Determination of *Heterodera glycines* Virulence Phenotypes Occurring in South Dakota

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Abstract

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the most important yield-limiting pathogen of soybean in the United States. In South Dakota, SCN has been found in 29 counties, as of 2016, and continues to spread. Determining the virulence phenotypes (HG types) of the SCN populations can reveal the diversity of the SCN populations and the sources of resistance that would be most effective for SCN management. To determine the HG types prevalent in South Dakota, 250 soil samples were collected from at least three arbitrarily selected fields in each of the 28 counties with fields previously found to be infested with SCN. SCN was detected in 82 fields (33%), and combined egg and juvenile counts ranged from 200 to 65,200 per 100 cm³ of soil. Eggs and juveniles were extracted from each soil sample and were used to infest seven SCN HG type test indicator soybean lines and 'Williams 82' as the susceptible check. A female index (FI) was calculated based on the number of females found on each

indicator line relative to those on the susceptible check. A FI equal to or greater than 10% in any line was assigned as that HG type. Out of 73 SCN populations for which HG type tests were done, 63% had FI $\geq$ 10% on PI 548316 (indicator line #7), 25% on PI 88788 (#2), 19% on PI 209332 (#5), 7% on PI 548402 (#1), 4% on PI 90736 (#3), and 4% on PI 89722 (#6). None of the SCN populations had FI $\geq$ 10% on PI 437654 (indicator line #4). The most prevalent HG types were 0, 2.5.7, and 7. These accounted for 81% of all the HG types determined for the samples tested. HG types with $\geq$ 10% reproduction on indicator lines PI 88788, PI 209332, and PI 548317 were most prevalent in the soil samples tested, suggesting that the use of these sources of resistance for developing SCN resistant cultivars should be avoided. For sustainable SCN management, use of resistant cultivars should be rotated with nonhost crops and cultivars with different sources of resistance.

Soybean [*Glycine max* L. (Merr.)] is the most important leguminous crop and is ranked second after corn in terms of production in South Dakota and in the United States (NASS 2015). Soybean production is limited by different abiotic and biotic factors. Abiotic factors include low temperature, salt toxicity, water stress, nutritional deficiency, and biotic factors include weeds, insect pests, soybean cyst nematode (SCN), and diseases (Hartman et al. 2011). Of the biotic factors, SCN is the most limiting factor for soybean production in the United States (Koenning and Wrather 2010; Wrather and Koenning 2009).

The soybean cyst nematode, *Heterodera glycines* (Ichinohe 1955) is an obligate, sedentary endoparasitic nematode that infects soybean roots. It was first discovered in northeast China in 1899 (Li et al. 2011b) and identified in Japan in 1915, in Korea in 1936, in Manchuria in 1938, and in the United States in 1954 in North Carolina (Riggs 1977; Winstead et al. 1955). SCN is thought to have been inadvertently introduced into the U.S. with the *Rhizobia* inoculum from China in the first half of the 19th century (Noel 1986). SCN has now been detected in 90% of the soybean producing states (Tylka and Marett 2014) and was first detected in South Dakota in 1995 in Union county (Smolik et al. 1996).

The extent of yield loss caused by SCN depends upon the level of infestation, environmental factors, and the cultivar planted. SCN can cause yield loss of up to 30% without showing any visible symptoms in soybeans (Wang et al. 2003). Yield loss of up to 75% in heavily infested fields is not uncommon (Wang et al. 2003; Young 1996). Yield loss can even be higher if SCN infection is associated with fungal pathogens such as *Fusarium* spp. (Xing and Westphal 2006).

Different practices have been used for SCN management, such as host resistance, chemical control, cultural methods and biological control (Auwal et al. 2014; Chen and Liu 2005; Concibido et al. 2004; Conley et al. 2011; Grabau 2013; Li et al. 2011a). Currently, growing resistant soybean cultivars and nonhost crops in rotation have proven to be the most effective, sustainable, and affordable methods of SCN management (Niblack et al. 2003; Oyekanmi and Fawole 2010; Schmitt et al. 2004). Recently, a few seed treatments have been introduced on the market that show some reduction of SCN population density in the soil (De Bruin and Pedersen 2008; Frye 2009; Grabau 2013; Wu et al. 2014).

