Dynamics of Population Density and Virulence Phenotype of the Soybean Cyst Nematode as Influenced by Resistance Source Sequence and Tillage

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Abstract

The soybean cyst nematode (SCN), *Heterodera glycines*, is the most damaging pathogen of soybean. Use of resistant cultivars is an effective strategy to manage SCN, but it also selects for virulent populations over time. A 12-year field experiment was initiated in 2003 to study how tillage and 11 different sequences of four cultivars impact SCN population dynamics and virulence. An SCN-susceptible cultivar and three resistant cultivars (R1, R2, and R3 derived from cultivars PI 88788, Peking, and PI 437654, respectively) were used. Tillage had minimal effect on SCN population density. Compared with no till, conventional tillage resulted in a faster increase of SCN virulence to Peking when the SCN was selected by R2 and virulence to PI 88788 by R3. Among the three SCN-resistant cultivars, R1 supported the greatest population density, R2 supported intermediate population density, and R3 supported the least SCN population density. The SCN populations selected by R1 overcame

the resistance in PI 88788 but not in Peking and PI 437654. R2 selected SCN populations that overcame the resistance in Peking but not in PI 88788 and PI 437654. In contrast, R3 selected SCN populations that overcame both PI 88788 and Peking sources of resistance. There was no increase of virulence to PI 437654 in any cultivar sequence. R1 in rotation with R2 or R3 had a negative effect on female index on Peking. Susceptible soybean reduced SCN virulence to Peking, indicating that there was fitness cost of the Peking virulent SCN type. These results suggest that rotation of Peking with PI 88788 is a good strategy for managing the SCN, and susceptible cultivar and no till may reduce SCN virulence selection pressure in some rotations.

Keywords: *Heterodera glycines*, HG type, host-parasite relationship, race, soybean cyst nematode, virulence phenotype

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pathogen of soybean, and it has been widely spread in most soybean-producing regions in the world. The United States is one of top soybean-producing countries, and most of its production is in the north central region. The extensive soybean production creates a high pressure of diseases and pests. The annual yield loss of soybean caused by SCN has been estimated to be >\$1 billion in the United States only (Allen et al. 2017; Koenning and Wrather 2010).

Growing resistant cultivars and rotations with nonhost crops have been major strategies to manage SCN in the United States (Chen et al. 2011; Niblack and Chen 2004; Niblack and Tylka 2008; Young 1998b). Effectiveness of using resistant cultivars depends on the genetic interaction between the cultivars and nematode populations. In the north central region, soybean is generally planted in annual rotation with corn, and most of the soybean fields are planted to SCN-resistant cultivars. In addition, most of the SCN-resistant commercial soybean cultivars in the north central region are derived from a single germplasm cultivar PI 88788. Only a small portion of cultivars planted are derived from the germplasm cultivars Peking (PI 548402), PI 437654, and others (Joos and Niekamp 2018; Lorenz et al. 2018; Tylka and Mullaney 2018). The extensive use of SCN-resistant cultivars with limited sources of resistance has created a high selection pressure on SCN toward increased

virulence to the resistant cultivars (Chen et al. 2010; Howland et al. 2018; Niblack et al. 2008).

Increase in virulence of SCN to the sources of resistance or change of the virulence phenotypes measured as races (Riggs and Schmitt 1988) or *Heterodera glycines* (HG) types (Niblack et al. 2002) following the use of resistant soybean cultivars has been demonstrated by a number of studies, including state, regional, and national surveys in the United States and greenhouse pot and field plot experiments. The national surveys in the late 1980s and early 1990s demonstrated that the frequency of SCN populations virulent to resistant cultivars was higher in the southern region than in the northern region, probably owing to longer use of resistant soybean cultivars in the southern states by that time (Anand et al. 1994; Kim et al. 1997). Increases of virulence of SCN on PI 88788 and/or Peking have been observed in state surveys in Tennessee (Young 1990), North Carolina (Koenning and Barker 1998), Illinois (Niblack et al. 2008; Sikora and Noel 1991), Missouri (Howland et al. 2018; Mitchum et al. 2007), Kentucky (Hershman et al. 2008), and Minnesota (Chen et al. 2010), and they have also been observed in fields of soybean cultivar trials over the years in Iowa (McCarville et al. 2017). For example, in Missouri, the percentage of populations that developed well on PI 88788 (female index [FI] > 10) increased from 58% in 1998 (Niblack et al. 2003) to 78% in 2005 (Mitchum et al. 2007) and to 100% in 2016 (Howland et al. 2018). In Minnesota, use of SCN-resistant cultivars in managing the SCN has a relatively short history compared with the southern states. The frequency of virulence phenotypes in Minnesota did not change significantly from 1997 to 2002, and most populations (>80%) did not reproduce well on PI 88788 and Peking (Zheng et al. 2006). However, two surveys showed that virulence level of the SCN populations has increased over the years in Minnesota, with 72.6% of the SCN populations in the state virulent to PI 88788 soybean and 12.1% virulent to Peking in 2007 and 2008 (Chen et al. 2010) and 68.5% virulent to PI 88788 and 15.1% virulent to Peking in 2013 (S. Chen, unpublished data).

Increase of SCN virulence to resistant cultivars has also been demonstrated in a number of greenhouse and field plot experiments. In a greenhouse experiment, PI 88788-selected SCN populations increased FI on PI 88788 but did not change FI on cultivar Pickett, which was derived from Peking; Pickett selected SCN

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populations with increased FI on Pickett but that did not affect FI on PI 88788 (Triantaphyllou 1975). In another greenhouse experiment, soybean lines (e.g., cultivar Bedford) containing resistance from PI 88788 selected SCN populations with increased FI on PI 88788 but reduced FI on Peking (Young 1984). In Tennessee field experiments with an initial SCN population of race 14 (HG type 1–), which is virulent to Peking, planting soybean cultivars derived from PI 88788 increased parasitic ability of the SCN populations on the selecting soybean cultivars and the source of resistance PI 88788, whereas planting cultivars derived from Peking did not change the level of virulence (Young 1994, 1998a; Young and Hartwig 1992; Young et al. 1986). Similar results were obtained in Missouri and Arkansas fields infested with race 14 (Anand et al. 1995; Young et al. 1986). In a Mississippi field experiment initially infested with race 3, which is avirulent to all sources of resistance in the commercial cultivars, after 10 years of various crop sequences parasitic ability of the nematode populations on Peking increased in the sequences with ≥ 6 years of planting a cultivar carrying resistance from Peking (Young and Hartwig 1988). A 2011 field plot experiment in Minnesota demonstrated that use of the cultivar Freeborn carrying PI 88788 resistance had a significant effect on virulence phenotype of SCN populations in the northern climate in fields initially infested with HG type 0 (race 3) populations (Zheng and Chen 2011). After 5 years, the original population of HG type 0 (race 3) became a population that was able to overcome the resistance of PI 88788 (Zheng and Chen 2011).

The selection pressure of resistant cultivars on the pathogens may depend on the genetic interaction between the hosts, pathogens, and environments. No-till was introduced into the north central United States a number of decades ago, but there is no report of a tillage effect on selection pressure for SCN virulence on resistant cultivars in this region and elsewhere. Commercial cultivars with SCN resistance derived from PI 88788 and Peking have been used in the region for a few decades. In the early 2000s, some cultivars derived from PI 437654 (CystX) were expected to have high potential in SCN management (Ferris et al. 2000), but CystX-derived cultivars are not accepted because of low yield potential. Rotation of cultivars with different sources of resistance is recommended for an effective management strategy (Niblack 2005; Young 1998b), although few sources of resistance in the commercial cultivars are available. Knowing the virulence phenotypes of SCN in fields and speed of change following the use of resistant cultivars in different rotations is important for strategically deploying and breeding resistant cultivars and making management recommendations to farmers. The objective of this study was to determine how growing the commercial SCN-resistant cultivars carrying resistance from PI 88788, Peking, and PI 437654 as well as a susceptible cultivar in monoculture or rotations and tillage affect SCN virulence phenotype and population density in the northern climate in Minnesota.

Materials and Methods

Field experimental design, site establishment, and maintenance.

