Effect of Planting Date, Seed Treatment, and Cultivar on Plant Population, Sudden Death Syndrome, and Yield of Soybean

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Abstract

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A 2-year study was conducted in Illinois, Indiana, Iowa, and Ontario in 2013 and 2014 to determine the effects of planting date, seed treatment, and cultivar on plant population, sudden death syndrome (SDS) caused by *Fusarium virguliforme*, and grain yield of soybean (*Glycine max*). Soybean crops were planted from late April to mid-June at approximately 15-day intervals, for a total of three to four plantings per experiment. For each planting date, two cultivars differing in SDS susceptibility were planted with and without fluopyram seed treatment. Mid-May plantings resulted in higher disease index compared with other planting dates in two experiments, early June plantings in three, and the remaining six experiments were not affected by planting date. Soil temperature at planting was not linked to SDS development. Root rot was greater in May plantings for most experiments. Resistant cultivars had significantly lower disease index than the susceptible cultivar in 54.5% of the experiments. Fluopyram reduced disease severity and protected against yield reductions caused by SDS in nearly all plantings and cultivars, with a maximum yield response of 1,142 kg/ha. Plant population was reduced by fluopyram seed treatment and early plantings in some experiments; however, grain yield was not affected by these reductions. Yields of plots planted in mid-June were up to 29.8% less than yields of plots planted in early May. The lack of correlation between early planting date and SDS severity observed in this study indicates that farmers do not have to delay planting in the Midwest to prevent yield loss due to SDS; cultivar selection combined with fluopyram seed treatment can reduce SDS in early-planted soybean (late April to mid May).

Sudden death syndrome (SDS), caused by *Fusarium virguliforme* O'Donnell and T. Aoki (Aoki et al. 2003), is one of the most important fungal diseases of soybean (Hartman et al. 2015b; Wrather et al. 2010) and is often ranked as one of the top five diseases in most soybean-producing areas in the United States (Wrather and Koenning 2009) and Ontario, Canada (Wrather et al. 2010). The disease can cause up to 100% yield loss on an individual plant, depending on the age of the plant when the disease appears and disease severity (Hartman et al. 2015a; Roy et al. 1997).

Foliar symptoms typically begin with interveinal chlorosis and mottling, progressing to necrosis, premature defoliation, and pod abortion (Hartman et al. 2015b; Roy et al. 1997). The fungus overwinters in soil and crop residues, and infects soybean seedling roots. The fungus does not infect foliar plant parts; the symptoms are the result of damaged foliar tissues from phytotoxins, mainly FvTox1 (Brar et al. 2011; Pudake et al. 2013). Once the plant is infected, the pathogen can reside in the plant with no noticeable symptoms, although early-season foliar symptoms occasionally may be seen. Foliar symptoms typically appear when the plant enters reproductive stages. If the cultivar is susceptible to SDS and weather conditions are favorable, symptoms can progress quickly.

Environment plays a major role in incidence and severity of SDS (Kandel et al. 2015). High soil moisture is favorable for root rot and increases the earliness and severity of foliar symptoms (Roy et al. 1997; Scherm and Yang 1996). Scherm and Yang (1996) reported that temperature differentially influences root and foliar symptoms, where low temperatures (15°C) during the early part of the season favor root rot, and moderate temperatures (approximately 25°C) optimize leaf necrosis. Root rot severity is higher when seedling infection occurs during cold soil conditions (Gongora-Canul and Leandro 2011), and it is generally believed that soybean crops planted in cold soils that favor *F. virguliforme* infection have more severe foliar SDS symptoms than soybean planted later in the season (Hershman et al. 1990).

Because no complete genetic cultivar resistance is available to manage SDS, farmers are encouraged to use practices that reduce the impact of the soil environment on SDS development, such as delaying planting until the soil is warm and dry and less conducive for *F. virguliforme* infection (Hershman et al. 1990; Navi and Yang 2008; Scherm and Yang 1996). However, in the Midwestern United States and Ontario, Canada, farmers are simultaneously encouraged to plant soybean early (late April or early May) to capture yield potential and improve seed quality. Soybean planted when soils are warmer and drier in the Midwest (late June or July) produce less yield and result in lower seed protein concentration than early plantings (Beatty et al. 1982; De Bruin and Pedersen 2008; Egli and Bruening 1992). This conflicting information makes it difficult for farmers to select an exact planting date that is early enough for them to optimize yield yet minimize the risk of SDS. Additionally, there are substantial variations in the range of soil temperature and moisture during late April through June in the Midwest and Ontario, and it is not known whether the very early soybean plantings (late April) have a different risk for SDS development compared with soybean plantings that occur in mid-May.

Many factors, including soybean cyst nematode (*Heterodera glycines*) populations, crop rotation, and tillage operations, influence SDS occurrence and severity (Westphal et al. 2014; Wrather et al. 1995; Xing and Westphal 2006), and farmers are encouraged to implement practices to limit the impact of these factors. However, foliar fungicide evaluations have not yielded any products with efficacy against SDS (Y. Kandel unpublished). Because *F. virguliforme* infects during seedling stages, fungicide seed treatment may be effective. In general, seed treatment protects against several soil and seedborne pathogens, and

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may provide protection against stand loss (Bradley 2008). However, Weems et al. (2015) tested several commercially available seed-applied fungicides registered for soybean, which include azoxystrobin, *Bacillus pumilus* GB34, fludioxonil, mefenoxam or metalaxyl, prothioconazole, thiophanate-methyl, thiophanate-methyl + pyraclostrobin, and trifloxystrobin + metalaxyl, and none effectively reduce root rot caused by *F. virguliforme* or SDS. In December 2014, fluopyram (ILeVO; Bayer CropScience, Research Triangle Park, NC) was registered as a seed treatment fungicide for the management of SDS. Fluopyram is classified as a succinate dehydrogenase inhibitor (Fungicide Resistance Action Committee Code 7) and effectively manages several soil, seedborne, and foliar diseases of horticultural and row crops (Fought et al. 2011; Labourdette et al. 2010; Meredith 2012; Scannavini et al. 2012). However, information about its efficacy against SDS and its effect on yield of soybean planted on different dates and under different management conditions is limited. Therefore, a detailed study that includes varied field conditions and cultivars is required to examine the effectiveness of fluopyram seed treatment for SDS management. The objective of this study was to evaluate the influence of planting date, seed treatment, and soybean cultivar on plant population establishment, SDS, and grain yield under a wide range of field conditions.