There are many SCN resistant soybean cultivars available in the United States, but more than 90% of the resistant cultivars have been derived from the resistance source PI 88788 (Concibido et al. 2004; Schmitt et al. 2004; Shannon et al. 2004). Continued planting of varieties with the same SCN resistance genes can result in SCN populations that are adapted to these sources of resistance (Niblack 2005; Tylka and Mullaney 2015). Several research reports suggested that commonly used resistance source PI 88788 is being overcome by SCN in different states such as Kansas (Rzodkiewicz 2010), Kentucky (Hershman et al. 2008), Missouri (Mitchum et al. 2007), Minnesota (Zheng et al. 2006), Wisconsin, and other states (MacGuidwin 2012). Knowledge of the occurrence and distribution of virulent phenotypes (*Heterodera glycines* [HG] types) can provide valuable information regarding sustainable and effective use of resistant cultivars (Conley et al. 2011).

Knowledge of SCN diversity adapted to specific areas is important for SCN management. Characterization of SCN populations currently found in South Dakota is necessary for developing improved management strategies that incorporate the use of resistant cultivars derived from durable resistance sources. The analysis of soil samples submitted to the Plant Diagnostic Clinic, South Dakota State University show varying levels of infestation in soybean fields in South Dakota (Acharya et al. 2014). Many factors can lead to high SCN population densities in fields infested with SCN, including adaptation of the nematode populations to SCN resistant cultivars. Thus, the objective of this study was to determine levels of SCN infestation and HG types occurring in South Dakota.

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Materials and Methods

Soil sample collection. Soil sampling was conducted across 28 counties of South Dakota in which soybean cyst nematode had been detected through voluntary SCN testing at the South Dakota State University Plant Diagnostic Clinic. At least three soybean fields were

Table 1. Frequency and population densities of *Heterodera glycines* in the soil samples collected from different South Dakota counties during 2013 and 2014

County	Soil samples ^a	% Positive ^b	Eggs and juveniles	
			Maximum no. ^c	Average no. ^d
Bon Homme	8	37.5	19,900	10,267
Brookings	13	46.2	12,000	6,875
Clay	21	71.4	65,200	10,827
Davison	8	25.0	2,300	2,300
Day	5	20.0	10,000	10,000
Deuel	10	50.0	16,850	4,850
Grant	10	20.0	6,550	7,425
Lake	3	33.3	350	350
Lincoln	14	64.3	8,800	2,711
McCook	8	25.0	7,600	4,175
Minnehaha	8	50.0	1,100	738
Moody	12	50.0	28,600	17,250
Roberts	8	25.0	23,750	12,775
Turner	15	66.7	7,100	2,920
Union	12	84.6	50,000	15,042
Yankton	8	37.5	1,600	1,233

^a Total number of soil samples collected in a county.

^b Percentage of samples positive for *H. glycines*.

^c Maximum number of *H. glycines* eggs per 100 cm³ of soil.

^d Average number of eggs and juveniles per 100 cm³ of soil (four replications) in positive soil samples from each county.

arbitrarily selected from each of the 28 counties positive for SCN. In each field, up to 8.8 × 10³ cm³ of soil was collected from field entrances, low spot areas, and along fence lines to obtain a representative soil sample that was most likely to have SCN. Soil was put into a plastic bag and labeled with the field number and collection date, then kept in the cooler before transportation and storage in the cold room at 4°C until SCN extraction was done. Each soil sample was manually mixed well and 100 cm³ of soil was taken for extraction of cysts using mechanical elutriation (Byrd et al. 1976). Extraction of eggs and juveniles from cysts was done by following the procedures as described by Faghihi and Ferris (2000). For samples with a sufficient number of eggs and juveniles (>10,000/100 cm³), extracted SCN eggs and juveniles (J2) were used for *H. glycines* typing. Samples with less than the required population density (<10,000) were increased by inoculating the susceptible cultivar ‘Williams 82’ in the greenhouse, before performing HG type testing.

