

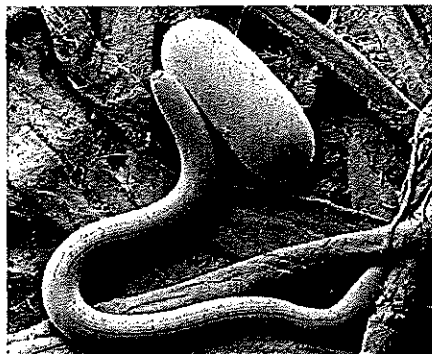
Proceedings

4th National Soybean Cyst Nematode Conference

**March 6-7, 2008
Embassy Suites Tampa – USF
Tampa, Florida**

Scientific program sponsored by
The Society of Nematologists
and
The NC 1035 Committee





4th NATIONAL

Soybean Cyst Nematode Conference

March 6-7, 2008 • Tampa, FL

Scientific Program - Society of Nematologists • Meeting Production - The American Phytopathological Society

Program Agenda

WEDNESDAY, MARCH 5

6:00 p.m. –	Registration	Foyer E
8:00 p.m.		
6:00 p.m. –	Poster Set-up	Foyer E
8:00 p.m.		

THURSDAY, MARCH 6

8:00 a.m.	Introduction – Welcome to the 4 th National SCN Conference	Salon E
	Current Research on SCN	
8:05 a.m.	Impact of SCN on World Soybean Supply	
	*Allen Wrather, <i>University of Missouri</i>	
8:50 a.m.	New Angles on Disease Interactions involving SCN	
	*Jason Bond, <i>Southern Illinois University</i>	
9:35 a.m.	Processed Biosolids: Unwanted Waste or Products for Soybean Cyst Nematode Control?	
	*Inga Zasada, <i>USDA-ARS Beltsville</i>	
10:20 a.m.	Break	Foyer E
10:30 a.m.	Use of Resistance for SCN management	Salon E
	*Greg Tylka, <i>Iowa State University</i>	
11:15 a.m.	Studies on the Effects of Major SCN Resistance Genes	
	*Brian Diers, <i>University of Illinois Urbana-Champaign</i>	
12:00 p.m.	Lunch (provided)	Atrium
1:00 p.m.	SCN Genetics and Genomics	
	*Kris Lambert, <i>University of Illinois Urbana-Champaign</i>	
1:45 p.m.	SCN Parasitism Genes	
	*Rick Davis, <i>North Carolina State University</i>	
2:30 p.m.	Soybean Cyst Nematode CLAVATA3/ESR-like (CLE) Peptides: A Cross-Kingdom Adaptation for Plant Parasitism	
	*Melissa Goellner Mitchum, <i>University of Missouri</i>	
15 min.	Break	Foyer E
3:00 p.m.	Commentaries	
3:30 p.m.	SCN Extension Education – Are We Meeting the Needs of the Northern Soybean Producer?	Salon E
	*Albert Tenuta, <i>Ontario Ministry of Agriculture, Food & Rural Affairs</i>	
3:45 p.m.	SCN Extension Education Needs, Points South	
	*Don Hershman, <i>University of Kentucky</i>	
4:00 p.m.	SCN – The Checkoff Strikes Back	
	*Mark Halsey, <i>United Soybean Board</i>	
4:15 p.m.	The North Central Soybean Research Program	
	*David Wright, <i>North Central Soybean Research Program</i>	
4:30 p.m.	Perspectives of Soybean Growers	
	*Ken Dalenberg, <i>Illinois soybean producer</i>	
4:45 p.m.	The Battle Continues: Commercial Breeding Approaches for Control of SCN	
	*Jeffrey Thompson, <i>Pioneer, Inc.</i>	
5:00 p.m.	Break	
5:30 p.m.	Contributed Poster Session with Reception	Foyer Area & Salon D

(over)

FRIDAY, MARCH 7

9:00 a.m.	Assessment of Resistance to SCN Assessment of SCN Resistance Salon E *Terry Niblack, <i>University of Illinois Urbana-Champaign</i>
9:30 a.m.	Evaluating Phenotypic SCN Screening Methods *Jennifer Hicks, <i>Monsanto, Inc.</i>
10:00 a.m.	A Proposal for Standardizing SCN Resistance Assessment – SCE07 *Ralph von Qualden, <i>ACTS, Inc.</i>
10:30 a.m.	Break Foyer E
11:00 a.m. -	Poster Take-down Foyer E
1:00 p.m.	
11:00 a.m.	Group Discussion Salon E
12:00 p.m.	Lunch (on your own)
1:00 p.m.	SCN Research and Education Needs – Group Discussion Salon E *Greg Tylka *David Wright
3:00 p.m.	Adjourn

Thank you to our Sponsors!

SCIENTIFIC PROGRAM



NC1035 Regional Technical Committee

MEETING SPONSORS



Bayer CropScience



MONSANTO
imagine*



syngenta

Publication of the Proceedings
sponsored by
The Illinois Soybean Association



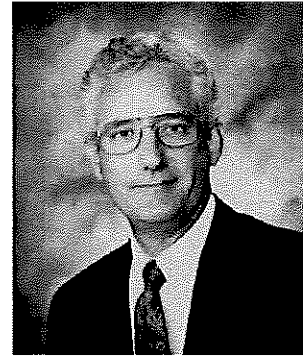
Printed on 100% post-consumer recycled paper, chlorine-free

Table of Contents

<i>Current research on SCN</i>		<i>Page</i>
Impact Of SCN On World Soybean Supply	<i>Allen Wrather</i>	1
New Angles On Disease Interactions Involving SCN	<i>Jason Bond</i>	4
Processed Biosolids: Unwanted Waste Or Products For SCN Control?	<i>Inga Zasada</i>	7
Use Of Resistance For SCN Management	<i>Greg Tylka</i>	11
Studies On The Effects Of Major SCN Resistance Genes	<i>Brian Diers</i>	15
SCN Genetics And Genomics	<i>Kris Lambert</i>	19
SCN Parasitism Genes	<i>Rick Davis</i>	23
Soybean Cyst Nematode CLAVATA3/ESR-Like (CLE) Peptides	<i>Melissa Mitchum</i>	29
 <i>Commentaries</i>		
Are We Meeting the Needs of the Northern Soybean Producer?	<i>Albert Tenuta</i>	34
SCN Extension Education Needs, Points South	<i>Don Hershman</i>	36
SCN – The Checkoff Strikes Back	<i>Mark Halsey</i>	39
Perspectives of Soybean Growers	<i>Ken Dalenberg</i>	41
 <i>Assessment of resistance to SCN</i>		
Assessment of SCN Resistance	<i>Terry Niblack</i>	42
Evaluating Phenotypic SCN Screening Methods	<i>Jennifer Hicks</i>	44
A Proposal For Standardizing SCN Resistance Assessment- SCE07	<i>Ralph von Qualen</i>	46
 <i>Abstracts of Contributed Posters</i>		49

Impact of SCN on World Soybean Supply

Allen Wrather
Professor
Division of Plant Sciences
University of Missouri
WratherJ@missouri.edu



High yields are critical to soybean producer profit margins, even during periods when soybean prices are high. Unfortunately, soybean yields in the US have been suppressed by diseases in the past (Wrather et al., 2001a), and income derived from this crop has been less than optimal. This financial loss is important to rural economies and to the economies of allied industries in urban areas.

Research must focus on management of diseases that cause extensive losses, especially when funds for research are limited. Clearly, knowledge of the losses caused by various soybean diseases is essential when prioritizing research budgets.

The impact of diseases on southern US soybean production from 1974 to 1994 (Wrather et al., 1995), and US soybean production for 1996 to 1998 (Wrather et al., 2001b), 1999 to 2002 (Wrather et al., 2003), and 2003 to 2005 (Wrather and Koenning, 2006) have been published. The impact of diseases on soybean production for the top ten soybean-producing countries during 1998 (Wrather et al., 2001a) was published.

The objective of this project was to compile estimates of soybean yields suppressed due to SCN and other diseases in the top eight soybean-producing countries for the 2006 harvested crop. The purpose is to provide this information to help

local and world agencies allocate funds for research and to help scientists focus and coordinate research efforts.

Methods used by scientists to estimate soybean yield suppression due to diseases in these countries were systematic field surveys, cultivar trials, and questionnaires sent to field workers and extension staff. Most of the scientists used several of these methods. These estimates for the United States were compiled from individual state estimates submitted by scientists in these states. Production losses were based on estimates of yield in the absence of disease. The estimates of soybean yield suppression due to diseases should not be construed as actual losses.

The total soybean production for the world during 2006 was 220.4 million metric tons (t). The top eight soybean-producing countries for the crop harvested during 2006 were the US (83.4 million t), Brazil (57.0 million t), Argentina (40.5 million t), China (16.4 million t), India (7.0 million t), Paraguay (3.6 million t), Canada (3.16 million t), and Bolivia (2.0 million t). These countries produced about 96.6% of the world supply for 2006.

Asian soybean rust caused more total yield suppression (13.2 million t) in these eight countries than any other disease during 2006

(Table 1). Next in decreasing order of total yield suppression were SCN (7.2 million t), brown spot (4.3 million t), seedling diseases (3.4 million t), anthracnose (2.5 million t), and charcoal rot (2.5 million t). Total estimated soybean yield suppression due to diseases in these countries during 2006 was 59.9 million t. Soybean yield suppression due to rust during 2006 was reported from all of these countries except Canada, but it was only reported from China during 1998.

Soybean cyst nematode caused more total yield suppression in these eight countries during 1998 than any other disease (Wrather et al., 2001a), and next in decreasing order of total yields suppression during this year were brown spot, charcoal rot, and Sclerotinia stem rot.

Soybean yield suppression due to SCN during 2006 occurred in Argentina (0.02 million t), Brazil (0.5 million t), Canada (0.09 million t), China (3.2 million t), and the US (3.4 million t). Scientists in Bolivia, India, and Paraguay reported no yield suppression due to SCN during 2006 and 1998. Soybean yield suppression due to SCN was similar during 1998 and 2006 for Brazil and Canada, it was greater during 2006 than 1998 in China, and it was less during 2006 than 1998 in Argentina and the US. The lower yield suppression due to SCN in these two countries for 2006 compared to 1998 may be due to greater farmer awareness of SCN and increased planting or SCN resistant cultivars in infested fields.

Clearly, SCN and other diseases caused extensive reductions in soybean yield in the top eight soybean-producing countries during 2006. Yield losses in some countries may have been worse if not for the use of disease management strategies and systems. Scientists at the universities

developed most of the disease-resistant cultivars and other disease management strategies and systems. To reduce disease losses, research and extension efforts must be expanded to provide more effective preventive and therapeutic strategies and systems.

References

- Wrather, J. A., Anderson, T.R., Arsyad, D.M., Tan, Y., Ploper, L.D., Porta-Puglia, A., Ram, H.H., and Yorinori, J.T. 2001a. Soybean disease loss estimates for the top 10 soybean producing countries in 1998. *Canadian Journal of Plant Pathology* 23:115-121.
- Wrather, J.A., Chambers, A.Y., Fox, J.A., Moore, W.F., and Sciumbato, G.L. 1995. Soybean disease loss estimates for the southern United States, 1974 to 1994. *Plant Disease* 79:1076-1079.
- Wrather, J.A., Koenning, S.R., and Anderson, T.R. 2003. Effect of diseases on soybean yields in the United States and Ontario (1999-2002) Online. *Plant Health Progress* doi:1..1094/PHP-2003-0325-01 RV.
- Wrather, J.A., Stienstra, W.C., and Koenning, S.R. 2001b. Soybean disease loss estimates for the United States from 1996 to 1998. *Canadian Journal of Plant Pathology* 23:122-131.
- Wrather, J.A., and Koenning, S.R. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. *Journal of Nematology* 38:173-180.

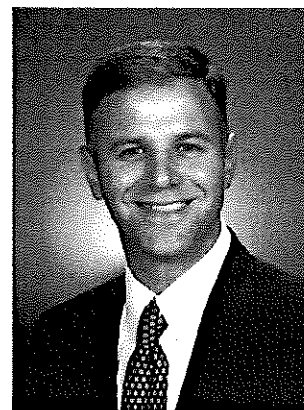
Table 1. Estimated reduction of soybean yields in thousand metric tons for the top 8 soybean-producing countries during 2006.

Disease	Argentina	Bolivia	Brazil	Canada	China	India	Paraguay	US	Total
Anthraxnose	45.3	Tr	220.0	0	1663.5	117.6	0.3	492.9	2,539.6
Bacterial diseases	22.6	Tr	130.0	0	570.3	19.6	0	101.5	844.0
Brown spot	1176.5	Tr	340.0	0.8	2186.3	19.6	0.1	536.6	4,259.9
Brown stem rot	22.6	0	40.0	3.3	998.1	0	0	497.5	1,561.5
Charcoal rot	905.0	500.0	360.0	1.6	0	39.2	1.6	697.6	2,505.0
Downy mildew	0	Tr	50.0	1.6	1996.2	0	0	147.4	2,195.2
Frogeye leaf spot	22.6	Tr	40.0	0.3	0	19.6	0	345.1	427.6
Fusarium root rot	0	Tr	40.0	19.6	1615.9	19.6	0	169.1	1,854.2
Phomopsis seed decay	45.3	0	130.0	16.3	95.1	0	0	122.0	408.7
Phytophthora root and stem rot	67.9	0	Tr	26.1	760.5	0	0	1464.2	2,318.7
Pod and stem blight	181.0	0	0	11.4	95.1	19.6	0.1	208.3	515.5
Powdery mildew	0	Tr	0	0	0	0	0	0	0
Purple seed stain & Cercospora leaf blight	1086.0	Tr	720.0	0.3	0	19.6	0.7	85.2	1,911.8
Rhizoctonia foliar blight	0	0	300.0	0	1188.2	39.2	0	12.5	1,539.9
Root-knot and other nematodes	22.6	Tr	260.0	0	2186.3	0	0.4	215.5	2,684.8
Rust	45.3	2000.0	4720.0	0	6368.9	78.4	1.9	24.5	13,239.0
Sclerotinia stem rot	135.7	Tr	200.0	4.9	1520.9	0	0	362.0	2,223.5
Seed diseases	45.3	0	70.0	16.3	1188.2	0	0.1	18.5	1,338.4
Seedling diseases	45.3	Tr	82.0	89.7	2043.8	19.6	0	1085.3	3,365.7
Southern blight	0	0	58.0	0	0	156.9	0	5.2	220.1
Soybean cyst nematode	22.6	0	520.0	97.8	3184.4	0	0	3368.1	7,192.9
Stem canker	0	Tr	0	11.4	0	0	0	211.7	223.1
Sudden death syndrome	769.3	0	320.0	16.3	0	0	0	743.4	1,849.0
Virus diseases	45.3	Tr	100.0	9.8	1568.5	196.1	0	202.7	2,122.4
Other diseases ^a	45.3	500.0	0	26.1	1948.7	58.8	0	0	2,578.9

^aOther diseases were Rhizoctonia root rot in Canada; target spot in Argentina and Bolivia; soybean leaf spot or grey spot in China; and Myrothecium leaf spot and target spot in India.

