Effect of Seed Treatment and Foliar Crop Protection Products on Sudden Death Syndrome and Yield of Soybean

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Abstract

Sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is an important soilborne disease of soybean. Risk of SDS increases when cool and wet conditions occur soon after planting. Recently, multiple seed treatment and foliar products have been registered and advertised for management of SDS but not all have been tested side by side in the same field experiment at multiple field locations. In 2015 and 2016, seed treatment fungicides fluopyram and thiabendazole; seed treatment biochemical pesticides citric acid and saponins extract of *Chenopodium quinoa*; foliar fungicides fluoxastrobin + flutriafol; and an herbicide, lactofen, were evaluated in Illinois, Indiana, Iowa, Michigan, South Dakota, Wisconsin, and Ontario for SDS management. Treatments were tested on SDS-resistant and -susceptible cultivars at each location. Overall, fluopyram provided the highest level of control of root rot and foliar symptoms of SDS among all the treatments. Foliar application of lactofen reduced foliar symptoms in some cases but produced the lowest yield. In 2015, fluopyram

reduced the foliar disease index (FDX) by over 50% in both resistant and susceptible cultivars and provided 8.9% yield benefit in susceptible cultivars and 3.5% yield benefit in resistant cultivars compared with the base seed treatment (control). In 2016, fluopyram reduced FDX in both cultivars by over 40% compared with the base seed treatment. For yield in 2016, treatment effect was not significant in the susceptible cultivar while, in the resistant cultivar, fluopyram provided 3.5% greater yield than the base seed treatment. In this study, planting resistant cultivars and using fluopyram seed treatment were the most effective tools for SDS management. However, plant resistance provided an overall better yield-advantage than using fluopyram seed treatment alone. Effective seed treatments can be an economically viable consideration to complement resistant cultivars for managing SDS.

Keywords: chemical, cultivar/resistance, disease management, field crops, fungi, oilseeds and legumes.

Sudden death syndrome (SDS) is one of the most destructive diseases of soybean (*Glycine max* (L) Merr.) in the United States and Canada (Allen et al. 2017; Koenning and Wrather 2010; Wrather and Koenning 2009; Wrather et al. 2010). The disease was first observed in Arkansas in 1971 (Hirrel 1983) and has since spread to nearly all major soybean-growing states in the United States and to Ontario, Canada (Anderson and Tenuta 1998; Hartman et al. 2015a,b).

In the United States and Canada, *Fusarium virguliforme* is the primary pathogen that causes SDS (Aoki et al. 2003; Hartman et al. 2015b). *F. virguliforme* resides in soil as thick-walled chlamydospores or in crop residue and on the cysts of soybean cyst nematode (SCN) as mycelium. The disease cycle begins with the colonization of the soybean root soon after the radicle emerges from seed under wet and cool conditions (Gongora-Canul and Leandro 2011; Huang and Hartman 1998; Scherm and Yang 1996; Wang et al. 2019). These initial root infections cause root rot, reducing the ability of the root to absorb water and nutrients

from the soil. The fungus also produces toxins that translocate through the xylem from the roots to the foliage, resulting in foliar symptoms (Brar et al. 2011; Chang et al. 2016; Pudake et al. 2013). Foliar symptoms are characterized by interveinal chlorosis and necrosis, followed by premature defoliation. In severe cases, flower and pod abortion and premature plant death may occur (Hartman et al. 2015b).

Host resistance is an effective tactic for SDS management. However, no lines with complete resistance have been identified in the soybean germplasm, and currently available commercial cultivars are only partially resistant to this disease. Resistance to SDS is polygenic, governed by quantitative trait loci (QTL), and, as a result, developing cultivars with improved SDS resistance is challenging (Brzostowski et al. 2018; Chang et al. 2018). When conditions are very favorable for SDS in a field infested with *F. virguliforme*, partial resistance may not prevent yield loss. Several cultural practices such as planting date, crop rotation, and tillage have been examined for their role in reducing SDS severity but the effects of these practices on SDS are not consistent or require further study (Kandel et al. 2016 a,b, 2019; Leandro et al. 2012, 2018; Marburger et al. 2016; Vosberg et al. 2017). SCN (*Heterodera glycines*) management is also important, because these nematodes have been found to play a role in increasing SDS severity (Kandel et al. 2017; McLean and Lawrence 1993; Melgar et al. 1994; Roy et al. 1989; Scherm et al. 1998; Xing and Westphal 2006); however, crop rotations with corn, which reduces SCN populations in fields over time, do not appear to help with management of SDS (Navi and Yang 2016a).

Previous studies (Gaspar et al. 2017; Kandel et al. 2016a,b, 2017, 2018a,b; Vosberg et al. 2017) showed that fluopyram fungicide as a seed treatment, a succinate dehydrogenase inhibitor (SDHI;

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Fungicide Resistance Action Committee [FRAC] group 7), reduced foliar and root symptoms of SDS and resulted in greater yields when compared with standard commercial base seed treatments that included a combination of fungicide, insecticide, and nematostat active ingredients. A seed treatment containing the active ingredient fluopyram known as ILeVO (BASF, Research Triangle Park, NC, U.S.A.) became commercially available for SDS management for U.S. soybean growers in 2015 and for Canadian farmers in 2017. Since 2015, seed treatments, including thiabendazole (Mertect 340-F; Syngenta Crop Protection, Greensboro, NC, U.S.A.), a methyl benzimidazole carbamate (FRAC group 1) fungicide, as well as foliar fungicides with different active ingredients such as fluoxastrobin + flutriafol (Fortix; Cheminova, Inc., Research Triangle Park, NC, U.S.A.), a premix of quinone outside inhibitor (FRAC group 11) and a demethylation inhibitor (FRAC group 3) are now registered and being marketed for SDS management.