HG type determination. To determine HG types, indicator lines PI 548402 (‘Peking’, indicator #1), PI 88788 (#2), PI 90763 (#3), PI 437654 (#4), PI 209332 (#5), PI 89772 (#6), PI 548316 (‘Cloud’, #7), and a susceptible check (‘Williams 82’) were used (Niblack et al. 2002). Two seeds of each indicator line were directly planted into the cone-tainer (3.8-cm diameter and 21-cm high, Stuewe and Sons, Inc., Tangent OR) filled with sterilized soil mixture (2 parts of sand and 1 part of soil by volume) and each line (indicator lines and susceptible check) was replicated four times for each SCN population. Infestation of the HG type indicator lines and the susceptible check with SCN was done at sowing following procedures by Niblack et al. (2002). After planting, the seven indicators and one susceptible check corresponding to SCN extracted from one soil sample were put in a 7.6-liter bucket filled with sand and then the bucket was placed in a water bath in the greenhouse. The water bath was maintained at 27–28°C and day length of 16 h. Only one seedling per cone-tainer was maintained. After 35 days, the cone-tainers were taken out of

Fig. 1. Distribution of *Heterodera glycines* types found in soil samples collected in 2013 and 2014 in South Dakota. Within each county, the number in parentheses refers to the number of soil samples screened for HG types.

Table 2. *Heterodera glycines* types in soil samples collected from different South Dakota counties during 2013 and 2014

