Characterization of Virulence Phenotypes of Soybean Cyst Nematode (*Heterodera glycines*) Populations in North Dakota

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Running head: Characterization of SCN virulence phenotypes in North Dakota.

ABSTRACT

Soybean cyst nematode (SCN; *Heterodera glycines*) continues to be the greatest threat to soybean production in the United States. Since host resistance is the primary strategy used to control SCN, knowledge of SCN virulence phenotypes (HG types) is necessary for choosing sources of resistance for SCN management. To characterize SCN virulence phenotypes in North Dakota (ND), a total of 419 soybean fields across 22 counties were sampled during 2015, 2016, and 2017. SCN was detected in 42% of the fields sampled and population densities in these samples ranged from 30 to 92,800 eggs and juveniles per 100 cm^3 of soil. The SCN populations from some of the infested fields were virulence phenotyped with seven soybean indicator lines and a susceptible check (Barnes) using the HG type tests. Overall, 73 SCN field populations were successfully virulence phenotyped. The HG types detected in ND were HG type 0 (frequency rate: 36%), 7 (27%), 2.5.7 (19%), 5.7 (11%), 1.2.5.7 (4%), and 2.7 (2%). However, prior to this study only HG type 0 was detected in ND. The designation of each of the HG types detected was then validated in this study by repeating the HG type tests for thirty-three arbitrarily selected samples. This research for the first time reports several new HG types detected in ND and confirms that the virulence of SCN populations is shifting and overcoming resistance, highlighting the necessity of utilization of different resistance sources, rotation of resistance sources, and identification of novel resistance sources for SCN management in ND.

Keywords: *Glycine max*, *Heterodera glycines*, HG types, North Dakota, resistance, soybean, soybean cyst nematode, indicator lines, survey, virulence phenotypes.

INTRODUCTION

Soybean (*Glycine max* L. (Merr.)) is a leguminous crop that plays a key role in achieving global food security (Hartman et al. 2011). It is an important source of protein and oil for

humans and animals. Processed soybean is the world's largest source of animal feed protein and the second largest source of vegetable oil (USDA-ERS 2019), and the United Sates is the leading producer of soybean in the world (FAOSTAT 2019). In the U.S., North Dakota ranks 4th in total soybean acres planted with over five million acres planted and harvested in 2019 and an annual value of production over $2 billion (USDA-NASS 2019).

Among the various biotic factors that threaten soybean production, soybean cyst nematode, *Heterodera glycines* (Ichinohe 1955) continues to be the most economically devastating pathogen for soybean producers worldwide. This sedentary endoparasitic nematode is responsible for more than 1.2 billion dollars in yield losses in the U.S. alone (Koenning and Wrather 2010). A more recent study revealed that soybean cyst nematode (SCN) caused yield losses of up to 617.4 billion bushels in 28 U.S. states and Ontario, Canada during 2010 to 2014 (Allen et al. 2017). However, losses often go unnoticed since SCN can cause more than 30% yield loss without any noticeable above-ground symptoms (Koenning and Wrather 2010; Wang et al. 2003).

In addition to causing serious yield loss, SCN has the ability to spread rapidly. Since this nematode's first discovery in 1899 in north-east China (Li et al. 2011) and characterization in Japan in 1915 (Hori 1916), it has spread to all the major soybean producing regions of the world (Yan and Baidoo 2018). SCN was first discovered in the U.S. during 1954 in North Carolina (Winstead et al. 1955) and by 2014, this nematode has spread to every soybean producing state in the U.S. except New York and West Virginia (Tylka and Marett 2014). Since then, SCN was detected in Cayuga County, New York in 2016 (Wang et al. 2017). SCN was first found in Richland County, North Dakota in 2003 (Bradley et al. 2004). Since then, infestation of SCN has spread to at least 19 soybean producing counties by 2015 (Yan et al. 2015) and the SCN

population densities in these fields were as high as 21,540 eggs and juveniles per 100 cm^3 of soil (Chowdhury et al. 2016). SCN continues to spread quickly as soybean production expands to other counties of North Dakota (Tylka and Marett 2017). Thus, SCN has the potential to become North Dakota soybean growers' most economically important soybean disease if it is not detected early and managed proactively.

Resistant cultivars combined with crop rotation are the two primary methods for controlling SCN (Mueller et al. 2016; Niblack et al. 2003). However, SCN is known to have genetically diverse nematode populations and can develop new virulent forms due to continuous use of the same resistance source (Colgrove and Niblack 2008; Yan and Baidoo 2018). These new virulent forms can have higher levels of resistance and can overcome the prevailing resistance genes that are commonly used for control (Colgrove and Niblack 2008). Thus, characterizing the occurrence and distribution of SCN virulence phenotypes is essential for developing management strategies based on the use of resistant cultivars derived from durable resistance sources.

To characterize the virulence phenotypes of SCN, a standardized classification scheme was proposed by Niblack et al. (2002) referred to as 'HG type' tests. HG refers to *H. glycines* and the type refers to seven different 'Plant Introductions' with various forms of resistance. These seven 'Plant Introductions' suggested by Niblack et al. (2002) are resistance sources which include PI 548402 ('Peking', indicator line #1), PI 88788 (#2), PI 90763 (#3), PI 437654 (#4), PI 209332 (#5), PI 89772 (#6), and PI 548316 ('Cloud', #7). Thus, the HG type tests classify SCN populations' virulence phenotypes by assessing the reproductive potential of SCN populations on the seven different indicator lines compared to a susceptible check, which is

77 referred to as female index (FI) that is expressed as a percentage (Howland et al. 2018; Niblack
78 et al. 2002).

79 Among the seven indicator lines, PI 88788 (#2) is the most commonly used source of
80 SCN resistance in North America. Additionally, Peking-type resistance derived from PI 548402
81 (#1) is the second most commonly used source of resistance, however, very few of the
82 commercially available cultivars derive resistance from other plant introduction lines
83 (McCarville et al. 2017; Yan and Baidoo 2018). In North Dakota the virulent type that has been
84 reported thus far is HG type 0, which is considered the least virulent type of SCN and does not
85 reproduce on any of the seven indicator lines. However, there has been several recent research
86 reports of commonly used resistance sources such as PI 88788 and Peking (PI 548402) being
87 overcome by SCN populations from other major soybean producing states in the Midwest, which
88 includes Kansas (Rzodkiewics 2010), Missouri (Howland et al. 2018), Iowa (McCarville et al.
89 2017), Nebraska (Broderick 2016) and Wisconsin (MacGudwin 2012). Additionally, the
90 neighboring states of Minnesota and South Dakota have also reported that the virulence of SCN
91 populations are shifting and the number of SCN populations that are virulent on PI 88788 is
92 increasing (Acharya et al. 2016, Chen et al. 2010, Zheng et al. 2006). Hence, the objective of this
93 study was to investigate the virulence diversity of SCN by characterizing the HG types occurring
94 in North Dakota to determine if SCN populations from North Dakota can overcome the
95 resistance provided by common resistance sources such as PI 878788 and Peking.

96 **MATERIALS AND METHODS**

97 **Soil sample collection.** During 2015, 2016, and 2017 soil samples were collected from
98 North Dakota soybean fields or fields with a history of soybean production. The sampled fields
99 were selected arbitrarily from each of the major soybean producing counties of North Dakota.

Sampling frequency from each county was weighted based on their soybean acreage (Niblack et al. 2003). Thus, greater number of samples were collected from counties with higher soybean production. Counties of western and south-western North Dakota were not sampled due to very low soybean acreage in these counties. Across the three years, a total of 419 soybean fields from 22 counties of North Dakota were selected and sampled. The majority of these samples were collected during April-May and August-September each year.