In addition to chemical fungicides, biopesticides such as citric acid (Procidic; Greenspire Global, Inc., Des Moines, IA, U.S.A.) and saponins extracted from *Chenopodium quinoa* (Heads Up; Heads Up Plant Protectant, Inc., Saskatchewan, Canada) and *Pasteuria nishizawae* Pn1 (Clariva PN; Syngenta Crop Protection) are also labeled for SDS management. Citric acid has been reported to have broad-spectrum bactericidal and fungicidal activity. Saponins act by activating systemic acquired resistance in plants and have been shown to be effective against SDS and Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) in soybean in one trial (Navi and Yang 2016b). In addition to products that are specifically labeled for SDS management, the herbicide lactofen, which can reduce Sclerotinia stem rot in soybean (Dann et al. 1999; Huzar-Novakowski et al. 2017; Willbur et al. in press), was also evaluated in this study. Examining labeled products side by side under diverse field environments and geographic regions is needed to determine which products are most efficacious against SDS. There is also a need to find chemistries with activity against SDS because reliance on fluopyram alone as a sole active ingredient may not be a sustainable SDS management tool due to the risk of fungicide resistance that can occur with SDHI fungicides (FRAC 2017; Wang et al. 2017).

The objectives of this study were to (i) examine the response of cultivars with different levels of SDS resistance across a broad geographic area and (ii) evaluate how seed treatment and foliar products

affect soybean plant populations, root rot and foliar symptoms of SDS, and yield in SDS-resistant and -susceptible cultivars.

Materials and Methods

Field experiments were established at seven locations in both 2015 and 2016. In 2015, experiments were conducted near Ames and Roland, IA; Decatur, MI; Dixon Springs, IL; and Wanatah, IN in the United States; and Highgate and Rodney, ON, Canada. In 2016, experiments were conducted near Ames and Roland, IA; Arlington, WI; Beresford, SD; Decatur, MI; and Wanatah, IN in the United States; and Highgate, Ontario, Canada (Table 1). In total, eight fungicide treatments, including commercial base seed treatments as controls, were tested at each location. The list of treatments with their application time and rates is shown in Table 2. In brief, treatments included (i) a commercial base seed treatment from Syngenta Crop Protection LLC (Syngenta base) that included a combination of insecticide and fungicide active ingredients: thiamethoxam + mefenoxam + fludioxonil + sedaxane (CruiserMaxx Vibrance); (ii) Syngenta base + *P. nishizawae* Pn1 (Clariva; Syngenta Crop Protection LLC) + thiabendazole (Mertect 340-F; Syngenta Crop Protection LLC); (iii) a commercial base seed treatment from Bayer CropScience (Bayer base) that included a combination of fungicide and insecticide active ingredients: prothioconazole + penflufen + metalaxyl (Evergol Energy), metalaxyl (Allegiance), and imidacloprid + ethoxylated polyarylphenol (Gaucho); (iv) Bayer base + fluopyram (ILeVO; BASF) + clothianidin + *Bacillus firmus* (Poncho VOTiVO; BASF); (v) Bayer base + saponins extracted from *C. quinoa* (Heads Up; Heads Up Plant Protectants, Inc.); (vi) Bayer base followed by (fb) lactofen (Cobra; Valent U.S.A. Corporation, Walnut Creek CA, U.S.A.); (vii) Bayer base fb fluoxastrobin + flutriafol (Fortix; Cheminova, Inc.); and (viii) Bayer base fb citric acid (Procidic; Greenspire Global, Inc.). Lactofen and fluoxastrobin + flutriafol were applied at R1 (beginning bloom) and citric acid was applied at R1 and R4 (full pod) growth stages. Lactofen was applied with crop oil concentrate at 1,169 ml/ha. A nontreated control was not included in this experiment because the fungicide active ingredients used in commercial base seed treatments from the two companies target seedling diseases and have no efficacy on SDS (Weems et al. 2015). Therefore, commercial base seed treatments served as surrogates for a control treatment. Each

Table 1. Field locations, soybean cultivars, seeding rates, and other field activities performed in experiments conducted in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016

Year, location	Cultivar ^x	SR (seed/ha) ^y	Infested ^z	Previous crop	Irrigated
2015					
Ames, IA	S: P25T51R (5), R: P93Y83 (7)	308,881	Yes	Corn	Yes
Roland, IA	S: P25T51R (5), R: P93Y83 (7)	308,881	No	Corn	No
Dixon Springs, IL	S: P33T72R (5), R: P36T86R (8)	345,947	Yes	Corn	Yes
Wanatah, IN	S: P25T51R (5), R: P93Y83 (7)	345,947	Yes	Corn	No
Decatur, MI	S: P25T51R (5), R: P93Y83 (7)	387,955	No	Corn	Yes
Highgate, ON	S: P25T51R (5), R: P93Y83 (7)	637,222	No	Corn	No
Rodney, ON	S: P25T51R (5), R: P93Y83 (7)	637,222	No	Soybean	No
2016					
Ames, IA	S: P22T41R2 (3), R: P22T69R (7)	308,881	Yes	Corn	Yes
Roland, IA	S: P22T41R2 (3), R: P22T69R (7)	308,881	No	Corn	No
Wanatah, IN	S: P22T41R2 (3), R: P22T69R (7)	345,947	Yes	Corn	Yes
Decatur, MI	S: P22T41R2 (3), R: P22T69R (7)	387,955	No	Corn	Yes
Beresford, SD	S: P22T41R2 (3), R: P22T69R (7)	407,723	No	Corn	No
Arlington, WI	S: P22T41R2 (3), R: P22T69R (7)	345,947	Yes	Corn	No
Highgate, ON	S: P22T41R2 (3), R: P22T69R (7)	637,222	No	Corn	No

^x Cultivar and sudden death syndrome (SDS) ratings in parenthesis: S = susceptible to SDS, R = resistant to SDS based on company standard, and P = Pioneer; cultivar ratings were provided on a 1-to-9 scale with 1 = susceptible.