County	Female index (FI) ^a							Mean ^b	HG type ^c
	1 (PI 548402)	2 (PI 88788)	3 (PI 90736)	4 (PI 437654)	5 (PI 209332)	6 (PI 89722)	7 (PI 548316)		
Bon Homme	1.3	4	0.5	0.5	1.3	0.2	10.7	143	7
Bon Homme	0.4	1.1	0	0.2	1.1	0.4	3.5	142	0
Bon Homme	10.3	0.1	2.6	0	0	0.1	6.1	172	1
Brookings	0	3.4	0	0	3.8	0.7	11.7	303	7
Brookings	46.9	6.5	31	1.7	1.8	31.1	8.5	176	1.3.6
Brookings	47.8	4.1	35.4	1	4.8	61.4	3.7	269	1.3.6
Brookings	0	9.5	0.3	0	8.4	0	36	92	7*
Brookings	1.7	11.1	1.9	0.1	11.9	1.9	26.1	189	2.5.7
Brookings	0	12.7	0.1	0.2	8.3	0.2	37.3	456	2.7
Brookings	1.3	2.6	0	0	1.7	0	15.4	59	7*
Clay	0.3	6.3	0	0	1.9	0.2	3.5	158	0
Clay	3	0.4	0	0.2	1.5	1.5	4.9	118	0
Clay	0.2	11.8	0.3	0	6.9	0.1	11.2	217	2.7
Clay	0.1	5	0	0.1	6.9	0.1	15.4	423	7
Clay	0.8	1.4	0.3	0.2	1.4	1.6	12.3	440	7
Clay	0.3	0.4	0.9	0	0	0.2	1.8	279	0
Clay	0.9	65.2	0	0	12.9	0	50.9	111	2.5.7
Clay	0.8	41.3	0.8	0.4	36	0	90.2	66	2.5.7*
Clay	0	0	0	0	0	0	0	96	0*
Clay	2.1	1.7	1.5	0	5.4	1	8.7	353	0
Clay	0	1.1	0	0	0.5	0.1	2.3	393	0
Clay	1	5.4	2	0	9.3	0.5	7.4	233	0
Davison	0	3.6	0	0	4	0.5	10.4	106	7
Davison	0	7.5	0	0	8	0	13.4	97	7*
Day	0.6	21	0	0	18.5	0.2	49	131	2.5.7
Deuel	0.7	7.3	0.2	0	9.5	0	17.9	134	7
Deuel	3.3	5.2	0.9	0	3.5	2	13.1	114	7
Deuel	0.2	13	0.9	0	4.7	0.2	6.7	112	2
Deuel	19.5	2.5	8	0.5	0	3.5	1	50	1*
Deuel	9.3	3.1	1.8	0.3	2.8	3.3	14.6	429	7
Grant	0	0	0	0	0	0	4.5	73	0*
Grant	0	4.3	0	0	1.2	0	8.8	226	0
Lincoln	1	11.4	0.1	0	22.7	2.2	22.9	169	2.5.7
Lincoln	4.2	0.8	3.3	0.1	3.4	3.8	6.7	183	0
Lincoln	1	17.8	0	0	21.4	0.3	23	329	2.5.7
Lincoln	0.8	2	0	0.4	2.4	0.6	13.8	125	7
Lincoln	0.4	6.7	0.1	0.1	14.9	0	13.8	432	5.7
McCook	0	2.3	0	0	0	0	5.3	296	0
McCook	0	0.7	0	0	2	0.1	11	205	7
Minnehaha	1.2	0.7	0	0	1.4	0	7.7	107	0
Moody	4.8	11.2	0	0.2	13	0.1	18.8	154	2.5.7
Moody	3.6	2.2	0	0	10.3	0.7	10.3	192	5.7
Moody	0.7	2.2	0	0	1.7	0	5	100	0
Moody	0	1.5	0	0	2.5	0	19.4	188	7
Moody	7.4	19.2	1.7	0.2	10.6	5.1	23.9	249	2.5.7
Moody	0	3.7	0	0	4.6	0	21.8	328	7
Moody	0.1	10.1	0	0.1	4.6	0.1	12.9	404	2.7
Roberts	2.6	5.6	0.3	0.4	4.6	0.2	19.8	244	7
Roberts	3.6	10.3	3.1	0.2	10.5	2.1	10.1	221	2.5.7
Turner	1.2	1.7	0	0.5	6.5	0.2	13.2	100	7
Turner	0.2	1	0	0	0	0.2	4.3	153	0
Turner	0	8.5	0	0.1	5	0.1	15.3	237	7
Turner	0	0.3	0	0	0.4	0.2	0.8	358	0
Turner	0.5	17.7	0	0.1	12.4	0.1	24.3	343	2.5.7
Turner	0	0.7	0	0.1	0.7	0	2.6	246	0
Turner	44.2	1.8	20.2	0	0.7	26.6	9	191	1.3.6
Turner	0	0	0.2	0	0.2	0	1	156	0
Turner	7.9	4.5	0.5	0	2	0.2	13.8	401	7
Turner	0.4	2.0	0	0	3.3	0.2	14.2	127	7
Union	0.1	14.7	0	0	5.5	0.4	26.2	183	2.7
Union	6.9	7	2.8	0.2	4.7	6.8	16.8	202	7
Union	5.8	26.7	1.2	0.4	33.4	3.7	42.1	168	2.5.7
Union	1.3	1.7	0.2	0	0.8	0.2	1.9	159	0
Union	8.8	21	1.3	0.2	12.9	0.3	26.2	250	2.5.7
Union	4.3	3.3	0.4	0.2	3.1	0.8	13.5	250	7
Union	5.9	2.8	3.3	0.2	1.2	3.1	4.7	207	0
Union	0.3	2.1	0.2	0	3.4	0.2	16.4	154	7
Union	2.1	2.3	1.5	0.4	3.7	1.5	11.4	260	5.7
Union	0.2	10.5	0	0	7.9	0	22.4	145	2.7
Union	0.2	4.1	0	0	1.6	0	11.1	153	7
Yankton	0.2	1.1	0	0	1.1	1.1	5.2	140	0
Yankton	1.2	3.5	0	0	1.9	0.3	17.3	145	7
Yankton	0.3	4.9	0.2	0	2.5	0.2	14.6	152	7

^a FI = (mean number of females on indicator line/mean number of females on 'Williams 82') × 100.^b Mean number of soybean cyst nematode females on 'Williams 82', the susceptible check.^c Asterisks (*) indicate HG types from tests in which the average number of cysts on 'Williams 82' was less than 100 (100 cysts on susceptible check is the desired level).

the bucket and soaked in water for 15 min and the soybean plants were uprooted gently, placed on a testing sieve and sprayed with a strong stream of water to dislodge the SCN females (cysts) from the roots. SCN females were collected in a 250- μ m-pore sieve nested under a 710- μ m-pore sieve. Released females were counted for the determination of the FI. The number of females present on the roots of each of the indicator lines and the susceptible line were used for calculating FI. The FI for each line was calculated as follows: FI = (average number of cysts found on indicator line/average number of cysts found on susceptible line) $\times$ 100.