This 12-year experiment was established in a field (44°04'21" N, 93°31'32" W) in Waseca, Minnesota in 2003. The soil at the site was a Nicollet clay loam (fine loamy, mixed, mesic Aquic Hapludoll). Prior to this study, the field was in corn and soybean annual rotation for a number of years. The field site was initially infested with the SCN HG type 0 (race 3), which is avirulent to the SCN-resistant cultivars carrying resistance genes from any available sources of resistance.

The experiment was a split-plot design, with tillage as main plots and cultivar sequences as subplots, and it was arranged in six complete blocks (replicates) (Supplementary Fig. S1). Main plot treatments included no-till (NT) and conventional tillage (CT). Each subplot, 7.31-m long and 3-m wide, consisted of four soybean rows. The subplot treatments included 11 cultivar sequences, each consisting of 1 or more of an SCN-susceptible soybean cultivar (S) and three cultivars carrying resistance from PI 88788 (R1), Peking

(R2), or PI 437654 (R3). The cultivars grown were susceptible cultivar Pioneer 92B13 (S), cultivar Latham EX547 RR N (R1) derived from PI 88788, cultivar Pioneer 91M90 (R2) derived from Peking, and cultivar Latham AR5084 (R3) derived from PI 437654 (Tables 1 and 2). The experiment was designed to determine how these three main sources of resistance influence the changes of the SCN population from avirulent to virulent types on cultivars carrying the resistance genes from these sources and whether any rotation of the different cultivars can slow or prevent development of virulent SCN populations in the NT and CT regimes.

The CT treatment was fall chisel plowing after harvesting and field cultivation in the spring followed by a finishing implement prior to planting. Soybean was planted from early May to early June and harvested from early October to mid-November. Seeding rate was 432,000 seeds per 1 ha. Fertilizer application was based on soil fertility test recommendations of the University of Minnesota Extension (Kaiser and Lamb 2012). Fertilizers P at 84 kg/ha and K at 168 kg/ha were applied in 2006. Fertilizers N at 22.4 kg/ha and P at 56 kg/ha were applied in 2009. No fertilizer was used in other years. All of the soybean cultivars were resistant to glyphosate (2-phosphonomethylamino acetic acid), and glyphosate was used for both preemergence and postemergence weed control. No insecticide or fungicide was used.

SCN population density assay. SCN population density in each subplot was determined at planting (Pi, initial population) and harvest (Pf, final population). A composite soil sample consisting of 20 cores was taken with a 2.5-cm-diameter soil probe to a 20-cm depth across the central area of approximately 1.5-m wide by 6.1-m long from each plot. The soil samples were stored in a cool room (4°C) before being processed. Each soil sample was thoroughly mixed, and cysts were initially extracted from a subsample of 100 cm³ of soil with a semiautomatic elutriator (Byrd et al. 1976). The cysts with some soil particles and debris caught on a 250- μ m-aperture sieve were collected, suspended in 63% (wt/vol) sucrose solution in a 50-ml tube, and centrifuged at 1,100 $\times$ g for 5 min. The cysts in the supernatant were collected and crushed in a motorized device to release eggs (Faghihi and Ferris 2000). The eggs were collected into a 50-ml tube and counted. Nematode population density was expressed as the number of eggs per 100 cm³ of soil.

Evaluation of SCN virulence phenotype. Virulence phenotypes of the SCN populations were evaluated on the sources of resistance PI 88788, Peking, and PI 437654 using FIs of the SCN in the soil samples taken in the spring of 2007, 2008, 2009, 2010, 2012, and 2014. Eight soil cores were taken with a 2.5-cm-diameter soil probe to a 20-cm depth from the two central rows of each subplot, and the soil cores from the six replicate subplots of the same tillage and cultivar sequence treatment were combined as one soil sample; a total of 22 composite soil samples were collected at each sampling time.

Each soil sample was placed in a clay pot with added autoclaved sands to increase drainage. The pots were planted to the SCN-susceptible soybean cultivar Sturdy. After culture for 3 months to half a year, the cysts and females were collected from the soybean and pot soil. Newly formed females and cysts were washed with a vigorously applied water stream through an 850- μ m-aperture sieve onto a 250- μ m-aperture sieve, and cysts in the soil were also collected by decanting the soil suspension to the sieves. The cysts and females were separated from soil particles and debris by centrifugation in 63% (wt/vol) sucrose solution. Eggs were released from the cysts by crushing the cysts on 150- μ m-aperture sieve with a rubber stopper mounted on a motor (Faghihi and Ferris 2000). An egg suspension of 800 eggs per milliliter was prepared and used for inoculation.

The virulence phenotype of each SCN population was tested on the three indicator soybean lines with cultivar Lee 74 as control following the procedures in previous studies (Niblack et al. 2002; Riggs and Schmitt 1988) with some modifications (Zheng et al. 2006). Each replicate included three soybean plants, one in each of three separate cone-tainers (4-cm diameter and 13.5-cm high) per soybean

line, and three replicates were used for each sample with a total of 36 cone-tainers for the four soybean lines. The cone-tainers were filled with autoclaved soil (80% sands + 20% field clay loam soil) to half, and 2,000 eggs in 2.5 ml of water were added. Additional soil was placed in the cone-tainer to approximately 2 cm from the top. A hole was made at the center to a depth of 3 cm with a glass stick (0.5-cm diameter). A soybean seedling (a germinated soybean seed) was placed in the hole with radical toward the ground. An additional 2,000 eggs in 2.5 ml of water were added near the seedling, and the seedling was covered with additional soil to about 1-cm depth. Although Niblack et al. (2002) recommended an inoculum level of 2,000 eggs per plant, 4,000 eggs per plant were used instead because 2,000 eggs did not consistently yield a female number of >100 females per plant on the susceptible control because of unknown environmental factors or the condition of the eggs. The cone-tainers were placed on a rack and maintained in the growth room for 35 days with temperature set at 28°C. Water was applied with a sprinkler irrigation system to maintain adequate soil moisture.

Cysts (females) were extracted from the roots and soil with the established procedures. Briefly, the soil and the plant roots were removed from the cone-tainer and placed in a beaker, and then, water was added. Any cysts on the wall of the cone were washed into the beaker. Soybean roots were removed, and females were washed off on an 850-µm-aperture sieve nested on a 250-µm-aperture sieve. In addition, the cysts in the soil were extracted by pouring soil suspension on the sieves. After rinsing the materials on the 850-µm-aperture sieve, the cysts with debris on the 250-µm-aperture sieve were collected. The cysts were separated from the debris by flotation-centrifugation in sucrose solution (63%) and counted under a dissecting microscope. FI of each plant was determined by comparing the SCN female number on the line with the female number on Lee 74:

$$FI = \frac{\text{female number on a given plant} \times 100}{\text{mean number of females on lee 74}}$$

Analysis of SCN population density. The SCN egg population densities were transformed with $x^{0.25}$ to improve the homogeneity for split-plot analysis of variance (ANOVA) with tillage as the main

plots and cultivar sequence as subplots in each sampling time using the general linear model (GLM) procedures in Statistical Analysis System (SAS) Version 9.2 (SAS Institute, Cary, NC). A mixed model with tillage and cultivar sequence as fixed factors and replicates as random factor was used in the GLM procedure. The tillage $\times$ replicate was used as the error term for calculating F value of the main plot tillage. The means were separated with least significant difference (LSD) test at $P < 0.05$. When interaction between main plot and subplot was significant, subsequent ANOVA was performed at one-factor level, tillage or cultivar sequence. In addition, ANOVA was performed using the pooled data of 2004, 2006, 2008, 2010, 2012, and 2014 for cultivar sequences 1, 5, 6, and 7 (Tables 1 and 2) to compare the effect of one preceding year of different cultivars on the SCN population densities on the susceptible soybean in the fall of the following year or the pooled data of 2005, 2008, 2011, and 2014 for cultivar sequences 1, 8, 9, and 10 to compare the effect of 2 preceding years of different cultivars on the SCN population densities on the susceptible soybean in the fall of the following year.