^y SR = seeding rate.

^z Field infestation was done at planting in Illinois, Indiana, Iowa, and Wisconsin. In Illinois, ground sorghum infested with *Fusarium virguliforme* isolate Mont-1 was applied at 4.1 cm³ per linear meter of row. In Indiana, sorghum inocula infested with the mixture of *F. virguliforme* isolates NRRL 22823, 00-11-183, and INS12-10 #3-1 were applied at 4.2 g/m. In Iowa, field experiments were inoculated with locally originated *F. virguliforme* isolate NE305 at 8.3 g of infested sorghum per linear meter of row. In Wisconsin, isolates Fv05, Fv06, and Fv07 were applied together at a rate of 8.2 g of infested sorghum per linear meter of row.

treatment was tested on susceptible and resistant cultivars at each location (Table 1). Pro-Ized red seed colorant and finisher (Peridiam Precise 1010; Bayer CropScience) was added on each seed treatment at the rate of 32.5 and 65 ml, respectively, per 100 kg of seed. Seed were treated with a Hege bowl seed treater (Wintersteiger, Salt Lake City, UT, U.S.A.).

Resistant and susceptible cultivars were tested separately in field experiments (Table 1). Treatments were arranged within each cultivar in a randomized complete block design with four or five replicates. Experiments were established in fields with a history of SDS or fields were infested with *F. virguliforme* isolates that originated locally. *F. virguliforme* inoculum was prepared by infesting autoclaved grain sorghum or oat grains following a previously published protocol (de Farias Neto et al. 2006). Infested grain sorghum or oat grains were applied in-furrow at planting. Individual plots were 5.3 to 9.1 m long and 4 to 6 rows wide with 38.1- to 76.2-cm interrow spacing. Corn was the previous crop in all the locations except in Rodney, Ontario, where soybean was planted in the previous year. Fields were cultivated in the spring with a field cultivator in all locations. Research trials were irrigated at the Illinois location, one location in Iowa, and at the Michigan location. Irrigation and other field operation details are provided in Table 1. Briefly, in Illinois, drip

irrigation was started from the beginning of June and ran for 6 weeks, with approximately 2.5 cm of water delivered weekly. In Indiana in 2016, overhead irrigation was started on 20 June and trials were watered at weekly intervals until 8 August. About 2.5 cm water was applied each time. In Iowa, drip irrigation was set up during the first week of August and ran three to four times each week; approximately 2.5 cm of water was delivered at each time until SDS ratings were completed in both years. In Michigan, up to 2.5 cm of water per week was applied starting from emergence through R5 using an overhead pivot irrigation system. Fertilizer applications and other field operations were performed in each state following their state extension recommendations. Pre- and postemergence herbicides were used as needed at recommended rates to manage weeds.

The foliar treatments were applied using different spray equipment across the experimental locations. In Illinois, Indiana, Michigan, Ontario, and South Dakota, a backpack sprayer with a CO₂-pressurized tank and handheld boom equipped with four to six nozzles was used. In Illinois, four nozzles (TJ-60 8002VS) spaced 48 cm apart were fitted in a 150-cm boom, which was calibrated to deliver 187 liters/ha at 276 kPa of pressure. In Indiana, four TJ-VS 8001 nozzles spaced 51 cm apart were fitted in the 150-cm boom and calibrated to deliver 140 liters/ha at 275 kPa of pressure. In Michigan,

Table 2. List of treatments with application timings and rates that were applied in field experiments performed at Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016

SN	Treatments ^z	Application method	Foliar application rate
1	Syngenta base (SB)	Seed treatment	...
2	SB + <i>Pasteuria nishizawae</i> + thiabendazole	Seed treatment	...
3	Bayer base (BB)	Seed treatment	...
4	BB + fluopyram + clothianidin + <i>Bacillus firmus</i>	Seed treatment	...
5	BB + saponins extract of <i>Chenopodium quinoa</i>	Seed treatment	...
6	BB fb lactofen	Foliar at R1	439 ml/ha
7	BB fb fluoxastrobin + flutriafol	Foliar at R1	365 ml/ha
8	BB fb citric acid	Foliar at R1 and R4	219 then 439 ml/ha

^z Treatments were SB that included a combination insecticide and fungicide active ingredients thiamethoxam + mefenoxam + fludioxonil + sedaxane (Cruiser-Maxx Vibrance; Syngenta Crop Protection LLC, Greensboro, NC, U.S.A.); SB + *Pasteuria nishizawae* Pn1 (Clariva; Syngenta Crop Protection LLC) + thiabendazole (Mertect 340-F; Syngenta Crop Protection LLC); BB = base seed treatment from Bayer CropScience, Research Triangle Park, NC, U.S.A. that included a combination of fungicide and insecticide active ingredients prothioconazole + penflufen + metalaxyl (Evergol Energy); metalaxyl (Allegiance) and imidacloprid + ethoxylated polyarylphenol (Gaucho); BB + fluopyram (ILeVO; BASF, Research Triangle Park, NC, U.S.A.) + clothianidin + *Bacillus firmus* (Poncho VOTiVO; BASF), BB + saponins extracted from *Chenopodium quinoa* (Heads Up; Heads Up Plant Protectants, Inc., Saskatchewan, Canada), BB followed by (fb) lactofen (Cobra; Valent U.S.A. Corporation, Walnut Creek CA, U.S.A.); BB fb fluoxastrobin + flutriafol (Fortix; Cheminova, Inc., Research Triangle Park, NC, U.S.A.); and BB fb citric acid (Procidic; Greenspire Global, Inc. Des Moines, IA, U.S.A.). Lactofen was applied with crop oil concentrate at 1,169 ml/ha.

