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RESEARCH

Impact of Soybean Cyst Nematode Resistance on Soybean Yield

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ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) of soybean [*Glycine max* (L.) Merr.] causes extensive yield loss, and host resistance has been an effective strategy to minimize this loss. However, shifts in SCN population virulence compatibility have resulted from the extensive use of PI 88788 as a source of resistance in the northern United States. The Northern Regional Soybean Cyst Nematode Tests offer a vast amount of yield testing, combined with resistance screening of entries and the characterization of the SCN infection of test fields. The objectives of this study were to use regional test results (i) to quantify the impact of resistance as the initial field SCN egg counts increase, (ii) to explore effects of maturity group (MG) on resistance, and (iii) to gain insights into how the ability of SCN to infect germplasm with resistance from PI 88788 affects yield. Yield tests across 11 yr were combined into a single dataset with over 1247 test–environment combinations. Analysis of these data showed that the yield advantage of SCN resistant entries increased as initial egg counts increased, and a larger advantage was found in early MGs (00–II) than in later MGs (III–IV). A yield advantage was documented at environments with an initial egg count as low as 100 eggs 100 cm^{-3} soil. At the levels of SCN infection on PI 88788 found in infested locations, breeding lines with resistance from PI 88788 yielded more than susceptible entries. Analysis of this dataset offers a unique view of the impact SCN resistance provides to soybean.

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Abbreviations: FI, female index; HG, *Heterodera glycines*; HR, highly resistant; LR, low resistance; MG, maturity group; MR, moderately resistant; NR, no effective resistance; R, resistant; P_i, initial egg count; QTL, quantitative trait loci; S, susceptible; SCN, soybean cyst nematode.

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is the pest estimated to cause the most damage to soybean [*Glycine max* (L.) Merr.] production in the United States due to its widespread distribution and ability to reduce seed yield. Losses in the United States were estimated to average 2,771,493 Mg annually during 2003 to 2005 (Wrather and Koenning, 2006) and 3,468,684 Mg annually during 2006 to 2009 (Koenning and Wrather, 2010). Growers are often slow to respond to SCN infestations in fields because yield losses often occur when above-ground symptoms, such as plant stunting, are not present (Young, 1996; Wang et al., 2003). Damage to soybean plants occurs when juvenile nematodes penetrate roots and feed from vascular tissue (Koenning, 2004). As the lifecycle of SCN continues, eggs are produced in cysts that protect the eggs for several years until optimum conditions are present for hatching (Koenning, 2004).

Populations of SCN are described by a *H. glycines* (HG) type classification system, which separates the major genetic groups on the basis of host compatibility (Niblack et al., 2002). The HG type designation of a nematode population identifies which standard indicator lines it can reproduce on. The indicator lines represent SCN-resistant sources that have been used in breeding-resistant cultivars. Therefore, an SCN population that can reproduce on a standard indicator line would be expected to reproduce on cultivars with resistance derived from this indicator

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line. Understanding the SCN HG type present in the soil is important when producers select a resistant cultivar.

The most effective methods to manage SCN are to use host resistance and to rotate with non-host crops (Niblack and Chen, 2004). Resistance to at least one SCN HG type has been found in 158 soybean accessions across the germplasm collection (NPGS, 2016; Crop: Soybean, Descriptors: Nematcyst_1, 2, 3, 4, 5, and 14, Observation: Resistant). Furthermore, resistance has been bred into cultivars; however, PI 88788 is the predominate source of SCN resistance for cultivars available to growers in the northern United States. For instance, the University of Illinois Department of Crop Science Variety Testing program evaluated 336 SCN-resistant entries within maturity groups (MGs) II, III, and IV in 2013, but only 10 contained sources other than PI 88788 (Joos et al., 2013). As a likely consequence of the abundant use of a single source of resistance, Niblack et al. (2008) found that 70% of the SCN-infested soil samples collected in Illinois had populations that could reproduce on PI 88788. Similar results were found in other soybean-producing regions as well (Mitchum et al., 2007; Faghihi et al., 2010). In contrast, resistance from other sources such as PI 437654 and Peking (PI 548402) remain effective, but it has been a challenge to combine resistance from these sources with the high yield and agronomic traits expected by soybean growers.

The genetic basis of resistance to SCN has been studied, and quantitative trait loci (QTL) controlling resistance have been mapped (Concibido et al., 2004; Guo et al., 2006). This research showed that the resistance sources PI 88788, Peking, and PI 437654 have a resistance allele at the major resistance locus *rhg1* (Concibido et al., 2004), and that PI 88788 has a different allele at *rhg1* compared with PI 437654 (Brucker et al., 2005). The allelic difference was found to result from differences in copy number of a 31.2-kb segment that contains four genes and from nucleotide diversity in the genes (Cook et al., 2012; Lee et al., 2015; Yu et al., 2016). Both PI 437654 and Peking have the same resistance allele for *rhg1* and have the gene *Rhg4*, which interacts with *rhg1* (Concibido et al., 2004). In addition, other QTL in the background of PI 437654 and Peking contribute to their resistance (Concibido et al., 2004).

Less is known about the basis for HG type diversity in *H. glycines*. It has been generally found that, in geographic regions that SCN-resistant cultivars have been grown the longest, field isolates increase in their likelihood in overcoming resistance in cultivars (Faghihi et al., 2010). Bekal et al. (2003) hypothesized that a chorismate mutase gene is a virulence gene, and that forms of the gene were responsible for parasitism of soybean with specific gene combinations. More recently, effector candidates have been identified from SCN (Noon et al., 2015), but their roles in parasitism remain to be discovered.