Materials and Methods

A 2-year study was established in 2013 and 2014 in Illinois, Indiana, and Iowa in the United States and Ontario, Canada. In total, 11 field experiments were completed (Table 1). In each site and year, soybean was planted at multiple dates. Target planting dates were 30 April, 15 and 30 May, and 15 June. However, actual planting dates and targets did not always match because of poor weather and field conditions. Therefore, the actual first planting was started in early May for most sites and plantings continued at approximately 15-day intervals for a total of three to four plantings through the middle of June (Table 2). Hereafter, planting dates are referred to as first planting through fourth planting, or early-May, mid-May, late-May, and mid-June. Two cultivars, one moderately resistant and the other moderately susceptible to SDS, were planted with two seed treatment levels: (i) the base seed treatment (control) consisted of prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg active ingredient [a.i.]/seed; Bayer CropScience), metalaxyl (Allegiance, 0.02 mg a.i /seed; Bayer CropScience), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i /seed; Bayer CropScience) and (ii) fluopyram (ILeVO, 0.15 mg a.i./seed; Bayer CropScience) in addition to the base seed treatment. Soybean cultivars used in this study varied across sites in 2013 but were consistent across sites in 2014 (Table 1). SDS severity ratings for each cultivar were provided by their respective companies (Table 1).

Treatments were arranged in a split plot, with complete randomized design for the whole-plot factor, planting date, and randomized complete block for split-plot factors. A two-by-two factorial combination of two cultivars and two levels of fungicide seed treatment was established as subplots within the main plot, with four replications.

Table 1. Year, location, cultivar, and management operations conducted for experiment sites in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

					Herbicide	
Year, Loc[v]	**Cultivar (SDS)[w]**	**Inoc[x]**	**Previous[y]**	**Irrig[z]**	**Preemergence**	**Postemergence**
2013						
Bo, IA	AG2031 (4)	Yes	Corn	No	S-metachlor	Glyphosate
	AG3231 (4)	Yes	Corn	No	S-metachlor	Glyphosate
Ur, IL	NK38H8 (5)	Yes	Soybean	Yes	Sulfentrazone + Cloransulam-methyl	Glyphosate
	NK39U2 (2)	Yes	Soybean	Yes	Sulfentrazone + Cloransulam-methyl	Glyphosate
La, IN	DSR3019 (2.5)	Yes	Soybean	Yes	Flumioxazin	Glyphosate and Fluazifop-P-butyl + Fenoxaprop-p-ethyl
	DSR3216 (1.8)	Yes	Soybean	Yes	Flumioxazin	Glyphosate and Fluazifop-P-butyl + Fenoxaprop-p-ethyl
Hg, ON	P31-10RY (2)	No	Soybean	No	None	Glyphosate
	P31-11RY (5)	No	Soybean	No	None	Glyphosate
Rd, ON	P31-10RY (2)	No	Soybean	No	None	Glyphosate
	P31-11RY (5)	No	Soybean	No	None	Glyphosate
2014						
Am, IA	P92Y60 (4)	Yes	Corn	No	Pendimethalin and Carfentrazone-ethyl Sulfentrazone	Glyphosate
	P92Y83 (7)	Yes	Corn	No	Pendimethalin and Carfentrazone-ethyl Sulfentrazone	Glyphosate
Ro, IA	P92Y60 (4)	No	Corn	No	None	Glyphosate
	P92Y83 (7)	No	Corn	No	None	Glyphosate
Ur, IL	P92Y60 (4)	Yes	Soybean	Yes	Sulfentrazone + Cloransulam-methyl	Glyphosate
	P92Y83 (7)	Yes	Soybean	Yes	Sulfentrazone + Cloransulam-methyl	Glyphosate
Wa, IN	P92Y60 (4)	Yes	Corn	Yes	Chlorimuron + Metribuzin	Glyphosate
	P92Y83 (7)	Yes	Corn	Yes	Chlorimuron + Metribuzin	Glyphosate
Hg, ON	P92Y60 (4)	No	Corn	No	None	Glyphosate
	P92Y83 (7)	No	Corn	No	None	Glyphosate
Rd, ON	P92Y60 (4)	No	Wheat	No	None	Glyphosate
	P92Y83 (7)	No	Wheat	No	None	Glyphosate

[v] Locations: Boone (Bo), IA; Urbana (Ur), IL; Lafayette (La), IN; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; Roland (Ro), IA; and Wanatah (Wa), IN.

[w] Cultivars and SDS rating scale: numbers in parenthesis are SDS rating scale provided by the respective companies. Asgrow (AG) cultivars and Northrup King (NK) cultivars: 1-to-9 scale, with 1 = most resistant; Dairyland seed (DSR) cultivars: 1-to-5 scale, with 1 = most resistant; Pioneer (P) cultivars: 1-to-9 scale, with 1 = susceptible.

[x] Inoculation: In Iowa, inoculated with the *Fusarium virguliforme* isolate NE 305, infested sorghum at 8.3 g/m of linear row; Illinois, inoculated with the isolate Mont-1, infested ground sorghum at 4.1 cm^3/m of linear row; Indiana, inoculated with a mixture of three isolates collected locally (NRRL 22823, 00-11-183, and INS12-10 #3-1), infested sorghum at 8.3 g/m of linear row.

[y] Previous crop.

[z] Irrigation: In Illinois, drip irrigation was set up starting in June and ran every week for 6 weeks; approximately 2.5 cm of water was delivered each week. In the second year in Iowa, drip irrigation was set up in the first week of August and ran six times at 1-week intervals; about 2.5 cm of water was delivered each time. In Indiana, between the growth stages V5 and R5, approximately 2.5 cm of water was delivered through overhead irrigation in weeks when natural precipitation did not occur.

Tillage was done prior to planting in all locations. Each plot was an experimental unit that consisted of four rows, with interrow spacing of 38.1 to 76.2 cm and length ranging from 5.3 m to 9.1 m. Planting population ranged from 344,444 to 590,477 seeds/ha.