Table 3. Dates of planting, foliar treatment application, disease rating, and harvest for field experiments established in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016^z

Year, location	DOP	Application GS		RR rating	SDS rating	GS	Harvest
		R1	R4				
2015							
Ames, IA	19 Apr	15 Jul	4 Aug	10 Aug	27 Aug	R5.8	22 Sep
Roland, IA	13 May	15 Jul	4 Aug	19 Aug	11 Sep	R6.0–R6.5	9 Oct
Dixon Springs, IL	2 May	22 Jun	15 Jul	22 Jun	21 Aug	R6.0	17 Oct
Wanatah, IN	1 Jun	21 Jul	11 Aug	12 Aug	9 Sep	R6.0	19 Oct
Decatur, MI	1 May	29 Jun	15 Jul	17 Jul	20 Aug	R6.0–R6.5	2 Oct
Highgate, ON	11 May	27 Jul	16 Aug	10 Aug	2 Sep	R6.0–R6.5	2 Oct
Rodney, ON	15 May	27 Jul	16 Aug	11 Aug	11 Sep	R6.0–R6.5	13 Oct
2016							
Ames, IA	6 May	30 Jun	9 Aug	17 Aug	1 Sep	R6.0	28 Sep
Roland, IA	6 May	30 Jun	15 Aug	17 Aug	1 Sep	R5.8	14 Oct
Wanatah, IN	23 May	2 Jul	4 Aug	5 Aug	6 Sep	R6.0	10 Oct
Decatur, MI	9 May	NA	NA	NA	23 Aug	R6.0	4 Oct
Beresford, SD	20 May	22 Jul	10 Aug	17 Aug	NA	NA	24 Oct
Arlington, WI	19 May	6 Jul	4 Aug	NA	NA	NA	11 Oct
Highgate, ON	31 May	19 Jul	15 Aug	17 Aug	31 Aug	R6.0	13 Oct

^z Abbreviations: GS = growth stages (R1 = beginning bloom, R4 = full pod, R5 = beginning seed, and R6 = full seed), DOP = date of planting, RR = root rot, SDS = sudden death syndrome, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, and NA = not available.

the spray boom consisted of six nozzles (TeeJet 11001VS) spaced 51 cm apart and was calibrated to apply 140 liters/ha. In Ontario and South Dakota, a 120-cm handheld boom with four 8002VS TeeJet nozzles spaced at 30 cm apart was calibrated to deliver 140 liters/ha at 241 kPa of pressure. In Iowa, foliar treatments were applied using a self-propelled small research plot sprayer equipped with six flat-fan nozzles (TeeJet 11015 XR). Nozzles were spaced 51 cm apart, which delivered 140 liters/ha at a pressure of 241 kPa. In Wisconsin, foliar treatments were applied using a 305-cm handheld boom equipped with eight flat-fan nozzles (TeeJet 8002XR). Nozzles were spaced 38 cm apart and delivered 187 liters/ha at 220 kPa of pressure.

Plant population, root rot severity, foliar SDS incidence and severity, yield, and moisture data were collected from each field experiment. The number of live plants was counted to calculate plant population from either a 3-m section or the entire two to four rows of each plot at early vegetative growth stages (V2 to V3) (Fehr et al. 1971). At approximately the R4 growth stage, 10 to 12 plants from outside rows (nonyield rows) were collected, roots were washed, and root rot severity was visually estimated as a percentage of root area with brown discoloration or with lesions. Root rot data were not collected at the Dixon Springs, IL location in 2015. Foliar SDS incidence and severity were recorded between R5 and R6 growth stages (Table 3). Foliar disease incidence (DI) was estimated as the percentage of plants with foliar symptoms in the middle two rows of each plot, and disease severity (DS) was recorded on a 1-to-9 scale based on area of chlorotic or necrotic lesions on the leaf and premature defoliation, where 1 = 1 to 10% leaf surface chlorotic or 1 to 5% necrotic and 9 = premature plant death (Gibson et al. 1994). Foliar SDS disease index (FDX) was calculated from DI and DS by using the following formula: $FDX = DI \times DS/9$. At or shortly after full maturity (R8) (Table 3), the two middle rows of each plot were harvested with a small plot combine, and yields were calculated and adjusted to 13% moisture.

Data analysis was performed using SAS (version 9.4; SAS Institute, Inc., Cary, NC, U.S.A.). Mixed-model analysis of variance was performed using PROC GLIMMIX for all measured variables. Treatment and cultivar were defined as fixed effects; location and replication within a location were used as random effects. Statistical models are given below by equation 1 (cultivars were combined) and equation 2 (cultivars were separate). Treatment least square means were obtained using the *lsmeans* statement. Fisher's protected least significant difference was used to compare treatment means at $\alpha = 0.05$. For root rot, only the five seed treatments were analyzed, excluding treatments containing foliar applications.

$$Y_{ijk} = \theta + L_i + \alpha_j + \beta_k + b(L)_{ir} + \alpha\beta_{jk} + e_{ijk} \quad (1)$$

$$L_i \sim N[0, \sigma_{(L)}^2], b(L)_{ir} \sim N[0, \sigma_{b(L)}^2], \text{ and } e_{ijk} \sim N(0, \sigma_e^2)$$

where Y = response (dependent) variable for the i th level of location, j th level of cultivar, k th level of crop protection products, and r th

level of block at i th level of location; θ = constant (intercept); L_i = effect of the i th level of location (random effect); α_j = effect of the j th level of cultivar (fixed effect); β_k = effect of the k th level of crop protection products (fixed effect); $b(L)_{ir}$ = effect of the r th level of block at i th level of location (random effect); $\alpha\beta_{jk}$ = interaction product (effect of j th level of cultivar and k th level of crop protection product, fixed effect); and e_{ijk} = residual error.

$$Y_{ijk} = \theta + L_i + \alpha_j + b(L)_{ik} + e_{ijk} \quad (2)$$

$$L_i \sim N[0, \sigma_{(L)}^2], b(L)_{ik} \sim N[0, \sigma_{b(L)}^2], \text{ and } e_{ijk} \sim N(0, \sigma_e^2)$$

where Y = response (dependent) variable for the i th level of location, j th level of crop protection products, and k th level of block at i th level of location; θ = constant (intercept); L_i = effect of the i th level of location (random effect); α_j = effect of the j th level of crop protection products (fixed effect); $b(L)_{ik}$ = effect of the k th level of block at the i th level of location (random effect); and e_{ijk} = residual error.