New angles on disease interactions involving soybean cyst nematode

Jason Bond
Associate Professor
Department of Plant, Soil, and Agricultural Systems
Southern Illinois University
jbond@siu.edu



The distribution and impact of soybean cyst nematode (SCN), *Heterodera glycines*, is unrivaled by any other soybean pathogen. In many cases, the direct impact of the pathogen and its ability to adapt to our best management options often overshadow the importance of interactions with other soybean pathogens. Assessing the damage of these multipathogen relationships is difficult, given that the crop encounters multiple biotic constraints throughout the growing season. In fact, the ubiquitous presence of *H. glycines* in many states allows great potential for interactions with fungi, bacteria, other nematodes, insects and weeds. Among these groups the greatest amount of research has focused on the interactions with soilborne fungi (Bond and Wrather, 2005).

In soybean, eight interactions involving *H. glycines* and fungal pathogens have been reported. Interactions can result in increased or decreased colonization of either the fungal or nematode pathogen. In addition the impact to plant health can be in the form of additive, synergistic or antagonistic interactions (Bond and Wrather, 2005). The widespread distribution of *H. glycines* and other soilborne pathogens has not contributed to a wealth of research in this area, and it is likely that interactions are greatly underestimated. One of the main reasons for the lack of research is that until recently, plant pathologists lacked the tools needed to accurately and precisely measure critical

aspects of infection, colonization, symptom production and reproduction of the pathogens.

For this presentation, two soybean fungal pathogens and their interaction with *H. glycines* will be used to describe new technologies and approaches used to decipher complex pathogen interactions. *Cadophora gregata* (Syn. *Phialophora gregata*), the causal agent of brown stem rot (BSR), was first reported in Illinois in 1944 (Abel, 1977; Allington and Chamberlain, 1948). The pathogen can be found in most production areas of the U.S., however environmental conditions required for moderate to severe disease are found mostly in the upper Midwest (Grau et al 2004). Sudden death syndrome (SDS), caused by *Fusarium virguliforme* (Syn. *F. solani* f. sp. *glycines*), was first reported in Arkansas in 1971, however it was many years later when the causal agent was identified (Aoki et al., 2003; Roy 1997). Today, SDS can be found in most soybean producing states along the Mississippi and Ohio Rivers and north into Canada.

Both BSR and SDS are caused by pathogens that overwinter in crop residue; however *F. virguliforme* can also overwinter in the soil. In the spring, soybean seedlings are attacked by the fungal pathogens soon after planting (Gao et al., 2006; Grau et al 2004; Njiti et al., 1998). Presumably, *H. glycines* attacks the plant soon

afterwards. Field and greenhouse studies indicate that SDS can be more severe when plants are parasitized by *H. glycines*. (McLean and Lawrence, 1993a, 1993b, 1995; Rupe et al., 1991; Hershman et al., 1990). The symptoms of SDS appeared earlier and were more severe in plants were attacked by both pathogens. Similar results have been found in studies with *C. gregata* and *H. glycines*. Plants attacked by both pathogens results in a greater severity of stem rot and colonization by the fungus when compared to plants infected only with *C. gregata* or *H. glycines* (Sugawara et al., 1997; Tabor et al., 2003, 2006).

One of the greatest limitations in past interaction studies was the lack of germplasm needed to precisely measure the impact of the pathogens. In the case of SDS, genetic resources are now available to allow the development of isolines to study the interaction between *F. virguliforme* and *H. glycines*. These isolines differ only in their resistance or susceptibility to the two pathogens. However, inadequate germplasm resources are not the only limitation in this area of research. While *F. virguliforme* can be readily isolated from soil or soybean roots, quantifying the pathogen in these two niches can be expensive, time consuming and a source of great frustration. Recently, advances in molecular technologies have allowed greater precision in studying infection and colonization by the pathogen. Techniques such as qPCR have been allow for quantification of *F. virguliforme* (Gao et al., 2004) and to confirm that *F. virguliforme* attacks the plant soon after the radical emerges (Gao et al., 2006). Molecular tools and new protocols will no doubt provide even greater value to studies that use isolines.

Recently, researchers were successful in transforming *F. virguliforme* and in generating transformed fungal strains that express a green fluorescing protein (GFP). The ability of these strains to express GFP allows researchers not only to quantify the fungus by the use of fluorometry, but also to assess the patterns and extent of fungal colonization in infected soybean roots by confocal microscopy. GFP-expressing strains with tagged

mutations were also generated. These strains exhibit various degrees of virulence on soybean as reflected by SDS root and foliar symptom development in greenhouse experiments. Research is also underway to assess changes in the expression profiles of *F. virguliforme* and soybean genes at different stages of colonization and in the presence or absence of stresses including co-inoculation with *H. glycines*. This will elucidate some of the aspects of the interaction between *H. glycines* and *F. virguliforme* such as how *H. glycines* and other factors influence fungal infection, colonization, symptom development and in determining how *F. virguliforme* affects SCN population dynamics. The available technologies will help to differentiate factors that govern the complex interactions between *F. virguliforme*, *H. glycines*, and soybean. The ability to manipulate fungal and nematode genes will facilitate the identification and characterization of genes involved in the interaction, and help to identify the plant processes involved in the response to the two pathogens.

Our current knowledge of the interactions between *H. glycines* and other biotic and abiotic constraints is woefully lacking when one considers that *H. glycines* has been reducing soybean yields for over 60 years in the U.S. In this time, millions of dollars have been spent on research and managing this pathogen. However, in many areas the nematode is now more difficult to manage and the potential for SCN to interact with other pathogens is greater than ever. Recent advancement in the tools that researchers use will no doubt help elucidate these interactions and facilitate management of *H. glycines*.

References

- Abel, G.H. 1977. Brown stem rot of soybean-*Cephalosporium gregatum*. Review of Plant Pathology 56:1065-1075.
- Allington, W.B., and Chamberlain, D.W. 1948. Brown stem rot of soybean. Phytopathology 38:793-802.

- Aoki, T., O'Donnell, K., Homma, Y. and Lattanzi, A.R. 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex-*F. virguliforme* in North America and *F. tucumaniae* in South America. *Mycologia* 95:660-684.
- Bond, J. and Wrather, J.A. 2004. Interaction with microorganisms. in *Biology and Management of the Soybean Cyst Nematode*. 2nd ed. Edited by D.S. Schmitt, J.A. Wrather, and R.D. Riggs. Schmitt and Associates of Marceline, Marceline, Missouri. pp. 111-129.
- Gao, X., Hartman, G.L., Niblack, T.L. 2006. Early infection of soybean roots by *Fusarium solani* f. sp. *glycines*. *Phytopathology*. 96:S38.
- Gao, X., Jackson, T.A., Lambert, K.N., Li, S., Hartman, G.L., and Niblack, T.L. 2004. Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. *Plant Disease* 88:1372-1380.
- Grau, C.R., Dorrance, A.E., Bond, J. and Russin, J.S. 2004. Fungal diseases. Pp. 679-763 in: *Soybeans: improvement, production, and uses*. 3rd edition. Madison, WI: ASA, CSSA, SSSA.
- Hershman, D.E., Hendrix, J.W., Stuckey, R.E., and Bachi, P.R. 1990. Influence of planting date and cultivar on soybean sudden death syndrome in Kentucky. *Plant Disease* 74:761-766.
- McLean, K.S., and Lawrence, G.W. 1993a. Interrelationship of *Heterodera glycines* and *Fusarium solani* in sudden death syndrome of soybean. *Journal of Nematology* 25:434-439.
- McLean, K.S., and Lawrence, G.W. 1993b. Localized influence of *Heterodera glycines* on sudden death syndrome of soybean. *Journal of Nematology* 25:674-678.
- McLean, K.S., and Lawrence, G.W. 1995. Development of *Heterodera glycines* as affected by *Fusarium solani*, the causal agent of sudden death syndrome of soybean. *Journal of Nematology* 27:70-77.
- Njiti, V.N., Shenaut, M.A., Suttner, R.J., Schmidt, M.E., and Gibson, P.T. 1998. Relationship between soybean sudden death syndrome (SDS) and yield components in F6 derived lines. *Crop Science*. 36:673-678.
- Roy, K.W. 1997. *Fusarium solani* on soybean roots: nomenclature of the causal agent of sudden death syndrome and identity and relevance of *F. solani* form B. *Plant Dis.* 81:259-266.
- Rupe, J.C., Gbur, Jr. E.E., and Marx, D.M. 1991. Cultivar responses to sudden death syndrome of soybean. *Plant Disease* 75:47-50.
- Sugawara, K., Kobayashi, K., and Ogoshi, A. 1997. Influence of the soybean cyst nematode (*Heterodera glycines*) on the incidence of brown stem rot in soybean and adzuki bean. *Soil Biology and Biochemistry* 29:1491-1498.
- Tabor, G.M., Tylka, G.L., Behm, J.E., and Bronson, C.R. 2003. *Heterodera glycines* infection increases incidence and severity of brown stem rot in both resistant and susceptible soybeans. *Plant Disease* 87:655-661.
- Tabor, G.M., Tylka, G.L., and Bronson, C.R. 2006. Soybean stem colonization by genotypes A and B of *Cadophora gregata* increases with increasing population densities of *Heterodera glycines*. *Plant Disease* 90: 1297-1301.

Processed Biosolids: Unwanted Waste or Products for Soybean Cyst Nematode Control?

Inga Zasada
Research Plant Pathologist
USDA-ARS Nematology Laboratory
Beltsville, MD
Inga.zasada@ars.usda.gov



Approximately 1 billion tons of organic and inorganic by-products are generated each year in the United States: 400 million tons are crop residues, 80 million tons are livestock and poultry manure; 7 million tons are biosolids (sewage sludge); and 300 million tons are municipal and industrial materials (USDA, ARS, 1999). Many of these waste products have been evaluated for their ability to control pests; however, their widespread implementation has for the most part not been realized. Lack of implementation as a pest management strategy is due to several reasons including: product inconsistency; availability and; cost. Despite these constraints, it would be advantageous to incorporate waste products into soybean production systems not only for pest management, but also to improve soil fertility, while sustainably utilizing local waste streams.

To successfully incorporate waste products into a *Heterodera glycines* (soybean cyst nematode) management program, a deep understanding of the mechanisms involved in nematode suppression will be required. Factors that require analysis and clarification include the concentration levels of waste products lethal to nematodes and the chemical composition of incorporation material, the fate of compounds released into the soil and consequent exposure to

nematodes, and environmental influences such as temperature, microbial community, and soil type. In this review, results from research evaluating an alkaline-stabilized biosolid (ASB) waste product to control plant-parasitic nematodes are presented to highlight the need to understand the mechanism(s) responsible for nematode suppression towards improving the consistency and efficacy of waste products.

Examples of waste products tested for plant-parasitic nematode management are abundant (Akhtar and Alam, 1993; Akhtar and Malik, 2000; Rodríguez-Kabána et al., 1987). They include sawdust, oilcakes, cellulosic waste, bone meal, chitin, manure, crop residues, yard wastes, and biosolids. These products have been tested against a range of plant-parasitic nematode with variable results. In general, Rodríguez-Kabána et al. (1987) noted that the most effective products for the management of plant-parasitic nematodes had narrow C:N ratios, usually below 20:1.

One waste product which has received attention as a nematode control product is biosolids (Barbosa et al., 2004; Mannion et al., 1994). Biosolids are the nutrient-rich, solid organic material recovered from treatment of domestic sewage in wastewater treatment facilities; 8.2 million dry tons will be

produced in the U.S. in 2010 (USEPA, 1999). Technologies developed for the treatment of biosolids yield a pathogen-free product that is stable during storage and transportation. One such process is the mixing of biosolids with alkaline reagents, including industrial by-products (Logan and Burnham, 1995). Industrial by-products include coal combustion byproducts, of which 129 million tons were produced in 2002 in the U.S. (ACAA, 2003). The final products are solid, granular materials with many positive agronomic properties. The use of ASB for plant-parasitic nematode management is appealing because two undesirable waste products (biosolids and industrial byproducts) would be consumed in a positive way.

Results of research conducted in six states using ASB to control *Meloidogyne* spp. and *Heterodera glycines* shared one common feature: inconsistency (Zasada et al., 2008). This is not surprising considering the diverse environments into which the amendment was added, and the different application rates and methods used. When ASB was applied to a loam or sandy loam in Iowa at a rate of 17 dry t/ha there was no reduction in *H. glycines* population densities. When a higher rate of ASB, 40 dry t/ha, was applied to a sandy soil in North Carolina there was a nonsignificant decrease in *H. glycines* populations densities compared with lower ASB application rates. A similar trend of lower numbers of *H. glycines* preadult stages and cysts with higher rates of ASB (40 dry t/ha) was observed in greenhouse studies conducted on a loamy sand in Michigan. One conclusion was evident from these multi-state results: high rates of ASB (at least 40 dry t/ha) were needed to effect a reduction in *H. glycines* population densities. This rate may not be realistic from environmental and economic perspectives; therefore a reduction of the rate of ASB required to suppress plant-parasitic nematodes would be desirable. One way to accomplish this is to gain a deeper understanding of how ASB controls nematodes in order to promote this mechanism. One mechanism by which biosolids, and other

nitrogenous amendments, suppress nematodes is through the production of gaseous ammonia (NH_3) at elevated soil pH (Meyer et al., 2005; Oka et al., 2006). Free NH_3 is nematicidal, whereas NH_4^+ is not. Many factors influence $\text{NH}_4^+/\text{NH}_3$ dynamics, with pH being the most widely recognized. According to the Henderson-Hasselbalch equation, at a pH of 9.3 (pK_a) the ratio of N present as $\text{NH}_4^+:\text{NH}_3$ is 1:1, with more NH_3 being generated as pH increases. While the use of nitrogenous fertilizers and amendments has received some attention (Rodríguez-Kabána, 1986; Walker, 1971), its adoption as a consistent and reliable pest management practice has not occurred because of incomplete and site-specific nature of disease control, large application rates, and the instability of NH_3 in soil (Oka et al., 2006). Identifying environments and management practices where this mechanism can be used is essential.