In each field, soil sampling was focused towards field entrances, along field borders and the edge of spots with yellow and stunted plants to increase the likelihood of detecting SCN in the representative soil sample. Soil sampling was conducted using a 2.5 cm diameter soil probe with a probe length of 30 cm (Gempler's model L sampler, Madison, WI), but when the field's soil was too compacted, a shovel was used. Approximately 100 to 200 cm^3 of soil were collected from each sampling point and each soil sample was a composite of the soil collected from 20 to 25 sampling points in each field, covering an area of 2 to 2.5 ha. For each field, soil samples from all the sampling points were then composited in a plastic bag and labeled with the field number and collection date. The samples were stored in a cooler during transport and then stored in a cold room at 4°C until processing. Latitude and longitude coordinates were recorded for each field in decimal degrees. Additionally, elevation and the nearest city were recorded for each location.

Soil sample processing, nematode extraction, and enumeration. Each soil sample was first thoroughly mixed by hand, and a 100 cm^3 subsample was collected from each sample for nematode extraction. Adult females and/or cysts of *H. glycines* were isolated from these subsamples using the sieving and decanting method described by Krusberg et al. (1994). According to this method, the 100 cm^3 subsample was stirred in a beaker with tap water (1000

ml) and after 30s the mixture was poured through a stack of sieves (710 µm and 250 µm apertures; VWR, Radnor, PA) to separate larger organic and inorganic soil particles from smaller soil particles and SCN adult females and/or cysts. Next, the smaller soil particles and adult females and/or cysts were transferred into a customized cylindrical PVC pipe that has an opening at the top but the bottom is lined 250 µm aperture sieve. The adult females and/or cysts at the bottom of the PVC pipe were then crushed using a rubber stopper attached to a drill press (MasterForce Drill Press, Menards, Fargo, ND) following the procedures described by Faghihi and Ferris (2000). Liberated eggs and juveniles (J2) were then collected from the crushed adult female and/or cysts in a 20 µm aperture sieve and enumerated using an inverted compound light microscope (at 100× magnification; Focus Precision Instruments, Cavaletti Court Victoria, MN).

Inoculum preparation and soil infestation. Eggs and juveniles collected from the samples with a low population density (less than 400 eggs and juveniles/100 cm^3 of soil) were arbitrarily selected for SCN population increase by inoculating the susceptible cultivar Barnes in controlled greenhouse conditions. Barnes, a local soybean cultivar developed and released at the North Dakota State University, is equally as susceptible as Lee 74, a standard cultivar that is widely used as a susceptible check for HG type testing (Helms et al. 2001; Niblack et al. 2002; Poromarto et al. 2015). After the population increase, the SCN white females from the susceptible check were collected and crushed. The liberated eggs and juveniles were then used as inoculum for HG typing tests that were conducted by artificially inoculating autoclaved sandy soil.

Samples with population densities higher than 300 eggs and juveniles per 100 cm^3 of soil were first manually mixed for 30 to 60 mins to break up soil clumps and then further mixed for 20 to 30 mins using a motorized cement mixer (Kushlan Products LLC, Houston TX) to ensure

uniform distribution of nematodes throughout the soil sample. These thoroughly mixed soil samples were then used for HG typing tests that were conducted with naturally infested soil. However, three of the field samples with population densities higher than 50,000 eggs per 100 cm^3 of soil were HG typed by artificially inoculating autoclaved sandy soil after eggs were extracted and collected from the samples. Overall, seventy-three SCN field populations were virulence phenotyped including assays conducted with artificially infested and naturally infested soil.

HG type determination. Modified HG typing tests were conducted according to standardized protocols suggested by Niblack et al. 2002. Each HG type test included four replications of each of the seven indicator lines, PI 548402 ('Peking', indicator line #1), PI 88788 (#2), PI 90763 (#3), PI 437654 (#4), PI 209332 (#5), PI 89772 (#6), and PI 548316 ('Cloud', #7), as test lines and a susceptible check (Barnes). Seeds of each indicator line and the susceptible check were pre-germinated for four to five days by placing them in Petri dishes (Falcon, Corning, NY) with wet filter paper (Whatman, Darmstadt, Germany). A single germinated seed for each experimental unit was then planted into a cone type container (3.8-cm in diameter and 21-cm in height; Stuewe and Sons, Inc. Tangent, OR) containing about 100 cm^3 of thoroughly mixed naturally infested soil. For artificial infestation, the cone type containers were filled up with autoclaved sandy soil and artificially inoculated with SCN eggs and juveniles obtained from crushed SCN adult females and/or cysts, following procedures suggested by Niblack et al. (2002). Inoculation was performed during planting by making 2 to 3 holes at a 5 cm depth around the seedling in the container and transferring a total of 5 ml nematode suspension that contained 2,000 SCN eggs and juveniles using a 5 ml pipette. The holes containing the inoculum as well as the seedling were then covered up with a thin layer of moist

sterilized sandy soil. The same procedure was also followed during the previously mentioned second round of HG type tests for confirmation purposes. After planting, the containers were put into plastic racks with 14 X 7 wells and arranged in a completely randomized design. They were then maintained in a greenhouse growth chamber (GR64, Conviron, Winnipeg, Manitoba, Canada) for 30 to 35 days at a constant temperature of 27 °C and a daylight period of 16 hours.

Out of the seventy-three field populations evaluated, fifty-one of the SCN field populations were HG typed by planting the HG type indicator lines and the susceptible cultivar into naturally infested soil (Table 1). Additionally, twenty-two SCN field populations with low density in naturally infested soil were first inoculated onto the susceptible soybean cultivar to increase the population density, then HG typed by artificially infesting autoclaved sandy soil (Table 2). To confirm the HG types and validate the accuracy of the first iteration, thirty-three SCN populations were arbitrarily selected and HG type tested for a second time (Table 3). These repeated HG types tests were all conducted by artificially infesting autoclaved sandy soil.

When the experiments were taken down, the above soil portion of the plants were removed and adult females and/or cysts from both roots and soil were collected using the sieving and decanting method described by Krusberg et al. (1994). The roots and soil from each container were emptied into a 4-liter bucket, which was then filled to ¾ of its capacity with water. This allowed the soil to easily dislodge from the root surface. The roots were then gently placed on top of a 710-µm-pore sieve that was stacked on top of a 250-µm-pore sieve, and subjected to high-pressure water spray to dislodge the SCN females attached to the roots; which were collected in the 250-µm-pore sieve. The remaining soil and water were then stirred and poured immediately through both the sieves, stacked in the same order, to collect the newly formed females dislodged from the roots and/or old cysts from the infested soil. The extracted

nematodes were then examined, and the newly formed white (majority) or light-yellow females (few) were enumerated under a dissecting microscope (SM 100 Series, Swift Optical Instrument, INC. TX, US) in a petri dish with grids containing the nematode suspension. For each indicator line, a female index (FI) was calculated as follows: FI = (average no. of white females found on the indicator line/average no. of females found on the susceptible check) x 100. A FI cutoff of 10 was used because a FI value of less than 10 on a test line would not be able to maintain themselves, at least within a single growing season and may result in false positive, as suggested by Golden et al. (1970) and Niblack et al. (2002). Therefore, the indicator lines with FI ≥ 10% were considered to have positive host compatibility, whereas the indicator lines that had FI < 10% were considered as negative host incompatibility.

Data analysis. The average number of white females from the four replicates of each indicator line and the susceptible check were used to calculate the FI value. The statistical software SAS 9.4 (SAS Institute, Cary, NC) was used to analyze the data and determine the descriptive statistics. Additionally, SAS 9.4 was used to determine the Pearson's product moment correlation between the FI values of different indicator lines (Acharya et al. 2016; Niblack et al. 2003). The samples in which SCN could not be detected were excluded from analysis. The mean population density in SCN positive fields and the HG types detected in each county were plotted into a North Dakota county map (Fig. 1) using geographic information system techniques with ArcGIS Pro software (Environmental Systems Research Institute, Redlands, CA).