For the cultivar sequences (1, 2, 3, and 4) of monoculture of S, R1, R2, and R3, regression was performed to determine the increase of SCN population densities over the years with the model modified from McSorley and Duncan (2004):

$$Y = a \cdot e^{b \cdot x + c \cdot z + d \cdot x \cdot z}$$

where Y is the average SCN egg population densities of the six replicates at harvest each year from 2005 to 2014; x is the number of years of SCN monoculture of an SCN-resistant cultivar; z is tillage when NT is zero and CT is one; a is a constant representing the SCN population density of NT in 2005; and b , c , and d are coefficients of the independent variable terms. Because the tillage did not affect the SCN egg population densities and because there was no interaction between tillage and years, subsequent regression was performed using the average of SCN population densities of the two tillage treatments with the model:

$$Y = a \cdot e^{(b \cdot x)}$$

where Y is the average SCN egg population densities of the six replicates of the two tillage treatments at harvest each year from 2005 to

Table 1. Effect of tillage and sequence of different sources of resistance on population density of the soybean cyst nematode (SCN) at planting (Pi) and harvest (Pf) from 2003 to 2008

Treatment	SCN population density (eggs per 100 cm ³ of soil) ^y										
	Pf03	Pi04	Pf04	Pi05	Pf05	Pi06	Pf06	Pi07	Pf07	Pi08	Pf08
Cultivar sequence ^w (2003–2008)											
1. S-S-S-S-S	8,485 a	5,844 a	5,231 a	2,899 a	4,725 a	3,207 a	4,136 b	4,752 b	3,623 a	4,195 a	4,929 bc
2. R1-R1-R1-R1-R1	2,335 bc	1,568 bcd	1,017	889 b	1,024 bc	1,010 bc	1,452 c	1,315 c	1,834 bc	1,830 b	1,820 d
3. R2-R2-R2-R2-R2	952 e	1,100 cde	267	313 bc	107 d	123 ef	128 e	81 e	140 f	95 e	286 e
4. R3-R3-R3-R3-R3	1,090 de	667 e	174	186 c	76 d	65 f	51 f	272 d	41 g	75 e	144 e
5. R1-S-R2-S-R1-S	1,966 b	1,735 b	5,402	2,463 a	820 c	749 cd	8,014 a	5,748 ab	2,184 b	2,291 b	7,609 a
6. R2-S-R3-S-R2-S	1,051 de	1,053 cde	4,020	1,834 a	801 c	334 de	7,138 a	6,106 ab	1,333 cd	1,568 b	7,638 a
7. R3-S-R1-S-R3-S	1,246 cde	1,168 bcde	5,718	2,624 a	1,746 b	1,210 b	6,317 ab	7,829 a	1,632 bc	1,544 b	8,844 a
8. R1-R3-S-R1-R3-S	1,567 bcd	1,457 bc	316	283 bc	5,538 a	3,323	1,075 c	1,178 c	436 e	398 d	4,811 c
9. R2-R1-S-R2-R1-S	798 e	1,181 bcde	871	746 bc	5,827 a	4,307 a	825 d	1,251 c	1,190 cd	870 cd	6,282 abc
10. R3-R2-S-R3-R2-S	1,289 de	1,000 de	281	195 c	6,671 a	3,918 a	1,488 cd	1,652 c	248 f	336 e	5,532 bc
11. R1-R2-S-R3-R1-S	2,353 bc	1,940 b	899	710 bc	7,317 a	5,020 a	1,335 cd	1,529 c	949 de	1,028 bc	7,020 ab
Tillage											
No till	2,252	1,791	2,012	1,229	3,982	2,702	3,307	3,388	1,485	1,501	5,191
Conventional tillage	1,954	1,612	2,388	1,160	2,318	1,528	2,504	2,378	990	1,086	4,793
ANOVA (F values) ^x											
Tillage	0.1	0.1	0.0	0.2	1.8	1.7	4.1	2.9	4.1	2.0	0.2
Sequence	16.0 ^y	16.0 ^y	40.9 ^y	15.8 ^y	50.7 ^y	30.3 ^y	56.0 ^y	46.5 ^y	56.0 ^y	21.1 ^y	47.5 ^y
Tillage $\times$ sequence	0.8	0.8	1.9	1.6	1.4	1.2	1.3	3.0 ^z	1.3	0.7	0.5

^v The values followed by the same letter(s) in the same column are not different according to least significant difference (LSD) test at $P > 0.05$. Pf03, Pi04 to Pi08, and Pf08 represent egg population densities (eggs per 100 cm³ of soil) at Pi and Pf from 2003 to 2008.

^w S is SCN-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^x ANOVA, analysis of variance.

^y Significant of the ANOVA F value at $P < 0.0001$.

^z Significant of the ANOVA F value at $P < 0.01$.

2014. In this model, the term e^b represents the end season egg population density annual change rate.

Analysis of FI. Similar to the analysis of SCN population density, the SCN FIs were transformed with $x^{0.25}$ to improve the homogeneity for split-plot ANOVA with tillage as the main plots and cultivar sequence as subplots in each sampling time using the GLM procedures in SAS Version 9.2 (SAS Institute). The means were separated with LSD test at $P < 0.05$.

To determine the effect of number of years of the four cultivars in a sequence and tillage on the SCN FI on PI 88788 or Peking, the data of all 6 years and 11 sequences each year were pooled, and regression was performed using the following model modified from Zheng and Chen (2011):

$$Y + 1 = \alpha \cdot e^{\beta_1 \cdot R_1 + \beta_2 \cdot R_2 + \beta_3 \cdot R_3 + \beta_4 \cdot S + \beta_5 \cdot T + \beta_6 \cdot R_1 \cdot T + \beta_7 \cdot R_2 \cdot T + \beta_8 \cdot R_3 \cdot T + \beta_9 \cdot S \cdot T}$$

where Y is the FI; R_1 , R_2 , R_3 , and S are the numbers of years of the soybean cultivars R_1 , R_2 , R_3 , and S , respectively, in a sequence (Supplementary Table S1); T is tillage when NT is 0 and CT is 1; α is a constant representing the FI ($\alpha - 1$) at the beginning of the study; and β_1 to β_9 are the coefficients of the independent variable terms. Because most of FIs on PI 437654 were 0 or 0.1, no regression was performed for those data.

Analysis of soybean yield. Similar to the above, split-plot ANOVA with tillage as the main plots and cultivar sequence as subplots each year was performed using the GLM procedures in SAS Version 9.2 (SAS Institute). Similarly, the yields of the same cultivars in different sequences were pooled each year, and the split-plot ANOVA with tillage as main plots and cultivar as subplots was also performed. When interaction between main plot and subplot was significant, subsequent ANOVA was performed at one-factor level. In addition, ANOVA was performed using the pooled data of 2004, 2006, 2008, 2010, 2012, and 2014 for sequences 1, 5, 6,

and 7 to compare the effect of one preceding year of different cultivars on the susceptible soybean yield in the following year or the pooled data of 2005, 2008, 2011, and 2014 for sequences 1, 8, 9, and 10 to compare the effect of 2 preceding years of different cultivars on the susceptible soybean yield in the following year.

Results

SCN population densities. The dynamics of SCN population density from 2003 to 2014 are summarized in Tables 1 and 2. Overall, egg population densities on susceptible soybean were greater than those on the SCN-resistant soybean cultivars, especially in earlier years. Initial SCN egg population density at the planting in 2003 was $4,579 \pm 1,539$ (mean $\pm$ standard deviation) eggs per 100 cm³ of soil in the field. In the fall of 2003, the average egg population densities (Pf03) after one growing season on S (susceptible Pioneer 92B13), R_1 (Latham EX547 RR N derived from PI 88788), R_2 (Pioneer 91M90 derived from Peking), and R_3 (Latham AR5084 derived from PI 437654) were 8,485, 2,055, 934, and 1,208 eggs per 100 cm³ of soil, respectively, indicating that all three cultivars (R_1 , R_2 , and R_3) were resistant to the original SCN population in the field. R_1 , however, had a lower level of resistance than R_2 and R_3 (Tables 1 and 2). Over the 12 years, the egg population densities in monoculture R_1 were higher compared with R_2 and R_3 monoculture in most sampling points, but the difference between R_1 and R_2 became smaller or insignificant toward the later years (Tables 1 and 2). The egg population densities in R_3 monoculture were similar to those in R_2 in first 3 years, and they became smaller than R_2 in most sampling points after that (Tables 1 and 2). After about 2 years of planting resistant cultivars, the SCN egg population density in the fall declined to about the lowest point (Pf04), and then, it increased gradually over the following 10 years. However, there were differences among the rates of increase among the three resistant cultivars.