Fig. 1. Box plots of the distribution of root rot raw data from experimental plots grouped by year and cultivar (S = susceptible and R = resistant to sudden death syndrome). Solid lines within a box represent the median and broken lines represent the mean. Top and bottom lines of the boxes are 75th and 25th percentiles, respectively, and the vertical lines extending the box are 90th and 10th percentiles. Locations: Am, IA = Ames, IA; Ro, IA = Roland, IA; Wa, IN = Wanatah, IN; De, MI = Decatur, MI; Hg, ON = Highgate, ON; and Rd, ON = Rodney, ON.

Table 4. Least square means of the plant population recorded in susceptible (S) and resistant (R) cultivars in the field experiments carried out in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016²

Treatments	Plant population (number of plants/ha)					
	2015			2016		
	S	R	Mean	S	R	Mean
Syngenta base (SB)	301,583 b	306,928 a	307,829 a	312,611 a	295,767 a	304,178 a
SB + <i>Pasteuria nishizawae</i> + thiabendazole	303,387 b	305,808 a	307,889 a	314,580 a	295,152 a	304,545 a
Bayer base (BB)	297,753 b	279,546 b	288,692 b	283,411 b	262,648 b	273,019 b
BB + fluopyram + clothianidin + <i>Bacillus firmus</i>	323,380 a	307,118 a	315,291 a	288,475 b	283,455 a	285,954 b
BB + saponins extract of <i>Chenopodium quinoa</i>	295,752 b	285,239 ab	290,271 b	279,428 b	275,501 ab	277,332 b
Mean	307,749 A	296,239 B	...	295,505 A	282,506 B	...
P > F	0.009	0.043	<0.01	0.001	0.008	<0.01

² Means were compared using Fisher's protected least significant difference at $\alpha = 0.05$. Means followed by the same lowercase letter within a column and the same uppercase letter between two cultivars within a year do not differ significantly.

Results

Plant population. In 2015, plant population was affected by seed treatment in both resistant and susceptible cultivars (Table 4). Bayer base + fluopyram + clothianidin + *B. firmus* had greater plant populations compared with all other treatments in the susceptible cultivar. However, in the resistant cultivar, Bayer base + fluopyram + clothianidin + *B. firmus* resulted in greater plant population compared with the Bayer base seed treatment only. Plant populations were similar across all other treatments in the resistant cultivar (Table 4). Bayer base + fluopyram + clothianidin + *B. firmus* resulted in greater plant population than the Bayer base in susceptible (8.6%) and resistant cultivars (9.9%) (Table 4).

In 2016, the highest plant populations were recorded in Syngenta base and Syngenta base + *P. nishizawae* + thiabendazole in the susceptible cultivar. All other treatments in the susceptible cultivar had statistically similar plant populations. In the resistant cultivar, the lowest plant population was observed in the Bayer base treatment, while the other treatments were not statistically different from each other (Table 4). The cultivar–treatment interaction was not statistically significant in any year. Resistant cultivars had 3.7% lower plant populations than the susceptible cultivars in 2015 and 4.4% lower in 2016.

Root rot severity. Box plots showing the distribution of root rot raw data in both cultivars across the experiment locations and years are given in Figure 1. In 2015, root rot severity was below 10% in control treatments in both cultivars (Table 5). The treatment effect for root rot was significant for the susceptible cultivars only (Table 5). Bayer base + fluopyram + clothianidin + *B. firmus* reduced root rot compared with Syngenta base and Bayer base + saponins extract of *C. quinoa*. Bayer base + fluopyram + clothianidin + *B. firmus*, Syngenta base + *P. nishizawae* + thiabendazole, and Bayer base had statistically similar levels of root rot in the susceptible cultivar (Table 5).

In 2016, the susceptible cultivar had a root rot severity of 15.6% for the Syngenta base and 13.7% for the Bayer base seed treatments. Treatment affected root rot severity in both cultivars (Table 5). In the susceptible cultivar, Bayer base + fluopyram + clothianidin + *B. firmus* reduced root rot compared with Bayer base, Syngenta base, and Syngenta base + *P. nishizawae* + thiabendazole; however, Bayer base + fluopyram + clothianidin + *B. firmus* and Bayer base + saponins extract of *C. quinoa* had similar levels of root rot. In the resistant cultivar, Bayer base + fluopyram + clothianidin + *B. firmus* reduced root rot compared with Bayer base, Bayer base + saponins extract of *C. quinoa*, and Syngenta base + *P. nishizawae* + thiabendazole; however, Bayer base + fluopyram + clothianidin + *B. firmus* and Syngenta base had similar root rot levels.

Bayer base + fluopyram + clothianidin + *B. firmus* reduced root rot severity by 53% in susceptible and by 45% in resistant cultivars compared with the Bayer base, which showed a root rot severity of 13.7% in susceptible and 11.7% in resistant cultivars. (Table 5). The cultivar–treatment interaction was not significant in any year.

