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Effect of Tillage and Cultivar on Plant Population, Sudden Death Syndrome, and Yield of Soybean in Iowa

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Accepted for publication 8 January 2019.

Abstract

Conservation tillage has become a common practice of soybean farming in the Midwestern United States owing to the benefits of soil and moisture conservation. Field trials were established in a field with a history of sudden death syndrome (SDS; caused by *Fusarium virguliforme*) in Iowa in 2011 and evaluated for five consecutive years to determine the impact of tillage on SDS and yield. The experiment was laid out in a split-split-plot design with four replicates. The main plot factor was tillage (no-till both crops, no-till corn and chisel plow soybean, and disc corn and chisel plow soybean), and each main plot was divided into subplots of corn or soybeans (in a 2-year rotation). Each subplot was again

divided into two subsubplots, in which two soybean cultivars, moderately susceptible (MS) and moderately resistant (MR) to SDS, were planted each year. Root rot and SDS disease index (FDX) differed among years, because some years were more favorable for the disease than the others. However, tillage did not affect any parameters, including yield, in any year ($P > 0.05$). Cultivar effect was significant for each parameter occasionally. When significant, the MR cultivar had lower root rot and FDX and greater yield than the MS cultivar. These data suggest planting resistant cultivars can be an effective management tactic, but tillage does not help for SDS management.

Sudden death syndrome (SDS) is an economically important disease of soybean in major soybean growing areas of the world (Hartman et al. 2015). SDS is caused by *Fusarium virguliforme* O'Donnell and T. Aoki (Aoki et al. 2003), a blue-pigmented slow-growing soilborne fungus, in the United States and Canada. SDS causes millions of tons of soybean yield loss in the United States and Canada annually. From 1996 to 2014, SDS was ranked one of the top 10 yield-reducing diseases of soybean in United States and Ontario, Canada (Allen et al. 2017; Wrather and Koenning 2009). Average annual yield loss estimates from SDS alone in 2010 to 2014 were nearly 1.14 million metric tons (Allen et al. 2017).

The fungus survives in crop residue as mycelium or as thick-walled survival structures (chlamydospores) in soil (Roy et al. 1997). The disease cycle begins with the infection of soybean roots soon after they emerge from the seeds (Gongora-Canul and Leandro 2011), within 15 to 30 days after planting, and continues throughout the season (Luo et al. 1999; Njiti et al. 1997). Along with root infection, the fungus produces toxins, mainly FvTox1, which causes foliar symptoms of SDS (Brar et al. 2011; Pudake et al. 2013). Foliar symptoms usually appear in early reproductive stages, around the R3 to R4 (beginning of pod) soybean growth stage (Fehr et al. 1971), as irregular chlorotic spots in between the veins. As the disease

progresses, the chlorotic lesions become necrotic with the veins remaining green, which causes premature defoliation, leaving petioles intact on the plant. In severe cases, pod abortion and premature plant death without pod set can be observed (Roy et al. 1997).

Soil temperature and moisture are two important factors for SDS epidemics (Chong et al. 2005; Scherm and Yang 1996). SDS was found to be positively correlated with moisture content at field capacity (Chong et al. 2005). Cool and wet conditions during planting and high soil moisture at early reproductive growth stages increase the earliness and severity of SDS (Kandel et al. 2016b; Leandro et al. 2013; Roy et al. 1997; Scherm and Yang 1996).

Reduced tillage, also known as conservation tillage, has become a common practice in the United States to minimize soil erosion, preserve soil moisture during drought, improve water quality, increase soil organic matter, reduce nutrient loss, and reduce the cost of irrigation and field activities (Anaele and Bishnoi 1992; Fawcett and Towery 2005). Long-term no-till increases soil carbon and nitrogen contents, viable microbial populations, and phosphatase activities in soil (Mathew et al. 2012). Another specific benefit of conservation tillage for soybean farmers is that soybean cyst nematode (SCN) populations can be reduced in no-till conditions when compared with the conventional tillage system (Westphal et al. 2009). This may influence SDS severity, because SCN populations are positively correlated with SDS severity (Kandel et al. 2017; McLean and Lawrence 1993; Workneh et al. 1999; Xing and Westphal 2009).