Resistant cultivars have repeatedly shown yield advantages compared with susceptible cultivars when SCN is present (Niblack et al., 1992; Young, 1996; Wheeler et al., 1997; Koenning, 2000; Chen et al., 2001; Brucker et al., 2005; Donald et al., 2006; De Bruin and Pedersen, 2008; Delheimer et al., 2010). Furthermore, as initial egg densities increase, greater yield differences between resistant and susceptible cultivars have been found (Niblack et al., 1992; Koenning, 2000; Chen et al., 2001). With the end goal of developing high-yielding resistant cultivars, the evaluation of breeding lines at many well-characterized locations is needed. For this reason, the Northern Regional Soybean Cyst Nematode Tests are conducted across the north-central soybean-producing region of the United States and southern Ontario to evaluate the yield performance of SCN-resistant germplasm from public soybean breeders. Yield test locations range from noninfested fields to fields with high initial SCN egg counts. Additionally, most locations were characterized by HG type testing field SCN populations. Data from these tests are a tremendous resource of replicated yield trial results from resistant and susceptible lines grown in MG 00 through IV locations.

The spatial variability of SCN in field environments has been documented (Francl, 1986; Donald et al., 1999; Avendaño et al., 2003), and controlling this variability is challenging to researchers. To address this spatial variability, researchers could either increase soil sampling at each site or increase the number of environments sampled. Data from the Northern Regional SCN Tests provide a benefit through the power gained by averaging across a large sample of environments to accurately calculate the impact of SCN resistance on yield.

Our objectives of this study are three-fold: (i) to quantify the impact of SCN resistance on yield by comparing SCN-resistant and susceptible breeding lines in fields that vary for SCN pressure, as measured by egg counts at planting, (ii) to explore the effect of MG on resistance, and (iii) to gain insights into how the ability of SCN to reproduce on PI 88788 affects yield. The Northern Regional Soybean Cyst Nematode Tests provide a rich resource to evaluate yield relationships over many locations and breeding lines.

MATERIALS AND METHODS

Description of Field Tests

The Northern Regional Soybean Cyst Nematode Tests (Cary and Diers, 2014) are conducted in the north-central United States and Canada across MGs 00 to IV through support provided by the United Soybean Board. Each year, public soybean breeders nominate experimental lines that are predicted to be SCN resistant, and these lines are organized, together with check cultivars, into separate MG tests. Seed of these experimental lines are distributed to the public breeder collaborators, who typically grow the tests at 6 to 20 locations annually for each MG (Table 1). After experimental lines are separated into tests by MG, the MG tests are also separated into conventional (nontransgenic) tests or transgenic tests and between preliminary tests, which include entries that were not previously tested in the regional tests, or uniform tests that include fewer entries that were previously evaluated in preliminary tests. Whether there were separate preliminary and uniform tests and conventional and Roundup Ready tests was determined on the basis of need, and not all tests were grown for each combination of year and MG. Field plots were replicated two to four times at each location and grown in multiple rows with spacing ranging from 19 to 76 cm, and the center rows were harvested for yield (Cary and Diers, 2014).

Soil Sampling and Analysis

Soil samples from each test location were collected at planting by taking cores 15 to 20 cm deep across the test area. The samples from each test location were composited into a single sample that represented the location and were shipped to the University of Illinois and analyzed by the nematology lab. Initial SCN egg counts were determined and reported as eggs per 100 cm^{-3} soil (P_i) (Faghihi and Ferris, 2000). The HG type of the SCN sample from each location was then determined according to Niblack et al. (2002) using seven indicator lines. Briefly, the nematodes were first allowed to reproduce on the susceptible cultivar Essex (Smith and Camper, 1973) to increase the population, if needed. One seedling from an indicator line was then placed in a tube filled with sterilized sandy soil and inoculated with 1000 eggs from the soil sample being tested. Tests were grown in the greenhouse with 27°C soil temperature, 16 h of light for 30 d, and each indicator line replicated six times (Cary and Diers, 2014). A female index (FI) was then calculated for each indicator line by dividing the mean number of cysts on the indicator line with the mean number of cysts on the susceptible cultivar Lee 74 (Caviness et al., 1975) and multiplying by 100. The FI on the indicator line PI 88788 was used in our analysis and is designated as FI_{88788}.

Table 1. Counts of environment and test combinations within Northern Regional Soybean Cyst Nematode Tests (2004–2014). Counts are summarized by state or province and different soybean cyst nematode infestation classifications within maturity groups.

	Maturity group					
State or province†	00	0	I	II	III	IV
ND	2	8				
MN	6	37	48	39		
ON	6	32	16	24		
SD			10	17		
NE			24	59	74	
IA			24	33	38	
IL			39	66	147	114
MI				11		
OH				17	28	
IN				13	24	1
TN				3	3	24
KS					49	39
MO					80	77
DE						2
KY						13
Environment classification						
Noninfested	8	41	18	45	112	88
Infested, HG‡ type 2 present	3	25	92	146	211	118
Infested, HG type 2 not present	3	5	32	53	47	17
Infested§	0	6	19	38	73	47
Total	14	77	161	282	443	270

† Locations with low overall yield (<673 kg ha^{-1}) and high CV (>20%) were removed.

‡ HG, *Heterodera glycines.*

§ Infested environments are lacking full HG type testing of soybean cyst nematode population present in the soil.

