefgh	110	bcde
FSG30	81	efgh	43	bcde	54	fghij	99	efghi
Mean	82		39		55		101	

[a] Means of root length and root dry weight of 10 plants for each isolate were calculated as percentage of the non-inoculated control. Means followed by the same letter are not significantly different by the least significant difference test ($P = 0.05$).
[b] Means of tap root lesion lengths of 10 plants for each isolate were calculated as a percentage of the root length.
[c] Total plant weight (sum of shoot and root weights).

cause shoot length reduction (105%) when compared with the control without inoculation (Table 3). The mean shoot weight as percentage of the non-inoculated control was 46%. Isolate FSG1 caused the lowest shoot weight (35%), followed by 38% for isolates FSG5, 12, and 16 (Table 3).

All *F. virguliforme* isolates caused root discoloration on soybean Great Lakes 3202. The root lesion length as percentage of the root length ranged from 26% to 52% with a mean of 39%. Isolate FSG3, FSG5, and FSG 21 had the greatest lesion lengths with values of 52, 51 and 50% of non-inoculated control, respectively (Table 4).

All *F. virguliforme* isolates also caused soybean root length reduction. The mean root length as percentage of the control without inoculation was 82%. Isolate FSG5 had the lowest root length of 67%, while isolate FSG 27 had the highest root length of 95%. The range of root weights as percentage of the control without inoculation was from 40% to 69% with a mean of 55%. FSG 5 caused the greatest root weight reduction, while isolates FSG10, FSG27, and FSG14 caused much less root weight reduction (Table 4). In the ranking of total weight (shoot and root weight as percentage of the control without inoculation), FSG5 had the lowest weight, while FSG10 had the greatest weight.

Using Pearson's correlation analysis, foliar severity was negatively correlated with shoot and root lengths and weights ($P \leq 0.01$), and positively correlated with root lesion length ($P \leq 0.001$). Shoot and root weights were significantly ($P < 0.001$) correlated with a value of correlation coefficient of 0.887. This was the highest correlation among the other severity correlation analyses besides the correlation between weights (Table 5).

DISCUSSION

Evaluations of *F. virguliforme* aggressiveness have been previously conducted with limited numbers of isolates (Gray and Achenbach, 1996; Huang and Hartman, 1998) or isolates from the same geographic location (Cho *et al*., 2001). Isolate FSG1 (Mont-1) was reported to cause the greatest disease severity based on foliar and root severity of nine isolates (Gray and Achenbach, 1996), and ranked second among four isolates based on the foliar severity (Huang and Hartman, 1996). In this study, 123 *F. virguliforme* isolates were tested, 30 isolates from different geographic origins with different levels of foliar severity were selected to further evaluate both foliar severity and root infection. Significant differences in aggressiveness among isolates were found. Compared to reference isolate FSG1, several isolates were more aggressive based on either foliar or root severity. Isolate FSG5 had not only higher values of foliar severity, but also caused smaller root length and lower root weight when compared to isolate FSG1. Treatment with isolate FSG5 also had the lowest root length and caused the highest root weight reduction among the 30 selected isolates. This result was support-

Table 5. Pearson's correlation coefficient values and significance for sudden death syndrome foliar severity rating, shoot length, root length, root lesion length, shoot weight, and root weight of soybean line Great Lakes 3202 inoculated with 30 *Fusarium virguliforme* isolates for 21 days in two replicated trials under greenhouse conditions.