Although there are advantages to using a conservation tillage system, there are disadvantages too. No-till fields may produce lower yields than the tilled fields (Grassini et al. 2015; Rattalino Edreira et al. 2017). Conservation tillage may increase surface bulk density, cause soil compaction on poorly drained soils, increase soil moisture content, and leave crop residues on the soil surface that are the primary source of inoculum for crop diseases (Anaele and Bishnoi 1992; Navi and Yang 2016). A greater incidence and severity of brown stem rot, soybean

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Funding: The study was partially supported by soybean checkoff programs through Iowa Soybean Association, USDA Capacity Hatch IOW03908, and North Central Soybean Research Program.

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

The author(s) declare no conflict of interest.

stem canker, and *Phytophthora* root rot were reported in conservation tillage compared with conventional tillage (Rothrock et al. 1988; Tyler et al. 1983; Von Qualen et al. 1989; Workneh et al. 1999).

SDS management is difficult owing to the lack of effective foliar fungicides, lack of completely resistant cultivars, environmental influence on the occurrence of disease, and inconsistent effects of cultural management practices on SDS development. A conventional tillage system buries infected crop residues after harvest under the soil, which reduces the inoculum on the soil surface and may decrease the risk of disease development. Soil compaction increases SDS (Roy et al. 1997; Vick et al. 2003). It is believed that tillage reduces SDS, presumably by reducing soil compaction, maintaining sufficient vertical water movement, and by providing more aerated root zone compared with no-till; however, previous results were not consistent, and the effect was influenced by planting dates and tillage types (Seyb et al. 2007; Vick et al. 2003, 2006; Von Qualen et al. 1989; Wrather et al. 1995). In addition, little information is available about the influence of tillage on root rot phase of the disease. This study was established in 2011 and evaluated for five consecutive years to determine (i) the impact of long-term tillage activity in a field with a history of SDS on the disease (root rot and foliar symptoms of SDS) severity and yield and (ii) if cultivars with differences in SDS susceptibility respond differently across different tillage practices.

Field Experiments

Field experiments were established in a field with a history of SDS in Boone County, Iowa (GPS: 42.010787, -93.794230) in 2011 and evaluated for five consecutive years from 2012 to 2016. The study site contains mainly Webster clay loam, Bemis moraine, and Nicollet loam soil, with 1 to 3% slopes, and is poorly drained. The soil salinity was characterized as nonsaline or slightly saline (0.0 to 2.0 mmhos/cm). The field was not irrigated. Monthly precipitation and soil temperature at planting time were measured throughout the study period.

The size of the experimental area was 98 × 98 m (9,604 m² = 0.96 ha). The experimental design was a split-split plot with four replications. The experimental area was divided into 12 main plots, 30.5 m (40 rows) wide and 12.2 m long, and three tillage treatments (no-till, no-till in corn and chisel plow in soybean, and disc in corn and chisel in soybean) were assigned in each main plot. Tillage plots were maintained the same each year. Each main plot was divided equally into two subplots 15.2 × 12.2 m. Each subplot was assigned to either corn or soybean and rotated each year. Each soybean plot

was again divided into subsubplots, and two cultivars differing in SDS susceptibility, moderately resistant (MR) and moderately susceptible (MS) to SDS, were planted each year. The size of individual plots or experimental units was 3.0 m (four rows with 76.2-cm intrarow spacing) wide × 12.2 m long. The cultivars were different across the years, because the same cultivars were not available (Table 1).

Data Collection and Analysis

Plant population data were collected at the early vegetative growth stages (V2 to V3) (Fehr et al. 1971) by counting the number of live plants in a 3.0-m section of the two center rows of each plot in 2014 through 2016. Root rot data were collected in 2013, 2015, and 2016. In 2012 and 2014, root samples were accidentally mixed up while processing in the greenhouse; therefore, root rot data were absent in those two years. In 2013, root samples were collected at V2 to V3 growth stages, whereas in the other two years the root samples were collected at reproductive stages (R4 to R5), because root rot at reproductive stage was found to be better correlated with foliar symptoms and yield than the early season root rot in our another evaluation (Sjarpe et al. *unpublished*). Ten to 12 plants were carefully uprooted from the two outside rows (non-yield rows) of each plot. Roots were gently washed with tap water to remove soil attached to the root, and root rot severity was estimated visually on a percent scale based on the rotted area compared with the total root area per plant.