RESULTS

Out of the 419 soybean fields sampled, 176 (42%) fields were tested positive for the SCN. The SCN positive samples came from 14 of the 22 counties sampled. The SCN population

densities of these samples ranged from 30 to 92,800 eggs and juveniles per 100 cm^3 of soil. The majority of the SCN positive counties were in the south-eastern part of North Dakota (Fig. 1), and the sample with the greatest population density was collected from a field in the south-eastern county, Richland. The average population density from the positive samples was 3,873 eggs and juveniles per 100 cm^3 of soil. Out of the 176 SCN positive fields detected, 79 (45%) had a population density greater than 500 eggs and juveniles per 100 cm^3 of soil. A total of 73 SCN field populations were successfully virulence phenotyped using HG typing tests. Although the remaining samples did have detectable levels of SCN eggs and juveniles, they did not produce enough eggs and juveniles for HG typing tests within the period of this study.

Out of the 73 SCN populations tested, 3 (4.1%) were able to parasitize PI 548402 (HG type indicator line #1) having a FI $\geq$ 10% (Table 1 and 4). Two of these populations originated from Traill County and the other one was from Richland County (Table 1). The FI for all three of these populations on PI 548402 were from 10 to 26% (Table 1 and 4). These three populations were also HG typed for a second time to confirm their HG type designations. In the repeated tests, the FI of the two samples from Traill County on PI 548402 were 27 and 39%, whereas the sample from Richland County had a FI of 12% (Table 3).

Twenty-six percent of the SCN populations tested were able to produce FI $\geq$ 10% on PI 88788 (#2). The FI among the SCN populations that were able to parasitize PI 88788 ranged from 11 to 40% (Table 1, 2, and 4). The highest FI on PI 88788 was produced by a SCN population from Traill County (Table 1). Moreover, this population produced the highest FI in HG type tests conducted for repetition purposes as well (Table 3). The remaining SCN populations that attacked PI 88788 were collected from Cass, Richland, Steele, and Traill counties, and the percentage of SCN populations that successfully parasitized PI 88788 from

each of these counties were 30, 21, 29, and 39%, respectively. Furthermore, three of the SCN populations from Traill County and one of the populations from Cass County had FI values ≥ 30% on PI88788 (Table 5).

The soybean line PI 209332 (#5) had the second-highest number of SCN populations from North Dakota that had a FI ≥ 10%. Thirty-four percent of the SCN populations tested had FI ≥ 10% on PI 209332. The highest FI among the SCN populations that reproduced on PI 209332 was 46% (Table 4), and this population was collected from a field in Richland County (Table 1). This SCN population had the third-highest FI (20%) among the repeated HG type tests conducted (Table 3). The remaining SCN populations that attacked PI 209332 had FI values between 10 and 42% (Tables 1 and 2). These field populations were collected from soybean fields in Cass, Grand Forks, Richland, Steele, and Traill counties. The percentage of SCN populations in each of these counties with FI ≥ 10% on PI 209332 were 30, 33, 35, 57, and 39%, respectively. Furthermore, three SCN populations from Cass County and one of the populations from each of Richland, Steele, and Traill counties produced aggressive response on PI 209332, having FI ≥ 30% (Table 5).

The HG type indicator line PI 548316 (#7) was the most commonly infected soybean indicator line in North Dakota. More than 64% of the SCN populations tested had FI values ≥ 10% on PI 548316. The highest FI among the SCN populations virulent on PI 548316 was 61% (Table 4), and two samples from Richland County had this FI value (Table 1). The HG type test for one of these SCN populations (sample ID: SCN 52*) was repeated. In the repeated experiment the SCN population with the sample ID 52* also had high FI value on PI 548316 (Table 3). The remaining SCN samples that attacked PI 548316 had FI values between 10 and 60% (Tables 1 and 2). These SCN samples originated from Cass, Grand Forks, Richland, Steel,

and Traill counties (Table 5). The percentage of SCN populations in each of these counties with FI ≥ 10% on PI 548316 were 60, 100, 59, 71, and 77%, respectively. Furthermore, 25, 33, 3, 43, and 15% of the SCN samples collected from Cass, Grand Forks, Richland, Steel, and Traill counties, respectively were found to have FI ≥ 30% on PI 548316 (Table 5). None of the SCN populations tested had a FI ≥ 10% on PI 90763 (#3), PI 437654 (#4), or PI 89772 (#6). The greatest FI produced on each of these indicator lines were 4, 3, and 7%, respectively (Table 1).

A total of six different HG types were detected in North Dakota and the most commonly detected SCN virulence phenotype was HG type 0. Out of the 73 SCN field populations tested, 36% were HG type 0, 27% were HG type 7, 19% were HG type 2.5.7, 11% were HG type 5.7, 4% were HG type 1.2.5.7, and 2% were HG type 2.7 (Fig. 2). Thirty-one of the 33 SCN populations that were HG type tested had the same HG type in both first and second tests (Table 3). One of the samples from Traill County was HG type 7 in the first test; however, in the second test, this sample was HG type 0 with a FI of 9.7% on PI 548316 (#7), barely short of the cutoff of FI ≥ 10% (Tables 1 and 3). Similarly, a sample from Steele County was HG type 5.7 in the first test, whereas, in the second test this sample's HG type was determined to be 7 with a FI of 9.6% on PI 209332 (#5; Tables 1 and 3)

Overall, 10, 8, 7, 3, 2, and 1 samples with HG type 0, 2.5.7, 7, 1.2.5.7, 5.7 and 2.7 in the first round of HG type tests, respectively, were repeated and confirmed through the second round of the HG type tests (Table 3). The diversity in virulence phenotypes of SCN populations from North Dakota varied between and within counties. Richland County had the greatest diversity in virulence phenotypes detected in North Dakota. In fact, each of the six different HG types detected in North Dakota was detected in Richland County. On the other hand, only HG type 0

was detected in Barnes County. Cass, Traill, and Steele counties had four different HG types detected, whereas, SCN populations of Grand Forks County only had two HG types (Fig. 1).

The FI values produced on the indicator lines PI 88788 (#2), PI 209332 (#5), and PI 548316 (#7) were positively correlated ($P < 0.0001$) with each other (Table 6). Additionally, there was a positive correlation between the FI values of PI 88788 (#2) and PI 548402 (#1) ($P < 0.0001$; Table 6). Although positive correlations ($P < 0.05$) were observed between the FI values of PI 548402 (#1), PI 90763 (#3) and PI 89772 (#6) (Table 6), stronger correlations were observed between the FI values of indicator lines #3 and #6 as well as #1 and #4 ($P < 0.0001$)

DISCUSSION

This study investigated and characterized SCN virulence phenotypes prevalent in North Dakota, one of the major soybean producing states in the U.S. SCN populations were demonstrated for the first time to have positive host compatibility with soybean indicator lines that have resistance to SCN. The indicator line PI 88788 (#2) is the most commonly used source of resistance in the U.S. (Joos et al. 2013; Mitchum 2016; Tylka and Mullaney 2020; Yan and Baidoo 2018) and our results indicated that 26% of the SCN populations tested were virulent against this line. Additionally, 4% of the SCN populations tested in this study were virulent on PI 548402 (#1), which is the second most utilized source of resistance (Joos et al. 2013; Mitchum 2016; Tylka and Mullaney 2020). Increased virulence on PI 88788 and PI 548402 can directly correlate with yield reduction in cultivars deriving resistance from them (Howland et al. 2018; McCarville et al. 2017). Very few SCN populations in the upper Midwest (Acharya et al. 2016; MacCarville et al. 2017; MacGuidwin 2012; Niblack et al. 2003; Zheng et al. 2006) and none of the populations from North Dakota were virulent against resistance sources such as PI 90763 (#3) or PI 437654 (#4). This is not surprising, as these sources of resistance are not commonly

306 deployed in commercial cultivars. Such results highlight the importance of rotating between
307 resistance sources in commercially available SCN-resistant cultivars for North Dakota growers
308 because SCN populations can become virulent on commonly used resistance sources such as PI
309 88788 (#2).

