Survey of *Heterodera glycines* Population Densities and Virulence Phenotypes During 2015–2016 in Missouri

Amanda Howland, Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211; Nick Monnig and Jeff Mathesius, DuPont Pioneer, Agronomy Sciences, Johnston, IA 50131; Manjula Nathan, Division of Plant Sciences, University of Missouri, Columbia, MO 65211; and Melissa G. Mitchum,[†] Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211

Abstract

The soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most important pathogens of soybean. Periodic monitoring of SCN population densities and virulence phenotypes is necessary for developing management strategies utilizing resistant cultivars, the primary strategy used to combat this pest. Therefore, we conducted a statewide survey of Missouri to determine SCN population densities and virulence phenotypes during 2015–2016 and compared these results with a similar survey conducted in 2005. SCN population densities were determined for 393 soil samples representing 74 soybean-producing counties across eight geographical regions of Missouri. Eighty-eight percent of samples tested positive for SCN, up from 50% in 2005, and population densities ranged from 125 to 99,000 eggs per 250 cm³ of soil. The virulence phenotypes of 48 SCN populations also were determined. For this, female indices (FI) were calculated by dividing the mean number of females that develop on

the roots of a set of resistant soybean indicator lines by the mean number of females that develop on the roots of susceptible cultivar Lee74 after 30 days in the greenhouse then multiplying by 100 to obtain a percentage. Notably, all SCN populations evaluated during 2015–2016 had a FI > 10 on PI 88788, the most widely used source of resistance in Missouri, in contrast to 78% in 2005. Moreover, 50% of these populations had a FI > 50 on PI 88788, up from 16% in 2005. Forty-three percent of populations tested also had a FI > 10 on Peking, the second most common source of resistance by farmers. Our results show that over the last decade, SCN has become more prevalent in Missouri fields. Additionally, the percentage of individuals within SCN field populations that are virulent on PI 88788 and Peking has markedly increased. The results stress the importance of rotating cultivars with different types of resistance when using resistant cultivars to manage SCN.

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the most economically important pathogen of soybean (*Glycine max* (L.) Merr.). SCN causes more than \$1.2 billion in yield losses every year (Koenning and Wrather 2010). The total estimated soybean yield loss attributed to SCN in 28 U.S. states and Ontario, Canada, from 2010 to 2014 was 617,405,000 million bushels, exceeding all other soybean diseases (Allen et al. 2017). SCN continues to spread throughout the United States wherever soybeans are grown. In Missouri, this pest has been found in 87 counties to date (Tylka and Marett 2017; this study). Management of SCN relies on the use of resistant cultivars. However, repeated use of the same resistant cultivars can select for virulent nematodes that can reproduce on these resistant cultivars and reduce their effectiveness. For this reason, regular monitoring of SCN population densities and virulence phenotyping is essential for devising management recommendations based on the use of resistant cultivars.

Classifying SCN populations based on their virulence phenotype involves assessing the reproductive potential of a given population on a set of soybean indicator lines used as sources of resistance in commercially available cultivars relative to a standard susceptible

and is expressed as a percentage called the female index (FI). The race scheme used a “+/-” rating system based on a FI $\geq$ 10 on four soybean indicator lines, including Pickett, and plant introductions (PI) 548402 (Peking), PI 88788, and PI 90763 (Riggs and Schmitt 1988). In 2002, the HG type (HG = *H. glycines*) test (Niblack et al. 2002) replaced the race scheme. Though the methodology used in both tests is similar, the HG type test was expanded to include seven PIs used as sources of SCN resistance in soybean germplasm lines or cultivars (Niblack et al. 2002), (1) PI 548402 (Peking), (2) PI 88788, (3) PI 90763, (4) PI 437654, (5) PI 209332, (6) PI 89772, and (7) PI 548316 (Cloud). An SCN population is given a numerical designation (i.e., HG type) based on the number associated with the soybean PI on which it is virulent (FI $\geq$ 10). More recently, a greater understanding of the genetics of SCN resistance has revealed similar resistance genes among indicator lines (Lee et al. 2015; Mitchum 2016). For this reason, a modified HG (SCN) type test, which includes a subset of the original seven indicator lines, has been adopted by some labs (Niblack 2005).