In laboratory experiments we determined that ASB did suppress *H. glycines* juvenile activity and egg hatch (Zasada and Tenuta, 2004). When ASB was applied to sand the concentration necessary to result in a 90% reduction in second-stage juveniles was 1.4% (equivalent to 28 dry t/ha) and > 3.0% for eggs (60 dry t/ha). *Meloidogyne incognita* was similar in its susceptibility to ASB. These results indicate that for ASB to effectively reduce *H. glycines* population densities application should coincide with the presence of juveniles in soil. In this same experiment *H. glycines* mortality was closely related ($r^2 > 0.72$) to the soil solution pH to which nematodes were exposed, and to a lesser extent NH_3 exposure ($r^2 < 0.54$). Similar relationships between soil solution pH/ NH_3 concentration and nematode mortality were observed in experiments that demonstrated that alkaline-stabilization of biosolids was necessary to achieve *H. glycines* suppression (Zasada, 2005).

The lack of a strong relationship between soil solution NH_3 levels and nematode mortality in initial experiments was most likely due to methodology (Zasada and Tenuta, 2004; Zasada,

2005). Ammonia levels were determined after 24 hrs from sand assayed in open containers. Subsequent experiments (Zasada and Tenuta, submitted; Zasada, unpublished) demonstrated that the generation of NH_3 in soil, and therefore exposure potential to nematodes, is a complex process requiring multiple measurements over time. For example, a sandy loam amended with the equivalent of 40 dry t/ha ASB was incubated either open or closed, and soil solution pH and NH_3 concentration were determined at 1, 3 and 5 days (Zasada and Tenuta, submitted). Nematodes were exposed to a higher peak concentration and total amount of NH_3 in a closed vs. open environment. Regardless of incubation environment the concentration of NH_3 over time peaked at day 3, and then gradually decreased to day 5. In our initial experiments either all of the NH_3 was rapidly lost from the open system or the incubation time was not long enough to get a good indication of NH_3 exposure to nematodes. Regardless, our research clearly demonstrates that understanding the relationship between pH and NH_3 is an important mechanism of nematode control by ASB.

Experiments were designed to determine how environmental factors influenced soil pH/ NH_3 dynamics and plant-parasitic nematode suppression after the application to soil of an ASB amendment. In all experiments, ASB was added to *M. incognita*-inoculated soil 5 days prior to planting of a nematode-susceptible plant. The pH and NH_3 concentrations in soil solution were measured at 0, 3 and 5 days, and nematode reproduction was determined after approximately 45 days. Manipulation of soil temperature and moisture of a loamy sand soil after the addition of ASB had a profound influence on pH/ NH_3 dynamics. Maximum and cumulative NH_3 concentrations in soil solution were highest at the lowest soil moisture (25% of water holding capacity) and highest temperature (31° C). The pH of the soil solution was not influenced by moisture or temperature. These results indicate that it may be necessary to manage the soil

environment after ASB application to maximize nematode suppression.

The generation of unwanted waste streams in the U.S. is only going to increase with time. The challenge is to incorporate the use of waste products into current soybean production systems in an environmentally and economically viable manner. From a nematode management perspective these waste products should be considered as part of a *H. glycines* management program, not as stand-alone practices. For example ASB maybe combined with *H. glycines*-resistant cultivars to provide additional nematodes suppression and potentially reduce rotation lengths. The most important consideration when using waste products for *H. glycines* management is that the mechanism of nematode suppression is understood and that these products are applied only in environments where suppression is obtainable.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

The author thanks Stephen Rogers (USDA-ARS Nematology Laboratory) for technical support and Mario Tenuta (University of Manitoba) for a productive collaboration.

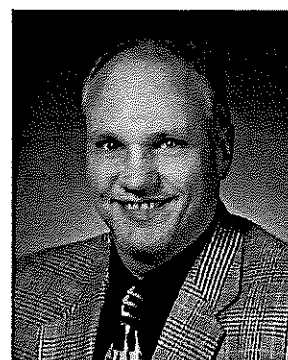
References

- Akhtar, M., and M. M. Alam. 1993. Utilization of waste materials in nematode control: a review. *Bioresource Technology* 45:1-7.
- Akhtar, M., and A. Malik. 2000. Roles of organic amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology* 74:35-47.
- Barbosa, G. M. de C., M. L. Mendes, J. Filho, P. B. N. Rodriguez, and E. Visoni. 2004. Effect of

- sewage sludge compost on *Meloidogyne javanica* on tomato. *Nematropica* 34:13-21.
- American Coal Ash Association (ACAA). 2003. 2002 coal combustion product (CCP) production and use survey. American Coal Ash Association, Syracuse, NY.
- Logan, T. J., and J. C. Burnham. 1995. The alkaline stabilization with accelerated drying process (N-Viro): An advanced technology to convert sewage sludge into a soil product. Pp. 209-223 in D. L. Karlen, D.L., R. J. Wright, and W. O. Kemper, W.O. eds. Agricultural utilization of urban and industrial by-products. ASA Special Publ. No. 58. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.
- Mannion, C. M., B. Schaffer, M. Ozores-Hampton, H. H. Bryan, and R. McSorley. 1994. Nematode population dynamics in municipal solid waste-amended soil during tomato and squash cultivation. *Nematropica* 27:181-189.
- Meyer, S. L. F., I. A. Zasada, M. Tenuta, and D. Roberts. 2005. Application of a biosolid soil amendment, calcium hydroxide, and *Streptomyces* for management of root-knot nematode on cantaloupe. *HortTechnology* 15:635-641.
- Oka, Y., N. Tkachi, S. Shuker, R. Rosenberg, S. Suriano, and P. Fine. 2006. Laboratory studies on the enhancement of nematicidal activity of ammonia-releasing fertilizers by alkaline amendments. *Nematology* 8:335-346.
- Rodríguez-Kabána, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology* 18:129-135.
- Rodríguez-Kabána, R., G. Morgan-Jones, and I. Chet. 1987. Biological control of nematodes: soil amendments and microbial antagonists. *Plant and Soil* 100:237-247.
- U.S. Department of Agriculture, Agricultural Research Service. 1999. FY-1999 Annual Report Manure and Byproduct Utilization, http://www.ars.usda.gov/research/programs/programs.htm?np_code=206&docid=246.
- U.S. Environmental Protection Agency. 1999. Biosolids generation, use and disposal in the United States. EPA530-R-99-009. Office of Solid Waste and Emergency Response, Washington, DC.
- Walker, J. T. 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. *Journal of Nematology* 3:43-49.
- Zasada, I. A., and M. Tenuta. 2004. Chemical-mediated toxicity of N-Viro Soil to *Heterodera glycines* and *Meloidogyne incognita*. *Journal of Nematology* 36:297-302.
- Zasada, I. A. 2005. Factors affecting the *Heterodera glycines* suppressiveness of N-Viro Soil. *Journal of Nematology* 37:220-225.
- Zasada, I. A., F. Avendano, Y. C. Li, T. Logan, H. Melakeberhan, S. R. Koenning, and G. L. Tylka. 2008. Potential of an alkaline-stabilized biosolid to manage nematodes: case studies on soybean cyst and root-knot nematodes. *Plant Disease* 92:4-13.
- Zasada, I. A., and M. Tenuta. submitted. Alteration of the soil environment to maximize *Meloidogyne incognita* suppression by an alkaline-stabilized biosolid amendment. *Applied Soil Ecology*.

Use of Resistance for SCN Management

Gregory L. Tylka
Professor
Department of Plant Pathology
Iowa State University
gtylka@iastate.edu



The soybean cyst nematode (SCN) has plagued United States soybean production since the 1950s. For all practical purposes, SCN can never be eliminated from a field. Effective management involves an integrated approach of scouting for early detection of infestations, when population densities are low, followed by proper use of SCN-resistant soybean varieties in rotation with nonhost crops. A few soil-applied nematicides are available for management of SCN, but the economics associated with field-wide application of such chemicals can make this an unfeasible option.

The Different Sources of SCN Resistance

Soybean varieties resistant to SCN possess resistance genes from one of several breeding lines, referred to as “sources of resistance”. These include PI (Plant Introduction) 88788, PI 548402 (also called Peking), and PI 437654. Resistance from PI 437654 sometimes is referred to as “Hartwig resistance”. SCN resistance in these sources is oligogenic (conferred by several genes).

Availability of SCN-resistant Soybean Varieties

Currently, there are hundreds of SCN-resistant soybean varieties available (Shier, 2007; Tylka, 2007) for soybean growers in the Midwest. In Iowa, there are 763 SCN-resistant soybean varieties in late maturity group 0 and maturity groups 1, 2, and 3 for 2008 (Tylka, 2007).

The availability of SCN-resistant varieties has increased greatly in the past two decades, but few of the varieties currently available possess a specific source of resistance other than PI 88788 (Figure 1). For 2008, only 16 of the 763 varieties (2%) available for Iowa have SCN resistance from a source other than PI88788. This is down from 15% of 586 SCN-resistant soybean varieties in 2004.

What is SCN Resistance?

SCN juveniles are unable to establish feeding sites in the roots of resistant soybean varieties. Depending on the type or source of resistance, the feeding site may start to develop and then deteriorate very quickly or it may develop for a few days then deteriorate. But the end result is the same – the SCN juveniles starve inside the root tissue.

In the soybean seed industry, SCN resistance usually is defined by the number of adult SCN females that develop on roots relative to the number that form on a standard, susceptible soybean variety (usually Lee 74) in a greenhouse experiment. If reproduction is $\leq 10\%$ of what occurs on a susceptible variety, a soybean line is considered resistant. If there is $>10\%$ but $\leq 30\%$ reproduction, the line is considered moderately resistant. If the soybean line supports $>30\%$ but $\leq 60\%$ reproduction, it is considered moderately

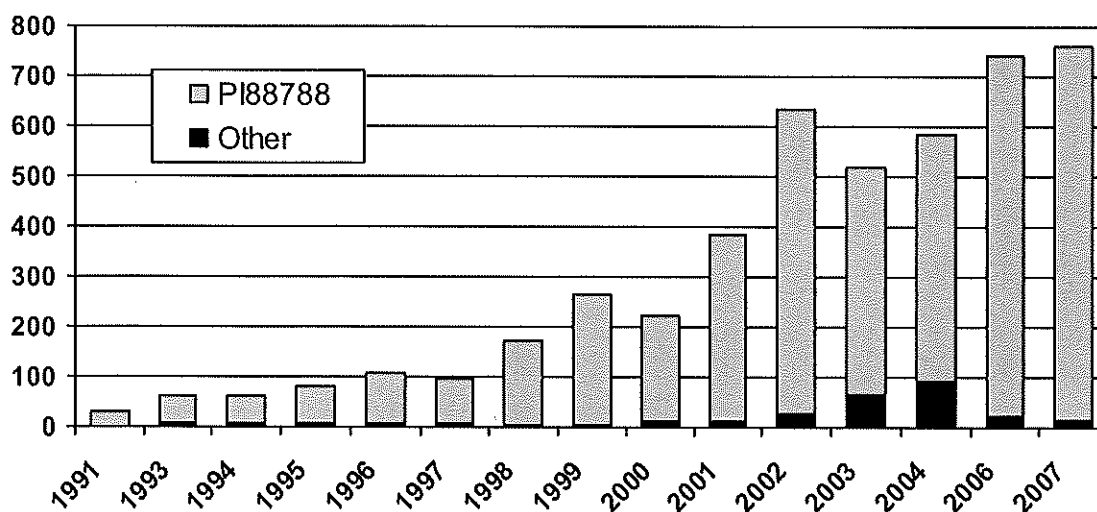


Figure 1. Number of SCN-resistant soybean varieties available for Iowa growers from 1991 to 2007. No data were available for 1992 or 2005. The black portion of each bar indicates the number of resistant varieties with SCN resistance other than PI88788; gray portion of each bar represents the number of varieties with SCN resistance from PI88788.

Table 1. Average yield of SCN-resistant and SCN-susceptible varieties and final SCN population densities at 10 locations of the ISU SCN-resistant Soybean Variety Trial Program in 2007.

Location	Initial SCN population density (eggs/100 cc)	Yield (bushels/acre)		Yield benefit (bushels/acre)	Final SCN population density (eggs/100 cc)	
		resistant varieties	susceptible varieties		resistant varieties	susceptible varieties
<i>----- northern district -----</i>						
Albert City	3,353	63.0	51.8	11.2	1,993	15,187
Manchester	301	58.9	60.2	- 1.3	205	1,575
Mason City	3,887	46.5	34.9	11.6	2,231	5,825
Vincent	4,001	45.4	31.2	14.2	2,138	2,738
<i>----- central district -----</i>						
Cambridge	3,156	59.3	55.1	4.2	642	2,119
Farnhamville	5,461	54.8	48.0	6.8	1,432	3,769
Urbana	5,369	59.5	52.6	6.9	1,226	4,781
<i>----- southern district -----</i>						
Council Bluffs	515	67.4	65.9	1.5	1,782	6,094
Crawfordsville	2,329	57.2	59.8	- 2.6	297	769
Melrose	5,242	60.9	50.8	10.1	1,199	10,294

susceptible. And a soybean line with >60% reproduction is considered susceptible (Schmitt and Shannon, 1992). The parasitic capabilities of the SCN population used in such greenhouse resistance evaluations will greatly affect how a soybean line is characterized.

Performance of SCN-resistant Soybean Varieties

The Iowa State University (ISU) SCN-resistant Soybean Variety Trial Program annually evaluates the yield and SCN control provided by many SCN-resistant soybean varieties in experiments at several sites throughout the state. Each variety is grown in four replicate plots at each experimental location, and soil samples are collected from each 4-row plot at planting and analyzed to verify the presence of SCN in every plot. At harvest, another soil sample is collected from each plot, and SCN population densities are determined to assess the SCN reproduction that occurred on the soybean variety grown in each plot. Commonly grown SCN-susceptible varieties are included in each of the variety trials. The results of these experiments illustrate what can be expected when growing a resistant soybean variety in an SCN-infested field.