Dataset Preparation

Data from the 2004 to 2014 Northern Regional Soybean Cyst Nematode Tests were maintained at the University of Illinois as the means of experimental lines and checks for each location. Yield data were combined with two more sets of data: (i) a characterization of the SCN population for locations (P_i and HG type, as mentioned above), along with (ii) the FI of experimental lines and checks from greenhouse SCN testing at the University of Illinois (2005–2014) and at the University of Missouri (2004). Each entry was evaluated separately with a SCN HG type 0 and a 2.5.7 population. A FI was calculated for each entry in the same manner as HG type testing and designated as FI_{entry}. Entries were rated as highly resistant (HR, FI_{entry} < 10), resistant (R, FI_{entry} =10–24), moderately resistant (MR, FI_{entry} of 25–39), low resistance (LR, FI_{entry} = 40–59), or no effective resistance (NR, FI_{entry} > 60) (Niblack, 2005) for each HG population. For entries tested in multiple years, an average FI_{entry} value was calculated across years and included in the overall dataset. *Heterodera glycines* Type 0 ratings from entries in the year 2008 were not included due to low cyst counts.

This screening and rating system provided two variables to characterize the resistance of entries. However, neither rating variable alone is fully informative of an entry's resistance to SCN populations commonly found in production fields. For example, entries with any level of resistance to HG type 0 may or may not have resistance to HG type 2.5.7. Also, entries with no effective resistance to HG type 2.5.7 may or may not contain some level of resistance to the HG type 0 screening population. Therefore, new rating classes were developed to better classify resistance to both screening populations. First, if entries were found to have high resistance or resistance to the HG type 2.5.7 population, they were classified as HR257 or R257. Next, those entries not placed in the first categories and found to have high resistance or resistance to the HG type 0 population were classified as HR0 or R0. Then, entries with no resistance to both screening populations were rated as susceptible (S). All other entries were rated as having a midlevel resistance, and this included entries with moderate or low resistance to either HG type 0 and/or moderate, low, or no resistance to HG type 2.5.7. This created a rating class system that, when summarized for resistance from greatest to least, is ordered as follows: HR257, R257, HR0, R0, midlevel, and S.

Steps were taken to account for yield data quality. First, entries and environments with average yield <673 kg ha^{-1} were removed, since these do not represent a typical production agriculture environment and were likely overly influenced by environmental factors, such as moisture, that were outside the scope of this research. Next, locations with high CV (>20%), as listed in the Northern Regional Soybean Cyst Nematode Tests Report, were removed. These high CV values indicate that a high level of variability exists among entries and across replications, caused by such factors as localized pests, unequal moisture within blocks, or plot errors. Overall, 6% of the data were removed on the basis of data quality parameters, primarily high CV. To evaluate the yield impact of resistant entries compared with susceptible entries, a new variable was calculated from the yield data generated. First, all susceptible (S) entries that have a $FI_{entry} > 60$ for HG type 0 and 2.5.7 were identified. The average yield of all susceptible entries was then calculated within each environment of that test. Next, the yield of each of the nonsusceptible entries were divided by the susceptible average within that environment and multiplied by 100 to create a new variable, yield as a percentage of susceptible.

Statistical Analysis

The initial egg counts (P_i) were transformed [$\log_{10}(P_i + 1)$], due to a non-normal distribution of raw P_i values. Our model to analyze the yield impact of resistance compared with susceptible entries included the independent variables of resistance class from the experimental lines and checks, the log-transformed egg counts from the field samples, the squared term of log-transformed egg counts, and the interactions of resistance class with both the log-transformed egg counts and the squared term. Means of the yields within each resistance class were also calculated at noninfested environments. To further explore the additional predictor variable of MG, a model was analyzed with this variable included. Since the variable nature of SCN populations combined with our data structure requires large datasets to identify trends, exploring all levels of MG and possible interacting factors was not feasible. To circumvent this, we further analyzed MG by grouping MGs into early (MGs 00, 0, I, and II) and late (III and IV) data subsets and analyzed the results from a model described above on each subset. Data were analyzed with Proc Mixed of SAS 9.3 (SAS Institute, 2011). Response mean yields at levels of specified P_i were calculated with an LSMEANS statement, and *P*-values were adjusted to control for Type I statistical errors by the Dunnett procedure.

Data of SCN population virulence on PI 88788 (FI_{88788}) from HG type tests were also non-normally distributed and transformed by $\log_{10}(FI_{88788} + 1)$. To gain insights into the effects of the SCN population virulence on PI 88788 in combination with P_i, we developed a separate model that included $\log_{10}(P_i + 1)$, $\log_{10}(FI_{88788} + 1)$, and resistance classes. Two resistance classes were used, and these were the susceptible class and a second class composed of the HR0 and R0 entries with resistance from PI 88788 (HR0 and R0). Data from noninfested sites were excluded from the analysis to gain better estimates of regression slopes at varying SCN infestations. Significance of factors and interactions were calculated in SAS Proc Mixed of SAS 9.3 (SAS Institute, 2011). Fitted regression functions were then graphed between P_i values of 100 and 10,000, where the majority of our SCN-infested dataset is located.

RESULTS

After data quality filtering was completed, the overall dataset from 11 yr of tests included six MGs, 1682 soybean entries, 408 environments, and over 25,000 observations. When considering multiple tests conducted in a single environment, 1247 different test–environment combinations were represented in the dataset (Table 1). After filtering to only include locations with HG type 2 ($FI_{88788} > 10$), 183 environments and 595 test–environments were represented (Table 1). The variable of P_i ranged from noninfested environments to >30,000 eggs 100 cm^{-3} of soil. The distribution of the test–environment's log-transformed P_i is shown in Fig. 1A. Yields ranged from 673 to 6893 kg ha^{-1}, and yield response as a percentage of the susceptible mean ranged from 23 to 256. The number of susceptible entries in a test varied from 1 to 12 with an average of 3.6, and there was a prevalence of fewer susceptible entries in MG IV tests compared with other MGs. The number of entries within our rating classes is as follows: HR257, 57; R257, 42; HR0, 679; R0, 333; mid-level, 316; and S, 193, whereas the remaining entries were found to have incomplete screening test results.