Table 2. Effect of tillage and sequence of different sources of resistance on population density of the soybean cyst nematode (SCN) at planting (Pi) and harvest (Pf) from 2009 to 2014

Treatment	SCN population density (eggs per 100 cm ³ of soil) ^y											
	Pi09	Pf09	Pi10	Pf10	Pi11	Pf11	Pi12	Pf12	Pi13	Pf13	Pi14	Pf14
Cultivar sequence ^w (2003–2014)												
1. S-S-S-S-S-S-S-S-S-S-S-S	4,019 bc	5,342 a	3,638 a	4,302 a	2,313 a	4,387 a	2,996 a	7,248 a	5,700 a	5,556 a	3,471 a	2,827 b
2. R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1	1,764 d	1,641 b	1,265 bc	1,023 b	781 b	2,056 b	1,365 b	2,546 bc	2,116 cd	2,115 bc	1,548 bc	1,944 c
3. R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2	308 e	344 c	652 d	392 de	323 cd	1,190 d	352 de	2,329 c	1,433 e	1,127 de	938 cd	1,175 c
4. R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3	216 e	195 d	104 e	199 e	245 d	226 e	244 e	483 d	350 f	410 f	333 e	369 d
5. R1-S-R2-S-R1-S-R2-S-R1-S-R2-S-R1-S-R2-S	6,748 a	1,766 b	1,399 bc	5,221 a	4,020 a	1,478 bc	1,060 bc	7,667 a	5,168 ab	2,450 b	1,540 bc	4,148 ab
6. R2-S-R3-S-R2-S-R3-S-R2-S-R3-S-R2-S-R3-S	5,843 ab	1,560 b	1,016 cd	3,538 a	2,576 a	847 cd	535 d	5,585 a	4,099 bc	2,098 cd	1,594 bc	4,531 ab
7. R3-S-R1-S-R3-S-R1-S-R3-S-R1-S-R3-S-R1-S	6,298 a	2,571 b	2,294 b	4,713 a	2,529 a	915 bcd	500 cd	8,377 a	6,142 a	3,523 b	2,721 ab	5,315 a
8. R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S	3,802 c	2,471 b	1,031 bc	399 cd	297 cd	4,069 a	3,354 a	2,306 bc	1,468 de	579 ef	779 cd	3,629 ab
9. R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S	5,973 ab	2,034 b	1,413 bc	797 bc	728 bc	6,131 a	3,913 a	1,529 c	1,051 e	931 def	975 cd	4,023 ab
10. R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S	5,024 abc	1,971 b	1,287 c	662 d	350 d	5,151 a	3,119 a	2,181 bc	1,428 de	696 ef	590 de	4,331 ab
11. R1-R2-S-R3-R1-S-R2-R3-S-R1-R2-S	7,369 a	2,752 b	1,734 bc	638 bcd	473 cd	5,459 a	4,504 a	3,183 b	2,886 cd	1,148 de	1,083 bcd	4,315 ab
Tillage												
No till	4,616	2,207	1,594	2,126	1,695	3,079	2,545	4,375	3,570	2,309	1,758	4,216
Conventional tillage	3,995	1,910	1,285	1,852	966	2,722	1,444	3,522	2,219	1,442	1,073	2,440
ANOVA (F values) ^x												
Tillage	0.2	0.2	0.3	0.4	1.7	0.2	2.5	3.4	9.7 ^y	6.2	3.4	6.3
Sequence	43.3 ^z	18.3 ^z	13.0 ^z	36.2 ^z	22.5 ^z	21.9 ^z	19.4 ^z	15.2 ^z	15.2 ^z	14.7 ^z	6.8 ^z	20.0 ^z
Tillage $\times$ sequence	0.9	0.5	1.1	1.6	1.7	1.0	2.1 ^y	0.6	1.6	2.3 ^y	1.6	2.3 ^y

^y The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$. Pi09, Pf09 to Pi14, and Pf14 represent egg population densities (eggs per 100 cm³ of soil) at Pi and Pf from 2009 to 2014.

^w S is SCN-susceptible cultivar Pioneer 92B13. R_1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R_2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R_3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^x ANOVA, analysis of variance.

^y Significant of the ANOVA F value at $P < 0.05$.

^z Significant of the ANOVA F value at $P < 0.0001$.

Regression analysis demonstrated that the change of Pf on the resistant cultivars in monoculture from 2005 to 2014 fits well in the exponential model. No effect of tillage or its interaction with year was detected. Subsequent regression analyses were performed using the average egg population densities of the two tillage treatments. A highly significant increase of egg population densities over years was detected on R2 ($P = 0.0001$) and R3 ($P = 0.004$), and a marginally significant increase was detected on R1 ($P = 0.0635$) (Fig. 1). Based on the regression analyses, the predicted egg population densities in the fall of 2005 were 1,206,

70, and 40 eggs per 100 cm³ of soil on R1, R2, and R3 monocultures, respectively. After 10 years, the predicted egg population densities in the fall of 2014 were 2,203, 1,983, and 525 eggs per 100 cm³ of soil on R1, R2, and R3 monoculture, respectively. The Pf annual change rate e^b was highest in R2 (1.398) followed by R3 (1.294), and it was lowest in R1 (1.0620). The annual change rate e^b is determined by b (Fig. 1), which may indicate the virulence phenotype selection pressure. No annual increase or decrease of SCN fall egg population density over the 10 years was detected on the SCN-susceptible soybean monoculture (Fig. 1).

Fig. 1. Predicted end season population densities (Pf) of the soybean cyst nematode (SCN) following monoculture of an SCN-resistant or -susceptible soybean cultivar. Year 0 was the fall of 2004, when the egg population densities were dropped to about lowest point. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654. S is SCN-susceptible cultivar Pioneer 92B13. Each data point is an average of two tillage treatments with six replicates.

Table 3. Interactive effect of cultivar sequence and tillage on the soybean cyst nematode (SCN) population density

Cultivar sequence* (2003–2014)	Pi07 ^a		Pi12		Pf13		Pf14	
	NT	CT	NT	CT	NT	CT	NT	CT
1. S-S-S-S-S-S-S-S-S-S	5,067 b	4,438 a	3,054 abc	2,938 a	5,879 a	5,233 a	2,896 bc	2,758 abc
2. R1-R1-R1-R1-R1-R1-R1-R1-R1-R1	1,279 d	1,350 b	1,100 def	1,629 ab	2,354 bcd	1,875 b	2,342 c	1,546 c
3. R2-R2-R2-R2-R2-R2-R2-R2-R2-R2	38 f ^y	125 d	238 gh	467 c	538 ef ^y	1,717 b	788 d ^y	1,563 bc
4. R3-R3-R3-R3-R3-R3-R3-R3-R3-R3	396 e	148 d	171 h	317 c	488 f	333 c	483 e	254 d
5. R1-S-R2-S-R1-S-R2-S-R1-S-R2-S	6,933 ab	4,563 a	1,533 cde ^y	588 bc	3,229 abc ^y	1,671 b	4,388 ab	3,908 ab
6. R2-S-R3-S-R2-S-R3-S-R2-S-R3-S	7,192 ab	5,021 a	671 fg	400 c	2,583 def	1,613 b	6,283 a	2,779 abc
7. R3-S-R1-S-R3-S-R1-S-R3-S-R1-S	8,275 a	7,383 a	567 efg	433 c	5,129 ab	1,917 b	6,188 a	4,442 a
8. R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S	1,509 cd	848 bc	4,121 ab	2,588 a	738 def	421 c	4,692 ab	2,567 abc
9. R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S	2,152 d	350 cd	5,796 ab ^y	2,029 a	1,558 cde ^y	304 c	5,850 a ^z	2,196 abc
10. R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S	1,788 cd	1,517 bc	3,646 bcd	2,592 a	979 def	413 c	5,846 a	2,817 abc
11. R1-R2-S-R3-R1-S-R2-R3-S-R1-R2-S	2,646 c ^z	413 cd	7,104 a ^y	1,904 a	1,929 cd ^y	367 c	6,621 a ^y	2,008 bc

^a S is SCN-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^x The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$. Pi07, Pi12, Pf13, and Pf14 represent egg population densities (eggs per 100 cm³ of soil) at planting (Pi) in 2007 and 2012 or harvest (Pf) in 2013 and 2014. CT, conventional tillage; NT, no tillage.