Experiments were established in locations where SDS had been observed in previous years; plots were also infested with local *F. virguliforme* isolates, except at the Roland, IA, and Ontario sites (Table 1). Where infested, sorghum or oat grains colonized with *F. virguliforme* were seeded with soybean. *F. virguliforme* isolates used to infest sorghum or oat were derived from a single spore and inoculum was prepared as described by de Farias Neto et al. (2006). Preceding crops were corn or soybean in all locations, except in Rodney, ON. Field experiments in Illinois and Indiana were irrigated while other field experiments occurred under natural precipitation. Pre- and postemergence herbicides were applied at the recommended rates throughout the study (Table 1).

Plant population and root rot data. Plant population and root rot severity data were collected at early vegetative (V2 to V3) growth stages (Fehr et al. 1971). Total live plants were counted in 3.0 to 6.1 m on one to two center rows of each plot. At the V2 to V3 growth stage, 12 plants were collected from the two outer rows of each plot to estimate root rot severity. Roots were gently washed and root rot severity was estimated visually as percentage of area covered by root lesions of the total root area.

Foliar disease assessment. Foliar symptoms were recorded between R5 and R6 growth stages (Table 2). Foliar SDS incidence was recorded as percentage of symptomatic plants in the middle two rows of the plot, and severity was visually estimated following a previously published 0-to-9 scale (0 = no disease and 9 = premature plant death) (Gibson et al. 1994; Kandel et al. 2015). Foliar SDS disease index (FDX) was calculated from the disease incidence and severity score by using the following formula: FDX = disease incidence × disease severity/9.

Yield data. The center two rows were mechanically harvested for grain yield at full maturity (R8), which ranged from the last week of September to the first week of November (Table 2). Grain moisture was also recorded while harvesting and grain yield was adjusted to 13% moisture.

Weather data. Monthly cumulative precipitation and soil temperature at planting for each experiment sites were obtained from the

Table 2. Planting dates, foliar disease scoring dates and growth stages, and harvesting dates for experiment sites in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

Year, loc[y]	Date[z]			
2013				
Planting				
Bo, IA	…	14 May	3 June	19 June
Ur, IL	…	14 May	30 May	12 June
La, IN	9 May	15 May	5 June	17 June
Hg, ON	8 May	17 May	4 June	22 June
Rd, ON	8 May	17 May	3 June	22 June
SDS				
Bo, IA	5 September (R6)	5 September (R6)	12 September (R6)	12 September (R6)
Ur, IL	…	13 August (R5.4)	19 August (R5.6)	29 August (R5.4)
La, IN	13 August (R6)	13 August (R6)	13 August (R5)	13 August (R5)
Hg, ON	27 August (R5)	6 September (R5)	6 September (R5)	6 September (R5)
Rd, ON	27 August (R5)	4 September (R5)	4 September (R5)	4 September (R5)
Harvest				
Bo, IA	…	14 October	14 October	4 November
Ur, IL	…	14 October	14 October	14 October
La, IN	26 September	26 September	11 October	11 October
Hg, ON	15 October	15 October	15 October	21 October
Rd, ON	23 October	23 October	23 October	23 October
2014				
Planting				
Am, IA	6 May	17 May	30 May	10 June
Ro, IA	9 May	24 May	30 May	10 June
Ur, IL	26 April	6 May	20 May	9 June
Wa, IN	6 May	20 May	6 June	18 June
Hg, ON	12 May	20 May	5 June	23 June
Rd, ON	12 May	20 May	5 June	23 June
SDS				
Am, IA	8 September (R6)	8 September (R6)	16 September (R6)	24 September (R6)
Ro, IA	8 September (R6)	15 September (R6)	24 September (R6)	24 September (R6)
Ur, IL	19 August (R6)	19 August (R6)	26 August (R6)	5 September (R5)
Wa, IN	12 August (R5)	28 August (R5.5)	28 August (R5)	5 September (R5)
Hg, ON	3 September (R5)	3 September (R5)	…	17 August (R5)
Rd, ON	3 September (R5)	3 September (R5)	12 September (R5)	17 September (R5)
Harvest				
Am, IA	8 October	8 October	8 October	21 October
Ro, IA	18 October	18 October	18 October	18 October
Ur, IL	12 October	12 October	12 October	12 October
Wa, IN	3 November	3 November	3 November	3 November
Hg, ON	23 October	23 October	…	3 November
Rd, ON	30 October	30 October	30 October	30 October

[y] Planting = actual planting date, SDS = SDS scoring date and growth stage, and Harvest = harvesting date. Targeted planting dates were 30 April, 15 May, 30 May, and 15 June for the first, second, third, and fourth planting dates, respectively. Locations (loc): Boone (Bo), IA; Urbana (Ur), IL; Lafayette (La), IN; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; Roland (Ro), IA; and Wanatah (Wa), IN; … indicates not available.

[z] Letters and numbers in parenthesis are soybean growth stages at the time of disease scoring, according to Fehr et al. (1971).

weather stations installed in the plots or from a station located near the experimental sites through public weather service websites (http://w2.weather.gov/climate/ or http://mesonet.agron.iastate.edu/).

Data analysis. Analysis of variance was performed using Proc GLIMMIX in SAS (version 9.3; SAS Institute Inc. Cary, NC) for plant population, root rot severity, FDX, and grain yield. Data analysis was performed individually for each experiment. Planting date, seed treatment, and cultivar were treated as fixed factors and replication within planting date was treated as a random factor. To test the effect of planting date, replication within planting date was used as an error term and the residual error was used to test the effect of seed treatment, cultivar, and interactions. Mean separation was performed using Fisher's protected least significant difference at $P = 0.05$. Pearson correlation coefficients were estimated to determine the relationships among root rot, foliar disease, and grain yield. Regression analysis was performed using Proc REG to establish the relationship between the soil temperature at plating and FDX.