Each year, the SCN-resistant varieties, as a group, produce greater yields than the widely grown, SCN-susceptible soybean varieties at most of the variety trial locations. In these experiments in 2007 (Tylka et al., 2008), the smallest positive yield difference between resistant and susceptible varieties, 1.5 bushels per acre, was at the Council Bluffs location, in southwest Iowa, where the initial SCN population density was 515 eggs per 100 cc (about a half cup) of soil (Table 1). The greatest yield difference, 14.2 bushels per acre, occurred at the Vincent location, in north central Iowa, where the initial SCN population density was 4,001 eggs per 100 cc of soil. And in two of the locations in 2007 (Crawfordsville and Manchester), the SCN-susceptible varieties yielded more than the SCN-resistant varieties as a

group (Table 1). Yield and SCN population density data for individual varieties evaluated from 1996 to 2007 at all locations of the ISU SCN-resistant Soybean Variety Trial Program are available on-line at www.isuscntrials.info.

Not only do SCN-resistant varieties produce greater yields than susceptible varieties, they also prevent large increases in SCN population densities in SCN-infested fields. Table 1 contains final (harvest) SCN population densities in plots planted with SCN-resistant and SCN-susceptible varieties at these locations in 2007. At every location in 2007, the final SCN population density under the SCN-resistant varieties was less than that under the susceptible variety, even at locations where there was no yield benefit associated with the SCN resistance. And at the Albert City and Melrose locations, there was a 7- to 9-fold difference in final SCN population densities under resistant versus susceptible soybean varieties. These data clearly illustrate that SCN-resistant soybean varieties pay economically significant dividends twice – in the form of increased yields and control of SCN population densities.

Recently, scientists have reported increases in the occurrence of SCN populations with greater than 10% reproduction on PI 88788 (Mitchum et al., 2007; Niblack et al., 2008). And it is not uncommon for Iowa SCN populations to have greater than 10% reproduction on PI 88788, too. The SCN populations in 11 of 23 locations (or 48%) of the ISU SCN-resistant Soybean Variety Trial Program from 2005 to 2007 had >10% reproduction on PI 88788. And almost all of the SCN-resistant varieties evaluated in the ISU SCN-resistant Soybean Variety Trial Program have SCN resistance genes from PI 88788. But most of the SCN-resistant varieties usually yielded greater than the susceptible varieties at these locations.

Summary

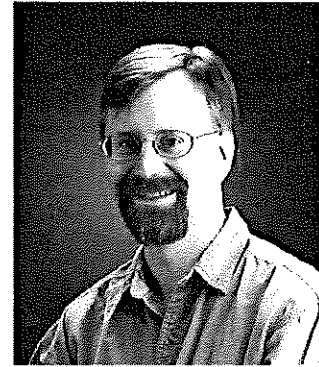
SCN is a very damaging, long-lived, and widespread pest of soybeans. SCN-resistant soybean varieties are an effective and affordable tool to manage SCN, providing greater yields than susceptible varieties and preventing increases in SCN population densities. Almost all SCN-resistant soybean varieties possess SCN resistance genes from PI 88788, and it is no longer uncommon for SCN populations to have 10% or greater reproduction on PI 88788. But varieties with the PI 88788 source of SCN resistance continue to yield greater than susceptible soybean varieties and also prevent increases in SCN population densities throughout the growing season. And considering that most companies do not charge a premium price for seed of varieties with the SCN resistance trait, SCN-resistant varieties are a great management tool to ensure long-term, profitable soybean production in fields infested with SCN.

References

- Mitchum, M. G., Wrather, J. A., Heinz, R. D., Shannon, J. G., and Danekas, G. 2007. Variability in distribution and virulence phenotypes of *Heterodera glycines* in Missouri during 2005. *Plant Disease* 91:1473-1476.
- Niblack, T. L., Colgrove, A. L., Colgrove, K., and Bond, J. P. 2008. Shift in virulence of soybean cyst nematode is associated with use of resistance from PI 88788. Online. *Plant Health Progress* doi:10.1094/PHP-2008-0118-01-RS.
- Schmitt, D. P., and Shannon, J. G. 1992. Differentiating soybean responses to *Heterodera glycines* races. *Crop Science* 32:275-277.
- Shier, M. 2007. 2007 SCN resistant soybean varieties. University of Illinois Extension, 47 pp. (<http://web.extension.uiuc.edu/livingston/reports/i120/index.html>)
- Tylka, G. L. 2007. Soybean cyst nematode-resistant soybean varieties for Iowa. Iowa State University Extension, Publication Pm-1649, 24 pp. (<http://www.extension.iastate.edu/Publications/P1649.pdf>)
- Tylka, G. L., Gebhart, G. D., and Marett, C. C. 2008. Evaluation of soybean varieties resistant to soybean cyst nematode in Iowa – 2007. Iowa State University Extension, Publication IPM 52, 27 pp. (<http://www.extension.iastate.edu/Publications/IPM52.pdf>)

Studies on the effects of major SCN resistance genes

Brian Diers
Professor
Department of Crop Sciences
University of Illinois
bdiers@uiuc.edu



Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is an important soybean [*Glycine max* (L.) Merrill] disease worldwide and is estimated to cause the greatest yield loss of any soybean pest or disease in the U.S. (Wrather and Koenning, 2006; Wrather et al., 2001). Once fields are infested with SCN, the pest cannot be eliminated, however, soybean producers can largely control SCN by planting resistant soybean cultivars and by rotating to nonhost crops. The development and widespread production of SCN resistant cultivars has been an important success of the soybean breeding industry because it has allowed the continued profitable production of soybean in infested soybean fields.

Most accessions in the soybean germplasm collection have been screened for resistance to SCN and at least 118 soybean plant introductions (PIs) with SCN resistance were identified (Rao Arelli et al., 1997). Although all of these resistance sources are available to breeders, PI 88788 is the predominant resistance source for most SCN resistant cultivars in the northern U.S. In a summary of soybean cultivars available for planting in Illinois during 2005 assembled by Marion Shier (University of Illinois Extension), PI 88788 was the only SCN resistance source for 93% of these cultivars, followed by PI 88788 resistance combined with Peking and or PI 437654 resistance (3%), Peking (1%), and Peking combined with PI

437654 resistance (3%). The high representation of PI 88788 resistance in soybean cultivars is in spite of repeated attempts by breeders to develop cultivars using other resistance sources. These attempts have largely failed because breeders have been the most successful in combining PI 88788 resistance together with the high yield needed for a successful cultivar.

The wide production of cultivars in the northern U.S. with SCN resistance from only PI 88788 is resulting in SCN population shifts in fields. During 1989-1990, the SCN race was determined from 44 SCN samples collected from fields in Illinois (Sikora and Noel, 1991). They found that 64% of these samples typed to Race 3, which can be controlled by PI 88788 resistance. To evaluate the extent of field population shifts that occurred since 1990, 218 soil samples were taken from Illinois fields in 2005 (Niblack et al., 2008). Eighty-three percent of these samples tested positive for SCN and of these positive samples, 70% had SCN populations that could overcome PI 88788 resistance. This shifting of the SCN populations was not surprising and it highlights the importance of developing cultivars with resistance from sources other than PI 88788.

Researchers have focused attention on mapping the locations of SCN resistant genes with the goal of being able to use marker-assisted selection for SCN resistance. Marker-assisted selection for SCN

resistance is especially attractive because of the expense of selecting for SCN resistance phenotypically. Many genes and quantitative trait loci (QTL) controlling SCN resistance have been mapped and the results from these mapping efforts were reviewed by Concibido et al. (2004). Their summary revealed that by 2004, quantitative trait loci (QTL) or genes had been mapped from eight resistance sources and from these sources, 61 resistance QTL were mapped onto 18 soybean linkage groups. Although this is a large number of mapped QTL, a few important trends emerged from their summary. One important trend is that the SCN resistance gene *rhg1* was mapped in almost all resistance sources. In the sources that *rhg1* was mapped, the gene was typically found to confer the greatest resistance of any of the resistance QTL. In addition, the resistance gene *Rhg4* also was found to be important in a number of resistance sources.

Because of the importance of the *rhg1* resistance gene across a large number of SCN resistance sources, my lab has conducted experiments to improve our understanding of this gene. To study the effect of this gene on resistance, we developed near isogenic line (NIL) populations that segregated for the *rhg1* resistance allele from PI 88788. When these NIL populations were tested for resistance to the SCN isolate PA 3 (HG Type 0, Race 3) in greenhouse tests, the homozygous resistant lines had a female index (FI) of 5, and the homozygous susceptible lines had a FI of 96 (Brucker et al., 2005a). This shows that under the right conditions, *rhg1* from PI 88788 can act as a qualitative resistance gene. This result is supported by Glover et al. (2004) who showed that in a cross between the SCN resistant cultivar Bell, which traces its SCN resistance to PI 88788, and the SCN susceptible cultivar Colfax, *rhg1* explained 68% of the variation for resistance in the population. A minor resistance QTL on LG J was the only other SCN resistance gene mapped in the population. The importance of *rhg1* has resulted in it becoming a focus of marker-assisted selection especially among breeders in private industry, which has lead

to the more widespread development SCN resistant cultivars.

In contrast to PI 88788, the genetic basis of resistance from PI 437654 and Peking is more complex. In addition to *rhg1*, both PI 437654 and Peking have been shown to have *Rhg4* and PI 437654 has been found to have at least four other resistance QTL (Webb et al., 1995; Webb, 2000). These additional QTL in PI 437654 are at least partially responsible for the more broad-based resistance observed in PI 437654 than in PI 88788.

The presence of *rhg1* in SCN resistance sources that show differences in their resistance reaction to a wide array of SCN isolates has caused researchers to hypothesize whether sources may have different alleles at *rhg1*. Brucker et al. (2005b) tested populations segregating for *rhg1* alleles from PI 88788 and PI 437654 with HG type 7 and HG type 1.2.5.7 SCN populations to test this hypothesis. They found that these sources had *rhg1* alleles that gave different resistant phenotypes and the allele from PI 437654 gave almost complete resistance to the HG type 1.2.5.7 isolate, while the PI 88788 allele gave almost no resistance. For the HG type 7 isolate, both the PI 88788 and PI 437654 alleles gave resistance but the PI 437654 allele was at least partially dependent on an interaction with *Rhg4*. These results show that the source of the *rhg1* allele in soybean genotypes is important in determining the SCN resistance profile of cultivars.

The importance of *rhg1* in most SCN resistance sources has brought focus to the need to breed resistance from sources that are not dependent on *rhg1*. The *Glycine soja* line PI 468916 is one resistance source that does not have *rhg1*, but instead carries major resistance QTL on LGs G and E (Wang et al., 2001). The effect of these resistance QTL were confirmed after backcrossing them into a soybean background and the QTL containing regions were found to have a positive effect on yield (Kabelka et al., 2005; 2006). These *G. soja* resistance QTL do not provide a high level of SCN resistance on their own, therefore we have

done tests to determine whether they would complement resistance alleles from other sources. Positive complementation was recently found in a population segregating for *rhg1* from PI 88788 and the two *G. soja* QTL. When this population was tested for resistance to a HG type 2.5.7 (Race 1) SCN isolate, lines with no major resistance QTL had a FI of 76, those with only resistance at *rhg1* had a FI of 64 and those lines with *rhg1* and the two *G. soja* QTL had a FI of 23. These results indicate that combining these genes could result in improved broad based resistance.

In summary, the past decade has led to many advances in our understanding of the genetic basis of SCN resistance. These advances have been applied by scientists who have used them in the development of high yielding, SCN resistant cultivars. The most important advance in breeding that has come from this research has been the widespread use of marker-assisted selection for SCN resistance, which has reduced the dependence of breeding programs on expensive greenhouse assays. As we look to the future, breeders will need to increase their focus on developing varieties with more broad-based SCN resistance to counter the increasing prevalence of SCN field populations that can overcome resistance from PI 88788.

References

Brucker, E., T. Niblack, F.J. Kopsch-Obuch, and B. Diers. 2005a. The effect of *rhg1* on reproduction of *Heterodera glycines* in the field and greenhouse and associated effects on agronomic traits. *Crop Sci.* 45:1721-1727

Brucker, E., S. Carlson, E. Wright, T. Niblack, and B. Diers. 2005b. *Rhg1* alleles from soybean PI 437654 and PI 88788 respond differentially to isolates of *Heterodera glycines* in the greenhouse. *Theor. Appl. Genet.* 111:44-49.

Concibido, V.C., B.W. Diers, and P. Arelli. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. *Crop Sci.* 44:1121-1131.

Glover, K.D., D. Wang, P.R. Arelli, S.R. Carlson, S.R. Cianzio, and B.W. Diers. 2004. Near isogenic lines confirm a soybean cyst nematode resistance gene from PI 88788 on linkage group J. *Crop Sci.* 44:936-941.

Kabelba, E.A., S.R. Carlson, and B.W. Diers. 2005. Marker saturation and the localization of two soybean cyst nematode resistance loci from *Glycine soja* PI 468916. *Crop Sci.* 45:2473-2481.

Kabelka, E.A., S.R. Carlson, B.W. Diers. 2006. *Glycine soja* PI 468916 SCN resistance loci associated effects on soybean seed yield and other agronomic traits. *Crop Sci.* 46:622-629.

Niblack, T.L., A.L. Colgrove, K. Colgrove, and J.P. Bond. 2008. Shift in virulence of soybean cyst nematode is associated with use of resistance from PI 88788. Online. *Plant Health Progress* doi:10.1094/PHP-2008-0118-01-RS.

Rao Arelli, A.P. J.A. Wilcox, O. Myers, Jr., and P.T. Gibson. 1997. Soybean germplasm resistant to races 1 and 2 of *Heterodera glycines*. *Crop Sci.* 37:1367-1369.

Wang, D., P.R. Arelli, R.C. Shoemaker, and B.W. Diers. 2001. Loci underlying resistance to Race 3 of soybean cyst nematode in *Glycine soja* plant introduction 468916. *Theor. Appl. Genet.* 103:561-566.

Webb, D.M., B.M. Baltazar, A.P. Rao-Areli, J. Schupp, K. Clayton, P. Keim, and W.D. Beavis. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in soybean PI437.654. *Theor. Appl. Genet.* 91:574-581.

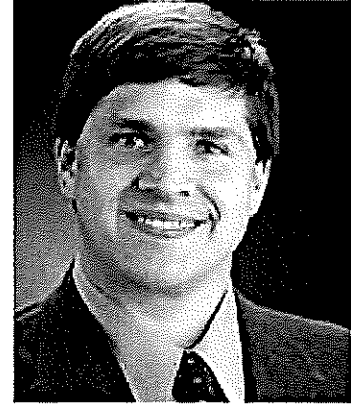
Webb, D.M. 2000. Positional cloning of soybean cyst nematode resistance genes. U.S. Patent 6162967.

Wrather, J. A., Anderson, T. R., Arsyad, D. M., Tan, Y., Portapuglia, A., Ram, H. H., and Yorinori, J. T. 2001. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. *Can. J. Plant Pathol.* 23:115-121.