310 Monitoring the diversity and distribution of SCN virulence phenotypes in the state is
311 crucial for directing breeding programs and establishing management recommendations for
312 growers (Howland et al. 2018; Niblack et al. 2002). Our results indicate there has been a shift in
313 SCN virulence phenotypes in North Dakota, since its first detection in Richland County during
314 2003 (Bradley et al. 2004). Prior to this study, only HG type 0 was identified in infested fields of
315 North Dakota (Poromarto and Nelson 2009). Since then, we detected a total of six different HG
316 types, including 0, 7, 2.7, 5.7, 2.5.7, and 1.2.5.7, in the soil samples collected during 2015, 2016,
317 and 2017. Although our results showed HG type 0 to be the most prevalent virulence phenotype
318 in North Dakota, HG types 7 and 2.5.7 were the second and third most prevalent HG types.

319 Our results were consistent with those of HG type surveys of the neighboring states of
320 South Dakota and Minnesota, where HG types 0, 7, and 2.5.7 were the three most prevalent HG
321 types (Acharya et al. 2016; Zheng et al. 2006). Additionally, in these neighboring states, PI
322 548316 (#7) was the most commonly parasitized indicator line followed by PI 88788 (#2) and PI
323 209332 (#5) (Acharya et al. 2016; Zheng et al. 2006), which is similar to this study. Thus, a
324 regional pattern in HG type diversity is observed and may be attributed to similarity in cropping
325 practices, available cultivars, and environmental conditions.

326 Four percent of the SCN populations tested in South Dakota had FI $\geq$ 10% on both PI
327 90763 (#3) and PI 89772 (#6) (Acharya et al. 2016), however, none of the SCN populations from
328 North Dakota had a FI $\geq$ 10% on either of these two indicator lines. A possible explanation for

329 this difference is that SCN was first detected in South Dakota in 1995 (Smolik et al. 1996),
330 whereas, SCN was first detected in North Dakota much later during 2003 (Bradley et al. 2004).
331 Consequently South Dakota may have a longer history of planting SCN-resistant cultivars
332 (Acharya et al. 2016; Smolik et al. 1996). In other midwestern states such as Kansas, Missouri,
333 and Nebraska that have a much longer history of SCN infestation and planting resistant cultivars,
334 several SCN populations that were virulent against all six of the seven indicator lines used in HG
335 type testing, except for PI 437654 (#4), have been detected (Broderick 2016; Mitchum et al.
336 2007; Rzodkiewicz 2010). Furthermore, a population of SCN that reproduced on all seven
337 indicator lines has been reported in China (Lian et al. 2017) that has the greatest history of SCN
338 infestation (Li et al. 2011). North Dakota has a shorter history of SCN infestation and subsequent
339 resistant cultivar use, which may partially explain why none of the SCN populations found in
340 this study having FI ≥10% were able to adapt to PI 90763 (#3) and PI 89772 (#6).
341 One distinction of this study compared to a majority of the previously published SCN
342 virulence phenotype surveys is the use of naturally infested soil as well as artificially inoculated
343 sterilized soil in which HG type indicator lines and the susceptible check were planted during the
344 HG type tests (Acharya et al. 2016; Howland et al. 2018; Niblack et al. 2002). Artificially
345 inoculating sterilized soil ensures only SCN is infesting the soil in which the soybean seedlings
346 are planted. However, it takes considerable time to increase the SCN population to a sufficient
347 level for inoculation purposes. Our results have shown if the field population density is higher
348 than 400 eggs/100 cm^3 of soil, HG type tests can be conducted with naturally infested soil.
349 Moreover, using naturally infested soil may better emulate field soil conditions and also make
350 testing results available to growers quicker without increasing population first. An advantage of
351 using artificial inoculum is that an equal amount of inoculum can be added to each of the

indicator lines as well as the susceptible cultivar. Although, it is difficult to achieve even distribution of inoculum in naturally infested soil, it can be obtained by thoroughly mixing the naturally infested soil prior to planting. For this study, we diligently ensured even distribution of inoculum by thoroughly mixing the soil by hand first to break up soil clumps, and then using a motorized cement mixer to mix the soil for 30 to 60 minutes depending on the soil texture.

Furthermore, to validate the designation of the HG types determined in this study, we repeated the HG type tests for 33 arbitrarily selected SCN populations. These repeated HG type tests were conducted by artificially inoculating sterilized sandy soil during planting. Although within the study period we were not able to repeat the HG type tests for all the SCN populations, the HG types detected in the second iteration of the tests were consistent with the first iteration. Thus, suggesting that naturally infested soil can be used to conduct HG type tests, since similar results were observed when the same population was HG typed by using naturally infested soil and artificially inoculated soil. However, three of the SCN field populations from North Dakota with population densities higher than 50,000 eggs per 100 cm^3 of soil were HG typed by artificially inoculating sterilized sandy soil during planting. These soil samples were not HG typed using naturally infested soil because we suspect that the sheer population pressure on the indicator lines may influence the HG type results. However, further research needs to be conducted to ascertain the effect of high SCN population densities on HG type results.

The occurrence and frequency of SCN virulence phenotypes varied among the counties in North Dakota. The greatest diversity in SCN virulence phenotypes was detected in the south-eastern county, Richland. This may be attributed to Richland County having one of the highest soybean acreages and the longest history of SCN infestation in North Dakota (Bradley et al. 2004; USDA-NASS 2019). Richland County also shares its border with South Dakota and

375 Minnesota, both of which has a longer history of SCN infestation than North Dakota (Macdonald
376 et al. 1980; Smolik et al. 1996). This may explain why Richland county is the first North Dakota
377 county to have detectable levels of SCN infestation (Bradley et al. 2004). Another such south-
378 eastern North Dakota county that shares a border with Richland is Cass County. This county has
379 the highest soybean acreage in North Dakota (USDA-NASS 2019) and the second highest
380 diversity in SCN virulence phenotypes according to our results. Furthermore, these eastern and
381 south-eastern counties of North Dakota are characterized by higher precipitation and warmer
382 climatic conditions than western counties of North Dakota (Chowdhury et al. 2020), which can
383 favor SCN populations and result in greater diversity of SCN virulence phenotypes under these
384 climatic conditions in these counties.

385 A statistically significant positive correlation was observed between the FI of the HG
386 type indicator lines #2, #5 and #7. Similar results were reported in previous studies where
387 significant positive correlations were found between the FI values on the indicator lines #2, #5,
388 and #7 (Acharya et al. 2016; Niblack et al. 2003; Zheng et al. 2006). The reason may be that
389 these three indicator lines belong to a group that share similar resistance mechanism and confer
390 similar type of resistance response to SCN. The HG type indicator lines #5 and #7 were reported
391 to belong to the PI 88788-type resistance group (Colgrove and Niblack 2008).

392 Previous studies also reported positive correlations between the FI values on the indicator
393 lines #1, #3, and #6 (Acharya et al. 2016; Niblack et al. 2003; Zheng et al. 2006). Moreover, the
394 indicator lines #3 and #6 belonged to the Peking-type (#1) resistance group because these three
395 indicator lines (#1, #3, and #6) shared similar resistance mechanism and conferred similar type
396 of resistance response to SCN (Colgrove and Niblack 2008). In this study, positive correlations
397 exist between the FI values of the indicator lines #1, #3, and #6. However, positive correlations

between the FI values of the indicator #1 and #2 as well as #1 and #4 were also observed at a high significance level. These results maybe be explained by the low number of populations from North Dakota (three) that attacked the indicator line #1 having a FI ≥ 10% and all three of these populations also attacked indicator line #2. It is possible that the fields from which these three populations were collected may have a history of using soybean cultivars that derived resistance from the indicator lines #1 as well as #2. Thus, the three SCN populations were able to overcome the resistance derived from both these indicator lines, as a result, they had higher FI on both the indicator lines in our HG type tests, leading to a stronger correlation between them in our correlation analysis. However, a majority of the SCN populations that attacked indicator line # 5 and #7 did not attack indicator lines #1. Thus, significant correlation between the FI values of indicator lines #1, # 5, and #7 were not observed.