Resistance Class and Initial Egg Count as Predictors of Yield

Initial egg count is an estimate of the number of eggs within a volume of soil and is a predictor of the amount of infection and subsequent yield loss. As expected and similar to previous studies, we found all resistant classes to have a yield advantage compared with the susceptible (S) class at high P_i (Fig. 2). The analysis of the whole dataset by resistance class shows that the variables resistance class, $[\log_{10}(P_i + 1)]^2$, and the interactions of both resistance class × $\log_{10}(P_i + 1)$ and resistance class × $[\log_{10}(P_i + 1)]^2$ were significant factors explaining yield ($P < 0.0001$) (Table 2). Interpretation of this polynomial model and effect estimates indicates that an overall upward trend of resistant classes for yield and differences among the slopes exist (Fig. 2). Additionally, all resistance classes except HR257 show an increased rate of yield gain compared with the susceptible class as P_i increases. As a result of this increase of yield that resistance provides, we find that breeding lines with any level of resistance show significantly higher yield than the susceptible at $P_i = 1000$ and 10,000 [$\log_{10}(P_i + 1) = 3$ and 4] (Dunnett adjusted $P < 0.0001$). The resistance class with the highest response mean yield over susceptible at $P_i = 10{,}000$ [$\log_{10}(P_i + 1) = 4$] was found to be the R0 class with 121% and at $P_i = 1000$ [$\log_{10}(P_i + 1) = 3$] the HR0 and R257 with 109%. The R257 and HR0 classes even show a significantly higher yield than the susceptible at $P_i = 100$ [$\log_{10}(P_i + 1) = 2$] (Dunnett adjusted $P < 0.05$).

In noninfested environments ($P_i = 0$), significant differences were found among resistance classes ($P < 0.0001$).

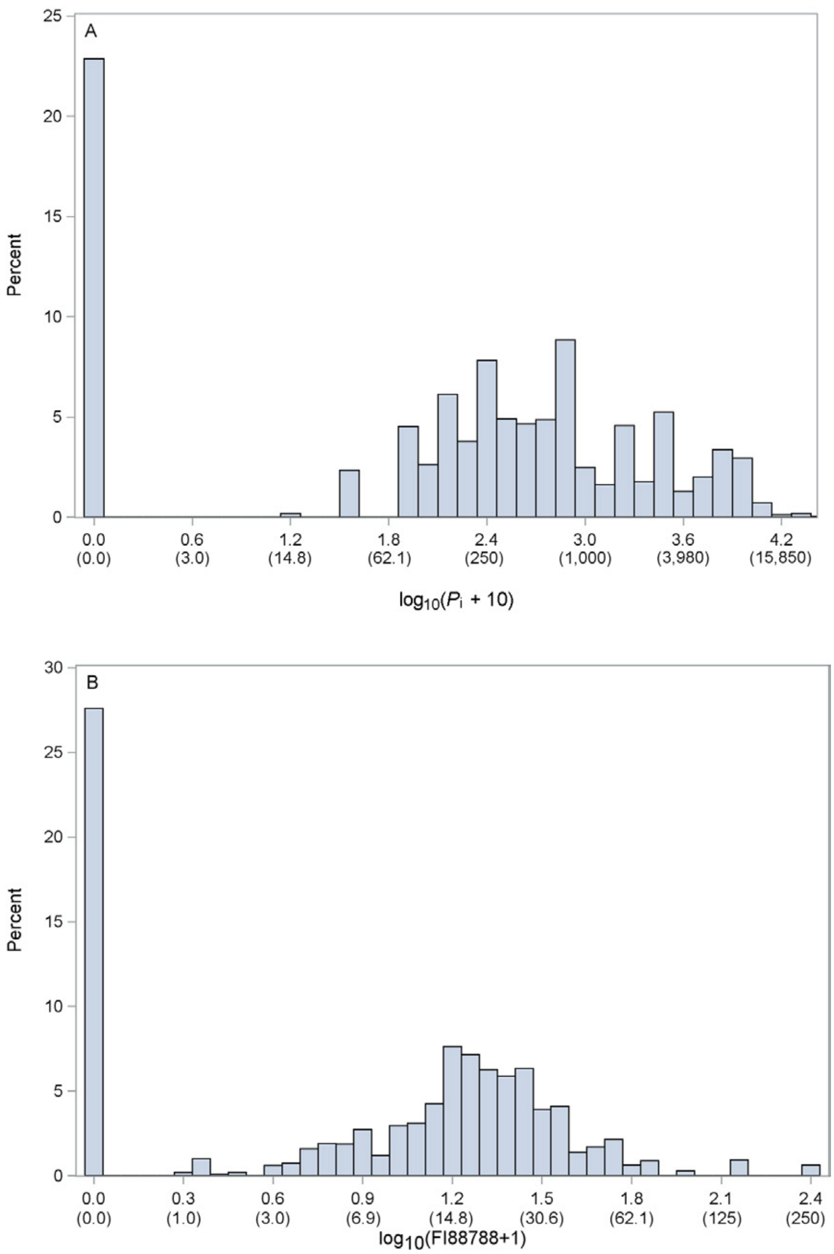

Fig. 1. Histograms of soybean cyst nematode (SCN) population characteristics included in Northern Regional Soybean Cyst Nematode Tests (2004–2014). The percentage occurrence is graphed on the *y*-axes. (A) Initial egg counts (P_i) of test locations are represented by the transformation $\log_{10}(P_i + 1)$. (B) Virulence phenotype on PI 88788 (FI_{88788}) of SCN samples at test locations determined by *Heterodera glycines* type testing are represented by the transformed variable $\log_{10}(FI_{88788} + 1)$. Values of $\log_{10}(FI_{88788} + 1) = 0$ represent sites without infestation or no virulence on PI 88788. Back transformed values are shown in parentheses along the *x*-axis.

