

## Shift in Virulence of Soybean Cyst Nematode is Associated with Use of Resistance from PI 88788

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### Abstract

The soybean cyst nematode (SCN) is the most economically important pathogen of soybean in the United States. Most of the SCN-resistant cultivars being grown in this region have resistance derived from a single source, Plant Introduction (PI) 88788. A survey conducted in 2005 showed that 83% of the soybean hectareage in Illinois is infested with SCN, with average population densities high enough to cause significant yield suppression (2,700 eggs/100 cm<sup>3</sup> soil). Further characterization of these populations showed that 70% have adapted to PI 88788 at some level, reducing the effectiveness of using SCN-resistant cultivars as a crop management tool. Rotation with alternative sources of resistance is recommended as a means to slow the adaptation to PI 88788.

### Introduction

*Heterodera glycines* Ichinohe, commonly known as the soybean cyst nematode (SCN), causes significant soybean yield suppression in many soybean-producing countries (14) and is the most economically important pathogen of soybean in the United States (15). First identified in the US in North Carolina in 1954 (12), SCN has since been found in most soybean production areas of this country (5). In 1999, 82% of the soybean fields in Illinois were reported to be infested with SCN (13). The primary measurable effect of infection by SCN is yield reduction in the absence of symptoms (11) (Fig. 1), although chlorosis and stunting may occur if nematode population densities are high or infection is associated with one or more secondary problems such as nutrient deficiency or root rot (5) (Fig. 2).



Fig. 1. SCN-resistant and -susceptible soybean cultivars growing in a replicated yield trial near Urbana, IL, in a field infested with ca. 10,000 SCN eggs/100 cm<sup>3</sup> soil. Note the absence of obvious visible symptoms.



Fig. 2. Visible symptoms on soybean plants heavily infested with soybean cyst nematode, and also exhibiting evidence of root rotting, in Champaign County, IL in 2007.

The most effective and typical means of managing soybean in SCN-infested fields involve host resistance and nonhost rotation (7). Nonhost rotation, usually to maize in Illinois, effectively decreases SCN population densities (4) and has few economic disadvantages. The use of host resistance is more problematic, because complete resistance to SCN has not been found or engineered, and the nematode readily adapts to resistance (5). Genes for resistance to SCN may be found in numerous accessions in the USDA Soybean Germplasm Collection (1), seven of which have actually been used in breeding programs directed at SCN management (6). Of these seven, only Plant Introduction (PI) 548402 (sometimes referred to as 'Peking') and PI 88788 have been used to much extent in commercially-available soybean cultivars, although an increasing number of cultivars are available with resistance genes derived from PI 437654. These three PIs are used as indicator plants for SCN virulence profiling by means of the Illinois SCN Type test (5).

In 1991, a survey was conducted in Illinois to determine the level of adaptation of SCN field populations to resistance in soybean (10). At that time, adaptation was measured by means of a race test (3,9), a greenhouse bioassay in which the nematode's ability to develop on a set of four resistant soybean lines was compared with its ability to develop on a standard susceptible line, the cultivar Lee. The objective of the 1991 survey was to determine the percentage of SCN populations that could be described as "race 3," defined as those without the ability to develop on any of the four differentials – in other words, those that could be managed through the use of any SCN-resistant cultivar. By 2005, over 90% of the SCN-resistant cultivars available in Illinois (and surrounding states) were derived from a single source, PI 88788.

We conducted a preliminary study of Illinois SCN Types in southern Illinois to determine whether a shift in virulence phenotypes had occurred, because cultivars with SCN resistance derived from PI 88788 have been used since the 1980s in that part of the state. Following the preliminary study, we conducted a survey of soybean fields throughout Illinois during 2005 and virulence phenotyping in 2005-2006 in order to determine whether the consistent use of this single source of resistance has resulted in an increase in the percentage of SCN populations that are adapted to PI 88788. This is important information for managing SCN, because if a population can parasitize PI 88788, then it can parasitize any cultivar derived from PI 88788 (5).