Foliar symptoms were recorded between R5 and R6 growth stages (Table 2). Foliar SDS incidence was recorded as the percentage of plants (0 to 100%) with foliar symptoms in the middle two rows of each plot, and severity was visually estimated only on diseased plants following a previously published 1 to 9 scale based on area of chlorotic or necrotic lesions on the leaf and premature defoliation, in which 1 = 1 to 10% leaf surface chlorotic or 1 to 5% necrotic and 9 = premature plant death (Gibson et al. 1994; Kandel et al. 2015a). 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. The middle two rows of each plot were mechanically harvested at full maturity (R8), which ranged from 21 September to 11 October (Table 1). Grain yield and moisture were recorded per plot, and the grain yield was adjusted to 13% moisture.

Analysis of variance was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Data from the experiments were analyzed in two ways: pooled and separately. Data

TABLE 1
Soybean cultivars and additional field activities performed in the field experiments in Iowa from 2012 to 2016

Year	Cultivar ^v		Seed treatment ^w	DOP ^x	Seed rate/ha	Root rot		SDS rating		DOH ^z
	MR	MS				Date	GS ^y	Date	GS	
2012	AG2632	AG2532	Accelaron standard	18 May	342,095	...	...	...	...	11 Oct
2013	AG2733	AG2431	Accelaron standard	29 Apr	342,095	17 Jun	V2–V3	5 Sep	R6.4	23 Sep
2014	AG2534	AG2931	Accelaron standard	8 May	380,380	...	...	29 Aug	R5.8	8 Oct
2015	AG2535	AG2431	Accelaron standard	22 Apr	322,335	18 Aug	R5.4	14 Aug	R5.4	21 Sep
2016	P22T41R2	P25T51R	Untreated	25 Apr	380,380	17 Aug	R5.2	26 Aug	R5.6	27 Sep

^v Cultivars were different across the years but were chosen based on susceptibility to sudden death syndrome (SDS). MS = moderately susceptible to SDS, and MR = moderately resistant to SDS.

^w Standard Accelaron seed treatment contains a combination of fungicides, metalaxyl, fluxapyroxad, pyraclostrobin, and insecticide (imidacloprid); treated by Monsanto Company.

^x DOP = date of planting.

^y GS = soybean growth stage.

^z DOH = date of harvest.

from all five years were combined for pooled analysis in which year, treatments, and their interactions were defined as fixed effect factors, and replication and replication $\times$ tillage interaction were used as random factors. Year was treated as a repeated measure in the model because the same plots were observed each year. Effect of year and year $\times$ cultivar interaction was significant for all parameters; therefore, the individual year was also analyzed separately. Individual year analysis also gives additional information on the effect of treatments in each year with different environmental conditions. For individual year analysis, tillage treatment and cultivar were considered fixed effects, tillage as main plot, and soybean cultivar as subplot factor; replication and replication $\times$

tillage interaction were defined as random factors in the model. The *lsmeans* statement was used to obtain treatment means. The level of significance (α) was set at 5%. Fisher's protected least significant difference was used to separate the treatment means at $\alpha = 0.05$.

Effect of tillage on root rot and FDX. Root rot severity was significantly different across the years ($P < 0.001$). Greater root rot was observed in wet years 2015 and 2016 compared with the dry year 2013. In 2013, the root rot severity was 48 and 59% less than 2015 and 2016, respectively. Effect of tillage ($P = 0.401$) and tillage $\times$ year interaction was not significant ($P = 0.396$), indicating tillage did not affect root rot and FDX in any year.