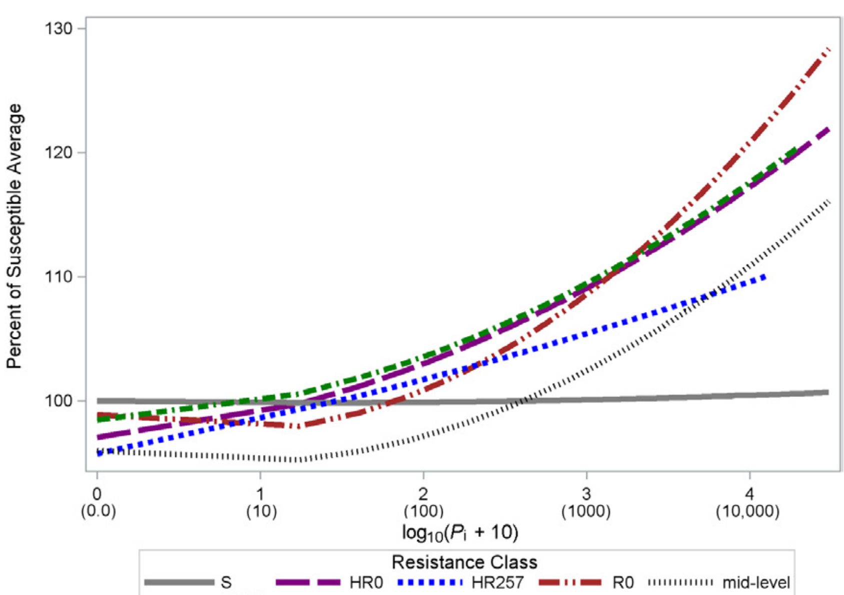

Fig. 2. Yields as a percentage of the average yield of susceptible entries graphed over the transformed initial egg counts (P_i) represented as $\log_{10}(P_i + 1)$. Back transformed values are shown in parentheses along the *x*-axis. Fitted regression functions are based on a model with Northern Regional Soybean Cyst Nematode Test data from 2004 to 2014 across maturity groups 00 through IV. Resistance class is determined from screening entries with *Heterodera glycines* (HG) type 0 and 2.5.7. Entries with high resistance (HR) or resistance (R) to the HG type 2.5.7 population were classified as HR257 or R257, and entries not placed in the first categories and found to have high resistance or resistance to the HG type 0 population were classified as HR0 or R0. Entries with no resistance to both screening populations were rated as susceptible (S). All other entries were rated as having a midlevel resistance.

Table 2. Analysis of variance results for percentage of susceptible average yield across all environments in the Northern Regional Soybean Cyst Nematode Tests from 2004 to 2014.

Source	df	Mean square	F value	Pr > F
Resistance classes	5	2,020	6.4	<0.0001
$\log_{10}(P_i + 1)$	1	245	0.8	0.3793
$[\log_{10}(P_i + 1)]^2$	1	18,954	59.8	<0.0001
$\log_{10}(P_i + 1) \times$ resistance classes	5	2,839	9.0	<0.0001
$[\log_{10}(P_i + 1)]^2 \times$ resistance classes	5	5,034	15.9	<0.0001
Residual	23,259	317		

Contrasts with the susceptible entries (S) show that the highly resistant classes (HR0 and HR257) and mid-level resistance yielded less than the susceptible entries at $P_i = 0$ (Dunnett adjusted $P < 0.001$, Table 3). The means of these classes were found to be 2.9 and 3.9% less than the susceptible entries. However, resistant entries (R257 and R0) were found to not be significantly different than the susceptible entries.

The effect of MG on yield was further explored in a separate model, and an interaction of MG $\times \log_{10}(P_i + 1)$ was found to be significant ($P < 0.0001$). Maturity group effect estimates indicated that the impact of resistance on yield was greater in earlier MGs than in later MGs in this study. This is shown by the greater average yields of the resistance classes, relative to susceptible entries, in the earlier MGs. To maintain adequate sample size within analyses, MGs were divided into early (MGs 00–II) and late (MGs III and IV) maturity subsets, and analyses were conducted to further explore the impact of resistance on yield within the subsets. In Fig. 3, the percentage of susceptible (y-axis) is graphed with the $\log_{10}(P_i + 1)$ (x-axis) of early and late subsets of data. Response means of resistance classes at $P_i = 1000$ and 10,000 [$\log_{10}(P_i + 1) = 3$ and 4] ranged from 104 to 131% in the early MGs (Fig. 3A) and 101% to 112% in the late MGs (Fig. 3B).

SCN Virulence on PI 88788

The abundant use of the PI 88788 source of SCN resistance warrants investigations into the ability of breeding lines

Table 3. Least squared means (LSM) of percentage susceptible average for resistance classes at sites without soybean cyst nematode infestation in the Northern Regional Soybean Cyst Nematode Tests (2004–2014).