^y Significant between the two NT and CT treatments in a year at $P < 0.05$.

^z Significant between the two NT and CT treatments in a year at $P < 0.01$.

The SCN Pf on susceptible cultivar in monoculture (sequence 1) did not differ from the Pf on susceptible cultivars following 1 year of resistant cultivar (sequences 5, 6, and 7) in 2004, 2010, and 2012, but it was lower than that in 2006 and 2008 (Tables 1 and 2). In 2014, significantly higher egg population density in the susceptible cultivar in annual rotation compared with monoculture was observed only in the R3-S-R1-S rotation (sequence 7) (Tables 1 and 2). No significant difference was detected in Pf between susceptible cultivar following 2 years of resistant cultivars (sequences 8 to 11 in years 2005, 2008, 2011, and 2014) and susceptible cultivar in monoculture.

Tillage had little effect on SCN egg population density based on ANOVA. Significant difference was observed only in Pi in 2013 when NT resulted in a little higher SCN population density compared with CT (Tables 1 and 2). However, significant interaction ($P < 0.05$) was detected in a few sampling points (Pi07, Pi12, Pf13, and Pf14) (Tables 1 and 2). When the data were analyzed for difference between the tillage treatments in each cultivar sequence, significance was detected in only 4 of the 11 cultivar sequences, with higher egg population densities in NT in nine sequence-time points in sequences 5, 9, and 11 (R1-S-R2-S, R2-R1-S-R2, and R1-R2-S-R3) and higher densities in CT at three sequence-time points in sequence 3 (R2 monoculture) (Table 3).

Virulence of the SCN populations. The soil samples collected in the spring in the years 2007, 2008, 2009, 2010, 2012, and 2014 were analyzed for FI on the indicator lines Peking, PI 88788, and PI 437654. There were large variances in the FI data, but general trends can be determined by the ANOVA and LSD tests (Supplementary Table S1). In 2007, the FIs were still low in all treatments. However, a slight increase in FI occurred in some cultivar sequences involving resistant cultivars compared with susceptible monoculture. Continuously planting PI 88788-derived cultivar EX547 RR N (R1) for 4 or more years significantly increased FI on the source of resistance PI 88788 compared with monoculture of susceptible soybean in all sampling years except 2010 (Supplementary Table S1). Monoculture of the Peking-derived cultivar Pioneer 91M90 (R2) for 4 or more years had higher FI on the source of resistance Peking compared with the monoculture of susceptible soybean. Monoculture of the PI 437654-derived cultivar (R3) also increased FI on both PI 88788 and Peking in some years (Supplementary Table S1). There was little increase in FI of SCN populations on PI 88788 and Peking following the sequences that included different resistant cultivars, but significantly higher FIs were detected in a few sequences and years (Supplementary Table S1). The FI on PI 437654 was 0 or <1 in most measurements. The FIs on PI 88788 and Peking were higher in CT than NT in some cultivar sequences and years (Supplementary Table S1).

Fig. 2. Predicted female index (FI) of the soybean cyst nematode (SCN) in different rotations of sources of resistance in no till (NT) and conventional tillage (CT). **A and B,** The model for FI on cultivar PI 88788 after a cultivar sequence is $FI = 11e^{(0.08779R1 + 0.05699T \times R3)} - 1$. **C and D,** The model for FI on cultivar Peking is $FI = 2.18e^{(0.14985R2 + 0.11135R3 + 0.083927 \times R2 - 0.08394R1 - 0.14204S)} - 1$. S is SCN-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654. In the models, R1, R2, R3, and S are numbers of years of the soybean cultivars in a crop sequence. T is tillage, where NT is zero and CT is one.

The regression analysis produced more informative results. The effect of cultivars and tillage on the FI of the SCN populations on PI 88788 can be described by the model (Fig. 2A and B):

$$FI = 11e^{(0.08779R1 + 0.05699T \times R3)} - 1$$

Significant effects of PI 88788-derived R1, PI 437654-derived R3, and tillage were observed on the FI on PI 88788. However, a significant effect of R3 was detected only in CT. Both R1 and R3 selected SCN populations toward increased virulence to PI 88788, and R1 had greater effect than R3. Based on this model, the

predicted FI of the initial population on PI 88788 was 10. Monoculture of R1 had the greatest selection pressure, and after 12 years of monoculture, the predicted FI on PI 88788 was 31 (Fig. 2A and B). Monoculture of R3 for 12 years selected the SCN population in CT soil that had FI 21 on PI 88788. Rotation of one or two SCN-resistant soybean cultivars with susceptible soybean slowed the change of FI on the PI 88788 (Fig. 2A and B). No effect of the susceptible soybean and Peking-derived R2 on the FI on PI 88788 was detected.

There was greater effect of the cultivars and tillage on the FI of the SCN populations on Peking than PI 88788 (Fig. 2C and D):

Table 4. Effect of tillage and sequence of different sources of resistance on soybean yield

Treatment	Yield (tons/ha) ^u											
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cultivar sequence ^v (2003–2014)												
No till	1.987	2.675	2.757 b	2.599 b	2.739	2.271	3.089	2.832	3.046	2.353	1.749	1.225 b
Conventional tillage	2.032	2.876	3.068 a	2.995 a	2.977	2.620	3.148	3.002	3.108	2.626	2.016	1.527 a
Cultivar sequence ^v (2003–2014)												
1. S-S-S-S-S-S-S-S-S-S-S-S	2.228 a	2.803 bc	2.935 cde	2.894 ab	2.962 b	2.630 ab	3.513 ab	2.970 bc	2.993 ab	2.390 de	1.894 abcd	1.384 cde
2. R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1	2.063 bc	2.976 ab	3.115 abc	2.827 b	3.050 ab	2.596 abc	3.313 abc	3.185 a	3.044 ab	2.623 bc	2.026 ab	1.475 abc
3. R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2	1.933 cd	2.777 c	2.928 de	2.856 ab	2.658 c	2.279 e	3.139 c	3.107 ab	3.229 a	2.429 cde	1.740 de	1.258 e
4. R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3	1.747 e	2.319 d	2.558 f	2.507 c	2.458 d	1.800 f	2.704 d	2.565 d	3.136 ab	2.400 de	1.834 bcde	1.030 f
5. R1-S-R2-S-R1-S-R2-S-R1-S-R2-S-R1-S-R2-S	2.179 ab	2.907 bc	2.938 cde	3.091 a	3.168 a	2.413 cde	3.078 c	2.923 c	3.124 ab	2.293 e	1.717 e	1.361 cde
6. R2-S-R3-S-R2-S-R3-S-R2-S-R3-S-R2-S-R3-S	1.951 cd	2.834 bc	2.368 f	2.932 ab	2.703 c	2.476 cd	2.699 d	2.976 bc	3.103 ab	2.355 de	1.785 cde	1.396 bcd
7. R3-S-R1-S-R3-S-R1-S-R3-S-R1-S-R3-S-R1-S	1.855 de	2.916 bc	3.240 a	2.888 ab	2.662 c	2.517 bcd	3.549 a	3.005 bc	2.887 b	2.425 cde	2.037 ab	1.436 abcd
8. R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S	2.189 ab	2.343 d	3.203 ab	3.108 a	2.695 c	2.683 a	3.271 bc	2.533 d	3.052 ab	2.861 a	1.830 bcde	1.557 a
9. R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S	2.052 bc	3.109 a	2.915 de	2.936 ab	3.163 a	2.462 cd	3.159 c	3.196 a	3.023 ab	2.553 bcd	2.086 a	1.520 ab
10. R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S	1.815 de	2.789 c	3.044 bcd	2.374 c	2.743 c	2.638 ab	2.771 d	3.055 abc	3.073 ab	2.312 e	1.924 abc	1.383 cde
11. R1-R2-S-R3-R1-S-R2-R3-S-R1-R2-S-R1-R2-S	2.093 abc	2.756 c	2.793 e	2.354 c	3.173 a	2.408 de	3.107 c	2.576 d	3.179 ab	2.742 ab	1.830 cde	1.333 de
ANOVA (<i>F</i> value)												
Tillage	0.1	5.6	12.6 ^w	17.4 ^x	3.3	5.5	0.1	1.8	0.7	3.1	2.4	8.1 ^w
Sequence	7.7 ^y	14.5 ^y	14.3 ^y	8.5 ^y	17.3 ^y	14.5 ^y	11.1 ^y	20.0 ^y	0.6	6.8 ^y	3.0 ^x	9.5 ^y
Tillage × sequence	1.0	1.0	0.6	1.5	1.4	1.7	1.0	1.9	0.8	2.0 ^w	0.7	2.0 ^w
Cultivar												
S	2.228 a	2.865 b	2.978 b	2.951 a	2.962 b	2.528 a	3.513 a	2.968 b	3.064 a	2.366 c	1.894 b	1.421 a
R1	2.131 a	3.042 a	3.177 a	2.967 a	3.139 a	2.596 a	3.378 a	3.191 a	3.084 a	2.742 a	2.050 a	1.475 a
R2	1.979 b	2.774 b	2.933 b	2.896 a	2.701 c	2.279 b	3.121 b	3.081 a	3.166 a	2.491 b	1.803 b	1.258 b
R3	1.806 c	2.331 c	2.463 c	2.412 b	2.605 c	1.800 c	2.725 c	2.558 c	3.012 a	2.356 c	1.816 b	1.030 c
ANOVA (<i>F</i> value)												
Tillage	0.2	5.4	9.3 ^w	12.3 ^w	3.9	2.9	0.1	1.5	0.2	2.6	1.8	5.8
Cultivar	15.0 ^y	34.2 ^y	20.7 ^y	34.8 ^y	35.5 ^y	44.5 ^y	35.3 ^y	61.0 ^y	0.6	23.3 ^y	9.4 ^z	28.5 ^y
Tillage × cultivar	0.4	1.0	0.6	5.3 ^x	1.3	0.0	0.3	2.8	0.6	3.6 ^w	0.3	2.3