Wrather, J. A., and Koenning, S. R. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. *J. Nematol.* 38:173-180.

SCN Genetics and Genomics: a model system for the molecular genetic analysis of plant parasitic nematodes

Kris Lambert
Associate Professor
Department of Crop Sciences
University of Illinois
knlamber@uiuc.edu



Heterodera glycines, the soybean cyst nematode (SCN), has a tremendous economic impact on US soybean production, causing significant losses each year. This nematode is a chronic and expanding pathogen of soybean that is difficult to manage with existing control technologies. Molecular genetic analysis may offer solutions for the control of SCN if vital molecular and biochemical data can be collected. For example, a better understanding of SCN parasitism genes, may allow the genetic engineering of SCN resistance in soybean. Likewise, a more complete picture of SCN metabolism may provide new, nematode specific, targets for the generation of effective SCN nematicides. Similarly, the understanding the underlying molecular mechanisms controlling important SCN phenotypic traits, such as SCN virulence (the ability of a nematode to grow on a resistant plant) and host range, may allow undesirable subpopulations of SCN to be monitored and maintained at low, non-damaging, numbers. In all cases, the more genomic data that is collected, the higher the probability that a molecular biology-based control strategy for SCN will be successful.

Fortunately, SCN is amenable to molecular genetic/genomic analysis and recent advances in

data collection promises to elevate SCN to the level of a model system for the study of plant-nematode interactions, making it easier and more efficient to study. SCN has many qualities that make it an ideal model plant parasitic nematode. Cyst nematodes have an obligate sexual reproductive cycle, so both male and female nematodes are present and easily separated, making them amenable to genetic analysis (Lambert *et al.*, 2005). The existence of a genetic map for SCN (Atibalentja *et al.*, 2005) indicates cyst nematodes are practically suitable for genetic studies. SCN has nine chromosomes and a genome size of 92 megabases, which is smaller than that of *C. elegans* (Opperman & Bird, 1998), thus sequencing its genome will not be prohibitively expensive.

To use SCN as a genetic/genomic system, an enormous amount of “genetic infrastructure” is required. To this end, our research team, as well as others in the nematology community, have produced a number of valuable resources such as: 1) a collection of well-characterized inbred SCN; 2) a mapping population of SCN F3-backcross lines; 3) a genetic map of SCN (Atibalentja *et al.*, 2005); 4) a SCN BAC library with end-sequences; 5) four-fold DNA sequence coverage of the SCN

genome; 6) a collection of SNPs between virulent and avirulent SCN biotypes (Bekal et al 2008) and 7) over 200,000 ESTs collected by our group and others in the community (Parkinson *et al.*, 2003).

In short, many of the tools required for the molecular and genomic analysis of SCN have been initiated and are well on their way to being completed. The description below details how some of these new genomic resources for SCN have been developed or their current state of development.

High-throughput sequencing of the SCN genome and transcriptome

454 Life Sciences Corporation has commercialized a rapid, high-throughput DNA micro-bead sequencing system that routinely produces 20-100 Mbp of sequence in a single sequencing run (Margulies *et al.*, 2005). This output is in excess of 100-fold faster than other conventional types of sequencing, drastically reducing time and costs.

454 Life Sciences performed 12 shotgun sequencing runs on the genomes of inbred SCN line TN20 and TN10. SCN inbred line TN20 is a highly virulent nematode that reproduces on all known SCN-resistant soybean, while inbred line TN10 is non-virulent and does not reproduce on resistant soybean. For TN20, 10 runs on a 454 sequencer were conducted producing a total of 379,047,339 bp of raw sequence data with an average read of about 116 bp. Since the SCN genome is about 92 mega bases, the TN20 454 sequence is about 4-fold coverage of the genome. For TN10, a total of 66,000,000 bp of sequence was collected from two runs on a 454 sequencer.

To determine what fraction of SCN genes were represented in our micro-bead sequence data, we compared the SCN micro bead sequence to 8415 SCN cDNAs using FASTA, 95% of the ESTs matched a 454 read, which is nearly identical to the predicted hit frequency for a 4 x genome coverage. Each cDNA matched multiple micro bead sequences with e-values $<1 \times 10^{-4}$ and with an overall accuracy of 99%. Most of the cDNAs that

did not match a 454 sequence appear to be contaminant non-nematode sequences or of low quality.

454-based micro-bead sequencing is not just limited to shotgun genome sequencing, but can also be applied to sequencing an organism's transcriptome. This type of project focuses on the portion of the genome that is translated into mRNA and has the advantage of collecting sequencing data on the genes expressed in the target genome. In our SCN EST project, five inbred biotypes of differing virulence will have one normalized cDNA library sequenced for each. To date, preliminary sequence data has been collected on two libraries, yielding 459,159 raw micro-bead cDNA sequences. After bioinformatic filtering, removing low quality sequences, the average sequence length for each 454 micro-bead sequence was over 225 bp. Preliminary searches of existing EST and protein databases indicate thousands of new SCN genes have been discovered.

Identification of SNPs in between SCN biotypes

The ability to use a genetic approach to study a given trait is a valuable tool. Since we obtained genomic DNA sequence from two SCN inbred lines, we were interested in determining if we could discover SNPs in the entire SCN genome via comparative whole genome analysis. To do this, we compared all TN20 micro-bead sequences to each other using BLASTN to identify SCN sequences that were present in low copy number in the genome. Using this approach, 193,623 TN20 micro-bead sequences that were low copy (less than 10 significant matches) were identified. These low copy TN20 sequences were then compared to all TN10 micro-bead sequences, also via BLASTN. This comparison resulted in 51,999 low-copy of TN20/TN10 matches containing sequence polymorphisms. These matching sequences were extracted and 9748 sequences with putative high-quality single SNPs were retained. PCR primers were designed flanking 96 putative SNPs, which were used to PCR amplify the selected region from genomic DNA from SCN TN10 and TN20. The resulting amplicons were sequenced using

conventional dideoxy sequencing. When the amplicons were re-sequenced, 64% SNPs were confirmed to be correct, indicating we currently have discovered ~ 6238 SNPs in the SCN genome. A similar SNP discovery approach is being used for polymorphism discovery in the SCN EST data. Since SNPs in cDNA sequences are from gene coding regions, they will facilitate map based-cloning of SCN genes of interest.

While putative SNP discovery in SCN is progressing well, the issue of SNP validation still remains. This is primarily a problem due to the low sequence coverage one obtains, even with the large amounts of 454 micro-bead sequence we have collected. However, new sequencing technologies have been commercialized that produce even more sequence data than a 454 machine. If a higher depth of DNA sequence coverage was obtained, this would enable SNP verification and it would also allow one to determine if a SNP is homozygous or heterozygous. To facilitate SNP validation, we decided to collect more SCN genomic sequence from SCN inbreds TN20 and TN10 using a different "next generation" DNA sequencing method developed by Applied Biosystems Inc (ABI). The ABI sequencing technology is called SOLiD (Sequencing by Oligonucleotide Ligation and Detection). This new sequencing project will generate well over one billion bases of sequence data for both TN20 and TN10 genomes. SOLiD sequencing technology is specifically designed for re-sequencing projects to find SNPs for comparative genomic analysis. The ABI SOLiD sequencing project is combined with a conventional SCN whole genome shotgun-sequencing project that is being conducted at the Joint Genome Institute (JGI) using conventional capillary sequencing in this community SCN genome sequencing project:

(<http://www.jgi.doe.gov/sequencing/why/CSP2008/hglycines.html>).

The JGI project will allow the assembly of large contigs of DNA sequence, since it will use an eight-fold deep sequence of the genome, using paired-end sequences from an SCN fosmid library.

This type of long read length DNA sequence data is critical for the assembly of the entire SCN genome. The JGI genome sequence will provide a reference genome for the SOLiD re-sequencing, this will allow the confirmation of the current SNPs we have discovered and the identification of tens of thousands of new SNPs.

In summary, we are continuing to collect significant amounts of SCN DNA sequence data, with this information we are discovering new SCN genes and SNPs from five different SCN biotypes that differ in their virulence profile and host range. We have a formal SCN genome sequencing project established that will help organize the SNP and EST data on the larger scaffold of the genome. The SCN genome sequencing project will provide very valuable data, in terms of SCN gene discovery, but it will be particularly powerful when combined with the SNP data to allow a whole genome genetic approach to be used to analyze SCN traits of interest, such as SCN virulence, host range, nematode aggressiveness and other nematode traits that may be useful in the management of SCN.

References:

- Atibalentja, N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, G. R. and Lambert, K. N. (2005) A genetic linkage map of the soybean cyst nematode *Heterodera glycines*. *Mol Genet Genomics*, **273**, 273-281.
- Lambert, K. N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, G. R. and Smyth, C. A. (2005) Selection of *Heterodera glycines* chorismate mutase-1 alleles on nematode-resistant soybean. *Mol Plant Microbe Interact*, **18**, 593-601.
- Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., *et al.* (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376-380.
- Opperman, C. H. and Bird, D. M. (1998) The soybean cyst nematode, *Heterodera glycines*: a

genetic model system for the study of plant-parasitic nematodes. *Curr Opin Plant Biol*, **1**, 342-346.

Parkinson, J., Mitreva, M., Hall, N., Blaxter, M. and McCarter, J. P. (2003) 400000 nematode ESTs on the Net. *Trends in parasitology*, **19**, 283-286.

SCN Parasitism Genes*

Eric Davis
Professor
Department of Plant Pathology
North Carolina State University
rick@ncsu.edu



*Co-authors: Eric L. Davis, Richard S. Hussey, Melissa G. Mitchum, and Thomas J. Baum

The soybean cyst nematode (SCN), *Heterodera glycines*, is a highly-specialized obligate parasite that transforms plant cells within the vascular and cortical tissues of susceptible soybean roots into an elaborate feeding site called a syncytium that is required for nematode growth and reproduction in sedentary parasitic stages (Endo, 1964; Ichinohe, 1961). Syncytium formation represents one of the most complex responses elicited in plant tissue by any parasite or pathogen. Secretions from the nematode's hollow oral spear (stylet) regulate, directly or indirectly, specific host genes affecting plant cell metabolism, protein synthesis, DNA endoreduplication, cell differentiation, and cell wall synthesis and degradation (Gheysen and Fenoll, 2002; Hussey and Grundler, 1998; Ithal *et al.*, 2007a,b). The coordinated dissolution of adjacent root cell walls produces a multinucleate feeding site where the central vacuoles of the parasitized cells decrease in size, the cytoplasm increases in volume and density, and the cell walls thicken to form elaborate ingrowths, giving the syncytium the phenotype of modified plant transfer cells.

The completion of post-embryonic development of SCN is dependent upon plant parasitism and is delineated by molts through four successive juvenile stages to reach reproductive maturity (see

Davis & Tylka, 2000). Infective second-stage juveniles (J2) of SCN hatch from eggs in the soil and are the infective stage. The infective J2 penetrate soybean roots, migrate intracellularly to the root vascular tissue, and must induce a syncytium for feeding to progress to the swollen, sedentary reproductive adult life stage. The parasitic J2 inserts its protrusible stylet into a selected vascular parenchyma cell and releases secretions that help transform a root cell into an initial syncytial cell that subsequently develops into the full syncytium (Davis & Mitchum, 2005; Hussey and Grundler, 1998). The syncytium serves as a nutrient sink for the nematode and leads to extensive plant disease upon multiple nematode infections of the root system.

SCN is well-adapted for plant parasitism by possessing, in connection to the stylet, three large esophageal gland cells, one dorsal (DG) and two subventral (SvG), that are the principal sources of protein secretions involved in plant infection and parasitism. Each esophageal gland is a single transcriptionally-active secretory cell (Hussey, 1989). The two SvG cells are the most active esophageal glands in infective J2 of SCN while the single dorsal gland cell becomes the predominate source of secretions released through the stylet in subsequent parasitic stages (Davis *et al.*, 2000,

2004; Hussey 1989). Interestingly, the secretory proteins synthesized within the different esophageal gland cells also change during the course of plant parasitism (Davis *et al.*, 2004). More than fifty different SCN parasitism genes expressed exclusively within the esophageal gland cells have been identified that encode secreted protein products (reviewed in Baum *et al.*, 2006; Mitchum *et al.*, 2007).

Identifying the potential functions of the products of cyst nematode parasitism genes in plants is a current challenge. Although the use of soybean hairy roots (Cho *et al.*, 2000) provides some utility, efficient experimental manipulations using the soybean – SCN pathosystem can be difficult (long soybean generation time, large size, determinate seed set, limited reverse genetics capabilities, expertise required to transform and regenerate, etc.). Since the beet cyst nematode (BCN), *Heterodera schachtii*, can infect *Arabidopsis thaliana* (but SCN cannot), an effort to isolate BCN homologs of the SCN parasitism genes was conducted. BCN homologs have now been identified for the majority of SCN parasitism genes that, remarkably, have greater than 90% identity in both nucleic acid and predicted sequence (Hussey *et al.*, 2007). As observed with the *Hg-SYV46* (CLAVATA3/ESR) CLE-like gene (Wang *et al.*, 2001, 2005), the utility of appropriate *Arabidopsis* mutants for functional analysis of parasitism genes has been demonstrated for several of the SCN parasitism genes identified by Gao *et al.* (2003). For the reasons above, the use of *Arabidopsis* for analyses of cyst nematode parasitism gene function through *in planta* expression and host-derived RNA interference (RNAi) has been a recent research focus (Baum *et al.*, 2006; Mitchum *et al.*, 2007).

The secreted nematode parasitism proteins with predicted nuclear localization in plant cells are of particular functional interest and were analyzed by direct expression as fusions with reporter proteins in plant cells. Plant cell nuclear localization of two full-length SCN parasitism proteins (Elling *et al.*, 2007) and four parasitism protein domains has

been demonstrated via tissue bombardment and in transformed *Arabidopsis*. Developmental profiles of SCN gene expression have recently identified other predicted plant nuclear cyst nematode parasitism proteins (A.A. Elling and T.J. Baum, unpublished). These discoveries suggest that plant feeding cell phenotype in some capacity may be regulated directly within the host cell nucleus by secreted nematode parasitism proteins. This assessment also is supported by the identification of nuclear plant proteins interacting with cyst nematode proteins that are predicted to be imported into the plant nucleus (see below).