An FI equal to or greater than 10% on any indicator line was assigned the number of the indicator line as the HG type.

Data analysis. Descriptive statistics for the FI data were obtained using SAS 9.3 (SAS Institute, Cary, NC). Pearson's product moment correlation was used for determining the association between the female indices for indicator lines. Average number of cysts from four replications was used for the calculation of FI for each soybean line.

Results

Of the 250 samples collected from the different counties previously known to have fields infested with SCN, 82 (33%) were positive for SCN. These came from 16 of 28 counties sampled. Combined egg and juvenile counts from at least four replications of each of the soil samples ranged from 200 to 65,200 per 100 cm³ of soil (Table 1). Most of the SCN positive counties were from the southeastern part of the state (Fig. 1). The highest average SCN population density was found in Moody county, followed by Union and Roberts counties. Of the 82 samples that were positive for SCN, 73 samples had sufficient eggs and juveniles or were increased to obtain sufficient eggs and juveniles to conduct HG type tests. Nine samples did not have the required population density even after increasing on 'Williams 82' (mainly due to very low initial SCN population density in these soil samples) and were, hence, not included in the study.

Three SCN populations reproduced on PI 548402 (HG type indicator line #1, 'Peking') with FI of 48, 47, and 44%. These came from Turner and Brookings counties (Table 2). This line had only two other SCN populations with FI of 20% (from Deuel county) and

10% (from Bon Homme county). PI 88788 (HG type indicator line #2) had the second highest number of SCN populations with a FI >10%. The highest FI among SCN populations reared on PI 88788 was 65% and this soil sample came from Clay county. The rest of the SCN populations reared on PI 88788 (16 populations) had FI values between 10 and 27%. PI 90736 (HG type indicator line #3) had three SCN populations with >10% FI (20, 31, and 35%). None of the SCN populations reared on PI 437654 (HG type indicator line #4) had more than 10% FI, the highest FI on this indicator line was 1.7%. On PI 209332 (HG type indicator line #5), three SCN populations had an FI of 33, 23, and 21%, and the rest of the SCN populations had a FI of >10% but <19%. PI 89722 (HG type indicator line #6) had three SCN populations with FI values of 61, 31 (both from Brookings county), and 27% (from Turner county). PI 548316 (HG type indicator line #7) had the highest number of SCN populations with FI >10%. The majority of the populations had FI values between 10 and 20%. Four SCN populations had FI between 37 and 51% on this PI line.

Most of the *H. glycines* populations reproduced on all the indicator lines. However, PI 437654 (HG type indicator line #4) had the lowest FI. Overall, 63% of SCN populations tested had FI $\geq$ 10% on PI 548316, 25% on PI 88788, 19% on PI 209332, 7% on PI 548402, 4% on PI 90736, and 4% on PI 89722 (Table 3). Among the SCN populations tested, a total of eight different HG types were found, i.e., HG type 0, 1, 2, 7, 2.7, 5.7, 1.3.6, and 2.5.7 (Table 4). HG type 7 ($\geq$ 10% FI on indicator line #7) was predominant (36%), followed by HG types 0 (29%) and 2.5.7 (16%). These HG types (7, 0, and 2.5.7) collectively accounted for 81% of *H. glycines* populations found in the soil samples tested (Tables 1 and 5). HG type 0 indicates *H. glycines* populations with <10% FI on any indicator line, whereas HG type 2.5.7 indicates *H. glycines* populations that had $\geq$ 10% FI on indicator lines 2, 5, and 7. The diversity of the *H. glycines* populations varied between and within the counties. Brookings, Clay, Turner, and Union counties had more diverse SCN populations than any of the other counties (Table 6).