Results

Plant population. The effect of planting date on plant population was significant at $P = 0.05$ in 8 of the 11 experiments (Table 3). In 2013, plant population was affected by planting date in two experiments in Lafayette, IN ($P < 0.01$) and Highgate, ON ($P < 0.01$). In 2014, plant population differed among the planting dates in all six experiments ($P < 0.01$). In all experiments, the fourth plantings had more plants than preceding planting dates, except at Lafayette, IN and Ontario locations. Treatments containing fluopyram reduced plant populations compared with the control by 8.1% in Rodney, ON in 2013 ($P = 0.03$), 3.3% in Urbana, IL in 2014 ($P = 0.03$), and 3.5% in Highgate, ON in 2014 ($P = 0.04$) (data not shown). Cultivar–seed treatment interaction was significant in Highgate, ON in 2013, where the susceptible cultivar treated with fluopyram reduced plant population by 5.1% compared with the base treatment of that cultivar. Seed treatment–planting date interaction was significant in both Ontario locations in 2014, where fluopyram seed treatment affected plant populations most when soybean cultivars were planted in mid-May. Planting date–cultivar interaction was significant in Ames, IA; Urbana, IL; and Rodney, ON ($P < 0.05$). In Ames, IA, significant difference between the cultivars was observed only in the second planting, where the SDS-resistant cultivar had greater plant populations than the susceptible cultivar. In Urbana, IL, greater plant populations were observed in the susceptible cultivar than the resistant cultivar at the first and third planting dates and the other two planting dates had no significant difference between cultivars. Similarly, significant differences between cultivars were observed in the first and third planting dates in Rodney, ON and the resistant cultivar had a greater plant population than the susceptible cultivar for both planting dates.

Root rot. For all 5 of the 11 experiments where root rot was scored, severity of root rot differed across planting dates. Typically, May plantings had higher root rot severity than June plantings (Table 4). Treatments including fluopyram had less root rot than the control in two of five experiments; 53.7% less in Highgate, ON in 2013 ($P < 0.01$) and 33.5% less in Roland, IA in 2014 ($P < 0.01$). Fluopyram did not significantly reduce root rot in the remaining three locations. Interactions of seed treatment–cultivar and seed treatment–planting date were also significant in Highgate, ON; however, the interactions were only quantitative. Fluopyram seed treatment resulted in less disease for nearly all planting dates and all cultivars, although the interaction was significant.

Table 3. Effect of planting date on plant population recorded at V2 to V3 growth stage in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

	Plant population (number of plants/ha) on dates of planting[y]				
Year, loc[z]	First	Second	Third	Fourth	$P > F$
2013					
Bo, IA	NA	241,507	262,303	273,707	0.06
Ur, IL	NA	324,532	331,529	312,557	0.19
La, IN	158,605 c	249,992 a	269,771 a	204,649 b	<0.01
Hg, ON	421,869 c	468,999 b	513,032 a	447,903 b	<0.01
Rd, ON	390,115	466,462	444,550	436,831	0.14
2014					
Am, IA	263,979 c	239,761 d	310,263 b	369,194 a	<0.01
Ro, IA	273,128 c	276,088 c	310,263 b	352,242 a	<0.01
Ur, IL	296,546 b	307,041 b	280,938 c	339,063 a	<0.01
Wa, IN	295,194 b	160,782 c	307,169 ab	325,736 a	<0.01
Hg, ON	440,110 a	364,964 c	NA	410,509 b	<0.01
Rd, ON	317,199 c	335,565 c	416,900 a	372,566 b	<0.01

[y] Specific dates of planting were different across the experiments. The first, second, third, and fourth planting dates ranged from 8 to 9 May, 14 to 17 May, 30 May to 4 June, and 12 to 22 June, respectively, in 2013. In 2014, the first, second, third, and fourth planting dates ranged from 26 April to 12 May, 6 to 20 May, 20 May to 6 June, and 9 to 23 June, respectively. Means separation was done by Fisher's protected least significant difference. Means followed by the same letter within a column do not differ significantly at $P = 0.05$. NA = not available.

[z] Locations: Boone (Bo), IA; Urbana (Ur), IL; Lafayette (La), IN; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; Roland (Ro), IA; and Wanatah (Wa), IN.

Table 4. Main effects of planting date, seed treatment, and cultivar on root rot cased by *Fusarium virguliforme* in soybean root tissue collected during V2 to V3 growth stage at five experiment sites during 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

	Root rot (%)[w]				
	2013			2014	
Variables	Ur, IL	Hg, ON	Rd, ON	Am, IA	Ro, IA
Planting date[x]					
First	NA	2.2 ab	3.9 b	26.3 a	5.6 b
Second	13.7 a	3.0 a	7.7 a	3.1 b	17.3 a
Third	11.1 a	0.7 b	1.2 c	1.7 b	9.8 b
Fourth	1.0 b	3.6 a	3.0 bc	1.5 b	6.2 b
$P > F$	<0.01	0.02	<0.01	<0.01	<0.01
Seed treatment[y]					
Control	10.0	3.2	4.8	9.1	11.7
Fluopyram	7.2	1.5	3.1	7.2	7.8
Difference (%)	27.9	53.7	35.3	21.5	33.4
$P > F$	0.24	<0.01	0.08	0.13	<0.01
Cultivar[z]					
SDS-MS	10.5	2.8	4.1	8.5	11.0
SDS-MR	6.7	1.9	3.8	7.8	8.5
Difference (%)	35.9	33.5	8.3	8.0	22.8
$P > F$	0.12	0.01	0.72	0.59	0.02

[w] Locations: Urbana (Ur), IL; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; and Roland (Ro), IA. Means separation was done by Fisher's protected least significant difference. Means across the planting dates followed by the same letter with in each location do not differ significantly at $P = 0.05$. NA = not available. Root rot severity was estimated visually on 0 to 100% scale based on the area covered by root lesions out of the total root area.

[x] Specific dates of planting were different across the experiments. The first, second, third, and fourth planting dates were ranged from 8 to 9 May, 14 to 17 May, 30 May to 4 June, and 12 to 22 June, respectively, in 2013. In 2014, the first, second, third, and fourth planting dates ranged from 26 April to 12 May, 6 to 20 May, 20 May to 6 June, and 9 to 23 June, respectively.

[y] Seed treatments: Control = base seed treatment by Bayer CropScience with prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed), metalaxyl (Allegiance, 0.02 mg a.i./seed), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed); and fluopyram (ILeVO, applied in addition to base seed treatment at 0.15 mg a.i/seed).

[z] Percent difference for seed treatment was estimated by using the formula Difference (%) = [(control mean − fluopyram mean)/control mean] × 100. Cultivar SDS-MS = moderately susceptible to SDS and SDS-MR = moderately resistant to SDS. Percent difference for cultivars was estimated by using the formula Difference (%) = [(MS mean − MR mean)/MS mean] × 100. Cultivars were different across the sites in 2013.

In two experiments, the resistant cultivar had less root rot compared with the susceptible cultivar ($P = 0.01$; Highgate, ON in 2013 and Roland, IA in 2014) but there was no effect of cultivar on root rot in the remaining three locations.