Year effect was also significant for FDX. No foliar disease symptoms were observed in 2012, and only trace amounts were observed in 2013 and 2016. In 2014, a high level of disease was observed in all tillage treatments (26 to 27% FDX), but the difference among the tillage treatments was not statistically significant (Table 3). In 2015, 5 to 7% FDX was observed in all three tillage treatments with no statistical difference among the treatments. Foliar symptoms of SDS were noticed around the beginning of the pod-filling stage (R5 growth stage). No other foliar diseases, in particular those causing similar symptoms to SDS such as brown stem rot caused by *Cadophora gregata* and stem canker caused by *Diaporthe caulivora*, were observed in any years. The presence of *F. virguliforme* was confirmed in roots collected from border rows by running a specific quantitative polymerase chain reaction protocol (Kandel et al. 2015b; Wang et al. 2015).

Our study showed that there were differences in root rot severity and foliar disease symptoms across the years and cultivars. The differences in both symptoms are more likely owing to the differences in rainfall during the season. The importance of high soil moisture for increased SDS incidence and severity has been explained in several previous studies as well (Chong et al. 2005; Leandro et al. 2013; Melgar et al. 1994; Roy et al. 1989; Scherm and Yang 1996). The largest differences in monthly rainfall amounts across the years occurred in the month of June. In 2014, when

TABLE 2

Least square means of plant population (per hectare) in different tillage treatments and cultivars evaluated in 2014 through 2016 in Iowa

Year	Cultivar ^y	Tillage treatment			Mean ^z
		No-till	Chisel	Disc + chisel	
2014	MR	377,805	364,889	383,187	375,294 a
	MS	306,765	368,118	348,743	341,209 a
2015	MR	296,001	319,681	303,536	306,406 a
	MS	286,314	258,328	290,619	278,420 b
2016	MR	379,958	377,805	370,809	376,191 a
	MS	333,674	327,216	319,143	326,678 b
Mean		330,086 A	336,006 A	336,006 A	

^y Cultivars were different across the years, but they were chosen based on the susceptibility to sudden death syndrome (SDS). MS = moderately susceptible to SDS, and MR = moderately resistant to SDS.

^z Cultivar means followed by the same lowercase letter within a year were not statistically different. Tillage treatment means within a row followed by the same uppercase letters did not differ statistically. Means were separated by Fisher's protected least significant difference at $\alpha = 0.05$.

TABLE 3

Least square means of root rot, foliar disease index (FDX), and yield of different tillage treatments evaluated in 2012 through 2016 in Iowa

Variable ^y	Treatment ^z	2012	2013	2014	2015	2016	2012–2016
Root rot	No-till	...	10.9	...	21.1	23.3	18.4
	Chisel	...	9.6	...	14.7	25.0	16.4
	Disc + chisel	...	8.2	...	19.3	21.3	16.3
	Mean	...	9.5	...	18.4	23.2	17.0
	P value	...	0.456	...	0.368	0.541	0.401
FDX	No-till	0.0	0.0	27.2	5.4	0.9	6.7
	Chisel	0.0	0.0	26.1	6.2	0.6	6.6
	Disc + chisel	0.0	0.0	27.4	7.3	0.6	7.0
	Mean	0.0	0.0	26.9	6.3	0.7	6.8
	P value	NT	0.084	0.996	0.853	0.873	0.989
Yield	No-till	3,419	2,798	3,771	4,238	3,028	3,451
	Chisel	3,410	2,947	3,737	3,947	2,973	3,403
	Disc + chisel	3,542	2,828	3,656	4,305	2,955	3,457
	Mean	3,457	2,858	3,722	4,163	2,986	3,437
	P value	0.867	0.813	0.657	0.364	0.923	0.913

^y Root rot was estimated as percentage of root area covered with necrotic lesions. Root rot data were not taken in 2012 and 2014. FDX of SDS was calculated using the formula FDX = disease incidence $\times$ disease severity/9. Disease incidence was estimated as percentage of symptomatic plants per plot in two middle rows of each plot, and SDS severity was scored within the symptomatic plants on a 1 to 9 scale based on percentage of the chlorotic and necrotic leaf area and defoliation (1 = 1 to 10% leaf area chlorotic or 1 to 5% necrotic, and 9 = premature death).