Since cyst nematode infection has profound effects on plant cellular morphology, it is reasonable to assume that *in planta* expression of parasitism genes will produce alterations in cell and tissue phenotype. We have constitutively expressed individual BCN parasitism genes in *Arabidopsis* and have obtained striking phenotypes in some transgenic lines. Remarkably, expression of cDNA of the 4G12 member (Gao *et al.*, 2003) of the SYV46 family restored the *clv3* mutant phenotype to wild-type, essentially proving the concept of ligand mimicry as a parasitic tool of cyst nematodes (Mitchum *et al.*, 2008; Wang *et al.*, 2005). Consistent with this observation, overexpression of 4G12 in wild-type *Arabidopsis* induced a phenotype and negative regulation of the *Arabidopsis wuschel* gene (Wang *et al.*, 2005) similar to that observed with overexpression of native *Arabidopsis clv3*. Over expression of the 4F01 (Gao *et al.*, 2003) SCN annexin-like parasitism protein in *Arabidopsis* induced no observable phenotype but was able to rescue annexin defective *annAt1* mutant of *Arabidopsis* (Lee *et al.*, 2004) from osmotic stress (N. Patel and E. Davis, unpublished). Several other overexpression plant phenotypes, ranging from accelerated root growth, to altered leaf size or shape, to a dramatically increased shoot length, have also been observed among a subset of parasitism genes analyzed (T. J. Baum and M.G. Mitchum, unpublished). The changes in plant phenotype observed at the tissue level provide an

indicator of potential cellular changes induced by nematode parasitism proteins in plant feeding cells.

The ability of a nematode parasitism protein to directly interact with a specific domain of a plant protein and induce an observable change in root cell growth has recently been reported (Huang *et al.*, 2006a). Potential direct protein-protein interactions in the cyst nematode – plant pathosystem are currently under analysis using selected parasitism proteins (from Gao *et al.*, 2003) as bait in yeast-two-hybrid (Y2H) assays of three high-quality prey libraries from cyst nematode-infected *Arabidopsis* roots at different time points after infection. Stringent Y2H screens (T. Hewezi and T.J. Baum, unpublished) have initially isolated a plant cell wall-modifying pectin methyl esterase enzyme that interacts with a SCN cellulose-binding parasitism protein (3B05), a plant kinase and transcription factor that interacts with a nuclear nematode parasitism protein (10A07), and three plant transcriptional regulators that bind to a predicted NLS-containing nematode parasitism protein (10A06). In complement, *Arabidopsis* knock-out mutants for the genes coding for the interacting plant proteins above have altered nematode susceptibilities. From this initial evidence it is logical that gaining an understanding of the plant interacting proteins can shed enormous light on the functions of nematode parasitism proteins

The ability to express double-stranded RNA (dsRNA) in plants that is complementary to a nematode gene and provide an observable RNAi effect (Fire *et al.*, 1998) on parasitism is a critical gene knockout strategy for functional analyses (Gheysen & Vanholme, 2007; Huang *et al.*, 2006b; Lilley *et al.*, 2007; Steeves *et al.*, 2006; Yadav *et al.*, 2006). Target-specific silencing of plant-parasitic nematode genes has been demonstrated by *in vitro* RNAi assays (reviewed in Lilley *et al.*, 2007), including quantitative reduction in parasitism gene transcripts of SCN (Bakhetia *et al.*, 2007; Sukno *et al.*, 2007). We have provided proof of concept that significant effects on parasitism can be achieved by *in planta* expression of dsRNA

species with homology to a single target parasitism gene (Huang *et al.*, 2006b, Hussey *et al.*, 2007). Observed phenotypes range from reduction in numbers of adult sedentary nematodes to failure of sedentary nematodes to develop past the early juvenile stages. Specific silencing of the target nematode parasitism gene transcripts by plant host-derived RNAi during nematode infection of plant roots has also been observed (A. Sindhu and T.J. Baum, unpublished). Histological analyses are under way to discern how and when parasitic success is impaired, which will reveal crucial insights into the function of individual parasitism proteins. These interactions can also be investigated at the molecular level through gene profiling assays of recipient host cells (Ithal *et al.*, 2007ab) compromised by host-derived RNAi effects of target SCN parasitism genes. In addition to elucidating parasitism protein functions, this approach also has the very real opportunity to generate plant genotypes that have broad resistance resistant to multiple cyst nematode genotypes, as has been documented successfully for the root-knot nematodes (Huang *et al.*, 2006b).

Biological relevance can be visualized by consolidating all data generated for a single parasitism gene, which is done here only for one parasitism gene (T. Hewezi and T.J. Baum, unpublished). The SCN parasitism gene 3B05 (Gao *et al.*, 2003) encodes a protein with significant similarity to bacterial cellulose-binding proteins (CBP). We subjected this gene to the experiments described above. A working hypothesis was that the CBP protein would be secreted by the nematode into the plant cell wall where it would function in consort with cell wall-modifying enzymes. This hypothesis was based on the previous discovery (Smant *et al.*, 1998) that some nematode endoglucanase proteins contain a cellulose-binding domain whereas others do not and that plant cell wall-modifying enzymes are upregulated in nematode feeding cells (Goellner *et al.*, 2001). Using 3B05 in yeast-two-hybrid assays, this cellulose-binding protein bound to a pectin-methyl-esterase (PME) enzyme, a plant protein involved in cell wall-modifications. When

overexpressing 3B05 in Arabidopsis, transgenic lines became almost twice as susceptible to cyst nematode infection as the wild-type. Similarly, overexpression of PME had the same effect. To the contrary, insertional mutagenesis of the PME gene resulted in significant reduction of plant susceptibility to nematodes. Interestingly, expression analyses revealed a significant mRNA increase of PME in cyst nematode-infected Arabidopsis plants. Finally, post-transcriptional silencing of 3B05 through *in planta* RNAi produced significant reductions in plant susceptibility to cyst nematode infection. Taken together, the (T. Hewezi and T.J. Baum, unpublished) data suggest that 3B05 serves a critical function in parasitism. This function involves the binding to PME, thereby, presumably promoting the activity of this enzymes in cell wall modifications required for cyst nematode parasitism to proceed. Similarly complete and compelling stories are beginning to materialize and represent breakthroughs in our fundamental understanding of cyst nematode parasitism.

Targeting essential mechanisms of parasitism identified for SCN can provide the foundation to develop desirable soybean cultivars with novel transgenic resistance that is broad-spectrum among SCN HG-types (Niblack et al., 2002). A research collaboration between the authors and scientists in the Soybean Tissue Culture and Genetic Engineering Center (the Center) sponsored by the United Soybean Board (St. Louis, MO) has been established to develop transgenic soybeans that provide resistance via expression of host-derived RNAi targeted to SCN parasitism genes. Vectors containing a promoter, a target gene flanking a soybean FAD intron, and a terminator have been developed by the Center to express dsRNA to selected SCN parasitism genes in transgenic soybean. The enhanced levels of constitutive transgene expression (as compared to the CaMV 35S promoter) observed using a *Glycine max* ubiquitin (Gmubi) promoter (Chiera et al., 2007) have made it the primary choice of promoter to drive host-derived RNAi to SCN in transgenic soybean. Targeting RNAi transgene expression

more specifically to feeding SCN is also being attempted in some constructs using the *Nicotiana tabacum* cellulase 7 (Ntcel7) promoter shown to be upregulated in nematode feeding cells in a number of heterologous plants (Goellner et al., 2001; Wang et al., 2007). Germplasm of 'Jack' soybean (containing the PI 88788 source of SCN resistance) and a soybean hybrid with no known SCN resistance have been chosen for their relative efficiency of biolistic transformation and regeneration (Meurer et al., 2001). Host-derived RNAi constructs targeted to eight different SCN parasitism genes in transgenic soybean are now currently under development in collaboration with the Center for pending assays of potential SCN resistance in the transgenic soybean lines.

References

- Bakhetia, M., Urwin, P.E., and Atkinson, H.J. 2007. qPCR analysis and RNAi define pharyngeal gland cell-expressed genes of *Heterodera glycines* required for initial interactions with the host. *Molecular Plant Microbe Interactions* 20:306-312.
- Baum, T.J., R.S. Hussey, and E.L. Davis. 2006. Root-knot and cyst nematode parasitism genes: the molecular basis of plant parasitism. Pp. 17-42 In: Setlow, J.K., ed. *Genetic Engineering Volume 28*, Springer.
- Chiera, J.M., Bouchard, R.A., Dorsey, S.L., Park, E.H., Buenrostro-Nava, M.T., Ling, P.P., and Finer, J.J. 2007. Isolation of two highly active soybean [*Glycine max* (L) Merr.] promoters and their characterization using a new automated image collection and analysis system. *Plant Cell Reports* 26:1501-1509.
- Cho, H.J., S.K. Farrand, G.R. Noel, and J.M. Widholm. 2000. High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. *Planta* 210:195-204.
- Davis, E.L., and M.G. Mitchum. 2005. Nematodes: sophisticated parasites of legumes. *Plant Physiology* 137:1182-1188.

- Davis, E.L., R.S. Hussey, and T.J. Baum. 2004. Getting to the roots of parasitism by nematodes. *Trends in Parasitology* 20 (3):134-141.
- Davis, E. L. and G. L. Tylka 2000. Soybean cyst nematode disease. *Plant Health Instructor*. DOI: 10.1094/PHI-I-2000-0725-01. www.apsnet.org/Education/LessonsPlantPath/SoyCystNema/Top.htm
- Davis, E. L., R. S. Hussey, T. J. Baum, J. Bakker, A. Schots, M.-N. Rosso, and P. Abad 2000. Nematode parasitism genes. *Annual Review of Phytopathology* 38:365-396.
- Elling, A. A., E. L. Davis, R. S. Hussey, T. J. Baum 2007. Active uptake of cyst nematode parasitism proteins into the plant cell nucleus. *International Journal for Parasitology* 37:1269-1279.
- Endo, B. Y. 1964. Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology* 54:79-88.
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, et al. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806-11.
- Gao, B., R. Allen, T. Maier, E. L. Davis, T. J. Baum, and R. S. Hussey. 2003. The parasitome of the phytonematode *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 16:270-276.
- Gheysen, G., and Vanholme B. 2007. RNAi from plants to nematodes. *Trends in Biotechnology* 25:89-92.
- Gheysen, G., and C. Fenoll. 2002. Gene expression in nematode feeding sites. *Annual Review of Phytopathology* 40:191-219.
- Goellner, M., X. Wang and E. L. Davis 2001. Endo-1,4-glucanase expression in compatible plant-nematode interactions. *Plant Cell* 13:2241-2255.
- Guo, H.S., J.F. Fei, Q. Xie, and N.H. Chua. 2003. A chemical-regulated inducible RNAi system in plants. *Plant Journal* 34:383-392.
- Huang, G., R. Dong, R. Allen, E.L. Davis, T.J. Baum, and R.S. Hussey. 2006a. A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. *Molecular Plant Microbe Interactions* 19:463-470.
- Huang, G., R. Allen, E.L. Davis, T.J. Baum, and R.S. Hussey. 2006b. Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. *Proceedings of the National Academy of Sciences* 103:14302-14306.
- Hussey R.S, Davis E.L., Baum T.J. 2007. Cyst nematode-resistant transgenic plants. PCT/US2007/000377.
- Hussey, R. S., and F. M. Grundler 1998. Nematode parasitism of plants. Pages 213-243 in: Physiology and biochemistry of free-living and plant parasitic nematodes. R. N. Perry and D. J. Wright, eds. CAB International Press, England.
- Hussey, R. S. 1989. Disease-inducing secretions of plant-parasitic nematodes. *Annual Review of Phytopathology* 27:123-141.
- Ichinohe, M. 1961. Studies on the soybean cyst nematode, *Heterodera glycines*. *Hokkaido National Agricultural Experiment Station Reports*. 56:80.
- Ithal, N., Recknor, J., Nettleton, D., Hearne, L., Maier, T., Baum, T.J., and Mitchum, M.G. 2007a. Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean. *Molecular Plant Microbe Interactions* 20:293-305.
- Ithal, N., Recknor, J., Nettleton, D., Maier, T., Baum, T.J., and Mitchum, M.G. 2007b. Developmental transcript profiling of cyst nematode feeding cells in soybean roots. *Molecular Plant Microbe Interactions* 20:510-525.
- Lee S, Lee E.J, Yang E.J, Lee J.E, Park A.R, Song W.H, and Park O.K. 2004. Proteomic identification of annexins, calcium-dependant membrane binding proteins that mediate osmotic stress and abscisic acid signal transduction in Arabidopsis. *Plant Cell* 16: 1378-1391.

- Lilley, C.J., Bakhetia, M., Charlton, W.L., and Urwin, P.E. 2007. Recent progress in the development of RNA interference for plant parasitic nematodes. *Molecular Plant Pathology* 8:701-711.
- Meurer, C.A., Dinkins, R.D., Redmond, C.T., McAllister, K.P., Tucker, D.T., Walker, D.R., Parrott, W.A., Trick, H.N., Essig, J.S., Finer, J.J., and Collins, G.B. 2001. Embryogenic response of multiple soybean [*Glycine max* (L.) Merr.] cultivars across three locations. *In Vitro Cellular & Developmental Biology-Plant* 37 (1): 62-67.
- Mitchum, M.G., X. Wang, and E.L. Davis. 2008. Diverse and conserved roles of CLE peptides. *Current Opinion in Plant Biology* 11:75-81.
- Mitchum, M.G., R.S. Hussey, E.L. Davis, and T. J. Baum. 2007. Application of biotechnology to understand pathogenesis of nematode plant pathogens. Pp. 58-86 In: Punja, Z.K., S. DeBoer, H. Sanfacon, eds, *Biotechnology & Plant Disease Management*. CABI Intl., Oxford, UK.
- Niblack, T.L., *et al.* 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *J. Nematol.* 34:279-288.
- Smant, G., J.P.W.G. Stokkermans, Y. Yang, J. M. de Boer, T. J. Baum, X. Wang, R. S. Hussey, F. J. Gommers, B. Henrissat, E. L. Davis, J. Helder, A. Schots, J. Bakker 1998. Endogenous cellulases in animals: isolation of *beta*-1,4-endoglucanases from two species of plant-parasitic nematodes. *Proc. Natl. Acad. Sci. USA*:95:4906-4911.
- Steeves, R.M., T.C. Todd, J.S. Essig, H.N. Trick. 2006. Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress *Heterodera glycines* reproduction. *Functional Plant Biology* 33:991-999.
- Sukno, S.A., McCuiston, J., Wong, M.Y., Wang, X., Thon, M.R., Hussey, R.S., Baum, T.J., and Davis, E.L. 2007. Quantitative detection of double-stranded RNA-mediated gene silencing of parasitism genes in *Heterodera glycines*. *Journal of Nematology* 39:145-152.
- Wang, X., Replogle, A., Davis, E.L., Mitchum, M.G. 2007. The tobacco *NtCel7* gene promoter is auxin-responsive and locally induced in nematode feeding sites of heterologous plants. *Molecular Plant Pathology* 8:423-436.
- Wang X., Mitchum M.G., Gao B., Li C., Diab H., Baum T.J., Hussey R.S., Davis E.L. 2005. A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR (CLE)* of *Arabidopsis thaliana*. *Molecular Plant Pathology* 6(2):187-191.
- Wang, X., R. Allen, X. Ding, M. Goellner, T. Maier, J. M. de Boer, T. J. Baum, R. S. Hussey and E. L. Davis. 2001. Signal peptide-selection of cDNA cloned directly from the esophageal gland cells of the soybean cyst nematode, *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 14:536-544.
- Wang, X., D. Meyers, Y. Yan, T. Baum, G. Smant, R. Hussey and E. Davis. 1999. *In planta* localization of a β -1,4-endoglucanase secreted by *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 12:64-67.
- Yadav BC, Veluthambi K, Subramaniam K. 2006. Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. *Molecular and Biochemical Parasitology* 148:219-222.