^u The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$.

^v S is soybean cyst nematode (SCN)-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^w Significant of the ANOVA *F* value at $P < 0.05$.

^x Significant of the ANOVA *F* value at $P < 0.01$.

^y Significant of the ANOVA *F* value at $P < 0.0001$.

^z Significant of the ANOVA *F* value at $P < 0.001$.

$$FI = 2.18e^{(0.14985R2 + 0.11135R3 + 0.08392T \times R2 - 0.08394R1 - 0.14204S)} - 1$$

R2 and R3 had positive effect and R1 and S had negative effect on the FI on Peking. Based on this model, the predicted FI of the SCN population at the beginning of experiment on Peking was only 1.18. There was greater effect of R2 on FI on Peking in CT than NT; monoculture of R2 for 12 years increased FI on Peking to 35 in CT soil and 11 in NT soil (Fig. 2C and D). Monoculture of R3 increased FI on Peking to 7.3 in both CT and NT. Rotation of one or two SCN-resistant soybean cultivars with the susceptible soybean resulted in only a little increase of FI on the Peking over the 12 years (Fig. 2C and D).

Soybean yield. Soybean yield differed among different soybean cultivars and among different sequences of soybean cultivars. The difference of yield in different cultivar sequences was mainly because of the difference of yield potential of the cultivars (Table 4). In 2003 when the initial SCN population densities were presumably the same at 4,579 eggs per 100 cm³ of soil among the treatments, average yield of SCN-susceptible cultivar Pioneer 92B13 (S) did not differ from the PI 88788-derived cultivar Latham EX547 RR N (R1), but it was higher than Peking-derived cultivar Pioneer 91M90 (R2) and PI 347654-derived cultivar Latham AR5084 (R3). Among the three SCN-resistant cultivars, R1 produced highest yield, R2 produced intermediate yield, and R3 produced the lowest yield (Table 4). In all following years when SCN population densities varied among treatments, R1 maintained the highest yield among the four cultivars. SCN-susceptible soybean either had similar or lower yield compared with R1. R2 produced lower yield in all years except 2006, 2010, and 2011, when the yield did not differ from R1. R3 produced the lowest yield all years except 2011 and 2013, when the yield did not differ from R2 and S. Average yields of the 12 years were 2.65, 2.75, 2.54, and 2.24 tons/ha for the cultivars S, R1, R2, and R3, respectively; 1 or 2 preceding years of resistant soybean did not have detectable effect on the yield of the susceptible soybean in the following year (Supplementary Fig. S2), although the resistant soybean lowered SCN population densities compared with the susceptible soybean.

The soybean yield was significantly higher in CT than NT in 2005, 2006, and 2014; in other years, the yield was also numerically higher in CT than in NT, although it was not statistically significant ($P > 0.05$) (Table 4). The average yield of 12 years was 8.35% higher in CT compared with NT.

Significant interaction between tillage and cultivar sequences on yield was detected only in 2012 and 2014 (Table 4). Significantly higher yield in CT compared with NT was observed only in the

sequences 4, 5, and 7 in 2012 and in the sequences 3, 6, 7, and 11 in 2014 (Table 5). The interaction between tillage and cultivars was observed in 2006 and 2012; higher yield in CT compared with NT was observed only in cultivars S, R1, and R2 in 2006 and in cultivars S and R3 in 2012 (Table 6). There was no case in which the yield was significantly higher in NT than CT.

Discussion

In this study, the effect of tillage and rotation of commercial soybean cultivars carrying resistance from different sources of resistance (PI 88788, Peking, and PI 437654) on SCN population density and virulence was evaluated in a 12-year field experiment with an initial population of HG type 0. By definition, this initial population was avirulent to the three sources of resistance used in this research. NT has been practiced in the north central United States for a number of decades, and commercial SCN-resistant cultivars derived from PI 88788 and Peking have been used in the region for a few decades. Some cultivars derived from PI 437654 (CystX) were expected to be commercially available when this experiment was initiated. Because of their poor yield potential, no commercial cultivar from this source has widely been accepted in the region. Rotation of cultivars with different sources of resistance remains the most effective management strategy, although few sources of resistance in the commercial cultivars are available. This study generated important data related to how the different rotations of cultivars from three sources of resistance and tillage affect SCN virulence and population density in field over a 12-year period.

Selection pressure may depend on many factors, including the genetic interaction between the SCN population and the soybean cultivars, SCN resistance level in the cultivars, virulence type and level of initial nematode population, and climate and soil conditions that may affect nematode parasitism and survival. Tillage had minimal effect on SCN egg population density in the northern climate in Minnesota, agreeing with a few previous field studies (Chen 2007b; Grabau et al. 2017). Interestingly, significant tillage effect on the virulence of SCN was detected in some cases, especially for the FI on Peking. There was higher selection pressure of planting the Peking-derived cultivar (R2) toward the virulence to Peking in the CT than in NT (Fig. 2C and D). For the virulence to PI 88788, there was a little bit of an increase of selection pressure of planting the PI 437654-derived cultivar (R3) in the CT compared with NT (Fig. 2A and B). This is probably the first study showing the tillage effect on SCN virulence phenotypes in fields planted to SCN-resistant cultivars. The mechanism of the effect cannot be determined in this study. However, it is possible that the movement of soil may redistribute the SCN virulent individual nematode eggs