Resistance class†	LSM estimate	SE	Number of observations	Contrast with S class, Dunnett adjusted P
S	100.0	0.46	892	–
HR257	96.1	0.93	211	0.0007
R257	98.6	1.36	99	0.8208
HR0	97.1	0.28	2296	<0.0001
R0	98.8	0.38	1249	0.1582
Midlevel	96.0	0.55	597	<0.0001

† S, susceptible; HR, highly resistant; R, resistant.

with this source of resistance to yield well in fields with SCN populations able to reproduce on PI 88788 ($FI_{88788} \geq 10$). Analyses were completed to gain insights into the impact of field P_i and the ability of nematode populations to reproduce on PI 88788 on the yield of breeding lines that are highly resistant (HR0), resistant (R0), and susceptible (S). Entry yield data were used in this analysis instead of yield as a percentage of the susceptible average, which was used in other analyses. This is due to a correlation found in HG type testing between the number of females on PI 88788 and the number of females on the susceptible check Lee74 ($r = 0.35$, $P < 0.0001$) for the SCN populations at locations. This shows that virulent populations on PI 88788 are also more virulent on susceptible entries when compared with populations that are not virulent on PI 88788. This means that SCN populations with greater virulence on PI 88788 could reduce yields of susceptible entries more than populations not virulent on PI 88788. Therefore, if yields are given as a percentage of susceptible yield, the impact of virulent populations may be underestimated.

We analyzed a model with initial egg counts [$\log_{10}(P_i + 1)$], virulence on PI 88788 [$\log_{10}(FI_{88788} + 1)$], resistance classes (contrast of susceptible group with the combined highly resistant and resistant classes, HR0 and R0), and all interacting factors. The interacting factors of resistance class $\times \log_{10}(FI_{88788} + 1)$ and the three-way interaction of resistance class $\times \log_{10}(P_i + 1) \times \log_{10}(FI_{88788} + 1)$ were found to be nonsignificant (simultaneous F-test, $P = 0.0821$) and were removed from the optimal model. Polynomial squares were evaluated for inclusion in the model; however, issues arose with inflated variances due to multicollinearity. Table 4 shows results from an optimal model in which all factors were found to be significant at the $P = 0.05$ level, except virulence on PI 88788 [$\log_{10}(FI_{88788} + 1)$] ($P = 0.0709$). The relationship found between $\log_{10}(P_i + 1)$, $\log_{10}(FI_{88788} + 1)$, and the response mean yield of highly resistant and resistant (HR0 and R0) and susceptible (S) classes is shown in Fig. 4 for three different $\log_{10}(FI_{88788} + 1)$ levels. Across all levels of virulence on PI 88788, resistant entries yielded more than susceptible entries, and as P_i increased, the yield advantage of resistant entries increased. In addition, as $\log_{10}(FI_{88788} + 1)$ increased, the slopes for the resistant (HR0 and R0) and susceptible classes were reduced, indicating that greater virulence reduced yield capacity.

DISCUSSION AND CONCLUSION

This research offers a unique opportunity to study the yield impact of SCN resistance across a wide range of environments. The scope of MG, genotypes, number of environments, and overall characterization of SCN populations included in this dataset is unmatched within public soybean yield testing efforts. This scope is needed to