FDX. Foliar SDS symptoms were observed at moderate to high levels in susceptible cultivars at many locations, indicating that conditions were favorable for SDS development in both years (Fig. 2) and FDX was not statistically different between the 2 years ($P = 0.68$). Other foliar and stem diseases, including those with similar symptoms to SDS such as brown stem rot (caused by *Cadophora gregata*) and southern stem canker (caused by *Diaporthe aspalathi*), were not observed in any of the trials.

The treatment effect was significant for FDX in both resistant and susceptible cultivars in both years (Table 6). In 2015, the overall FDX in susceptible cultivars was 17.9 in Bayer base and 21.8 in Syngenta base. The Bayer base + fluopyram + clothianidin + *B. firmus* seed treatment had the lowest FDX among all treatments in the susceptible cultivars, with a 50% reduction compared with Bayer base. No significant difference between the Syngenta base and Syngenta base + *P. nishizawae* + thiabendazole seed treatment was observed in any cultivar. Similarly, Bayer base + saponins of *C. quinoa* and foliar applications with lactofen, fluoxastrobin + flutriafol, and citric acid did not result

Fig. 2. Box plots of the distribution of foliar disease index (FDX = disease incidence $\times$ disease severity/9) from experimental plots grouped by year and cultivar (S = susceptible and R = resistant to sudden death syndrome). Solid lines within a box represent the median and broken lines represent the mean. Top and bottom lines of the boxes are 75th and 25th percentiles, respectively, and the vertical lines extending from the box are 90th and 10th percentiles. Locations: Am, IA = Ames, IA; Ro, IA = Roland, IA; Ds, IL = Dixon Springs, IL; Wa, IN = Wanatah, IN; De, MI = Decatur, MI; Hg, ON = Highgate, ON; and Rd, ON = Rodney, ON.

Table 5. Least square means of the root rot recorded in susceptible (S) and resistant (R) cultivars in the field experiments carried out in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016^z

Treatments	Root rot (%)					
	2015			2016		
	S	R	Mean	S	R	Mean
Syngenta base (SB)	9.3 a	6.7	8.3 a	15.3 a	8.7 ab	12.0 a
SB + <i>Pasteuria nishizawae</i> + thiabendazole	8.3 ab	6.3	7.5 a	14.8 a	9.5 a	12.0 a
Bayer base (BB)	6.6 ab	6.2	6.5 a	13.7 a	11.7 a	12.7 a
BB + fluopyram + clothianidin + <i>Bacillus firmus</i>	4.2 b	4.0	4.3 b	8.4 b	5.5 b	7.0 b
BB + saponins extract of <i>Chenopodium quinoa</i>	9.3 a	6.1	7.8 a	10.7 ab	9.9 a	10.2 a
Mean	7.9 A	5.9 B	...	12.5 A	9.0 B	...
$P > F$	0.042	0.166	0.002	0.045	0.016	<0.01

^z Means were compared using Fisher's protected least significant difference at $\alpha = 0.05$. Means followed by the same lowercase letter within a column and the same uppercase letter between two cultivars within a year do not differ significantly.

in lower FDX than the Bayer base alone in susceptible cultivars (Table 6). Disease levels were overall much lower in resistant cultivars, with an overall FDX in Bayer base of 3.1% and in Syngenta base of 3.8%. In resistant cultivars, Bayer base + fluopyram + clothianidin + *B. firmus* and Bayer base fb lactofen had lower FDX than Syngenta base + *P. nishizawae* + thiabendazole and Bayer base fb citric acid. All other treatments were statistically similar.

In 2016, no SDS symptoms were observed in Beresford, SD and Arlington, WI, whereas all other locations recorded over 25% FDX in Bayer base and Syngenta base in susceptible cultivars, except Highgate, ON (FDX < 5%). In the susceptible cultivar, Bayer base + fluopyram + clothianidin + *B. firmus* reduced FDX by 41% compared with Bayer base alone. Foliar products did not affect FDX when compared with Bayer base. Bayer base + fluopyram + clothianidin + *B. firmus* had lower levels of FDX compared with all treatments, except Bayer base + saponins extract of *C. quinoa* and Bayer base fb lactofen. (Table 6). In the resistant cultivar, disease severity was much lower compared with the susceptible cultivar, with an average FDX of 4.1 in the Bayer base and 4.3 in the Syngenta base treatments. Bayer base + fluopyram + clothianidin + *B. firmus* reduced FDX compared with all treatments except Bayer base fb lactofen. No foliar products reduced disease compared with Bayer base alone (Table 6).

Cultivars were significantly different for FDX in both years ($P < 0.01$). Resistant cultivars reduced FDX by 75% in 2015 and by 84% in 2016, compared with the susceptible cultivars, which had 18.9 FDX in 2015 and 24.1 in 2016 (Table 6). Cultivar–treatment interaction was not statistically significant in any year.

Yield. In 2015, treatment significantly affected grain yield in the susceptible cultivar (Table 7). In the susceptible cultivar, the Bayer base + fluopyram + clothianidin + *B. firmus* seed treatment had the highest yield, with an 8.9% increase over the Bayer base treatment. (Table 7). Bayer base fb lactofen treatment had significantly lower yield compared with all other treatments. All other treatments had similar yields. (Table 7). In the resistant cultivars, treatment effect was marginally significant ($P = 0.061$).

In 2016, soybean yield was greater than 2015, although similar levels of SDS were observed in both years. Treatments affected grain yield only in the resistant cultivar (Table 7). Syngenta base had statistically greater yield than the Bayer base, Bayer base fb lactofen, Bayer base + saponins of *C. quinoa*, and Bayer base fb citric acid. Syngenta base and Syngenta base + *P. nishizawae* + thiabendazole were not statistically different and no foliar treatments resulted in greater yield than the Bayer base treatment.