Soybean Cyst Nematode CLAVATA3/ESR (CLE)-Like Peptides*

Melissa Mitchum
Assistant Professor
Plant Sciences
University of Missouri
goellnerm@missouri.edu



Co-authors: Melissa G. Mitchum, Xiaohong Wang, Eric L. Davis

Soybean cyst nematode (SCN; *Heterodera glycines*) and other species of cyst nematodes feed as obligate sedentary endoparasites of plant roots. Sedentary endoparasitism is an evolutionarily advanced form of plant parasitism that requires modulation of host cellular developmental pathways to form specialized feeding cells that provide short distance solute transfer to the feeding nematodes (reviewed in Gheysen and Fenoll, 2002; Davis and Mitchum, 2005). The multinucleate feeding cells induced by cyst nematodes, termed syncytia, form by extensive cell wall dissolution through the activation of cell wall modifying proteins (Goellner et al., 2001; Wang et al., 2007; Wieczorek et al., 2006; 2008) that leads to the coalescence of adjacent cells. Moreover, syncytia become metabolically highly active, cytoplasmic volume and density increase, the large central vacuole is reduced in size, organelles proliferate, and nuclei become enlarged and polyploid. Secondary cell wall deposition adjacent to vascular tissues results in wall thickening and ingrowth formation that increases the plasma membrane surface area for solute uptake. Syncytia are so far unique to plant-nematode interactions, however, they appear to share developmental characteristics with meristematic cells (reviewed in Bird, 2004),

developing xylem (Bird, 1996), seed endosperm (Lohe, 2002), and transfer cells (Jones and Northcote, 1972). Presumably, cyst nematodes have evolved mechanisms to manipulate plant developmental programs to dedifferentiate selected plant cells to create a novel cell type.

Parasitism genes expressed within the esophageal gland cells encode stylet-secreted proteins that provide the primary signals in feeding cell formation (Davis et al., 2004; Mitchum et al., 2007). Recent efforts to identify SCN parasitism genes using yeast secretion signal peptide selection of a cDNA library generated from microaspirated esophageal gland cell mRNA resulted in the discovery of *HgCLE*, the first nematode *CLE* gene (Wang et al., 2001, 2005; Olsen and Shriver, 2003). The full length *HgCLE* gene encoded a protein of 139 amino acids including a predicted 22-aa N-terminal signal peptide (Wang et al., 2001) and a conserved 14-aa C-terminal motif characteristic of the plant *CLE* signaling peptide family (Olsen and Shriver, 2003). *HgCLE* was expressed exclusively within the single dorsal esophageal gland cell of parasitic nematodes (Wang et al., 2001). Detection of *HgCLE* protein along the dorsal gland extension to the base of the

nematode stylet in parasitic life stages strongly suggests that HgCLE is secreted into plant tissues during syncytium formation (Wang et al., 2005).

Prior to the identification of HgCLE, CLEs were thought to be a plant-specific peptide family proposed to function as secreted ligands that act through leucine-rich repeat receptor-like kinases (LRR-RLKs), a large receptor gene family in plants (> 200 members in *Arabidopsis*), to activate a variety of signaling pathways (reviewed in Fiers et al., 2007; Mitchum et al., 2008). Plant CLE peptides have a hydrophobic N-terminal signal peptide, a highly variable domain among family members of unknown function, and a conserved 14 amino acid (aa) consensus sequence (KRXVPXGPNPLHNR) at or near the C-terminus of the protein, called the CLE motif. A large body of evidence now supports the conserved CLE motif as the major functional domain of CLE peptides (Fiers et al., 2005; 2006; Ito et al., 2006; Kondo et al., 2006; Ni and Clark, 2006) although the nature and regulation of CLE processing is not yet understood (Ni and Clark, 2006). CLAVATA3 (CLV3), the founding member of the *Arabidopsis* 31-member CLE gene family functions by signaling a stem cell-restricting pathway in shoot and floral meristems through the CLV1/CLV2 receptor complex (Rojo et al., 2002; Fiers et al., 2007). CLV1 is a LRR-RLK family member and CLV2 is a LRR protein lacking a cytoplasmic kinase domain. Recently, the *Arabidopsis* CLV3 12-aa CLE motif was shown to bind directly to the LRR domain of the CLV1 receptor (Ogawa et al., 2008). More recent studies point towards conserved factors regulating shoot and root meristem maintenance (Sarkar et al., 2007), including a CLV-like signaling pathway (Casamitjana-Martinez et al., 2003), providing further support that nematode CLEs may functionally mimic a plant CLE peptide ligand in roots to trigger a developmental cascade required for feeding cell differentiation.

To test the hypothesis of ligand mimicry, *HgCLE* was expressed in wild-type and *clv3* mutant *Arabidopsis* to determine if it had any functional

similarity to CLV3 (Wang et al., 2005). Remarkably, the nematode CLE peptide caused premature termination of shoot and floral meristems, a phenotype similar to CLV3 overexpressing plants, and was able to rescue the *clv3* mutant phenotype of enlarged shoot and floral meristems (Wang et al., 2005). These data provided the first indication that cyst nematodes secrete ligand mimics of plant CLE peptides to modify selected host cells to form syncytia.

Further studies have shown that SCN CLEs belong to a polymorphic gene family. DNA blots probed with *HgCLE* revealed complex banding patterns among different SCN inbred lines (A. Replogle and M.G. Mitchum). An intraspecific comparative genomic study to examine the molecular diversity of *HgCLEs* among SCN populations that differ in virulence on resistant soybean (Niblack et al., 2002) identified more than twenty different *HgCLE* sequences, likely representative of different gene family members and allelic variants, with a genomic structure consisting of four exons and three introns (J. Wang and M.G. Mitchum, unpublished). The majority of polymorphisms were detected in coding sequences. Sequence alignments of the predicted HgCLE protein sequences revealed a highly variable domain in the HgCLE proteins that appears to be under diversifying selection (J. Wang and M.G. Mitchum, unpublished). The three introns delineated four distinct domains in the HgCLE proteins: a hydrophobic N-terminal signal peptide, domain I, variable domain II, and the CLE domain. Interestingly, only two forms previously identified from gland-enriched cDNA libraries (Wang et al., 2001, 2005; Gao et al., 2001; 2003) were found to be expressed in parasitic life stages. The two HgCLEs are identical except for the highly variable domain II immediately N-terminal of the conserved CLE domain. Both *HgCLE* genes, referred to as *HgCLE-1* and *HgCLE-2* hereafter, are highly induced at the onset of parasitism and their expression is restricted to feeding life stages (J. Wang and M.G. Mitchum, unpublished). As previously reported, constitutive overexpression of *HgCLE-1* in *Arabidopsis*, a non-host for SCN,

resulted in shoot and floral meristem defects similar to that of overexpression of *CLV3* and other plant *CLEs* (Strabala et al, 2006), with the severity of the phenotype correlating with the expression level of the transgene (Wang et al., 2005). Roots of *HgCLE-1* overexpression lines also exhibited premature termination of the primary root meristem resulting in a short root phenotype. Overexpression of *HgCLE-1* without the CLE motif demonstrated its essential function (J. Wang and M.G. Mitchum, unpublished). Similarly, exogenous application of a synthetic peptide corresponding to the 12-aa HgCLE motif was sufficient to induce a root phenotype similar to that observed for *Arabidopsis HgCLE-1* overexpression lines (A. Replogle and M.G. Mitchum, unpublished). Interestingly, despite the identical CLE motifs of *HgCLE-1* and *HgCLE-2*, no phenotypes were observed in *Arabidopsis* overexpressing *HgCLE-2* (J. Wang and M.G. Mitchum, unpublished). Consistent with this result, *HgCLE-1* but not *HgCLE-2* complemented an *Arabidopsis clv3* mutant (J. Wang and M.G. Mitchum, unpublished). In contrast, expression of *HgCLE-2* in soybean, a major host for SCN, caused premature termination of the primary root meristem indicating that although *HgCLE-2* is not functional in *Arabidopsis*, it is functional in soybean (A. Replogle and M.G. Mitchum, unpublished) implicating an important role for variable domain II in HgCLE localization or function. Unlike *CLV3*, for which the variable domain is presumably dispensable for function (Ni and Clark, 2006; Fiers et al., 2006), preliminary results of *HgCLE-1* deletion analysis suggest that the variable domain is indeed required for HgCLE function (J. Wang and M.G. Mitchum, unpublished). We are currently investigating whether the observed differences in variable domain II between these two peptides is subject to host-specific processing activity to explain why *HgCLE-2* is functional in soybean, but not *Arabidopsis*. We hypothesize that *HgCLE-2* may have a function unique to soybean. (J. Wang and M.G. Mitchum, unpublished). Interestingly, in a survey of 15 SCN inbred populations that differ in their virulence on a set of resistant soybean lines,

HgCLE-1 was found to be expressed in all populations examined, but *HgCLE-2* was only expressed in a subset of populations with a wider range of virulence on resistant soybean germplasm (J. Wang and M.G. Mitchum, unpublished). In fact, *HgCLE-2* was absent from the genomic DNA of some SCN populations suggesting that it is not required for parasitism, but may have evolved a specific function related to SCN virulence. Future studies will be required to confirm this possibility.

In addition, the identification of nematode CLEs raises important questions that form the basis for ongoing investigations: Do nematode CLEs really function as ligand mimics, if so, which plant CLEs do they mimic? Where do nematode CLEs function, in the apoplast or cytoplasm? What plant receptors perceive nematode CLEs? How and where does nematode CLE processing occur? What are the downstream signaling events of nematode CLE perception and how does this contribute to feeding cell formation? How did nematode *CLE* genes evolve (i.e. cross-kingdom DNA transfer or convergent evolution) and does this relate to host-range specificity of cyst nematodes? Our observation that only one of the *H. glycines CLEs* is functional in *Arabidopsis*, a non-host for SCN, indicates that there is likely some host-specific control of CLE peptide recognition. Thus, nematode *CLE* gene evolution may be one of the underlying mechanisms driving the specific adaptation of cyst nematodes to parasitize particular host plant species. The recent identification of beet cyst nematode (*H. schachtii*) (J. Wang and M.G. Mitchum, unpublished) and potato cyst nematode (*Globodera rostochiensis*) (S. Lu and X. Wang, unpublished) *CLE* genes provides an opportunity to address this question in more detail.

The answers to these questions promise to provide exciting new knowledge in our understanding of signaling between cyst nematodes and plants. Ultimately, the application of our knowledge of nematode CLEs in plant parasitism may provide targets useful for designing novel strategies to engineer cyst nematode resistant crop plants.

References

- Bird, D.M. 1996. Manipulation of host gene expression by root-knot nematodes. *Journal of Parasitology* 82:881-888.
- Bird D.M. 2004. Signaling between nematodes and plants. *Current Opinion in Plant Biology* 7:372-376.
- Casamitjana-Martinez, E., Hofhuis, H.F., Xu, J., Liu C.M., Heidstra, R., Scheres, B. 2003. Root-specific *CLE19* overexpression and the *sol1/2* suppressors implicate a CLV-like pathway in the control of *Arabidopsis* root meristem maintenance. *Current Biology* 13:1435-1441.
- Davis, E.L., Hussey, R.S., Baum, T.J. 2004. Getting to the roots of parasitism by nematodes. *Trends in Parasitology* 20:134-141.
- Davis, E.L., Mitchum, M.G. 2005. Nematodes. Sophisticated parasites of legumes. *Plant Physiology* 137:1182-1188.
- Fiers, M., Golemic, E., Xu, J., van der Geest, L., Heidstra, R., Stiekema, W., Liu, C.M. 2005. The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in *Arabidopsis* through a CLAVATA2-dependent pathway. *Plant Cell* 17:2542-2553.
- Fiers, M., Golemic, E., van der Schors, R., van der Geest, L., Li, K.W., Stiekema, W.J., Liu, C.M. 2006. The CLAVATA3/ESR motif of CLAVATA3 is functionally independent from the nonconserved flanking sequences. *Plant Physiology* 141:1284-1292.
- Fiers, M., Ku, K.L., Liu, C.M. 2007. CLE peptide ligands and their roles in establishing meristems. *Current Opinion in Plant Biology* 10:39-43.
- Gao, B.L., Allen, R., Maier, T., Davis, E.L., Baum, T.J., Hussey, R.S. 2001. Identification of putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 14:1247-1254.
- Gao, B., Allen, R., Maier, T., Davis, E.L., Baum, T.J., and Hussey, R.S. 2003. The parasitome of the phytonematode *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 16:270-276.
- Gheysen, G., Fenoll, C. 2002. Gene expression in nematode feeding sites. *Annual Review of Phytopathology* 40:191-219.
- Goellner, M., Wang, X., and Davis, E.L. 2001. Endo-1,4-glucanase expression in compatible plant-nematode interactions. *Plant Cell* 13:2241-2255.
- Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N., Fukuda, H. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* 313:842-845.
- Jones, M.G.K., Northcote, D.H. 1972. Nematode-induced syncytium - multinucleate transfer cell. *Journal of Cell Science* 10:789-809.
- Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H., Sakagami, Y. 2006. A plant peptide encoded by CLV3 identified by *in situ* MALDI-TOF MS analysis. *Science* 313:845-848.
- Lohe, A.R., Chaudhury, A. 2002. Genetic and epigenetic processes in seed development. *Current Opinion in Plant Biology* 5:19-25.
- Mitchum, M.G., Hussey, R.S., Davis, E.L., and Baum, T.J. 2007. Application of biotechnology to understand pathogenesis of nematode plant pathogens. Pp. 58-86 In: Punja, Z.K., S. DeBoer, H. Sanfacon, eds, *Biotechnology & Plant Disease Management*. CABI Intl., Oxford, UK.