FDX. Growth stage was consistently linked to foliar symptom expression rather than calendar date of rating. Symptoms of SDS were observed in seedlings at V2 from the first planting in Iowa in 2014 (data not provided) whereas, in other experiments, the symptoms were observed during and after flowering. Correlation between the root rot severity and FDX was not significant ($r = 0.35$, $P = 0.14$). In 2013, foliar symptoms were observed in three of the five locations and disease severity was low to moderate. Foliar symptoms were not observed in Boone, IA and Lafayette, IN in 2013. In 2014, all six locations reported foliar SDS symptoms and the mean FDX in 2014 was greater than in 2013 (Table 5). Other diseases, in particular brown stem rot (*Cadophora gregata*) and northern stem canker (*Diaporthe caulivora*), that exhibit similar symptoms to SDS were not observed in any of the field experiments.

Planting date had a significant effect on FDX in 5 of 11 experiments (Table 5). In 2013, significant differences ($P < 0.05$) for FDX across the planting dates were observed in Urbana, IL and Highgate, ON. In Urbana IL, the maximum FDX was observed for the 14 May planting whereas, in Highgate, ON, May plantings had lower FDX than June plantings. In 2014, a significant effect of planting date on FDX was observed in three of the six experiments: Roland, IA, Urbana, IL, and Wanatah, IN. Among the three locations, two locations showed the highest disease in mid-May plantings (Urbana, IL and Wanatah, IN, each planted on 20 May) and the third (Roland, IA) showed the highest disease when planted in late May (30 May; Table 5). The FDX in final planting dates, which were from 9 through 23 June across experiments, was not different from the second (mid-May) plantings in any experiments except Urbana, IL in 2013 and Wanatah, IN in 2014 and was not different from third plantings in all locations except in Roland, IA and Urbana, IL in 2014 (Table 5). The first plantings did not result in higher FDX than later plantings in any locations where a significant planting date effect was observed. Soil was cooler in early plantings compared with late plantings (Supplementary Table S1); however, the higher FDX was not associated with cool soil temperature at planting (Table 5). Regression analysis of FDX against soil temperature at planting showed no significant association between soil temperature and SDS with R^2 value of 0.02.

Fluopyram seed treatment reduced the FDX in six of the nine experiments where SDS was reported. Reductions ranged from 38.7 to 94.3%, averaged over planting dates and cultivars (Table 5). Interaction of seed treatment–planting date was significant ($P < 0.01$) in Urbana, IL in 2013; Wanatah, IN in 2014; and Rodney, ON in 2014. Seed treatment–cultivar interaction was significant in Urbana, IL in 2013 and Wanatah, IN in 2014 ($P = 0.03$ and $P < 0.01$, respectively). Where the interaction was significant, effects of seed treatment on each planting date and cultivar were analyzed separately; fluopyram seed treatment resulted in less disease for nearly all planting dates and all cultivars, although interaction was significant.

Susceptible cultivars had higher FDX in six of nine experiments (Table 5) where SDS was reported. Even when interactions of cultivar–planting date or cultivar–seed treatment were significant (Urbana, IL in 2013 and Wanatah, IN in 2014), susceptible cultivars had higher FDX than resistant cultivars in all planting dates and control plots had higher FDX than fluopyram treated plots in both cultivars (data not provided).

Yield. Planting date had a significant ($P < 0.05$) effect on soybean yield in 8 of 11 experiments (Table 6). Late-planted soybean produced less grain yield regardless of SDS severity. Grain yields ranged

Table 5. Main effects of planting date, seed treatment, and cultivar on foliar disease index (FDX) caused by *Fusarium virguliforme* at experiment sites in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

	Least square means of FDX[w]								
	2013			2014					
Variables	**Ur, IL**	**Hg, ON**	**Rd, ON**	**Am, IA**	**Ro, IA**	**Ur, IL**	**Wa, IN**	**Hg, ON**	**Rd, ON**
Planting date[x]									
First	…	1.1 b	0.3	33.6	43.2 b	0.3 b	1.5 c	1.6	0.3
Second	3.0 a	2.3 b	4.4	33.8	35.6 b	0.9 b	16.6 a	0.6	0.6
Third	0.9 b	5.4 a	0.4	23.3	67.3 a	4.5 a	2.5 bc	…	3.6
Fourth	0.1 b	3.7 ab	0.6	30.7	31.1 b	0.7 b	7.6 b	2.3	1.0
Mean	1.3	3.1	1.4	30.3	44.3	1.6	7.0	1.5	1.4
$P > F$	0.01	0.03	0.22	0.78	<0.01	0.02	<0.01	0.25	0.09
Seed treatment[y]									
Control	2.5	3.3	2.7	42.6	54.9	2.4	11.5	1.9	2.5
Fluopyram	0.1	2.9	0.1	18.1	33.7	0.8	2.6	1.1	0.3
Diff (%)	94.3	12.5	95.2	57.5	38.7	66.2	77.3	40.7	89.4
$P > F$	<0.01	0.68	0.09	<0.01	<0.01	0.02	<0.01	0.15	<0.01
Cultivar[z]									
SDS-MS	2.2	5.0	2.3	45.1	59.5	2.4	9.9	1.0	1.5
SDS-MR	0.4	1.3	0.5	15.6	29.1	0.8	4.2	1.9	1.3
Diff (%)	82.1	74.5	79.8	65.5	51.1	66.9	58.1	-86.5	17.3
$P > F$	0.01	<0.01	0.22	<0.01	<0.01	0.02	<0.01	0.09	0.67

[w] FDX was calculated using the formula FDX = disease incidence × disease severity/9; disease incidence was estimated as percentage of symptomatic plants per plot, and foliar SDS severity was scored on a 0 to 9 scale (0 = no disease and 9 = premature death) based on percentage of chlorotic and necrotic leaf area, and defoliation. Locations: Urbana (Ur), IL; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; Roland (Ro), IA; and Wanatah (Wa), IN. Foliar symptoms of SDS were not observed in two experiments (Boone, IA and Lafayette, IN) in 2013. Means separation was done by Fisher's protected least significant difference. Means across the planting dates followed by the same letter with in each location do not differ significantly at $P = 0.05$.