The cultivar–treatment interaction was not statistically significant in any year. Resistant cultivars produced a greater yield in both years, with an increase of 11% in 2015 and 10% in 2016 over the

susceptible cultivars, which produced 3,451 kg/ha in 2015 and 4,629 kg/ha in 2016.

Discussion

Overall, fluopyram was the most effective treatment for SDS management, supporting previous studies (Gaspar et al. 2017; Kandel et al. 2016a,b, 2018a,b; Vosberg et al. 2017). None of the other fungicide or biological seed treatments evaluated in this study were significantly better than their respective base seed treatment in terms of their ability to reduce root rot and foliar symptoms of SDS. The disease response to fluopyram, as reflected by percent reduction in root rot and FDX, was not significantly different between susceptible and resistant cultivars. However, the yield benefit was greater in susceptible cultivars than resistant cultivars in 2015. A previous report analyzing over 200 studies from multiple field experiments also reported that the disease and yield response to fluopyram was not influenced by cultivar susceptibility; however, disease severity greatly influenced the yield response to fluopyram, with a greater yield response in higher disease conditions (Kandel et al. 2018a).

Resistant cultivars resulted in lower root rot, lower FDX, and greater yield than the susceptible cultivars, which is similar to what has been reported previously (Kandel et al. 2016b); however, in this study, the resistant cultivars performed better than the previous evaluations (Kandel et al. 2016b). There were significant differences between the susceptible and resistant cultivars in FDX (above 75% in both years) and yield (above 10%) in this study. The cultivar reaction to foliar symptoms of SDS was also consistent across the locations and follows the company ratings for these cultivars. These results reinforce the integrated pest management concept that the selection of a cultivar with SDS resistance is the primary management strategy for SDS. However, under high disease pressure, resistant cultivars may result in a level of disease similar to susceptible cultivars and suffer a significant yield loss (Kandel et al. 2016b). In addition, some resistance QTL that confer resistances against leaf symptoms are not associated with decreased root rot (Njiti et al. 1998). Those cultivars that are advertised as moderately resistant to SDS may not have resistance to early season root rot, which can also reduce yield due to poorly developed root systems. Therefore, under some circumstances, coupling the use of the most resistant cultivar available with an efficacious seed treatment may be warranted.

Fluopyram seed treatment was followed by foliar application of lactofen at R1 to R2 in an attempt to reduce FDX. Although the overall mean was not statistically different from Bayer base, the lactofen treatment did show the same level of FDX as the fluopyram seed treatment and lower FDX than some other treatments in most cases, meaning it may also have some effect against SDS. Similarly, a previous study by Sanogo et al. (2000) also reported a reduction in foliar

Table 6. Least square means of the foliar disease index (FDX) recorded in susceptible (S) and resistant (R) cultivars in the field experiments carried out in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016^y

Treatments ^z	2015			2016		
	S	R	Mean	S	R	Mean
Syngenta base (SB)	21.8 ab	3.8 ab	14.1 a	31.7 a	4.3 ab	18.0 a
SB + <i>Pasteuria nishizawae</i> + thiabendazole	21.4 ab	5.3 a	14.8 a	25.9 abc	4.1 ab	15.0 ab
Bayer base (BB)	17.1 ab	3.1 ab	10.7 a	25.7 abc	4.1 ab	14.8 ab
BB + fluopyram + clothianidin + <i>Bacillus firmus</i>	8.0 c	1.6 b	5.0 b	15.2 d	1.7 c	8.4 c
BB + saponins extract of <i>Chenopodium quinoa</i>	16.1 b	5.2 a	11.0 a	20.6 bcd	4.9 ab	13.3 abc
BB followed by (fb) lactofen	19.2 ab	2.0 b	10.7 a	19.1 cd	3.6 bc	11.3 bc
BB fb fluoxastrobin + flutriafol	22.4 ab	4.0 ab	13.4 a	26.2 abc	3.8 b	15.0 ab
BB fb citric acid	24.1 a	5.0 a	14.7 a	27.6 ab	5.8 a	16.7 ab
Mean	18.9 A	4.7 B	...	24.1 A	4.0 B	...
P value	0.002	0.015	<0.01	0.001	0.010	<0.01

^y Means were compared using Fisher's protected least significant difference at $\alpha = 0.05$. Means followed by the same lowercase letter within a column and same uppercase letter between two cultivars within a year do not differ significantly. FDX of sudden death syndrome (SDS) was calculated combining disease incidence, estimated as percentage of plants with SDS symptoms per plot, and severity, scored on 1-to-9 scale based on the percentage of leaf area with chlorosis, necrosis, and premature defoliation.

^z Lactofen and fluoxastrobin + flutriafol were applied at R1 (beginning bloom) and citric acid was applied at R1 and R4 (full pod) growth stages. Other treatments were applied on seed as seed treatments.

Table 7. Least square means of the soybean yield recorded in susceptible and resistant cultivars in the field experiments carried out in the states of Illinois, Indiana, Iowa, Michigan, South Dakota, and Wisconsin in the United States and Ontario, Canada during 2015 and 2016^y

Treatments ^z	Yield (kg/ha)					
	2015			2016		
	S	R	Mean	S	R	Mean
Syngenta base (SB)	3,468 b	3,858	3,621 b	4,618	5,238 a	4,929
SB + <i>Pasteuria nishizawae</i> + thiabendazole	3,553 b	3,713	3,596 b	4,778	5,115 abc	4,952
Bayer base (BB)	3,556 b	3,872	3,712 ab	4,605	5,038 bcd	4,822
BB + fluopyram + clothianidin + <i>Bacillus firmus</i>	3,904 a	4,012	3,956 a	4,650	5,226 ab	4,938
BB + saponins extract of <i>Chenopodium quinoa</i>	3,472 b	3,977	3,731 ab	4,688	4,943 cd	4,813
BB followed by(fb) lactofen	3,069 c	3,519	3,285 c	4,532	4,909 d	4,722
BB fb fluoxastrobin + flutriafol	3,509 b	3,969	3,737 ab	4,682	5,109 abc	4,896
BB fb citric acid	3,489 b	3,794	3,639 b	4,545	5,017 cd	4,781
Mean	3,451 B	3,868 A	...	4,629 B	5,085 A	...
P value	0.001	0.061	<0.01	0.593	<0.01	0.120

^y Means were compared using Fisher's protected least significant difference at $\alpha = 0.05$. Means followed by the same lowercase letter within a column and same uppercase letter between two cultivars within a year do not differ significantly.