Nonetheless, correlation analysis findings suggest, for example, the SCN populations that are able to overcome the resistance conferred by PI 88788 (#2) will most likely overcome the resistance conferred by PI 209332 (#5) and PI 548316 (#7). Thus, breeding efforts should consider resistance sources other than PI 209332 and PI 548316 to effectively manage SCN populations that are virulent against PI 88788. Furthermore, rotating between sources of resistance may delay the shift in SCN virulence phenotypes as the chances of SCN adapting to any particular resistance group get reduced (Acharya et al. 2016).

Several studies have been conducted to explore the genetic basis of SCN resistance (Brucker et al. 2005; Cook et al. 2014; Meksem et al. 2001; Yu et al. 2016). Briefly, the soybean indicator lines belonging to the PI 88788-type resistance group utilizes *Rhg1-b* resistance allele to confer resistance (Brucker et al. 2005; Cook et al. 2014). Cook et al. (2014) demonstrated that copy number variation of *Rhg1-b* allele played a significant role in conferring resistance with

susceptible soybean genotypes having lower copy numbers of the allele. Cook et al. (2014) further reported that the indicator line #7 contained seven copies of the *Rhg1-b* allele, whereas, indicator lines PI 88788 (#2) and PI 209332 (#5) contained nine and 10 copies of the allele, respectively. These findings can help explain why the soybean indicator line PI 548316 (#7) was the most commonly parasitized indicator line in our study. In contrast, the soybean indicator lines belonging to the Peking-type resistance group utilize low copy number of the *Rhg1-a* resistance allele and another resistance allele located at the *Rhg4* locus. Thus, both *Rhg1-a* and *Rhg4* alleles are required for soybean genotypes to confer Peking-type resistance (Brucker et al. 2005; Cook et al. 2014; Yu et al. 2016).

In conclusion, this study reports a shift in SCN virulence phenotypes in North Dakota has occurred since its first detection in 2003 (Bradley et al. 2004). This is a first report of five new HG types, namely 7, 2.5.7, 1.2.5.7, 5.7, and 2.7, that were detected in SCN field populations from North Dakota, in addition to HG type 0. The repeated HG type tests validated the occurrence of each of these HG types in North Dakota. Repetition of the HG type tests proved that there are several SCN populations in North Dakota that can successfully reproduce on indicator line #2 (PI 88788), the most widely used source of resistance (Yan and Baidoo 2018). This underscores the importance of further utilizing resistance sources other than PI 88788 in future breeding efforts. However, correlation analysis suggests that SCN populations that are adapted to PI 88788 are more likely to adapt to resistance from PI 209332 and PI 548316, since they belong to the same resistance group. Thus, highlighting the importance of rotating between resistance groups to delay the adaptation of SCN (Acharya et al. 2016; Colgrove and Niblack 2008). Since only three populations from North Dakota had FI $\geq$ 10% on PI 548402 (Peking), the indicator lines in the Peking-type resistance group can be an effective source of resistance to

444 rotate with cultivars deriving resistance from PI 88788-type resistance group. Growers can also
445 use SCN-resistant cultivar such as Hartwig (Anand 1992), which derives resistance from PI
446 437654 since none of the populations tested in this study had a FI ≥ 10% on PI 437654.
447 However, previous greenhouse studies have shown that SCN populations can adapt to resistant
448 cultivars that derives resistance from PI 437654 within a few successive generations (Colgrove
449 and Niblack 2008). An integrated approach that includes crop rotation, utilization of SCN-
450 resistant cultivars, rotation between sources of resistance in SCN-resistant cultivars, and the
451 emerging nematicide seed treatments is highly recommended (The SCN Coalition 2020).

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TABLES

Table 1. *Heterodera glycines* virulence phenotypes detected in North Dakota determined by HG type tests conducted with seven indicator lines and the susceptible check planted in naturally infested field soil[a]

Sample ID	County	Egg count[b] (100 cm^3)	Female index (FI)[c] PI 548402 (#1)	PI 88788 (#2)	PI 90763 (#3)	PI 437654 (#4)	PI 209332 (#5)	PI 89772 (#6)	PI 548316 (#7)	No. females on susceptible[d]	HG type[e]
Ken	Richland	2,800	10.0	25.5	2.2	0.1	46.3	0.3	40.3	583	1.2.5.7
N 32	Richland	12,200	1.6	9.7	0.3	0.2	2.0	0.2	14.0	668	7
N 48	Cass	12,200	5.8	14.1	0.4	0.0	19.4	0.6	25.1	509	2.5.7
SCN 2EF	Cass	4,200	0.6	2.9	0.2	0.1	1.5	1.3	2.7	981	0
SCN 3	Cass	9,600	1.1	2.7	1.3	0.3	5.4	1.5	10.4	968	7
SCN 4	Cass	13,600	2.4	8.5	3.7	0.5	5.6	1.4	10.3	1,568	7
SCN 7	Cass	700	1.0	11.9	0.3	0.7	22.0	0.7	38.0	296	2.5.7
SCN 14	Cass	750	0.0	0.4	0.0	0.0	3.5	0.0	7.3	391	0
SCN 22	Cass	1,800	0.0	0.7	0.0	0.0	2.2	0.3	11.2	381	7
SCN 24	Cass	840	0.0	0.0	0.0	0.0	0.0	0.0	0.1	617	0
SCN 49	Richland	450	1.1	3.8	0.0	0.0	2.5	0.0	26.7	275	7
SCN 52	Richland	630	5.7	16.4	0.2	0.0	2.8	0.2	26.7	307	2.7
SCN 52*	Richland	3,800	0.1	6.5	0.0	0.0	14.0	0.0	60.7	541	5.7
SCN 55	Richland	460	1.4	11.5	0.0	0.0	26.2	1.0	34.7	234	2.5.7
SCN 55*	Richland	12,300	2.0	9.9	0.0	0.0	15.5	0.0	61.1	461	5.7
SCN 59	Richland	780	1.1	6.0	3.9	0.1	5.1	1.3	7.6	340	0
SCN 61	Richland	1,190	0.1	2.4	0.0	0.0	1.4	0.0	0.7	549	0
SCN 62	Richland	2,700	0.0	1.7	0.0	0.0	10.3	0.0	10.5	332	5.7
SCN 63	Richland	1,050	1.6	23.5	0.0	0.0	18.7	0.7	25.3	352	2.5.7
SCN 78	Richland	1,368	0.0	22.3	0.0	0.0	13.8	0.0	21.1	220	2.5.7
SCN 79	Richland	3,630	0.1	9.7	0.1	0.1	10.2	0.0	15.2	534	5.7
SCN 81	Richland	1,260	0.0	0.1	0.0	0.0	0.2	0.0	0.1	518	0
SCN 82	Richland	610	0.0	1.2	0.0	0.0	1.6	0.0	0.2	620	0
SCN 95	Richland	720	0.0	8.8	0.0	0.0	5.9	0.0	16.0	190	7
SCN 102	Richland	1,020	0.0	0.0	0.0	0.0	0.0	0.0	0.3	223	0
SCN 103	Richland	1,680	0.0	0.9	0.0	0.0	3.5	0.0	4.9	143	0
SCN 131	Cass	400	0.0	2.4	0.0	0.1	2.2	0.0	1.9	247	0
SCN 154	Cass	500	0.0	2.4	0.0	0.0	0.6	0.0	7.4	116	0
SCN 156	Cass	8,700	1.9	7.0	0.0	0.0	27.0	0.7	60.0	541	5.7
SCN 180	Cass	664	2.0	39.1	0.0	0.0	38.9	0.0	56.9	172	2.5.7