^z P value within each variable refers to the probability of rejecting the null hypothesis that the tillage treatments were not statistically different. NT = not tested.

highest FDX was recorded, there was 78% more rainfall than the 30-year average and about three times greater rainfall than the 2012 and 2013 June rainfall. Consistent with this result, previous studies (Chong et al. 2005; Vick et al. 2003, 2006) have also indicated that increased levels of rainfall in June resulted in increased incidence and severity of SDS.

Tillage in our study did not affect root rot or foliar symptoms of SDS. This result was consistent across all cultivars and years with high and low disease conditions. No cultivar $\times$ tillage interaction was significant in any year for both variables. This result supports previous reports showing that chisel and disc tillage did not reduce root infection (Vick et al. 2006) and nonsignificant differences in SDS between no-till and tilled plots (Seyb et al. 2007). In contrast, SDS foliar symptoms were affected by tillage in some previous studies (Vick et al. 2003, 2006; Wrather et al. 1995) that showed chisel tillage lowered SDS compared with no-till plots under both low and high disease conditions. Vick et al. (2003) reported that the subsoiling at the depth of 40 to 45 cm reduced foliar SDS symptoms by reducing the soil compaction and moisture. In general, tillage reduces soil moisture that may enable rapid and expansive root growth and delay the root infection or reduce the pathogen's ability to colonize the roots because of drier soil conditions in the root zone (Vick et al. 2003); however, the effect of tillage on root rot has never been observed.

Although we did not observe higher disease in no-till plots in our study, plant residue left on the soil surface in reduced- or no-till fields may increase the risk of other seedling and root diseases. Some pathogens survive one or more years on infected plant residue and/or on seed left in the field after harvest. The inconsistency across the studies could be owing to the difference in cropping system, latitude, and other soil and environmental factors. In some soils, no-tillage can be beneficial in reducing the severity of SDS compared with plow or chisel-plow tillage (Seyb et al. 2007), whereas in other soils, intensive tillage reduces SDS compared with no-till (Vick et al. 2006).

Tillage did not affect plant population and soybean yield.

Plant population was lower in 2015 than the other two years, 2014 and 2016, primarily owing to difference in seeding rate (Table 1), but tillage treatment was not significant for plant population in any year.

Tillage also had no effect on yield. The result was consistent across all years and cultivars. This result was in agreement with the previous evaluations (Tyler et al. 1983; Vick et al. 2003, 2006; Westphal et al. 2009) in which no yield difference was observed in no-till compared with tilled systems. In contrast, a previous study reported that no-till plots produced lower yield than conventional tillage plots as a result of lowered plant population in no-tilled plots (Anaele and Bishnoi 1992; West et al. 1996). Seeds used in our experiments were treated with a standard seed treatment (Table 1) in most cases, which may have managed the soilborne pathogens that cause stand loss and, consequently, no tillage effect on plant population was observed.

Yield was different across the years. Yield was greater in 2014 and 2015, when SDS was the most prevalent. This result should be interpreted cautiously and not as a suggestion that SDS increases or does not affect yield. Instead, this increase in yield is likely owing to sufficient rainfall in those two years, which is also critical for soybean growth and development, suggesting high-yielding environments also favor SDS. This result supports the previous conclusions (Roy et al. 1997) that the SDS is more severe in environments that favor high yield. SDS can cause yield loss up to 80% depending upon the onset time, severity, and cultivar susceptibility to the disease (Roy et al. 1997).

Cultivar affects plant population, SDS, and yield. Cultivars MS to SDS had a significantly lower plant population than the MR

cultivars in 2015 and 2016 ($P < 0.001$). Because the tillage $\times$ cultivar interaction was significant in 2015 ($P = 0.036$), cultivar effect was analyzed separately for each tillage treatment. Cultivar difference was significant only in the disc + chisel treatment with greater plant population in MR.