Table 5. Interactive effect of cultivar sequence and tillage on soybean yield

Cultivar sequence ^w (2003–2014)	Yield (tons/ha) ^x			
	2012		2014	
	NT	CT	NT	CT
1. S-S-S-S-S-S-S-S-S-S	2.195 de	2.586 cd	1.221 bc	1.546 bcd
2. R1-R1-R1-R1-R1-R1-R1-R1-R1-R1	2.558 abc	2.688 bc	1.350 ab	1.600 abcd
3. R2-R2-R2-R2-R2-R2-R2-R2-R2-R2	2.328 bcd	2.529 cd	1.057 cd ^y	1.458 d
4. R3-R3-R3-R3-R3-R3-R3-R3-R3-R3	2.253 bcde ^y	2.546 cd	0.976 d	1.085 e
5. R1-S-R2-S-R1-S-R2-S-R1-S-R2-S	1.939 e ^y	2.646 c	1.230 bc	1.493 cd
6. R2-S-R3-S-R2-S-R3-S-R2-S-R3-S	2.283 bcde	2.427 d	1.259 bc ^z	1.534 bcd
7. R3-S-R1-S-R3-S-R1-S-R3-S-R1-S	2.185 de ^y	2.665 bc	1.166 bcd ^y	1.706 a
8. R1-R3-S-R1-R3-S-R1-R3-S-R1-R3-S	2.889 a	2.833 ab	1.498 a	1.617 abc
9. R2-R1-S-R2-R1-S-R2-R1-S-R2-R1-S	2.459 bcd	2.646 c	1.358 ab	1.681 ab
10. R3-R2-S-R3-R2-S-R3-R2-S-R3-R2-S	2.196 cde	2.427 d	1.259 bc	1.507 cd
11. R1-R2-S-R3-R1-S-R2-R3-S-R1-R2-S	2.593 ab	2.891 a	1.097 cd ^z	1.569 abcd

^w S is soybean cyst nematode (SCN)-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^x The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$. CT, conventional tillage; NT, no tillage.

^y Significant between the two NT and CT treatments in a year at $P < 0.05$.

^z Significant between the two NT and CT treatments in a year at $P < 0.01$.

or cysts so that they may have better chance to obtain food for survival and reproduction. It is also possible that the relocated virulent nematodes may have better chances to mate with avirulent SCN individuals, and the heterozygous progenies may have less fitness cost and better reproductive potential or survival ability on the resistant cultivar and/or susceptible cultivar. The tillage effect was more evident in FI on Peking selected by R2 than any FI selected by the other cultivars, probably owing to the fact that R2 had higher selection pressure than the other cultivars. Although the effect of tillage on virulence was detected, this effect was not reflected in the SCN population densities over time because generally, there was no difference in SCN population density between NT and CT, particularly in the monoculture of R2. This was probably because of large variability of SCN egg population densities in the field plot setting.

This study demonstrated that all three cultivars carrying the resistance from Peking, PI 88788, or PI 437654 had an impact on the SCN virulence to Peking and PI 88788, and the effect varied between FI on Peking and FI on PI 88788 and between the two tillage treatments. Peking and PI 88788 are the two germplasm lines that have been most frequently used in the commercial SCN-resistant cultivars. These two lines have two distinct types of genetics (Lee et al. 2015; Liu et al. 2012) that resist two different types of SCN (Niblack et al. 2002; Riggs and Schmitt 1988). In this study, the Peking-derived cultivar selected SCN populations that could not overcome the resistance in PI 88788, and likewise, the PI 88788-derived cultivar selected SCN populations that could not overcome the resistance in Peking. This result was similar to that in previous greenhouse (Triantaphyllou 1975; Young 1982) and field studies (Young and Hartwig 1988; Zheng and Chen 2011). Interestingly, the PI 88788-derived cultivar in a rotation with Peking-and/or PI 437654-derived cultivars had negative effect on FI on Peking. This result was similar to previous greenhouse pot (Young 1984) and field plot experiments (Young 1994) in which soybean cultivars Bedford and Nathan reduced FI on Peking. Bedford and Nathan were derived from PI 88788 and Peking, but apparently, the Peking resistance was lost. These results suggest that rotation of Peking with PI 88788 is a good strategy for managing the SCN HG type 0 populations and that planting PI 88788-derived cultivars in a field infested with SCN HG type 1– (race 14 or 9) may be able to reduce FI or change the population from virulent to avirulent on Peking.

PI 437654 had a broad spectrum of SCN resistance, and very few field populations have been shown to be able to reproduce on it (Gardner et al. 2017; Lian et al. 2017). In four state-wide surveys in the past two decades in Minnesota, only 1 (in 1998) of 307 SCN populations tested was observed to have positive response on PI 437654 (FI = 12.7) (Zheng et al. 2006). In the present study, the SCN populations selected by Peking- and PI 88788-derived cultivars each alone were unable to reproduce on PI 437654, agreeing with a previous study (Anand et al. 1985). Moreover, continuously planting a PI 437654-derived cultivar for 12 years was also unable to select SCN populations to overcome the resistance in PI 437654. This indicates that PI 437654 contains additional different types of SCN resistance that were not in the PI 437654-derived cultivar (R3) or that not all genes in SCN that are necessary for virulence to PI 437654 (Gardner et al. 2017) existed in the SCN populations in this field. In contrast, the PI 437654-selected SCN populations had increased FI on both Peking and PI 88788, indicating that the cultivar may carry similar SCN resistance genes or have function of the genes in both or either PI 88788 and Peking.

Little is known about the molecular mechanisms of the change of virulence of SCN populations on different sources of resistance over time. Nevertheless, there are a number of studies of parasitism genes in the SCN and of SCN resistance quantitative trait locus (QTL)/genes in soybean; >80 proteins or effectors from SCN have been identified as associated with SCN parasitism on soybean (Masonbrink et al. 2019). A large number of effectors, probably including many unidentified effectors, are needed for a successful parasitism of soybean by SCN.

In the host soybean, >200 SCN-resistant QTLs have been reported (<https://www.soybase.org/>), among which 2 major QTLs, *rhg1* and *Rhg4*, have been used in the commercial SCN-resistant cultivars (Mitchum 2016). The *rhg1* is located at the 10-cM region on chromosome 18 (Guo et al. 2006; Kim et al. 2010; Lee et al. 2015). Each copy of *rhg1* is a 31.2-kb unit containing four genes: an amino acid transporter, an N-ethylmaleimide-sensitive factor attachment protein (α -SNAP) protein, a W112 (wound-inducible domain) protein, and an leucine-rich repeat transmembrane receptor-kinase; however, only the former three genes contribute to the SCN resistance (Bayless et al. 2016; Cook et al. 2012, 2014; Mitchum 2016). The *Rhg4* QTL has frequently been mapped at around 50 cM on chromosome 8, and it encodes serine hydroxymethyltransferase (Kandoth et al. 2017; Liu et al. 2012; Patil et al. 2019; Yu et al. 2016).

The number of copies of the *rhg1* region varies among soybean germplasm lines. There is only one copy in SCN-susceptible soybean, and two or more copies of *rhg1* genes confer SCN resistance (Cook et al. 2012). In addition, the types of the *rhg1* copies are important in conferring SCN resistance (Lee et al. 2015; Yu et al. 2016). Interestingly, there also are copy number variations in the *Rhg4* gene region (Patil et al. 2019). The most commonly used source of resistance PI 88788 contains 9 (8.7) copies of repeat of *rhg1-b* genes and 1 copy of *Rhg4* gene, and only the three genes in the *rhg1-b* contribute to SCN resistance (Bayless et al. 2018; Cook et al. 2012; Patil et al. 2019). Peking has 3 (3.2) copies of repeat of *rhg1-a* genes and 2 (2.35) copies of the *Rhg4* gene. The combination of the *rhg1-a* and *Rhg4-a* genes confers the Peking-type SCN resistance (Lee et al. 2015; Liu et al. 2017; Meksem et al. 2001; Patil et al. 2019). The genetics of SCN resistance in PI 437654 are complex. Other than the *rhg1* and *Rhg4* loci, there are a number of other QTLs associated with SCN resistance in PI 437654, and there are various interactions among the QTLs (Lee et al. 2015; Wu et al. 2009). PI 437654 has 3 (3.3) copies of *rhg1-a* and 4 (4.3) copies of *Rhg4-c* genes (Patil et al. 2019). The high *Rhg4* copy number in PI 437654 may be responsible for the broad resistance to SCN (Patil et al. 2019). However, little is known about the molecular mechanism of interaction between *rhg1-a* and *Rhg4* genes for SCN resistance, and it is not known whether any other genes are important in contributing the SCN resistance in the three sources of resistance.