SCN 246	Cass	400	3.8	10.7	0.0	0.0	3.6	0.0	16.1	93	2.7*
SCN 280	Barnes	680	0.0	0.0	0.0	0.0	0.0	0.0	0.1	236	0
SCN 285	Steele	2,583	0.5	3.5	0.0	0.0	10.8	0.0	31.6	1,430	5.7
SCN 325	Grand Forks	300	0.0	0.0	0.0	0.0	0.0	0.0	49.8	54	7
SCN 329	Traill	5,200	0.8	8.2	0.0	0.0	4.4	0.1	6.8	1,603	0
SCN 331	Traill	1,200	0.0	5.3	0.1	0.0	0.2	0.0	5.8	384	0
SCN 333	Traill	2,800	0.0	3.1	0.0	0.0	1.1	0.0	11.3	331	7
SCN 334	Traill	7,600	1.7	4.4	0.1	0.0	1.0	0.1	17.3	1,954	7
SCN 339	Traill	1,200	0.5	7.3	2.5	0.1	2.8	0.4	6.3	371	0
SCN 342	Traill	42,500	25.5	39.7	0.0	0.2	10.5	0.0	35.5	325	1.2.5.7
SCN 344	Traill	4,600	25.4	30.5	3.0	2.6	10.0	2.7	31.4	742	1.2.5.7
SCN 350	Traill	8,600	0.0	17.7	0.0	0.0	14.6	0.0	28.1	1,037	2.5.7
SCN 351	Traill	2,400	0.0	2.4	0.0	0.0	2.1	0.0	12.6	731	7
SCN 352	Traill	4,100	0.1	2.2	0.0	0.1	0.0	0.0	23.4	491	7
SCN 355	Steele	15,500	0.1	0.5	0.0	0.0	0.5	0.0	0.0	425	0
SCN 357	Steele	19,200	0.0	8.5	0.0	0.1	12.8	0.0	55.5	1,252	5.7
SCN 370	Grand Forks	1,100	0.0	4.0	0.0	0.0	0.0	0.0	20.2	100	7
SCN 390	Steele	700	9.5	27.7	4.1	0.0	31.2	6.7	19.1	227	2.5.7
SCN 391	Steele	2,300	0.0	0.2	0.0	0.0	0.0	0.0	0.0	712	0
SCN 392	Steele	5,600	0.1	18.1	0.0	0.0	26.4	0.0	46.0	346	2.5.7
SCN 394	Steele	2,200	0.2	1.3	0.1	0.0	8.1	0.0	27.7	907	7

[a] Samples with population densities higher than 400 eggs and juveniles per 100 cm^3 of soil were HG typed by planting the seven indicator lines and the susceptible check in naturally infested soil. Average no. of white females for each of the seven indicator lines and the susceptible check were calculated from four replicates.

[b] Density of SCN eggs and juveniles in 100 cm^3 of naturally infested original samples.

[c] FI = (average no. of white females found on the indicator line/average no. of white females found on the susceptible check) x 100.

[d] Average number of white females in the susceptible check Barnes.

[e] HG types from assays in which the average number of white females on the susceptible check was greater than 50 but less than 100 are indicated with the asterisks (*) symbol.

Table 2. *Heterodera glycines* virulence phenotypes detected from additional field soil samples in North Dakota determined by HG type tests conducted with seven indicator lines and the susceptible check planted in artificially infested sandy soil[a]

		Female index (FI)[b]								
Sample ID	County	PI 548402 (#1)	PI 88788 (#2)	PI 90763 (#3)	PI 437654 (#4)	PI 209332 (#5)	PI 89772 (#6)	PI 548316 (#7)	No. females on susceptible[c]	HG type[d]
Arne	Richland	1.3	4.8	0.2	0.0	2.6	0.0	8.8	898	0
Castleton	Cass	0.0	1.1	0.0	0.0	0.2	0.1	1.2	568	0
GregShort	Richland	0.8	10.8	0.0	0.0	11.6	0.1	14.8	955	2.5.7
GregTall	Richland	0.0	7.0	0.0	0.0	9.5	0.0	23.3	831	7
Lee	Richland	0.0	1.1	0.0	0.0	2.0	0.0	4.7	261	0
Miller	Richland	0.0	5.9	0.0	0.0	6.1	0.0	8.9	110	0
Prosper	Cass	0.0	0.5	0.0	0.0	0.7	0.0	4.9	919	0
SCN 2WF	Cass	5.6	3.9	0.0	0.0	2.2	0.0	11.7	205	7
SCN 16	Cass	0.0	27.4	0.0	0.0	37.0	0.0	55.0	173	2.5.7
SCN 38	Cass	0.0	17.7	0.0	0.0	33.8	0.0	44.0	233	2.5.7
SCN 46	Richland	0.0	5.0	0.1	0.0	3.5	0.0	18.6	762	7
SCN 48	Richland	0.0	1.7	0.0	0.0	0.0	0.0	10.7	121	7
SCN 50	Richland	0.0	0.6	0.2	0.0	0.0	0.0	15.9	131	7
SCN 53	Richland	0.0	8.0	0.0	0.0	0.0	0.0	0.0	477	0
SCN 101	Richland	0.0	4.5	0.0	0.0	2.0	0.0	4.0	100	0
SCN 157	Richland	0.0	0.0	0.0	0.0	5.8	0.0	6.8	51	0*
SCN 182	Cass	0.1	5.9	0.1	0.0	3.5	0.1	2.9	963	0
SCN 220	Cass	0.0	2.6	0.0	0.0	1.6	0.0	12.1	64	7*
SCN 326	Grand Forks	0.0	3.0	0.0	0.0	13.4	0.0	25.4	209	5.7
SCN 330	Traill	0.6	11.6	0.2	0.0	15.3	0.0	19.1	238	2.5.7
SCN 332	Traill	0.3	1.9	0.1	0.0	1.3	0.5	29.0	405	7
SCN 353	Traill	0.6	32.6	0.2	0.0	42.0	0.0	13.6	216	2.5.7

[a] Additional samples with population densities lower than 400 eggs and juveniles per 100 cm^3 of soil were HG typed by planting the seven indicator lines and the susceptible check in artificially inoculated soil. Average no. of white females for each of the seven indicator lines and the susceptible check was calculated from four replicates.

[b] FI = (average no. of white females found on the indicator line/average no. of white females found on the susceptible check) x 100.

[c] Average number of white females in the susceptible check Barnes.

[d] HG types from assays in which the average number of white females on the susceptible check was greater than 50 but less than 100 are indicated with the asterisks (*) symbol.