^z Lactofen and fluoxastrobin + flutriafol were applied at R1 (beginning bloom) and citric acid was applied at R1 and R4 (full pod) growth stages. Other treatments were applied on seed as seed treatments.

disease severity, frequency of *F. virguliforme* isolation from roots, conidial germination, mycelial growth, and sporulation by lactofen. Lactofen was also reported to be effective in reducing Sclerotinia stem rot and protecting soybean from yield loss due to this disease (Dann et al. 1999; Huzar-Novakowski et al. 2017; Willbur et al. in press; Yang et al. 1998). Although lactofen has a potential benefit of controlling Sclerotinia stem rot and may have some effect on SDS, it produced the lowest yield. When combining all site-years, lactofen resulted in a yield loss of about 261 kg/ha (6.1%) compared with the Bayer base. A similar result has also been reported in previous studies (Dann et al. 1999; Nelson and Renner 2001; Nelson et al. 2007; Willbur et al. in press), where a 10 to 15% yield reduction was reported when Sclerotinia stem rot did not develop. However, under high Sclerotinia stem rot pressure, lactofen was shown to effectively reduce damage caused by the disease and not negatively affect yield loss. While under low Sclerotinia stem rot severity, the damage from lactofen was greater than that from the disease and resulted in yield reductions over multiple site-year studies (Willbur et al. in press). Lactofen toxicity in plants reduces leaf area index, causes leaf distortion, and reduces plant height, which consequently delays reproductive development (Huzar-Novakowski et al. 2017; Nelson et al. 2002) and is linked to a negative impact on yield in the absence of Sclerotinia stem rot. Some soybean farmers feel that lactofen application at the early-vegetative stage causes stress to the plants that leads to increased branch development and, subsequently, increased yield. Previous studies (Gregg et al. 2015; Mangialardi et al. 2016; Orłowski et al. 2016a,b) that investigated the effect of lactofen application at the early vegetative stage for yield purposes did not find any effect on soybean branching or yield.

Amendment of Syngenta base with *P. nishizawae* and thiabendazole did not provide any additional advantage in terms of reducing disease (root rot and FDX) or increasing yield than the Syngenta base. However, we did not evaluate their efficacy on SCN management in this study. Similarly, seed treatment with the saponins of *C. quinoa* and foliar applications of fluoxastrobin + flutriafol or citric acid did not reduce SDS significantly in any cultivars or years compared with Bayer base. In contrast, a previous study (Navi and Yang 2016b) has reported that saponins of *C. quinoa* significantly suppressed SDS in soybean compared with the untreated control. Saponins of *C. quinoa* were also reported as effective against other crop diseases such as dry rot, common scab, and black scurf diseases of potato (Al-Mugharabi et al. 2010).

Use of the fluopyram seed treatment has been a concern to some farmers due to the obvious phytotoxicity, which is particularly apparent on the cotyledons. Fluopyram has demonstrated some minor reductions in plant populations in some situations (Gaspar et al. 2017); however, the reduction in plant population has not been shown to affect yield (Kandel et al. 2016b). In this study, the fluopyram seed

treatment did not result in lower plant populations than the Bayer base in any case; in fact, the fluopyram seed treatment resulted in increased plant populations by nearly 9 to 10% in resistant and susceptible cultivars compared the Bayer base seed treatment. This response might be due not only to fluopyram's ability to protect seedlings from *F. virguliforme* infection but also activity against other seedling pathogens, which were not evaluated in the present study. A previous report has shown the efficacy of fluopyram in controlling other diseases such as soybean brown leaf spot (Batzner et al. 2016). Similarly, the Syngenta base and Syngenta base + *P. nishizawae* + thiabendazole resulted in greater soybean plant populations than Bayer base in many cases, which may be due to a better control of seedling pathogens.

The use of resistant cultivars and the fluopyram seed treatment were the two most effective treatments in reducing SDS in our research. Fluopyram seed treatment is typically sold in combination with other seed treatment active ingredients and adds approximately U.S.\$30 to 32/ha at the current price to the cost of the basic seed treatment. The use of fluopyram seed treatment as a management practice without incorporating plant resistance does not always provide a sufficient level of SDS control. Yield benefits associated with fluopyram seed treatment also varies among cultivars, locations, and years. Previous research demonstrated that the chance of not earning back the cost of the fluopyram seed treatment is high when no foliar disease symptoms are observed (Kandel et al. 2018a). Currently, there is no SDS forecasting system in place, meaning farmers cannot predict if SDS will develop in a particular season or field. Because the risk of economic loss is high in the absence of disease, a fluopyram seed treatment likely will not be warranted if the field has no history of SDS. Selectively using fluopyram seed treatment on high-risk fields may help to ensure a return on investment and preserve fungicide activity against *F. virguliforme* (Gaspar et al. 2017). The FRAC has categorized SDHI fungicides, including fluopyram, as medium risk for the development of fungicide resistance (FRAC 2017). Nonetheless, a fungicide resistance management plan must be taken into account because the repetitive use of a single mode-of-action fungicide such as fluopyram may likely select isolates of *F. virguliforme* to have reduced sensitivity or resistance to fluopyram over time.

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