### **Preliminary Study of Virulence Phenotypes in Southern Illinois**

During 2003, soil samples were collected from arbitrarily-selected fields in southern Illinois in order to determine the distribution of southern root-knot nematode, *Meloidogyne incognita*. The samples were processed for SCN as well as *M. incognita*, and 28 of those found to be positive for SCN were subjected to Illinois SCN Type tests. The materials and methods were described previously (6), with the exception that the infestation level was 2,500 eggs/100 cm<sup>3</sup> soil. The results (Table 1) showed that 82% of the SCN populations tested were able to develop on PI 88788, which demonstrated that adaptation to this source of resistance had occurred in southern Illinois, and encouraged us to survey the entire state.

Table 1. Virulence phenotypes (Illinois SCN Types) of soybean cyst nematode populations collected during a preliminary survey in southern Illinois in 2003.

Number of samples	Mean number of females on Lee 74	Female Indices <sup>x</sup>			Illinois SCN type <sup>y</sup>	Percentage of total samples
		PI 548402	PI 88788	PI 437654		
5	124 - 316 (218)	0 - 9 (3)	2 - 9 (7)	0 (0)	0	18
17	113 - 403 (277)	0 - 8 (3)	17 - 37 (27)	0 - 6 (0)	2	61
6	127 - 308 (203)	10 - 31 (17)	13 - 52 (32)	0 - 2 (0)	1.2	21

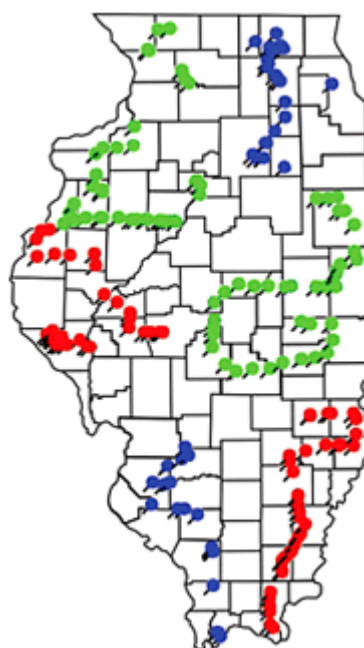
<sup>x</sup> The Female Index (FI) is a measurement of the ability of a SCN population to develop on a resistant soybean line. It is calculated from a replicated bioassay as the mean number of SCN females produced on an indicator line (in this case, PI 548402, 88788, or 437654) divided by the mean number of females produced on the standard susceptible cultivar Lee 74, multiplied by 100. Hyphenated numbers in these columns give the range of female numbers or FI values for each Illinois SCN Type observed, and parenthetical numbers directly below the ranges are the overall means for the SCN Type.

<sup>y</sup> The Illinois SCN Type is determined by the ability of the nematode population to produce FI values greater than 10 on each of three indicator lines, PI 548402, 88788, or 437654. A Type 0 cannot develop on any of the three; a Type 1 develops on PI 548402, a Type 2 on PI 88788, and a Type 4 on PI 437654 (there is no Type 3 in this test, although there is in the full HG Type test). A Type 1.2 develops on both PI 548402 and 88788.

### Distribution and Population Densities of SCN Populations

The purpose of this part of the survey was to verify that our sampling method would result in reliable data, by comparison with results obtained with the area-frame sampling method employed by Workneh et al. (13). We assigned soil sampling sites in the laboratory in the following manner: we arbitrarily divided the State of Illinois into six sections by drawing a line longitudinally through a state map, and dividing each half into thirds horizontally. We identified roads (excluding controlled-access highways) transecting each section and generated arbitrary GPS coordinates (Fig. 3) along each road with Mapsource software (Garmin International, Olathe, KS).

Fig. 3. Map of Illinois showing the locations of survey sample sites in six arbitrarily-designated sections (differentiated by contiguous dots of the same color) of the state. Soil samples were collected at these locations for analysis of soybean cyst nematode populations in 2005.