HG type 7 was the most predominantly detected phenotype, followed by HG types 2 and 1. Although several SCN populations had a FI >10% on HG type indicator line #5, these populations also had a FI >10% on HG type indicator lines #2 and #7. Similarly, SCN populations that had a FI >10% on HG type indicator line #6 also had a FI >10% on HG type indicator lines #3 and #1. There was also a tendency for HG type 2 to have a FI >10% on one or both HG type indicator lines #5 or #7. A positive correlation ($P \leq 0.05$) was found between the FIs of some soybean indicator lines used in the HG type experiment. Indicator lines PI 88788, PI 209332, and PI 548316 were correlated with each other, and PI 548402, PI 90736, and PI 89722 were also correlated among each other (Table 6).

Table 3. Univariate analysis of female indices (FIs) of *Heterodera glycines* populations that had a FI >10% on soybean indicator lines with resistance to *H. glycines*

Soybean indicator line ^a	FI >10% ^b	FI			
		Minimum observed	Maximum observed	Mean	Standard deviation
1 (PI 548402)	6.8	10.3	47.8	33.7	17.5
2 (PI 88788)	24.7	10.1	65.2	19.2	13.8
3 (PI 90736)	4.1	20.2	35.4	28.8	7.8
5 (PI 209332)	19.1	10.3	36.0	17.2	8.4
6 (PI 89722)	4.1	26.6	61.4	39.7	19.0
7 (PI 548316)	63.0	10.1	90.2	20.8	14.3

^a None of the *H. glycines* populations inoculated to PI 437654 (indicator line #4) had a FI >10%.

^b Percentage of populations having >10% FI.

Table 4. Frequencies of *Heterodera glycines* types in soil samples collected in 2013 and 2014 in South Dakota

Type	No. of populations ^a	Frequency (%)
0	21	28.8
1	2	2.7
1.3.6	3	4.1
2	1	1.4
2.7	5	6.8
5.7	3	4.1
7	26	35.6
2.5.7	12	16.4

^a Number of soybean cyst nematode populations (out of 73).

Table 5. County-wise distribution of *Heterodera glycines* types in soil samples collected from different South Dakota counties in 2013 and 2014

County	No. tested ^a	HG types ^b
Bon Homme	3	0, 1, 7 (3)
Brookings	7	1.3.6, 2.5.7, 2.7, 7 (4)
Clay	12	0, 2.5.7, 2.7, 7 (4)
Davison	2	7 (1)
Day	1	2.5.7 (1)
Deuel	5	1, 2, 7 (3)
Grants	2	0 (1)
Lincoln	5	0, 2.5.7, 5.7 (3)
McCook	2	0, 7 (2)
Minnehaha	1	0 (1)
Moody	7	0, 2.5.7, 2.7, 7 (4)
Roberts	2	7, 2.5.7 (2)
Turner	10	0, 1.3.6, 2.5.7, 7 (4)
Union	11	0, 2.5.7, 2.7, 5.7, 7 (5)
Yankton	3	0, 7 (2)

^a Number of soybean cyst nematode populations tested.

^b The number in parentheses refers to the number of *H. glycines* populations found in that county.

Table 6. Correlation coefficient among soybean indicator lines with resistance to *Heterodera glycines* based on female indices (FIs)^a from 73 *H. glycines* populations collected in 2013 and 2014

Indicator line	PI 548402	PI 88788	PI 90736	PI 437654	PI 209332	PI 89722
PI 88788	-0.049 ns ^b					
PI 90736	0.959 **	-0.057 ns				
PI 437654	0.683 **	0.045 ns	0.73108 **			
PI 209332	-0.084 ns	0.705 **	-0.088 ns	0.069 ns		
PI 89722	0.900 **	-0.049 ns	0.958 **	0.645 **	-0.05287 ns	
PI 548316	-0.124 ns	0.800 **	-0.137 ns	0.043 ns	0.796 **	-0.118 ns

^a FI = (mean number of females on indicator line/mean number of females on 'Williams 82') × 100.