SCN is widespread in Missouri and continues to spread throughout the state (Allen et al. 2017; Mitchum et al. 2007; Niblack et al. 1993, 2003). However, the last SCN statewide survey in Missouri was conducted in 2005 (Mitchum et al. 2007). Therefore, the objective of this study was to determine SCN population densities and virulence phenotypes across the state of Missouri a decade later and directly compare these data with the results of the survey conducted in 2005 (Mitchum et al. 2007).

Materials and Methods

Soil samples were collected from fields in Missouri planted to soybean in 2015 and 2016. The fields that were sampled were selected in an unbiased fashion by University of Missouri regional agronomy specialists and industry personnel located throughout the state. Different fields from each of 74 counties representing eight geographic regions of the state of Missouri (Fig. 1) were sampled. The south central (Ozark) region of the state was not sampled due to the low

[†]Corresponding author: M. G. Mitchum; E-mail: goellnrm@missouri.edu

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percentage of soybean fields in this region. During 2015 and 2016, 393 fields were sampled.

Soil samples included a composite of 20 soil cores (15 cm deep $\times$ 2.0 cm diameter) (Barker et al. 1978), which were processed by SCN Diagnostics, the University of Missouri's Plant and Nematode testing lab. Cysts of *H. glycines* were isolated from 100 cm³ subsamples of soil from each composite sample using a semiautomatic elutriator (Byrd et al. 1976) and captured on a no. 60 (250- μ m) sieve. Cysts were crushed using a rubber stopper attached to a drill press and the eggs collected on a no. 500 (25- μ m) sieve (Faghihi and Ferris 2000). Eggs were stained with acid fuchsin stain (Byrd et al. 1972) and counted under a dissecting microscope.

As in past surveys, cysts isolated from soybean fields were only visually inspected for consistency with *H. glycines*. Therefore, we cannot exclude the possibility that some of these samples contained cysts from other cyst nematode species known to occur in Missouri (Niblack et al. 2003). Population densities are reported per 250 cm³ of soil for comparison with prior survey results, but also per 100 cm³ in the supplemental data tables to allow for comparison with more recent research papers.

Forty-eight samples were arbitrarily selected for virulence phenotyping to include samples from each geographic region and spanning a range of population densities. To acquire sufficient inoculum for testing and minimize any selection pressure on the SCN population, eggs extracted from cysts isolated from these soil samples were used to inoculate a pot of susceptible soybean seedlings containing a mix of cultivars Lee 74, Essex, and Williams 82. All of these SCN populations increased on soybean after 1 to 2 generations, further confirming their identity as *H. glycines*. Modified HG (SCN) type tests were conducted according to standardized protocols (Niblack et al. 2002) and included four replications of each of the following soybean lines: susceptible check Lee74, and PI 548402 (Peking), PI 88788, PI 90763, and PI 437654. Pickett, an indicator line for the SCN race test (Riggs and Schmitt 1988), was also included so that race determinations could be made and used for comparison with past survey data.

Results

SCN population densities were determined for 393 soil samples across soybean-producing regions of Missouri. Three hundred forty-six (88%) samples tested positive for SCN. The highest population density detected was 99,000 eggs/250 cm³ (Table 1). SCN population densities were $\geq$ 500 eggs/250 cm³ in 75% of samples (Supplementary Table S1).

The virulence phenotypes of 48 SCN populations were determined (Supplementary Table S2). The predominant HG types were 2- and 1.2-, which accounted for 52 and 46% of the SCN populations tested, respectively (Table 2). HG types 2- and 1.2- were widely distributed throughout the state (Fig. 1). Only one SCN population (2%) from

Table 1. Frequency and population densities of soybean cyst nematode (SCN) in Missouri during 2015–2016

Region	Soil samples ^a	No. positive ^b	Positive (%) ^c	No. tested ^d	Max. no. of eggs ^e
Northwest	35	33	94	8	48,000
North-central	39	34	87	4	19,500
Northeast	108	89	82	9	64,875
West-central	25	25	100	5	8,625
Central	52	45	87	5	99,000
East-central	105	94	90	6	28,500
Southwest	9	9	100	4	61,125
Southeast	20	17	85	7	93,000
Summary	393 ^a	346 ^a	91 ^f	48 ^a	52,828 ^f

^a Number of soil samples.