During July and August 2005, we traveled to each section, completing the sampling for that section in 1 day if possible. To collect samples, we stopped at each previously-identified GPS coordinate, and if a soybean field was accessible from that location, we entered the field and collected approximately 1 liter soil with 2.5-cm-diameter soil probes by combining individual cores taken to a depth of ca. 15 cm from arbitrarily-selected points in an area no larger than 0.4 hectares. Symptoms of disease were rarely visible, and the occurrence of soybean aphids and other insects was noted but infrequent. We collected a total of 219 samples, with varying numbers from each section (Table 2). Each sample was stored at 4°C until processed according to the procedure described by Faghihi and Ferris (2).

Table 2. Distribution of soybean cyst nematode in soil samples collected during a 2005 survey of Illinois. Statistics were calculated based on samples testing positive for cyst nematodes.

Section	No. samples collected	Positive for cyst nematodes (%)	Eggs/100 cm <sup>3</sup> soil			
			Mean	Median	Maximum	SD
Northwest	32	88	1,933	240	18,720	4,693
Northeast	30	97	2,074	760	8,000	2,745
West Central	56	73	2,864	960	17,680	4,031
East Central	40	78	2,849	1,520	36,000	6,293
Southwest	20	95	5,587	1,200	42,800	10,274
Southeast	40	85	1,915	380	13,840	3,215
Overall	218	83	2,700	800	42,800	5,305

We found SCN in 73 to 97% of the samples collected from different regions of Illinois (Table 2). The overall incidence of 83% agreed very well with the estimate of 82% published by Workneh et al. (13), therefore we considered the sampling method to be reasonably reliable. For further evaluation of the results, there are a few facts that should be taken into account. First, although many factors affect the economic injury threshold for SCN, it is generally measured in the hundreds, not thousands, of eggs (5); both the mean and median egg population densities in Illinois are well above the threshold. Second, we have no way to know whether the soybean cultivar growing in a sampled field was SCN-resistant or susceptible. In a survey of farmers in early 2007, 62% of farmers reported that they planted SCN-resistant cultivars in 75% or more of their acreage (Niblack, *unpublished data*; 568 respondents). Finally, we did not do any further testing to verify that the cyst nematodes extracted from the sample were actually *H. glycines* (8); however, it is highly likely that a cyst nematode found in a soybean field in Illinois is SCN (personal observation), and further evidence for this was provided by the fact that every sample chosen for virulence profiling, as described in the following section, produced females and eggs on a susceptible soybean cultivar, which would not have been the case with the other cyst nematodes known to occur in Illinois.

### Virulence Profiling

Of the 218 samples collected, 156 had high enough egg densities to allow virulence profiling after a brief period of population increase in the greenhouse. The SCN populations in these samples were subjected to virulence profiling during 2005-2006 (Table 3) as described previously (6).

Table 3. Virulence profiles of 156 populations of soybean cyst nematode collected during a 2005 survey and subjected to the Illinois SCN Type tests.

Section of Illinois	No. samples tested	SCN Type <sup>x</sup> (frequency)					Development <sup>y</sup> on PI 88788 (%)	Range of FI <sup>z</sup> on PI 88788	
		0	1	2	1.2	4		Min	Max
Northwest	31	13	0	17	1	0	58	10	35
Northeast	28	12	0	15	1	0	57	13	48
West Central	22	11	0	11	0	0	50	11	27
East Central	23	1	2	20	0	0	87	10	55
Southwest	20	1	0	15	4	0	95	10	52
Southeast	32	7	0	21	4	0	78	11	98
Overall	156	45	2	99	10	0	70	10	98

<sup>x</sup> The Illinois SCN Type is determined by the ability of the nematode population to develop (see definitions in footnotes *b* and *c*) on three indicator lines, PI 548402, 88788, or 437654. A Type 0 cannot develop on any of the three; a Type 1 develops on PI 548402, a Type 2 on PI 88788, and a Type 4 on PI 437654 (there is no Type 3 in this test, although there is in the full HG Type test). A Type 1.2 develops on both PI 548402 and 88788.

<sup>y</sup> Development is considered positive when the FI (defined in footnote *c*) is 10 or higher.