[x] Specific dates of planting were different across the experiments. The first, second, third, and fourth planting dates were ranged from 8 to 9 May, 14 to 17 May, 30 May to 4 June, and 12 to 22 June, respectively, in 2013. In 2014, the first, second, third, and fourth planting dates ranged from 26 April to 12 May, 6 to 20 May, 20 May to 6 June, and 9 to 23 June, respectively.

[y] Seed treatments: Control = base seed treatment by Bayer CropScience with prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed), metalaxyl (Allegiance, 0.02 mg a.i./seed), and Clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed); and fluopyram (ILeVO, applied in addition to base seed treatment at 0.15 mg a.i/seed). Percent difference (Diff) for seed treatment was estimated by using the formula Difference (%) = [(control mean − fluopyram mean)/control mean] × 100.

[z] Cultivar SDS-MS = moderately susceptible to SDS and SDS-MR = moderately resistant to SDS. Percent difference (Diff) for cultivars was estimated by using the formula Difference (%) = [(MS mean − MR mean)/MS mean] × 100. Cultivars were different across the sites in 2013.

from 2,179.7 to 4,792.2 kg/ha; average yield difference between first and last plantings ranged between 5.4% (Lafayette, IN in 2013) and 29.8% (Rodney, ON in 2014) (Table 6).

Fluopyram seed treatment significantly increased yield in Urbana, IL in 2013 and Roland, IA; Wanatah, IN; and Rodney ON in 2014 (Table 6). The yield increase by fluopyram seed treatment ranged from 5.7% (Urbana, IL in 2013) to 20.8% (Roland, IA in 2014) in the experiments where significant effect was observed. Overall, a greater yield response was observed in 2014, when SDS severity was higher than in 2013 (Table 6). The yield response to fluopyram seed treatment for each experiment and planting date is shown in Figure 1. Yield response to fluopyram seed treatment was less for the final planting date compared with earlier planting dates (Fig. 1). Some yield responses (21.9%) to fluopyram were negative, and more negative responses were observed from the final planting.

Cultivars resistant to SDS yielded more than susceptible cultivars in three locations in 2013 and two locations in 2014 where significant differences in cultivar resistance were observed (Table 6). A cultivar–seed treatment interaction was detected in Ames, IA in 2014, where yield response to seed treatment was observed only in the susceptible cultivar. A weak correlation between yield and FDX was observed ($r = -0.30$, $P = 0.05$) based on correlations analysis of yield and FDX from all experiments.

Weather. Monthly precipitation during the season had different trends in 2013 and 2014. All locations received more precipitation in 2014 than 2013. Indiana, Iowa, and Ontario all experienced excessively wet conditions in 2014. In Iowa, 2014 precipitation levels over the growing season (May to September) were approximately 33% more than the 30-year average. In Indiana, the cumulative precipitation for the season in 2014 was 87% more than the 30-year average. In Ontario, the month of July was especially wet at both research locations.

Soil temperature differed across the range of planting dates and across locations and years. In general, April and May soil temperatures were cooler than June soil temperatures. However, in Iowa in 2014, soil temperature increased until late May, then decreased during the last planting.

Discussion

Many studies have demonstrated that cool, wet soil conditions favor infection by *F. virguliforme* and, subsequently, increase the risk for SDS and yield loss due to disease. Based on these findings, farmers are encouraged to delay planting soybean until soil is warm and dry to minimize risk of infection. Our research across multiple locations and years indicates that planting soybean early (late April to early May) in the Midwestern United States and Ontario, Canada is not always correlated with higher levels of SDS.

Our research indicates that rainfall during the reproductive phase of the crop was critical to SDS foliar symptom development, regardless of soil temperature at planting. Although planting date significantly affected FDX in 50% of the locations, there was no clear link between lower temperatures at planting and SDS development during reproductive stages. For example, soil temperature was cooler in early plantings compared with late plantings in most locations but SDS was not significantly higher in first plantings compared with late plantings. Regression analysis between soil temperature at planting and FDX did not show significant association. However, soil temperature was measured only at planting in this study. It would be interesting to determine how soil temperature during germination and early vegetative stages will affect SDS in future experiments.

During the 2 years of this study, planting earlier than mid-May reduced the likelihood that high soil moisture conditions, which are favorable for SDS development, would occur during the reproductive stages. In all experiments, early-May plantings resulted in lower or

Table 6. Main effect of planting dates, seed treatment, and cultivar on soybean yield at experiment sites in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on sudden death syndrome (SDS) of soybean

	Least square mean yield (kg/ha)[w]										
	2013					2014					
Variables	Bo, IA	Ur, IL	La, IN	Hg, ON	Rd, ON	Am, IA	Ro, IA	Ur, IL	Wa, IN	Hg, ON	Rd, ON
Planting[x]											
First	…	…	3,636.1 b	4,368.1 a	3,636.1	3,556.9 bc	3,311.7	4,792.2 a	3,884.4	3,894.1 a	4,320.8 a
Second	3,004.8 a	4,164.5 a	4,172.5 a	4,353.3 a	3,673.9	3,962.3 a	2,916.1	4,639.7 a	3,300.8	3,928.0 a	4,033.3 ab
Third	2,756.1 ab	3,909.3 ab	4,209.6 a	4,376.5 a	3,691.8	3,731.2 ab	2,883.5	4,177.9 b	3,304.3	…	3,817.5 b
Fourth	2,179.7 b	3,670.2 b	3,438.9 b	3,522.8 b	3,430.59	3,323.8 c	2,687.0	3,500.4 c	3,006.1	3,169.9 b	3,033.3 c
Diff (%)	…	…	5.4	19.4	5.7	6.6	18.9	27.0	22.6	18.6	29.8
Mean	2,646.9	3,914.7	3,864.3	4,155.2	3,608.1	3,643.5	2,949.6	4,277.5	3,373.9	3,664.0	3,801.2
$P > F$	0.04	0.02	0.01	<0.01	0.73	0.02	0.26	<0.01	0.06	<0.01	<0.01
Seed[y]											
Control	2,570.5	3,806.4	3,584.3	4,092.7	3,529.9	3,526.0	2,671.7	4,256.7	3,119.9	3,660.7	3,581.3
Fluop	2,723.1	4,023.0	3,629.5	4,217.6	3,685.7	3,761.1	3,227.5	4,298.4	3,627.9	3,667.3	4,021.2
Diff (%)	5.9	5.7	1.3	3.1	4.4	6.7	20.8	1.0	16.3	0.2	12.3
$P > F$	0.16	<0.01	0.65	0.13	0.28	0.07	<0.01	0.35	<0.01	0.95	<0.01
Cultivar[z]											
SDS-MS	2,770.2	3,617.2	3,636.6	4,020.1	3,159.1	3,541.8	2,745.3	4,284.2	3,294.9	3,765.7	3,727.2
SDS-MR	2,523.4	4,212.1	3,577.2	4,290.2	4,056.5	3,745.6	3,154.0	4,270.9	3,452.9	3,562.3	3,875.3
Diff (%)	−8.9	16.4	−1.6	6.7	28.4	5.8	14.9	−0.3	4.8	−5.4	4.0
$P > F$	0.10	<0.01	0.53	<0.01	<0.01	0.11	<0.01	0.76	0.02	0.07	0.09