Table 3. Repeated HG type tests conducted to confirm the virulence phenotypes detected in North Dakota by planting soybean lines and the susceptible check in artificially infested sandy soil[a]

		Females index (FI)[b]								
Sample ID	County	PI 548402 (#1)	PI 88788 (#2)	PI 90763 (#3)	PI 437654 (#4)	PI 209332 (#5)	PI 89772 (#6)	PI 548316 (#7)	No. females on susceptible[c]	HG type[d]
Arne's	Richland	1.6	3.7	0.0	0.0	6.2	0.0	7.3	109	0
Castleton	Cass	0.0	5.2	0.0	0.0	1.8	0.0	3.6	217	0
Greg Short	Richland	6.2	15.5	0.0	0.0	24.0	0.0	24.0	60	2.5.7*
Greg Tall	Richland	0.0	8.9	0.0	0.0	9.8	0.0	27.4	156	7
Ken's	Richland	12.2	17.9	1.6	0.0	20.1	0.4	19.8	92	1.2.5.7*
Lee's	Richland	0.1	4.7	0.0	0.0	6.4	0.1	6.8	409	0
Prosper	Cass	0.0	0.0	0.0	0.0	0.5	0.0	1.4	105	0
SCN 2WF	Cass	0.0	2.5	0.0	0.0	1.5	0.0	25.7	99	7*
SCN HG 7	Cass	0.7	10.0	0.0	0.0	16.3	0.0	26.6	335	2.5.7
SCN 16	Cass	0.3	13.7	0.0	0.0	24.6	0.0	50.6	181	2.5.7
SCN 38	Cass	0.0	27.0	0.0	0.0	35.0	0.0	24.0	53	2.5.7*
SCN 46	Richland	0.0	7.1	0.0	0.0	5.9	0.0	28.0	511	7
SCN 48	Richland	0.0	0.0	0.0	0.0	0.1	0.0	13.3	218	7
SCN 50	Richland	0.2	1.6	0.0	0.0	2.3	0.1	17.8	490	7
SCN 52*	Richland	0.0	4.4	0.0	0.0	10.3	0.0	27.7	120	5.7
SCN 101	Richland	0.0	1.9	0.0	0.0	3.8	0.0	3.7	431	0
SCN 102	Richland	0.0	5.9	0.0	0.0	0.3	0.0	44.3	81	0*
SCN 103	Richland	0.0	6.6	0.0	0.0	7.0	0.0	8.6	198	0
SCN 180	Cass	0.4	10.0	0.2	0.0	15.7	0.0	24.7	352	2.5.7
SCN 246	Cass	0.2	10.1	0.5	0.0	4.3	0.1	11.1	222	2.7
SCN 280	Barnes	0.0	0.0	0.0	0.0	0.0	0.0	0.3	106	0
SCN 285	Steele	0.0	0.0	0.0	0.0	10.3	0.0	15.0	104	5.7
SCN 325	Grand Forks	0.0	0.0	0.0	0.0	0.0	0.0	49.8	54	7*
SCN 330	Traill	0.0	11.7	0.0	0.0	10.0	0.0	13.9	81	2.5.7*
SCN 333	Traill	0.0	0.0	0.0	0.0	4.3	0.0	15.5	55	7*
SCN 339	Traill	0.0	9.3	0.1	0.0	7.4	0.0	3.8	210	0
SCN 342	Traill	27.2	48.8	1.2	0.0	40.7	3.4	34.2	81	1.2.5.7*
SCN 344	Traill	39.0	30.1	0.1	0.3	39.4	3.1	29.8	281	1.2.5.7
SCN 350	Traill	0.0	10.1	0.0	0.0	10.1	0.0	14.4	232	2.5.7
SCN 351	Traill	0.0	1.2	0.0	0.0	8.6	0.0	9.7	185	0

SCN 355	Steele	0.0	0.0	0.0	0.0	1.9	0.0	3.9	52	0*
SCN 357	Steele	0.0	7.2	0.0	0.3	9.6	0.0	12.5	234	7
SCN 392	Steele	0.3	13.5	0.1	0.0	19.0	0.0	30.9	173	2.5.7

[a] Within the study period we were not able to repeat the HG type tests for all 73 of the SCN populations tested, however the occurrence of each of the HG types detected was validated, by repeating the HG type tests for thirty-three arbitrarily selected samples. Average no. of white females for each of the seven indicator lines and the susceptible check was calculated from four replicates.

[b] FI = (average no. of white females found on the indicator line/average no. of white females found on the susceptible check) x 100.

[c] Average number of white females in the susceptible check Barnes.

[d] HG types from assays in which the average number of white females on the susceptible check was greater than 50 but less than 100 are indicated with the asterisks (*) symbol.

Table 4. Univariate descriptive statistics of female index (FI) of *Heterodera glycines* populations from North Dakota that produced FI ≥ 10 on soybean indicator lines with resistance to *H. glycines*[a]

		Female index			
Soybean indicator line[b]	FI ≥ 10%[c]	Minimum observed	Maximum observed	Mean	Standard deviation
PI 548402 (#1)	4.1	10.0	25.5	20.3	7.3
PI 88788 (#2)	26.0	10.8	39.7	21.5	9.1
PI 209332 (#5)	34.2	10.0	46.3	21.7	11.0
PI 548316 (#7)	64.4	10.3	61.1	27.3	15.4

[a] Female indices from the repeated HG type tests were not included in the analysis as they are repeat experiments conducted for the confirmation purpose.

[b] None of the *H. glycines* populations were able to produce FI ≥ 10 on soybean indicator lines PI 90763 (#3), PI 437654 (#4) and PI 89772 (#6). Therefore, they were not included in the table.

[c] Percentage of population having a female index greater than or equal to 10.

Table 5. Percentage of *Heterodera glycines* populations from North Dakota with female index (FI) ≥ 10% or ≥ 30% on resistant soybean lines in different counties of North Dakota[a]

Soybean indicator lines	Counties											
	Barnes		Cass		Grand Forks		Richland		Steele		Traill	
	FI ≥ 10	FI ≥ 30	FI ≥ 10	FI ≥ 30	FI ≥ 10	FI ≥ 30	FI ≥ 10	FI ≥ 30	FI ≥ 10	FI ≥ 30	FI ≥ 10	FI ≥ 30
PI 548402 (#1)	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	15.4	0.0
PI 88788 (#2)	0.0	0.0	30.0	5.0	0.0	0.0	20.7	0.0	28.6	0.0	38.5	23.1
PI 209332 (#5)	0.0	0.0	30.0	15.0	33.3	0.0	34.5	3.4	57.1	14.3	38.5	7.7
PI 548316 (#7)	0.0	0.0	60.0	25.0	100.0	33.3	58.6	3.4	71.4	42.9	76.9	15.4
Total[b] (HG tested)[c]	3 (1)		58 (20)		8 (3)		66 (29)		7 (7)		16 (13)	

[a] Female indices from the repeated HG type tests were excluded from this table as they are repeat experiments conducted for the confirmation purpose.

[b] Total number of *Heterodera glycines* positive samples are outside the parenthesis.

[c] Total number of samples used in HG typing assays are in parenthesis.

Table 6. Correlation coefficient among soybean lines with resistance to soybean cyst nematode based on female index (FI)[a] from 73 SCN field populations in North Dakota

Soybean lines	PI 548402 (#1)	PI 88788 (#2)	PI 90763 (#3)	PI 437654 (#4)	PI 209332 (#5)	PI 89772 (#6)
PI 88788 (#2)	0.61**[b]					
PI 90763 (#3)	0.41*	0.27*				
PI 437654 (#4)	0.64**	0.30*	0.44**			
PI 209332 (#5)	0.21ns	0.80**	0.18ns	0.03ns		
PI 89772 (#6)	0.41*	0.31*	0.74**	0.37*	0.25*	
PI 548316 (#7)	0.22ns[c]	0.50**	-0.03ns	0.11ns	0.64**	0.04ns

[a] Female index = (average no. of white females found on the indicator line/ average no. of white females on the susceptible check) x 100.