<sup>z</sup> FI = Female Index, a measurement of the ability of a SCN population to develop on a resistant soybean line. It is calculated from a replicated bioassay as the mean number of SCN females produced on an indicator line divided by the mean number of females produced on the standard susceptible cultivar Lee 74, multiplied by 100.

These results show clearly that most of the SCN populations in Illinois have adapted to some extent to PI 88788. Very few populations were able to develop on PI 548402 (SCN Types 1 and 1.2), and none were found that could develop on PI 437654 (SCN Type 4) during this survey (although 30% of the populations were able to produce one or more viable females on this PI; Colgrove and Niblack, *unpublished data*). Cultivars with resistance derived from PI 437654 are not yet deployed widely in Illinois.

A graphical comparison of the results of the survey conducted by Sikora & Noel in 1990 (10) and the results of our survey show a distinct virulence shift (Fig. 4). This shift has most likely been due to the widespread planting in Illinois of SCN-resistant cultivars derived from PI 88788.

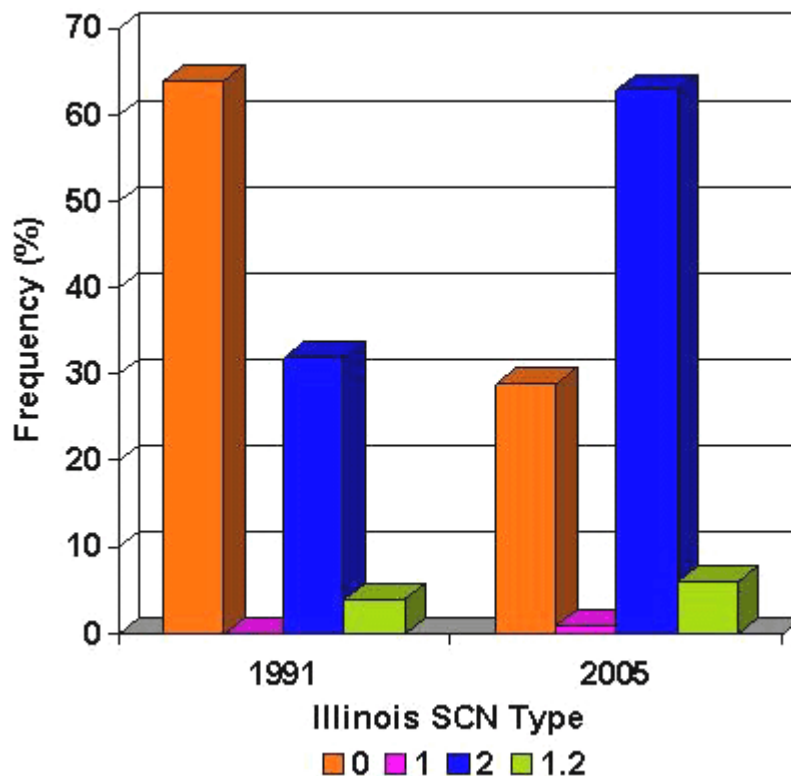


Fig. 4. Comparison of the results of surveys of soybean cyst nematode populations in Illinois. The 1991 data were reported by Sikora and Noel (10) as "races," and were expressed here as Illinois SCN Types (6) in order to make the comparison clearer.

### Conclusions and Recommendations

Despite the common use of nonhost rotation and host resistance to reduce the impact of the pathogen, SCN population densities remain at levels capable of causing significant yield loss in Illinois. The lack of obvious symptoms reduces recognition of the extent of SCN infestations, and the adaptation of SCN populations to most resistant cultivars reduces their effectiveness in alleviating damage. However, this survey showed that most populations of SCN in Illinois have not adapted to PI 548402 or 437654, so the option to use these sources of resistance remains open and should be encouraged, especially when SCN population densities are high. The use of alternative sources of resistance will benefit farmers with heavy infestations of SCN Type 2 (HG Type 2-) in the short term. For the long term, we recommend rotation of sources of resistance to slow the nematodes' adaptation to resistance and preserve the utility of alternative sources (6). We also encourage continuing research to identify new sources and approaches to SCN resistance.

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