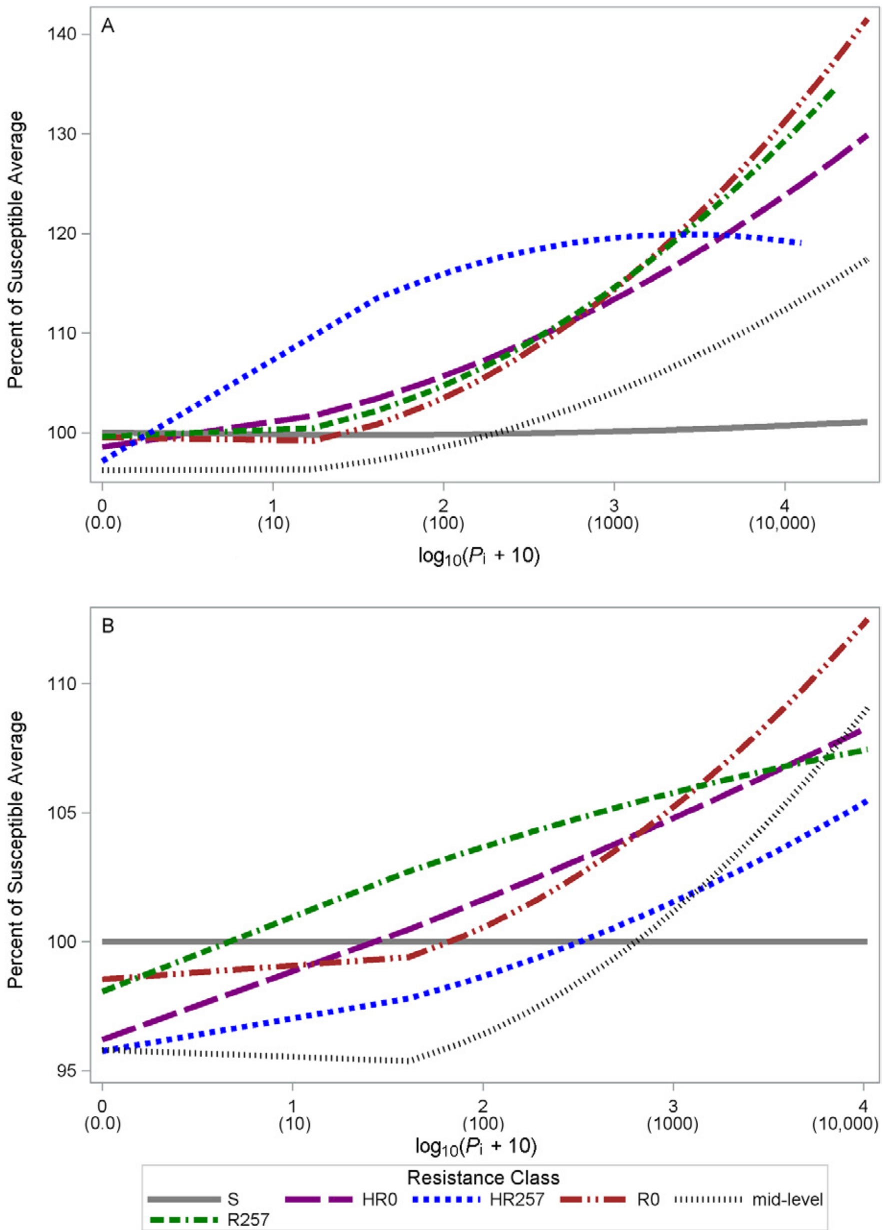

Fig. 3. Yields as a percentage of the average yield of susceptible genotypes graphed over the transformed initial egg counts (P_i) represented as $\log_{10}(P_i + 1)$. Back transformed values are shown in parentheses along the *x*-axis. Fitted regression functions are based on a model with Northern Regional Soybean Cyst Nematode Test data from 2004 to 2014 across (A) maturity groups 00 through II and (B) maturity groups III and IV. Resistance class is determined from screening entries with *Heterodera glycines* (HG) type 0 and 2.5.7. Entries with high resistance (HR) or resistance (R) to the HG type 2.5.7 population were classified as HR257 or R257, and entries not placed in the first categories and found to have high resistance or resistance to the HG type 0 population were classified as HR0 or R0. Entries with no resistance to both screening populations were rated as susceptible (S). All other entries were rated as having a midlevel resistance.

analyze yields resulting from complex interactions between genotypes and environments, which include differing SCN populations. Our model R^2 values from Fig. 2–4 are each 0.01. Although this presents a low amount of variation explained, the number of genotypes and environments sampled provides us precision in the response means graphed. Soybean cyst nematode effects on yield measured in our study have biological meaning to producers striving to protect yield by choosing SCN-resistant cultivars. In addition to a greater precision offered by this dataset, we have the opportunity to explore relationships among MGs and varying differences in the ability of field SCN populations to infect PI 88788.

Table 4. Analysis of variance results for entry yield of breeding lines grown in Northern Regional Soybean Cyst Nematode Tests from 2004 to 2014. Only entries with resistance from PI 88788 and susceptible entries at infested sites were included.

Source	df	Mean square	*F* value	Pr > *F*
Resistance class	1	6,469	35.9	<0.0001
$\log_{10}(P_i + 1)$	1	1,697	9.4	0.0022
$\log_{10}(FI_{88788} + 1)$	1	588	3.3	0.0709
Resistance class × $\log_{10}(P_i + 1)$	1	17,746	98.4	<0.0001
$\log_{10}(P_i + 1) \times \log_{10}(FI_{88788} + 1)$	1	4,038	22.4	<0.0001
Residual	12,321	180		

As expected, we found that entries resistant to SCN, either HG type 0 or 2.5.7 populations, yielded greater than susceptible entries at high levels of initial egg counts [$\log_{10}(P_i + 1) > 3$]. It is important to note that, even at P_i of 100 eggs cm^{-3} [$\log_{10}(P_i + 1) = 2$), yields of HR0 and R257 were significantly higher than the susceptible average. In addition, just as other researchers have reported, we found that, as SCN P_i increases, so does the impact of resistant entries compared with susceptible entries (Niblack et al., 1992; Koenning, 2000; Chen et al., 2001;). Although we found significant differences in yield at noninfested sites, our data are not well suited to evaluate a yield drag