The SCN has evolved virulence genes to overcome the resistance in soybean (Gardner et al. 2017). Dong and Opperman (1997) demonstrated that a single dominant gene designated *ror-1* from an inbred SCN line overcame the resistance in PI 88788 and that another independent single recessive gene overcame the resistance in Peking. In addition, they reported another two independent alleles of genes in another SCN inbred line for virulence to PI 88788 (Dong and Opperman 1997). In 2017, Gardner et al. (2017) studied

Table 6. Interactive effect of cultivar and tillage on the soybean yield

Cultivar ^a	Yield (tons/ha) ^w			
	2006		2012	
	NT	CT	NT	CT
S	2.662 a ^x	3.241 a	2.150 c ^x	2.581 b ^x
R1	2.766 a ^y	3.169 a	2.680 a	2.804 a
R2	2.671 a ^z	3.121 a	2.394 b	2.588 b
R3	2.355 b	2.469 b	2.225 bc ^z	2.487 b

^a S is soybean cyst nematode (SCN)-susceptible cultivar Pioneer 92B13. R1 is SCN-resistant cultivar Latham EX547 RR N derived from cultivar PI 88788. R2 is SCN-resistant cultivar Pioneer 91M90 derived from cultivar Peking. R3 is SCN-resistant cultivar Latham AR5084 derived from cultivar PI 437654.

^w The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$. CT, conventional tillage; NT, no tillage.

^x Significant between the two NT and CT treatments in a year at $P < 0.0001$.

^y Significant between the two NT and CT treatments in a year at $P < 0.05$.

^z Significant between the two NT and CT treatments in a year at $P < 0.01$.

virulence genes in an inbred SCN line named TN20, which could parasitize all HG type indicator lines (HG type 1.2.3.4.5.6.7 or race 4). They found that a single dominant gene in TN20 controlled the virulence to PI 88788 and that the gene may correspond to the *ror-1* (Gardner et al. 2017). The virulence in TN20 to Peking is controlled by a recessive gene *ror-3*, and the virulence to PI 437654 is controlled by multiple recessive genes. Dong et al. (2005) reported that extensive variations in SCN virulence genes exist among field populations. These previous studies indicate that multiple genes may exist in a field population for SCN virulence to each of the resistance in PI 88788, Peking, and PI 437654. Although the SCN populations in the field of the present study were phenotyped and the original population (HG type 0) was avirulent to the three sources of resistance used, the genetic background of the SCN populations in the field is unknown. The results of this study indicated that multiple virulence genes existed at low frequencies in the initial SCN population in this field. However, the type and number of virulence genes to each of the resistant cultivars in the SCN populations in the field were undetermined.

Rotation of susceptible soybean with resistant cultivars has been considered a proper choice for managing SCN (Niblack 2005; Niblack and Chen 2004). Presumably, the benefits of planting SCN-susceptible soybean can be (1) to avoid yield suppression by a genetic factor associated or linked with the SCN resistance genes (Kopisch-Obuch et al. 2005; Mudge et al. 1996) or (2) to reduce the amount of time of planting SCN-resistant cultivars and subsequently reduce selection pressure on SCN virulence. Additionally, (3) there is a fitness cost of SCN virulent genotypes, and susceptible soybean may select an SCN population with reduced virulence on SCN-resistant soybean cultivars (Young 1998b). The result that susceptible soybean reduced FI of SCN populations on Peking provides evidence that there is fitness cost for the Peking virulent SCN type. However, the fitness cost was not detected for the PI 88788 virulent SCN type because there was no significant reduction of FI on PI 88788 by planting the susceptible soybean in the field.

The trends of SCN density of the field populations were similar to those of the FI on the sources of resistance germplasm lines of PI 88788 and Peking. Based on the data of Pf in monoculture of the resistant cultivars, the Pf annual change rate e^b was highest in R2 followed by R3, and it was lowest in R1. The annual change rate e^b is determined by b , which may indicate the virulence phenotype selection pressure in the field. The difference in selection pressure may be mainly owing to the level of resistance and the interaction between the SCN-resistant soybean and SCN population genotypes. After 2 years of monoculture, the predicted SCN population densities were changed from initial density of 4,579 eggs per 100 cm³ of soil to densities of 1,206, 70, and 40 eggs per 100 cm³ of soil by R1, R2, and R3, respectively, indicating that the resistance level to the initial population was greatest in R3, intermediate in R2, and lowest in R1 (Fig. 1). The lower b value and the low P value of the regression of Pf on R1 were probably because of the lower level of SCN resistance in R1. However, the b value was greater in R2 than in R3, whereas R3 had a greater level of SCN resistance to the original population. This was probably because of the fact that there were fewer SCN resistance genes in the R2 compared with R3, and it may be easier for the nematode population to overcome a single or few SCN resistance genes than the greater number of genes in a resistant cultivar, like the PI 437654-derived cultivar R3.

The PI 88788-derived cultivar EX547 RR N used in this study had less selection pressure on the SCN population compared with another PI 88788-derived cultivar Freeborn used in a previous study (Zheng and Chen 2011). In the previous study, planting Freeborn for 10 years changed the SCN population FI on PI 88788 from 2.8 to 47 (Zheng and Chen 2011), whereas in the present study, 12 years of monoculture changed FI on PI 88788 from 10 to 30. This difference suggests that some genes involved in SCN resistance might be different between Freeborn and EX547 RR N, and the level of resistance was greater in Freeborn than in EX547 RR N. It is also possible that this difference was because of different

virulence genes in the two different SCN populations. The result also suggests that it may be useful to rotate cultivars with the same source of SCN resistance.

It is noticeable that the SCN Pf on the susceptible cultivar following 1 year of a resistant cultivar was statistically greater in 2006 and 2008 and numerically greater in 2004, 2010, and 2012 compared with the Pf in monoculture of susceptible cultivars (Tables 1 and 2). There were similar trends in 2 years of SCN-resistant cultivar rotations (Tables 1 and 2). The results suggested that the SCN population was able to reach carrying capacity on the susceptible soybean within one season following 1 to 2 years of resistant soybean. SCN-susceptible soybean yields following susceptible soybean did not differ from those following 1 or 2 years of resistant soybean, suggesting that the rebound of SCN population density following the rotation was not because of the plant biomass. The rebound of SCN population density was probably because of the fact that the resistant cultivar lowered the SCN population density as well as the biological antagonists in the preceding years, resulting in the soil being less suppressive to the SCN. This experimental site was located in the same field of the previous studies of SCN-suppressive soil in which fungal and bacterial microbes were involved in suppression of the SCN population densities (Chen 2007a; Hu et al. 2017, 2019). The site used in this study was also suppressive to SCN based on the overall low-SCN population densities on the susceptible soybean compared with some other fields in the region.

The soil suppressiveness of SCN population densities was also reflected by the fact that, generally, there was little change of SCN egg population densities from spring (Pi) to fall (Pf) in individual years in the sequence of continuous SCN-susceptible soybean S (Tables 1 and 2). On the S in monoculture, significant increase of egg population densities from Pi to Pf was observed only in 4 (2005, 2006, 2011, and 2012) of the 11 years, and in other years, no significant difference was detected (statistical analysis is not shown). It is unknown whether soil suppressiveness impacts the selection pressure on SCN virulence from the use of SCN-resistant cultivars. The reasons for the differences in SCN reproduction among different years were probably owing to some undetermined environmental factors.

Conclusion. This study demonstrated that tillage had minimal effect on SCN population density, but CT resulted in a faster increase of SCN virulence to Peking by the Peking-derived cultivar (R2) and the virulence to PI 88788 by the PI 437654-derived cultivar (R3). The SCN populations selected by the PI 88788-derived cultivar (R1) overcame the resistance in PI 88788 but not Peking and PI 437654. R2 selected SCN populations that overcame the resistance in Peking but not PI 88788 and PI 437654. In contrast, R3 selected SCN populations that overcame both PI 88788 and Peking sources of resistance. R1 in rotation with R2 or R3 had a negative effect on FI on Peking. Susceptible soybean reduced SCN virulence to Peking, indicating that there was a fitness cost of the Peking virulent SCN type. These results suggest that rotation of Peking with PI 88788 is a good strategy for managing the SCN, and susceptible cultivars and NT may reduce SCN virulence selection pressure.

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