^b Numbers of samples positive for SCN.

^c Percent of samples positive for SCN.

^d Number of samples tested for HG type (HG = *Heterodera glycines*) and race.

^e Maximum number of SCN eggs/250 cm³ of soil.

^f Average of samples.

Fig. 1. Map of counties in Missouri divided into nine geographical regions. Counties where soybean cyst nematode (SCN) has been found in at least one field in this survey or past surveys are highlighted in green. HG type (HG = *Heterodera glycines*) data are depicted in each region (FI = female index).

the southeast region of the state was found to be HG type 1.2.3-. No HG type 0 populations were among the 48 samples tested.

All SCN populations tested during 2015–2016 had a FI > 10 on PI 88788. More than half, 58%, of the populations tested had a FI > 50 on PI 88788. Fifty percent of the SCN populations also had a FI > 10 on PI 548402 (Peking), but only three populations exceeded a FI > 50. One population tested had a FI > 10 on PI 90763 (FI = 22) and none on PI 437654. Populations with a FI > 10 on PI 88788 and PI 548402 (Peking) were found in all sampled regions of Missouri. The only population found to have a FI > 10 on PI 90763 was from a field in the southeast region of the state (Table 3).

Discussion

In our 2015–2016 survey, SCN was detected in 88% of soil samples collected from eight geographic regions of the state of Missouri. In our prior survey in 2005 (Mitchum et al. 2007), SCN was only detected in 50% of soil samples collected from the same geographic regions of Missouri. Seventy-five percent of samples had ≥ 500 eggs/250 cm³ in 2015–2016 compared with 37% in 2005 (Mitchum et al. 2007). Although this number has been previously reported to represent the state average SCN population level at which yields may be significantly ($P \leq 0.05$) reduced (Niblack et al. 2003), yield loss can occur at lower population densities and is highly dependent on soil type and environmental conditions (Niblack 2005; Niblack et al. 1992; Wang et al. 2003). The United States Department of Agriculture National Agricultural Statistics Service area frame probability sampling design strategy used in past surveys (Mitchum et al. 2007; Niblack et al. 2003) was not employed here. However, samples were taken from a minimum of two separate fields representing 74 soybean-producing counties across eight geographic regions of the state to provide broad coverage. The higher percentage of fields testing positive for SCN and rising population densities during 2015–2016 is likely due to the widespread and repeated use of SCN-resistant cultivars with resistance derived from PI 88788. An increase in virulence of SCN populations on PI 88788 has been reported previously in the north-central United States (Mitchum et al. 2007; Niblack et al. 2008) and was recently shown to directly correlate with reduced yields of PI 88788-type resistant cultivars (McCarville et al. 2017).

An awareness of the predominant SCN virulence phenotypes in the state is essential for guiding breeding efforts and establishing management recommendations for farmers. This survey shows that the virulence phenotypes of SCN in Missouri have shifted since 2005 (Table 2). In 2005, the most common HG types of the SCN populations sampled were 2- (49%), 0 (22%), 1.2- (22%), and 1.2.3- (7%). In 2015–2016, the percentage of SCN populations sampled that were HG type 2- (52%) and 1.2- (46%) increased, whereas HG type 0 populations went undetected and only 2% of populations tested were HG type 1.2.3-. Significantly, 100% of the SCN populations tested in 2015–2016 had a FI > 10 on PI 88788 compared with 78% in 2005 (Mitchum et al. 2007). High female indices on PI 88788 were not restricted to soil samples with high population densities. SCN populations from seven soil samples with <1,000 eggs/250 cm³ had female indices ranging from 36 to 69% on PI 88788. Similarly, the percentage of SCN populations with a FI > 10 on PI 548402 (Peking) increased in 2015–2016 (48%) compared with 2005 (29%). In contrast, the percentage of populations with a FI > 10 on PI 90763 and PI 437654 were similar in both surveys, likely due to limited availability of these sources of resistance.