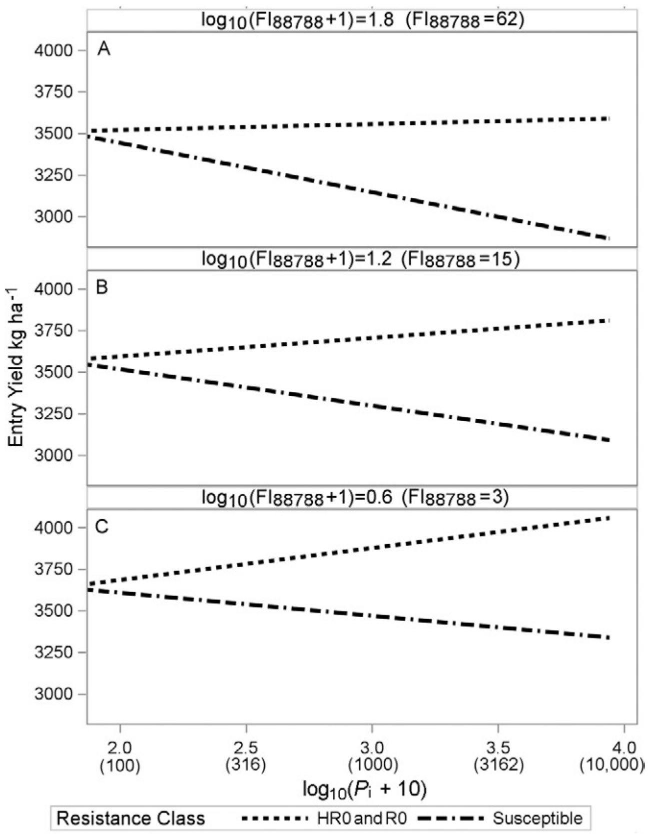

Fig. 4. Entry yields (kg ha^{-1}) of soybean entries are shown at multiple levels of virulence on PI 88788 (FI_{88788}) and initial egg counts (P_i). A model was fitted to data from infested sites and included P_i, represented by the transformation $\log_{10}(P_i + 1)$; FI_{88788}, represented by the transformation $\log_{10}(FI_{88788} + 1)$; and the resistance class of soybean entries. The relationship of susceptible entries and entries with resistance derived from PI88788 (HR0 and R0) are shown at three levels of $\log_{10}(FI_{88788} + 1)$. These levels are represented at $\log_{10}(FI_{88788} + 1)$ = (A) 1.8, (B) 1.2, and (C) 0.6. Back transformed values are shown in parentheses along the *x*-axis and for each panel. Fitted regression functions are based on data from Northern Regional Soybean Cyst Nematode Test 2004 to 2014 across maturity groups 00 through IV. Resistance class is determined from screening entries with *Heterodera glycines* (HG) type 0 and 2.5.7. Entries with high resistance (HR) or resistance (R) to the HG type 0 population but lacking resistance to the HG type 2.5.7 population were classified as HR0 or R0. Entries with no resistance to both screening populations were rated as susceptible.

associated with resistant breeding lines. Susceptible check entries have been selected for high yield with multiple years of advanced yield testing, whereas breeding line entries have not.

While analyzing data across MGs, we were able to show that the impact of resistance on yield is greater in early MGs (MGs 00–II) compared with later MGs (MGIII and IV). Care should be taken when interpreting our analysis, however. Since we measured yield as a percentage of the susceptible entries within a test, there is the possibility that differences among early versus late MGs could be due to high-yielding resistant breeding lines, low-yielding susceptible breeding lines, or both occurring in the early MGs, compared with the late MGs. In addition, the SCN populations in early and late MGs could vary considerably. Soybean cyst nematode has been identified in soils of the MG III and IV region for a longer time than earlier MGs (Riggs, 2004). Subsequently, PI 88788 has been used as a source of resistance for a longer timeframe in late MGs compared with early MGs (Faghihi et al., 2010). To explore the possibility that differences in SCN population between the early versus late MGs could cause the differences in yield, we tested for differences in SCN population virulence on PI 88788 [$\log_{10}(FI_{88788} + 1)$] between the early MGs (00–II) and late MGs (III–IV). No significant ($P = 0.2662$) difference was found. However, a significant ($P < 0.0001$) difference was found between the P_i for the early MG locations and late MG locations. The average P_i of observations in locations of early MGs is 1693, whereas the average P_i in locations of late MGs is 1072. When coding the MGs as an ordinal variable, a correlation of $r = -0.10$ ($P < 0.0001$) was found with P_i. Although higher P_i levels were found in sites of early MGs in this study, when evaluating specific levels of P_i, a greater percentage of susceptible lines was found in early MGs compared with later groups. In addition to differences among SCN populations, differences may exist among susceptible entries grown in early and late MGs. Susceptible entries developed in regions where SCN has been present for many years may have built up a small level of resistance that is not differentiated by the rating of NR (FI > 60) in screening efforts. To test this, we compared the FI of susceptible entries screened with HG type 0 and 2.5.7 for early and late MGs; however, no differences were found. Another possibility is that, because of the history of growing soybean in the presence of SCN for a longer period of time in the later maturities compared with earlier maturities, the lines classified as susceptible in the later maturities may have more tolerance to SCN than the early maturities.

Examination of the relationship between initial egg counts and virulence on PI 88788 (FI_{88788}) revealed that growing resistant varieties remains important as P_i and FI_{88788} increase. Breeding lines with resistance from PI 88788 remain higher yielding than susceptible entries, even at high FI_{88788} levels. One explanation for why there was limited yield loss on entries with PI 88788 resistance at $FI_{88788} > 10$ [$\log_{10}(FI_{88788} + 1) > 1$], is that many of the SCN populations present at these environments are infecting PI 88788 at rates lower than the susceptible control Lee 74 within HG type testing. Evidence of this is shown in Fig. 1B, where the majority (97%) of SCN populations in this study are virulent on PI 88788 at $\log_{10}(FI_{88788} + 1) < 1.8$ ($FI_{88788} = 62$). This means that SCN populations with $FI_{88788} = 62$ would infect the roots of PI 88788 at 62% of the level of infection on the susceptible Lee74. Therefore, it is expected that entries with resistance derived from PI 88788 and showing no resistance to the HG type 2.5.7 in greenhouse tests would show some resistance to SCN populations found in the soil of most production fields in this study labeled with the presence of HG type 2 ($FI_{88788} > 10$).

We did not find criteria to recommend that resistance sources other than PI 88788 should be used on the sole basis of yield. Although locations did exist where entries with sources of resistance other than PI 88788 yielded more than others, the highly variable nature of these data suggests that more observations are needed from breeding lines with resistance sources other than PI 88788. In addition, more observations are needed from all resistance sources in environments with $FI_{88788} > 40$ [$\log_{10}(FI_{88788} + 1) > 1.6$] (Fig. 1 B). Within our dataset, only 21 of 408 environments exceed this level of FI_{88788}. The occurrence of environments capable of reproducing on PI 88788 ($FI_{88788} > 10\%$) has been reported to be increasing (Mitchum et al., 2007; Niblack et al., 2008; Faghihi et al., 2010), which suggests that an increase in environments with $FI_{88788} > 40$ could be occurring as well. A focused effort to test multiple genotypes with varying resistance levels is needed to improve estimations of performance at environments from which we expect PI 88788 derived resistance to break down. An additional consideration is that the use of only PI 88788 as a source of resistance will continue to expose SCN populations to high selection pressure. The pressure could result in SCN populations that are better able to infect PI 88788 than found in this study, which would result in greater SCN problems in the future. Rotation of resistant sources and non-host crops remains important.

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