Values[a]	Foliar severity[b]	Shoot length[c]	Root length[c]	Lesion length[d]	Shoot weight[c]	Root weight[c]	Total weight[d]
Foliar severity[b]	1.000	-0.548	-0.652	0.632	-0.719	-0.810	-0.796
		(<0.002)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
Shoot length[c]		1.000	0.651	-0.437	0.455	0.502	0.492
			(<0.001)	(0.016)	(0.012)	(0.005)	(0.006)
Root length[c]			1.000	-0.749	0.603	0.675	0.662
				(<0.001)	(<0.001)	(<0.001)	(<0.001)
Lesion length[d]				1.000	0.379	-0.532	-0.479
					(0.039)	(0.003)	(0.008)
Shoot weight[c]					1.000	0.887	0.963
						(<0.001)	(<0.001)
Root weight[e]						1.000	0.979
							(<0.001)
Total weight[e]							1.000

[a] Values in parentheses are probabilities.
[b] Foliar severity ratings were based on a 1 to 5 scale, where 1 = no foliar symptoms; 2 = light symptom development with mottling and mosaic (1 to 20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21 to 50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51 to 80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81 to 100% foliage affected). Data were converted to percentage using the midpoint value (Campbell and Madden, 1990; Hartman *et al.*, 1997; Mueller *et al*., 2003) based on the range within each severity rating scale.
[c] Shoot and root length and shoot and root dry weight were calculated as percentage of the non-inoculated control.
[d] Tap root lesion lengths were calculated as percentage of the root length.
[e] Total plant weight (the sum of shoot and root dry weights).

ed by our previous root colonization assays, in which isolate FSG5 was identified as the most aggressive root colonizer based on *F. virguliforme* DNA accumulation and colony-forming units in infested soybean roots (Li *et al.,* 2008a). In addition, isolates FSG3, FSG6, FSG21, FSG12 and FSG25 caused the greatest foliar severity among the 30 selected isolates. Identification of these five very aggressive isolates will provide a useful tool for evaluating soybean breeding lines for SDS resistance.

Because of concerns over the identity and nomenclature of the SDS pathogen, a study was initiated to examine variation among *F. virguliforme* and other isolates of *F. solani* by Rupe *et al.* (2001). Differences among isolates were found based on the ability to cause SDS and the effect on plant mass of soybean (Rupe *et al*., 2001). The SDS isolates generally caused high levels of root rot and reduced root and top masses compared with non-SDS isolates. In another study, Cho *et al.* (2001) isolated and identified 112 isolates from two fields in Arkansas using modified Nash and Snyder's medium. Three *F. virguliforme* isolates were found that caused greater SDS foliar symptoms than the Arkansas standard isolate 171. In our study, there was no association found between the geographic origins of the isolates and their respective measures of aggressiveness. For example, both FSG15 and FSG 21 isolates were from Illinois, but FSG 15 caused the lowest foliar severity, while FSG 21 had the most severe foliar symptoms. Based on the root length calculated as percentage of the control without inoculation, the Missouri isolate FSG 7 caused the second shortest root length, but another Missouri isolate FSG 9 had the second longest root length. The top five isolates causing the most severe foliar severity were originally from three different countries.

In addition, it is time consuming to measure all different disease severities especially when evaluating many isolates. Based on the correlation analysis in this study, all weight data (shoot, root, or total plant weight) had higher correlation coefficients with foliar severity than other disease measurements, such as the shoot, root and root lesion length. Determination of plant weight has potential as a simple and fast method for initial comparison of isolate aggressiveness.

F. virguliforme has been isolated from roots, but not leaves of soybean plants. The foliar symptoms have been reported to be caused by toxins that are translocated to the leaves from infected roots (Hartman *et al*., 2004; Jin *et al*., 1996; Li *et al*., 1999). Based on this assumption, it may be possible that certain isolates are good root colonizers but may or may not be good toxin producers, and conversely, some isolates may be relatively poor root colonizers but may or may not be good toxin producers. In our study, the poor root colonizing isolates usually caused less foliar symptoms. It is likely to have good root colonizers with poor foliar symptoms, but it is less likely that poor root colonizers would cause much in the way of foliar symptoms. Further research is needed to determine the relationship of root colonization to toxin production and disease severity. This would help answer the question of why some isolates are more aggressive than others.

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Trade and manufacturer names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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