^b ns = not significant, $P > 0.05$; ** = $P < 0.001$.

Discussion

This study investigated the occurrence and distribution of HG types in the major soybean growing counties in South Dakota. Our results showed that SCN populations in soil samples collected in 2013 and 2014 had a total of eight different HG types, namely 0, 1, 2, 7, 2.7, 5.7, 1.3.6, and 2.5.7. HG types 0, 2.5.7, and 7 were the most prevalent among the different HG types, suggesting that most of the SCN populations in the collected samples are virulent on PI 548316 ('Cloud', #7), PI 88788 (#2), and PI 209332 (#5). Similar results were observed in Minnesota and Kentucky, where PI 548316 (#7) was found to be most supportive for SCN reproduction. However, in Missouri and Kansas, HG type 2 (virulent on PI 88788) was most prevalent compared with other HG types (Hershman et al. 2008; Mitchum et al. 2007; Niblack et al. 2003; Rzdokiewicz 2010; Zheng et al. 2006). These results might suggest that some of the SCN populations in South Dakota are adapted to the commonly used SCN resistance source PI 88788 (#2).

The frequencies of virulence phenotypes varied among the counties. SCN populations in some counties, such as Brookings, Turner, Union, and Clay, had more than three HG types detected. This may be partly due to a longer history of SCN infestation in these counties. Moreover, these counties may be planting resistant cultivars, hence, are likely to have higher SCN diversity as the nematode adapts to the SCN resistant cultivars (Kim et al. 1997).

We observed a significant correlation for FI between HG type indicator lines #1 (PI 548402), #3 (PI 90763), and #6 (PI 89772). Also, significant correlation was observed between HG type indicator lines #2 (PI 88788), #5 (PI 209332), and #7 (PI 89772). Similar results have been reported for two HG types, 1.3.6 and 2.5.7 (Colgrove and Niblack 2008). This implies that future breeding efforts should consider sources of resistances other than PI 209332 and PI 89772, because SCN populations adapting to PI 88788 will also most likely adapt to resistance from PI 209332 and PI 89772.

The available resistance sources for SCN are categorized into two groups, 'Peking' and PI 88788, based on their different resistance response (Colgrove and Niblack 2008; Kim et al. 1987). HG type indicator lines # 3 (PI 90763) and #6 (PI 89772) are considered part of the 'Peking' group, and their resistance induces early plant tissue response resulting in necrosis. HG type indicator lines #2 (PI 88788), #5 (PI 209332), and #7 (PI 548316) are considered as PI 88788 group, and their resistance induces slower nuclear degradation of plant cells at the nematode feeding site (Colgrove and Niblack 2008; Kim et al. 1987). These groups indicate the risk of using a similar type of resistance source. Rotating within sources of resistance may delay the adaptation of SCN on these lines.

All the indicator lines showed some level of SCN adaptation, except PI 437654 (#4), which was the indicator line that showed the best resistance to all the populations of SCN tested in this study. Due to the high level of resistance to most of the populations of SCN in the United States, PI 437654 (#4) was used to develop resistant cultivar Hartwig (Anand 1992). However, SCN populations adapted to the resistant varieties derived from the resistance source PI 437654 (#4) after a few generations in greenhouse studies (Colgrove and Niblack 2008). This underscores the need for an integrated approach to effectively manage SCN, as no one method can sustainably control SCN populations within a field.

Considering the frequency of *H. glycines* adapted on soybean indicator lines, PI 548316 ('Cloud', #7) may not be preferred for breeding for SCN resistance because of higher SCN adaptability (more than 63% FI). PI 88788 (#2) and 'Peking' (#1) had 25 and 7%, respectively, of the SCN populations tested with FI $\geq 10\%$, indicating a good level of resistance to most SCN populations tested. While these sources of resistance can still be used in SCN management, rotation with a nonhost crop and rotation between the different SCN-resistant cultivars that have been derived from different resistance sources is highly recommended.

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