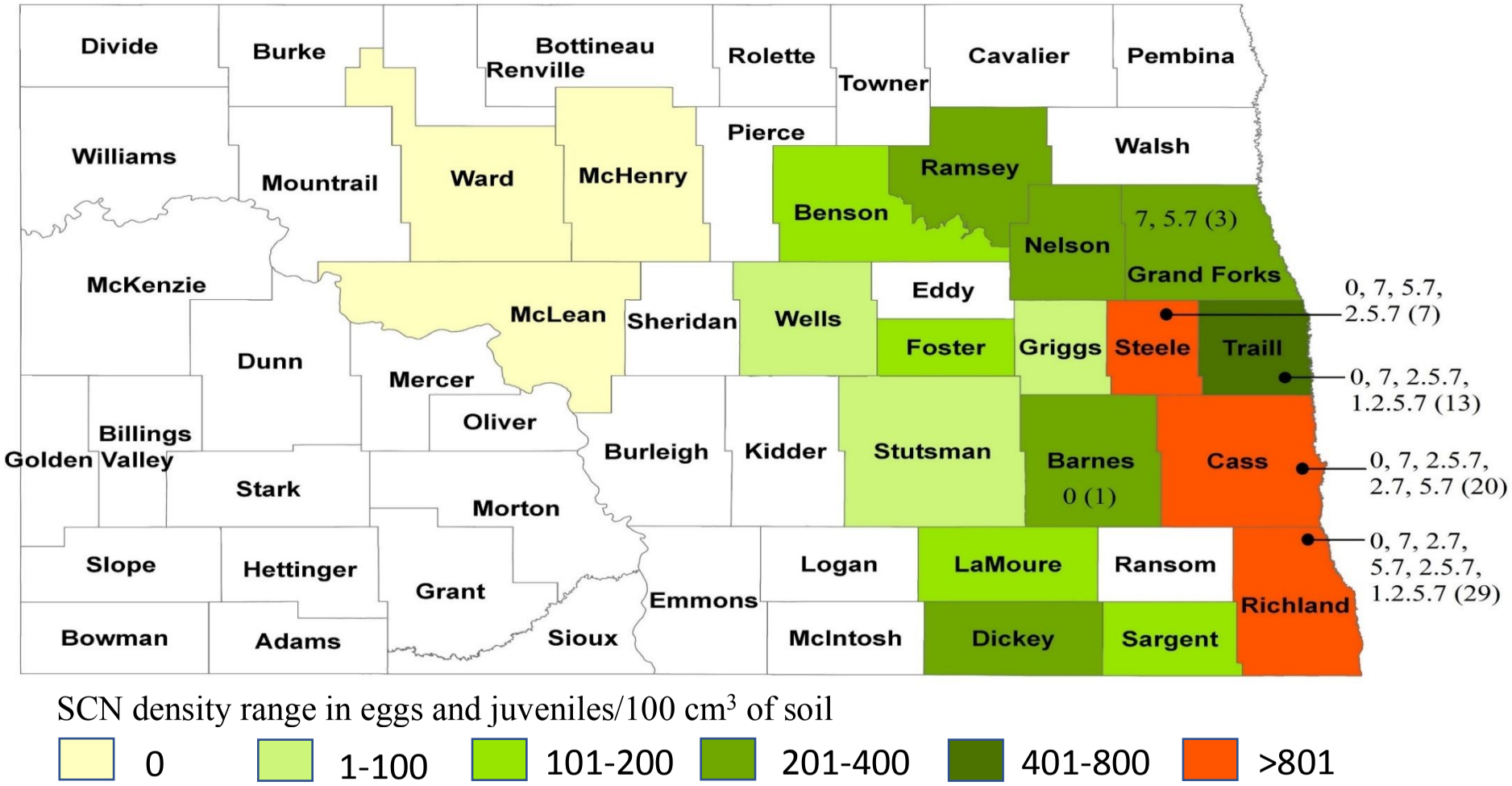
[b] * = $P < 0.05$, ** = $P < 0.0001$.

[c] ns = not significant, $P > 0.05$.

FIGURE LEGENDS

Figure 1. Distribution of *Heterodera glycines* and their virulence phenotypes (HG types) in North Dakota (ND). Each of the 22 major soybean producing counties, that were surveyed, were color coded based on their mean SCN population density in SCN positive fields. The color codes and their corresponding mean SCN population density range in eggs and juveniles/100 cm^3 of soil are shown on the bottom of the map. The population density of SCN eggs and juveniles in SCN positive individual fields of ND ranged from 90 to 92,800 eggs and juveniles per 100 cm^3 of soil. The numbers within each county represents the HG types found in those counties, and the number inside the parenthesis represents the number of samples tested from those counties.

Figure 2. Frequency of *Heterodera glycines* types detected in North Dakota from the 73 field populations tested. The x-axis represents *Heterodera glycines* types detected in North Dakota, and the y-axis represents the frequency of those HG types.



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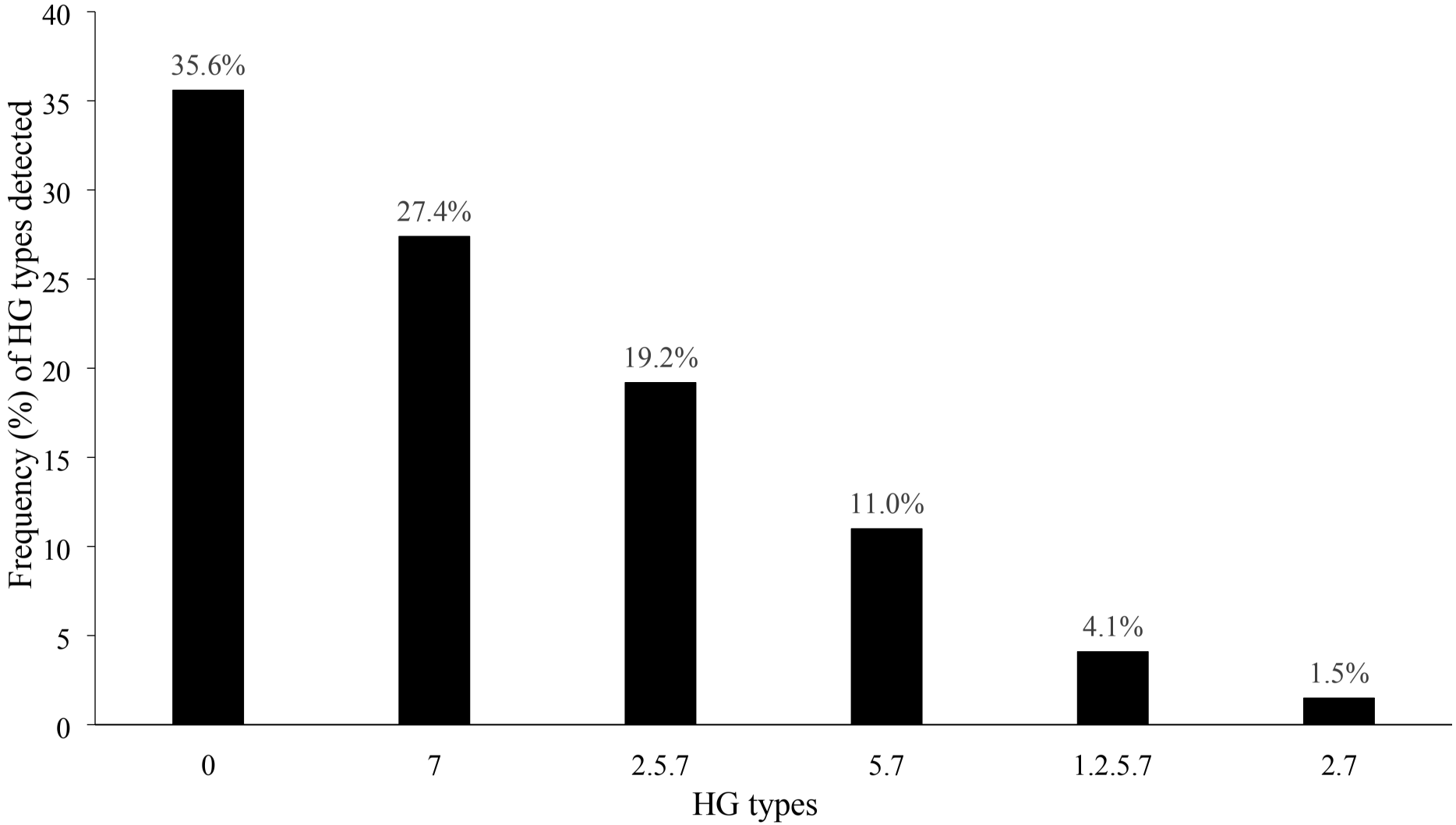

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