Cultivar effect and cultivar $\times$ year interaction were significant for both root rot and FDX ($P < 0.001$). Individual year analyses showed that cultivar effect for root rot was significant in 2015, when MR cultivars had nearly 61% less root rot than the MS cultivars (Fig. 1).

FIGURE 1

Root rot, foliar disease index (FDX), and yield for moderately resistant (MR) and moderately susceptible (MS) cultivars in 2012 to 2016 in Iowa. Means were pooled across the tillage treatments. Cultivars were statistically different for root rot in 2015 ($P < 0.001$), for FDX in 2014 ($P < 0.001$) and 2015 ($P = 0.048$), and for yield in 2012, 2015, and 2016, with P values < 0.001 , 0.004, and 0.048, respectively.

The cultivar effect for FDX was significant in 2014 and 2015. FDX in MS cultivars was about 39 and 9% in 2014 and 2015, respectively. The MR cultivars had over 60% less disease than the MS cultivar in both years. In 2016, a very low level of disease was observed in both cultivars, and the difference was not statistically significant ($P = 0.559$). No significant interaction between the tillage and cultivar was observed in any year for root rot and FDX, indicating the response of cultivar did not change with the tillage practices. The MR cultivars had less root rot and less foliar symptoms than the MS cultivars regardless of the tillage type. The difference was more prominent in years when SDS was more severe. MR cultivars resulted in greater plant population and greater yield along with less root rot and less foliar symptoms than MS regardless of the tillage type. MS cultivars may be susceptible to other soil pathogens in addition to *F. virguliforme* that resulted lower plant population. However, it would be interesting to isolate and identify the root pathogens that cause greater stand loss in susceptible cultivars in future studies.

Effects of cultivar and cultivar $\times$ year interaction also were significant ($P < 0.001$) for yield. Separate analysis for each year showed that cultivar effect was significant in three of the five years (2012, 2015, and 2016) (Fig. 1); however, there was a consistent trend across the years showing MR cultivars produced greater yield ranging from 54 to 538 kg/ha (Fig. 1). When all years were combined, MR cultivars produced about 11% (342 kg/ha) more yield than MS cultivars (Fig. 1).

Weather patterns across the years. Soil temperatures ($^{\circ}\text{C}$) at planting and monthly cumulative precipitation (cm) data were collected from weather stations that are closest to the experimental site using public weather service websites (<http://mesonet.agron.iastate.edu>) (Supplementary Table 1). Soil temperatures were collected at planting from a 10-cm depth.

Rainfall pattern was different across the years. Total seasonal precipitation (April to September) across the study period ranged from a minimum of 41.8 cm in 2012 to a maximum of 86.6 cm in 2015. Throughout the season, 2012 and 2013 were drier than 2014 and 2015. Total rainfall amount during the season was similar (79 to 86 cm) in 2014 through 2016. The largest difference in rainfall between years was in June; about 78% more rainfall occurred in June 2014 than the 30-year average and three times more than 2012 and 2013. The months of July and August in 2014 were drier than 2015 and 2016.

Conclusions and Recommendation

Our present study showed that tillage had no effect on SDS severity, although MR cultivars produced greater yield with decreased root rot and foliar symptoms. Farmers are not recommended to till in order to manage SDS; however, they can adopt practices that are more effective to manage SDS. This study also confirms that the use of resistant cultivars should be the primary means of SDS management. Other practices, such as seed treatment (Kandel et al. 2016a, b, 2018), SCN management (Kandel et al. 2017), and diversification in cropping system (Leandro et al. 2018; Xing and Westphal 2009) can complement host resistance. This study clearly and consistently shows that the tillage has no effect on SDS, although this study was performed in only one location. Similar studies at more locations may be useful to verify if the effects are broadly applicable. Research is in progress that compares soil physical and chemical properties across the tillage treatments. Further investigations are warranted to determine the influence of tillage on soil microbial population and their activities. The interaction of tillage with other crop management practices such as delayed planting, nonhost crop rotation, and use of cover crops also needs to be investigated.

Acknowledgments

We thank S. Wiggs, D. Sjarpe, and A. Haafke from Iowa State University for their support in trial establishment, trial management, and data collection.

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