[w] Locations: Boone (Bo), IA; Urbana (Ur), IL; Lafayette (La), IN; Highgate (Hg), ON; Rodney (Rd), ON; Ames (Am), IA; Roland (Ro), IA; and Wanatah (Wa), IN.

[x] Specific dates of planting were different across the experiments. The first, second, third, and fourth planting dates were ranged from 8 to 9 May, 14 to 17 May, 30 May to 4 June, and 12 to 22 June, respectively, in 2013. In 2014, the first, second, third, and fourth planting dates ranged from 26 April to 12 May, 6 to 20 May, 20 May to 6 June, and 9 to 23 June, respectively. Means separation was done by Fisher's protected least significant difference. Means across the planting dates followed by the same letter with in each location do not differ significantly at $P = 0.05$. Percent difference (Diff) measured across the first to fourth planting dates.

[y] Seed treatments: Control = base seed treatment by Bayer CropScience with prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed), metalaxyl (Allegiance, 0.02 mg a.i./seed), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed); and fluopyram (Fluop) (ILeVO, applied in addition to base seed treatment at 0.15 mg a.i/seed). Percent difference (Diff) for seed treatment was estimated by using the formula Difference (%) = [(fluopyram mean − control mean)/control mean] × 100.

[z] Cultivar SDS-MS = moderately susceptible to SDS and SDS-MR = moderately resistant to SDS. Percent difference (Diff) for cultivars was estimated by using the formula Difference (%) = [(MR mean − MS mean)/MS mean] × 100. Cultivars were different across the sites in 2013.

similar FDX compared with treatments planted in mid-May or early June. When rainfall around flowering was constant (12 to 15 cm/month) in early and late plantings, FDX levels were the same or higher in late-planted soybean as in early planted soybean. Hershman et al. (1990) reported that SDS was reduced by late plantings in 2 of 3 years; however, when conditions were wet, SDS symptoms in early and late plantings were the same, and some cultivars had more severe SDS in late plantings. Similarly, Wrather et al. (1995) reported an inconsistent effect of planting date on SDS under tilled and no-till conditions. Multiple factors, including weather during reproductive stages, soil conditions, cultivar, preceding crop, tillage operation, inoculum density, and site geography, may have obscured the impact of planting date on SDS development in our study. Part of the variation could also be attributed to the difference in plant ages at scoring times across the experiments. Although we used a different range of planting dates than these previous studies, in general, we did not observe less disease in June-planted than in May-planted soybean in many sites.

Soybean yield was affected by planting date, seed treatment, and cultivar, whereas yield was weakly correlated with FDX because of mild or no disease in some of the sites in 2013. Hershman et al. (1990) observed that yield was not affected if SDS symptoms were low or developed late, during or after pod-filling. Correlations of SDS with yield ranged from high to low in previous studies (Anderson et al. 2015; Hershman et al. 1990; Rupe et al. 1993).

Yield declined sharply when soybean crops were planted after the last week of May in all experiments, regardless of seed treatment and cultivar. This included experiments where SDS was not evident, probably because of premature flowering and shorter insolation associated with late plantings. Licht at al. (2013) concluded that early-planted soybean produced a denser crop canopy that increased photosynthesis by capturing more available sunlight and resulted in higher yield. In our study, we observed up to 29.8% yield increase when soybean cultivars were planted in early May compared with mid-June. Beatty et al. (1982) reported a 50% yield reduction when soybean was planted in July compared with April or May, as did others (Egli and Bruening 1992; Wrather et al. 1995). However, the lack of correlation between planting date and SDS severity observed in this study indicates that farmers do not have to delay planting in the Midwest to prevent yield loss due to SDS.

In most experiments, greater root rot was observed in May compared with June plantings, which is likely due to high soil moisture