The increased percentage of Missouri SCN populations with a FI > 10 on PI 88788 from 2005 to 2015–2016 is not surprising due to the fact that the majority of SCN-resistant cultivars grown in Missouri fields carry PI 88788 resistance. There were 226 soybean cultivars entered into the 2017 MU Variety Testing Program. Companies provided SCN resistance source information for 138 cultivars. Companies claimed that two cultivars (1.4%) had SCN resistance from Hartwig, one cultivar (0.7%) had SCN resistance from Peking, and 135 (97.8%) had SCN resistance from PI 88788 (Wiebold et al. 2017). The same holds true for other states in the north-central United

States, such as Illinois and Iowa, where more than 95% of the market share has shifted to PI 88788 resistance (Joos et al. 2013; McCarville et al. 2017). Considering the low percentage of commercial varieties with Peking-type resistance, the observed widespread increase in HG type 1.2- populations in Missouri is interesting, and warrants further investigation to determine the factors contributing to this shift. Our data indicate that farmers in Missouri who are using cultivars with resistance from PI 88788 and PI 548402 (Peking) should monitor SCN population densities and virulence phenotypes in their fields, and use this information to guide rotation with nonhost crops and cultivars with resistance from other sources, if available.

Our survey results emphasize the importance of incorporating resistance from other sources into cultivars adapted to conditions throughout Missouri. Based on the 2015–2016 survey, PI 437654 is an excellent source of resistance to SCN in Missouri. Several varieties with resistance from this source have been developed by the University of Missouri Southern Soybean Breeding program and released to Southern growers (Shannon et al. 2007a, b). Although SCN greenhouse populations with a FI > 10 on PI 437654 overcome all current sources of resistance, a recent study that evaluated SCN adaptation to this broad-based resistance suggested virulence on this source is a recessive, multigenic trait (Gardner et al. 2017). This characteristic may slow adaptation in field populations, especially when rotated with other sources. Similarly, few populations from Missouri collected during 2015–2016 had a FI > 10 on PI 90763. Although 15 of the 48 populations tested showed a low level of reproduction (FI < 4) on PI 90763, only one population exhibited a FI > 10. Rotating cultivars with resistance from PI 88788 and PI 90763 may be a useful strategy based on the genetics of SCN virulence (Gardner et al. 2017). In addition, stacking of resistance alleles from the wild soybean *Glycine soja* with PI 88788 and Peking resistance provides added protection against SCN that has adapted to these sources (Kim and Diers 2013; Kim et al. 2011). Soybean breeders should continue to focus on developing SCN-resistant cultivars using these or other sources of resistance and/or creating resistance gene stacks to

Table 2. Comparison of the percentage of soybean cyst nematode (SCN) populations designated as a particular HG type (HG = *Heterodera glycines*) or race between 2005 and 2015–2016

HG type ^a	Population (%)		Race	Population (%)	
	2005	2015-16		2005	2015-2016
0	22	0	1	45	33
2-	49	52	2	22	46
1.2-	22	46	3	22	0
1.2.3-	7	2	4	7	2
			5	4	19

^a Modified HG (SCN) type tests, $n = 45$ (2005); $n = 48$ (2015–2016).

Table 3. Percentage of soybean cyst nematode (SCN) populations during 2015–2016 with a female index > 10 on resistant soybean lines by region of Missouri

Soybean line	Region ^a								Total
	NW	NC	NE	WC	C	EC	SW	SE	
Pickett	63	50	89	20	60	50	100	86	67
PI 548402 (Peking)	63	25	67	20	40	17	100	57	50
PI 88788	100	100	100	100	100	100	100	100	100
PI 90763	0	0	0	0	0	0	0	14	2
PI 437654	0	0	0	0	0	0	0	0	0
Total ^b (HG tested)	33 (8)	34 (4)	89 (9)	25 (5)	45 (5)	94 (6)	9 (4)	17 (7)	346 (48)

^a Percentage; NW = northwest, NC = north-central, NE = northeast, WC = west-central, C = central, EC = east-central, SW = southwest, and SE = southeast.

^b Total number of SCN-positive soil samples; number of SCN-positive samples HG (HG = *Heterodera glycines*) tested.

provide more rotation options for growers. Future studies to evaluate how SCN adapts to new sources of resistance and various resistance gene combinations are highly recommended to determine how best to deploy resistance in rotation for strategic management.

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