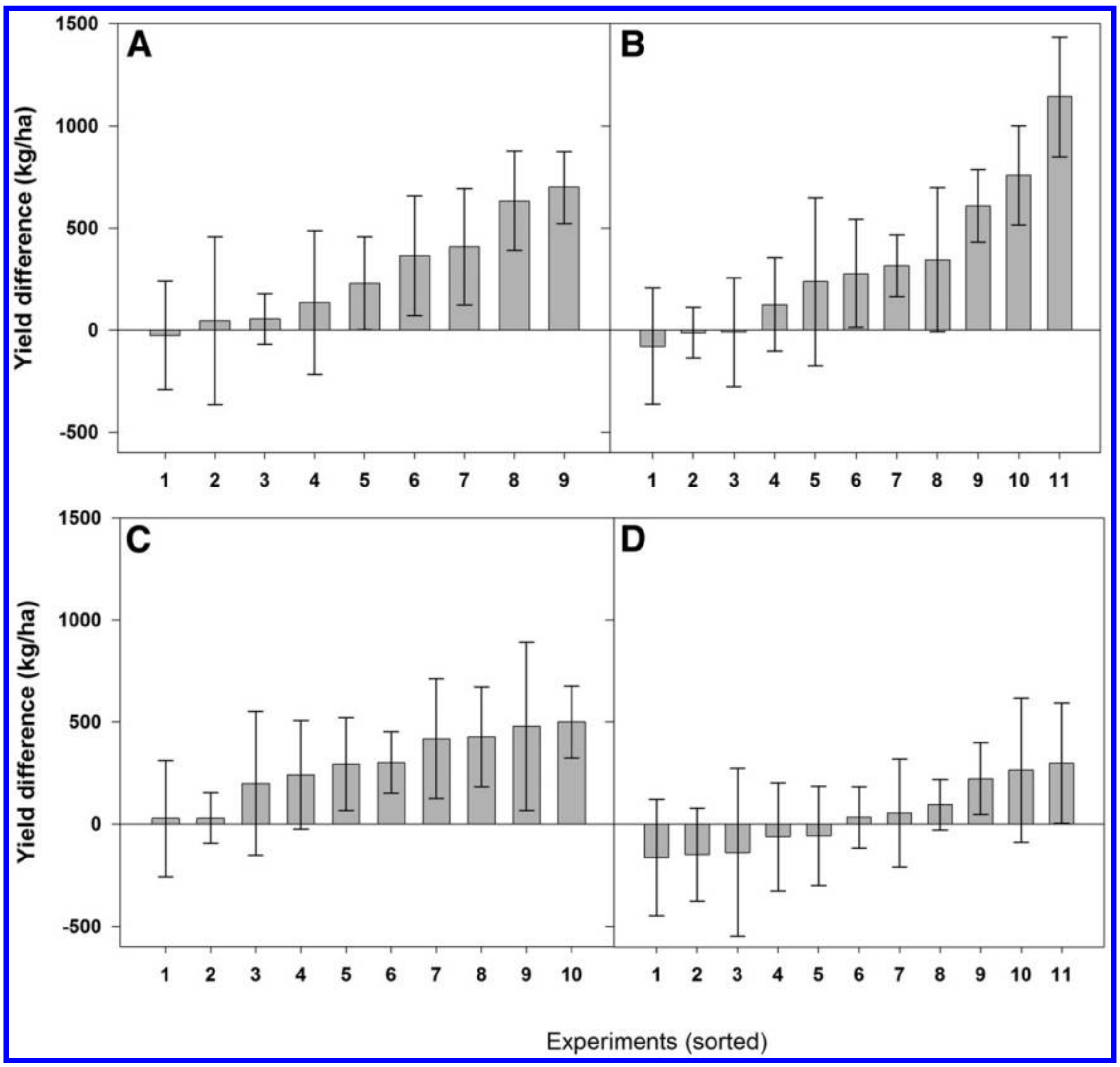

Fig. 1. Yield response to fluopyram (fluopyram mean – control mean) sorted from lowest to highest for four planting dates: **A,** first planting (early May); **B,** second planting (mid-May); **C,** third planting (early June); and **D,** fourth planting (mid-June) at the experiment sites in three states of the United States and Ontario, Canada from 2013 to 2014, examining the impact of planting date, cultivar, and seed treatment on soybean sudden death syndrome (SDS) of soybean. Each bar represents yield difference between fluopyram and control treatments and the vertical lines extending the bar represents the standard error of difference. Seed treatment means were combined over two varieties and four replications. Control = base seed treatment by Bayer CropScience with prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed), metalaxyl (Allegiance, 0.02 mg a.i./seed), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed); and fluopyram (ILeVO, applied in addition to base seed treatment at 0.15 mg a.i/seed). Experiment locations were Ames, Boone, and Roland, IA; Lafayette and Wanatah, IN; Urbana, IL; and Highgate and Rodney, ON.

and cool temperatures during the beginning of crop development in early-planted soybean. However, there was no significant correlation between root rot severity and foliar SDS. Previous studies (Scherm and Yang 1996; Wrather et al. 1995) also reported that root symptoms were not correlated with foliar symptoms of SDS. This could be due to the fact that the environment or physiological conditions that favor these two symptoms are different. For instance, the environment or host physiology that favors toxin translocation is more important for foliar symptom expression versus root rot development. Additionally, other soilborne pathogens which were not evaluated in this study could also be responsible for some of the root damage observed.

Seedling populations generally were lower when soybean was planted early, perhaps because cool soil temperatures suppressed seed germination or favored root rot pathogens (Rizvi and Yang 1996). Wrather et al. (1996) also observed a reduction in seedling establishment in early plantings when soils were cold. Despite the lower plant population, yields in early-planted plots exceeded yields in late plantings.

Fluopyram seed treatment reduced SDS in 66.7% of the experiments where SDS was observed and increased overall yield 6.8% compared with the control. Fluopyram efficacy was more consistent in 2014, when conditions favored SDS development and higher FDX was observed among locations. Fluopyram reduced root rot in two of the five locations. Seed treatment–cultivar and seed treatment–planting date interactions were significant at one location for root rot but, in general, fluopyram-treated plots had less root rot. The efficacy of fluopyram for SDS is novel, because previous evaluations of other seed treatment active ingredients for SDS were ineffective (Weems et al. 2015). Fluopyram seed treatment reduced seedling populations in three experiments. This reduction in initial plant establishment could be related to the phytotoxicity observed in fluopyram-treated seed. Field observations suggest that fluopyram enters the germinating seed and moves systematically through the plant (J. Riggs, personal communication), which causes phytotoxicity on the cotyledons. Despite stand reduction, yield was not affected, and soybean plants may have compensated for the plant population loss (Board 2000).

Cultivars rated as susceptible to SDS had greater FDX in most experiments, which is similar to previous reports (Hartman et al. 1997; Hershman et al. 1990; Melgar and Roy 1994; Rupe et al. 1991; Wrather et al. 1995). Moderately resistant cultivars had less root rot in two of the five experiments where root rot was rated. However, in some locations, the same moderately resistant cultivars did not express resistance against root rot or SDS. This supports the findings of Anderson et al. (2015), which suggested that resistance expression might be influenced by environmental conditions. As expected, moderately resistant cultivars yielded more than susceptible cultivars in all the experiments where significant differences in SDS severity were observed.

This study found that planting soybean in early May and choosing moderately resistant cultivars were incrementally beneficial to SDS management and yield enhancement. Although host resistance is the preferred way to manage SDS, cultivars with a high level of resistance are not always available and, in years when environmental conditions are highly conducive for disease development, resistance alone may not provide adequate control of SDS. Fluopyram suppressed SDS more consistently in years where high moisture occurred during soybean reproductive stages of soybean and may be an option for farmers who are planting into fields at high risk for SDS. Our research suggests that delayed planting should not be recommended for management of SDS. Although the general effect of high soil moisture on SDS incidence and severity is well documented (Hershman et al. 1990; Leandro et al. 2013; Wrather et al. 1995), understanding the specific relationship between reproductive plant stages and high soil moisture conditions is crucial to SDS management strategies aiming to disrupt this association.

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