- Mitchum, M.G., Wang, X., and Davis, E.L.. 2008. Diverse and conserved roles of CLE peptides. *Current Opinion in Plant Biology* 11:75-81.
- Ni, J., Clark, S.E. 2006. Evidence for functional conservation, sufficiency, and proteolytic processing of the CLAVATA3 CLE domain. *Plant Physiology* 140:726-733.
- Niblack, T.L., Arelli, P.R., Noel, G.R., Opperman, C.H., Ore, J.H., Schmitt, D.P., Shannon, J.G., Tylka, G.L. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of Nematology* 34:279-288.
- Ogawa, M., Shinohara, H., Sakagami, Y., Matsubayashi, Y. 2008. *Arabidopsis* CLV3 peptide directly binds CLV1 ectodomain. *Science* 319:294.
- Olsen, A.N., Skriver, K. 2003. Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to CLAVATA3. *Trends in Plant Science* 8:55-57.
- Rojo, E., Sharma, V.K., Kovaleva, V., Raikhel, N.V., Fletcher, J.C. 2002. CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. *Plant Cell* 14:969-977.
- Sarkar, A.K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R., Laux, T. 2007. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* 446:811-814.
- Strabala, T.J., O'Donnell, P.J., Smit, A.M., Ampomah-Dwamena, C., Martin, E.J., Netzler, N., Nieuwenhuizen, N.J., Quinn, B.D., Foote, H.C.C., Hudson, K.R. 2006. Gain-of-function phenotypes of many *CLAVATA3/ESR* genes, including four new family members, correlate with tandem variations in the conserved CLAVATA3/ESR domain. *Plant Physiology* 140:1331-1344.
- Wang, X.H., Allen, R., Ding, X.F., Goellner, M., Maier, T., de Boer, J.M., Baum, T.J., Hussey, R.S., Davis, E.L. 2001. Signal peptide-selection of cDNA cloned directly from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. *Molecular Plant-Microbe Interactions* 14:536-544.
- Wang, X.H., Mitchum, M.G., Gao, B.L., Li, C.Y., Diab, H., Baum, T.J., Hussey, R.S., Davis, E.L. 2005. A parasitism gene from a plant-parasitic nematode with function similar to CLAVATA3/ESR (CLE) of *Arabidopsis thaliana*. *Molecular Plant Pathology* 6:187-191.
- Wang, X., Replogle, A., Davis, E.L., Mitchum, M.G. 2007. The tobacco *NtCel7* gene promoter is auxin-responsive and locally induced in nematode feeding sites of heterologous plants. *Molecular Plant Pathology* 8:423-436.
- Wieczorek, K., Hofmann, J., Blochl, A., Szakasits, D., Bohlmann, H., Grundler, F.M. 2008. *Arabidopsis* endo-1,4-beta-glucanases are involved in the formation of root syncytia induced by *Heterodera schachtii*. *Plant Journal* 53:336-351.
- Wieczorek, K., Golecki, B., Gerdes, L., Heinen, P., Szakasits, D., Durachko, D.M., Cosgrove, D.J., Kreil, D.P., Puzio, P.S., Bohlmann, H., Grundler, F.M.W. 2006. Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. *Plant Journal* 48:98-112.

SCN Extension Education – Are We Meeting the Needs of the Northern Soybean Producer?

Albert Tenuta
Field Crop Plant Pathologist
Ontario Ministry of Agriculture,
Food, and Rural Affairs
albert.tenuta@ontario.ca



Without doubt one of the most frustrating and challenging aspects in my role as a Field Crop Extension Plant Pathologist comes from the “confusion” surrounding Soybean Cyst Nematode (SCN). My frustration does not arise from a lack of management options or tech transfer materials for delivery to producers. In actuality the opposite is true. Many management tools have been developed and for one reason or another, adoption by some producers and industry has been slow, inconsistent and disappointing. Unfortunately, this situation is all too common and a recent survey (fall 2007) conducted by the North Central Soybean Research Program (NCSRP) confirms the majority of nematologists, plant pathologists and agronomists interviewed from the northern states are equally frustrated (David Wright, Research Director, NCSRP, personal communication).

Why has SCN extension education and outreach efforts not been as successful as we anticipated? There are a number of factors responsible for impeding SCN education outreach and adoption of good best management practices for SCN. Until they are resolved the outcome will remain the same and unfortunately producers will continue to lose money unnecessarily. What are some of these factors?

“Resistant Varieties” – There are many different methods used to label and market SCN resistant

varieties and this has led to significant confusion amongst producers, industry and even some extension people on how they should be utilized and what does it all mean. This is especially important since SCN populations capable of reproducing on PI88788 are building in the north-central U.S. and southwestern Ontario. This is regrettable since one of the pillars of SCN management is the incorporation of SCN resistant varieties into the rotation. Until a standardized testing protocol and a uniform reporting system is in place such as the one proposed by the NC 1035 (“Standard Cyst Evaluation 2007 (SCE07) method), it is buyer beware!

“Not all SCN resistant varieties are created equally” – There are many good SCN resistant varieties available in the market place but unfortunately some varieties with moderate, low or no resistance are promoted inadvertently to producers as “highly resistant” or “immune” to SCN. I have had growers say “they tried a SCN variety but it didn’t yield”. As a result, they incorrectly concluded SCN varieties were not worth the effort or “they did not do what they were suppose too!” Characterizing the genetic diversity within PI 88788 needs to be addressed. From an extension education point of view, it is very difficult to correct a misconception once it has been established.

H. glycines classification – The replacement of the SCN race test with the HG Type Test although makes it simpler to predict SCN reproduction potential on resistant soybean varieties, many growers, industry and some extension staff still use the “old” SCN races. The lack of understanding around HG Typing continues to be an obstacle and must be addressed through a significant extension education effort.

“SCN Soil Testing” – After detecting SCN, the first step in managing it is to determine the respective population levels within the field and if possible the SCN virulence phenotype. But there continues to be a great reluctance by producers to “take the test, beat the pest”. Money is not the issue since many of the free testing programs continue to have the same challenges. Many producers would much rather plant a resistant variety than soil sample. Increasing linkages with industry could be pursued as a means to increase SCN soil testing. Industry partners should be encouraged to sample and submit the soil samples for testing on their client’s behalf. This situation would not only benefit extension education efforts and industry but meet grower needs.

Include Industry Stakeholders – Industry stakeholders play a vital role in aiding in the delivery of SCN extension education but we need to minimize conflicting messages between industry and extension (“one voice, one message”). Consultants, dealers and other industry stakeholders provide added exposure of extension education resources.

A 2004 Iowa State University study (Greg Tylka, personal communication) demonstrates how industry stakeholders can extend the information beyond the traditional extension network. ISU randomly asked 400 growers and 100 CCAs to identify their primary provider of crop production information. The vast majority of growers (over 90%) referred to their private sector crop advisers and only 5% to extension. On the other hand, 80% of the Certified Crop Advisors identified Iowa State University extension as their primary source of information. “Train the trainer programs” are very effective technology transfer vehicles and need to be encouraged. A regional educational effort aimed at “influencers” would benefit extension education by

providing a consistent message to producers regardless of their geographical location.

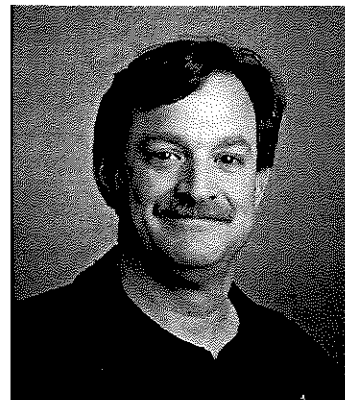
“Different Areas, Different Messages” – Expansion of SCN into new areas or previously uninfested areas requires a different message than established areas. Early detection of SCN into these new or expanding soybean production areas is critical. This provides an opportunity to target extension activities/demonstrations specifically for these areas with the goal to minimize SCN economic losses and maintain producer profitability. Areas where SCN populations capable of reproducing on PI88788 are building will require a more in-depth outreach message.

Extension Recommendations Lack Consistency across Region - Growers, crop consultants, and others have a multitude of local, regional and international resources available to them today to assist in developing their SCN management strategies. There exists considerable variability in SCN recommendations across the region which range from extensive to non-existent (Loren Giesler at the University of Nebraska (personal communication)). Some of this variability in recommendations is a direct result of the inherent variability amongst regional SCN populations. This inconsistency in recommendations has to be addressed and one approach would be the establishment of a regional SCN education outreach project.

As a group, extension education/research outreach has provided most of the “tools” necessary to successfully manage SCN and therefore has met the needs of the northern soybean producer. However, as outlined above there is still a lot of work to be done to make sure our messages are adopted and minimize confusion. This is essential to meeting producer, industry and extension/research needs into the future. In order to meet these obligations it is necessary to develop not only local information but present a consistent regional message through new or “refocused” extension education outreach delivery mechanisms. Is it time for a new “SCN Coalition”?

SCN Extension Education Needs: Points South

Don Hershman
Extension Plant Pathologist
Department of Plant Pathology
University of Kentucky
don.hershman@uky.edu



Soybean Cyst Nematode extension programs have been active in the southern U.S for over 40 years. During this time, southern producers have harvested untold millions of additional bushels of soybean as a result of their having implemented research-based SCN management recommendations. With this storied past, one would assume that 2008 would find us at the zenith of SCN extension programming in the South. Unfortunately, this is not the case - the health of SCN extension programs in the South is not uniformly good.

I recently conducted an ad hoc survey of state extension specialists with responsibility for SCN programs. The survey indicated that many specialists lack confidence that their SCN extension programs are meeting the needs of stakeholders. Put simply, many specialists are not quite sure what to tell producers beyond making general SCN management recommendations. The most significant concern was the inability to make specific recommendations to producers on how to effectively deploy SCN-resistant cultivars. The following are some barriers to effective SCN extension programming in the South:

Undefined SCN Populations. SCN populations in grower fields are largely undefined. Few producers use state SCN testing services to monitor SCN densities in fields, and almost none have had a race or HG-Type test conducted. In

addition, recent surveys from several states indicate that many current SCN populations can reproduce on PI548402 (Peking) and/or PI88788. However, reproduction (i.e., Female Index) is highly variable, ranging from just above 10% to 80+%, depending on the SCN population and PI. Finally, many states have not conducted SCN population surveys for years. Specialists in these states have limited information on the current SCN population structure in their state. These variables would make selection of the best SCN-resistant cultivar difficult even if we had detailed information on performance of resistant cultivars, and a range of SCN resistance sources was available in commercial cultivars (neither of which is the case). The end result is that specialists can only prescribe general recommendations to producers on how to select SCN-resistant cultivars. Site-specific recommendations cannot be made due to the lack of site-specific information on SCN populations.

Uniformity of SCN Resistance Source in Available Cultivars. Almost all SCN-resistant cultivars derive their resistance from PI88788; Peking is a very distant second. Thus, even if field populations of SCN were generally well defined, there is very little a producer could do if a field was infested with an HG-Type that had, for example, an FI of 51% on Peking and 70% on PI88788 (*Note: this is a real example from a field in west KY*). We can talk all we want about

switching to a different resistance source, but there is little practical value in making this suggestion due to the lack of cultivars derived from non-PI88788 and -Peking sources. True, current work suggests that PI88788-based cultivars still provide excellent yields in spite of significant reproduction by some SCN populations. In addition, simply planting different resistant cultivars, even if derived from PI88788, seems to provide acceptable results. However, if things don't change and producers continue to plant cultivars based on PI88788 (due to lack of choice), how long will we continue to be able to say, "all is well"? In 10 years, we could be in a situation where yield losses are high and there is nothing specialists can tell producers other than "grow a non-host crop". That is a good recommendation, but there are practical limitations to growing non-host crops. The bottom line is that specialists cannot provide much practical guidance to producers who have rogue SCN populations on their farm.

Performance Variability of SCN-Resistant Cultivars. Even though the genetic basis of SCN resistance is nearly homogenous in PI88788, there is still tremendous variability in cultivar performance (yield and SCN reproduction) to known SCN populations. Available resistant cultivars run the gamut from having little discernable resistance to having excellent resistance. Unfortunately, few comprehensive field or greenhouse tests are conducted to assess cultivar performance in the presence of SCN. Abundant yield information is available, but SCN population densities are rarely systematically recorded. Consequently, growers have to take seed dealers "at their word" that the SCN resistance in this or that cultivar will perform well in their specific situation. Except in rare cases, specialists cannot provide more guidance.

SCN Analysis Laboratories Are Having an "Identity Crisis". The original purpose of most SCN testing services - to identify fields where SCN-susceptible cultivars should *not* be grown - is a thing of the past in the South. Most southern soybean producers are aware of SCN and many

(most?) know which fields are infested; the discovery era ended years ago. In most cases, SCN analysis results now serve a forensic function by identifying fields where SCN management tactics are failing. This is good. However, beyond advising producers to plant a non-host crop or a different resistant cultivar, what other advice can we give producers who are having difficulties managing SCN? Specialist confidence is at an all time low that established SCN damage thresholds have much meaning. This situation is made worse by the oversimplification of current soybean production systems (e.g., plant it, spray it, harvest it). The end result is that growers do not use SCN testing services and we only weakly encourage them to do so.

Confusion Abounds. There is general confusion "in the field" about SCN terminology and management recommendations. The source of this confusion is manifold. In my survey, specialists who are not nematologists said they have difficulty staying current with SCN. Also, SCN information abounds on the web, but much of it is outdated and often contradictory. To a large extent, this situation exists due to a reduction in the number of nematologists and overwhelmed specialists responsible for keeping publications current. Information provided to stakeholders by industry and universities does not always agree. This is especially true when it comes to SCN-resistant cultivars. Finally, nematologists within the SCN community do not agree on some basic recommendations to producers. This situation has caused many southern soybean producers to disconnect from core SCN management principles.

Suggestions for improving the state of SCN extension educational programs in the South include:

Identify a funding structure to support SCN work and push to fill vacant nematology positions.

Formulate guidelines and standards for developing and testing SCN-resistant cultivars. This could be done through a cooperative public-private consortium, like the old SCN Coalition. Strategic

plans for cultivar development and testing could be devised and implemented. This group could also address sustainable use of SCN-resistant cultivars much like fungicide manufactures do with fungicide resistance (i.e., NAFRAC).

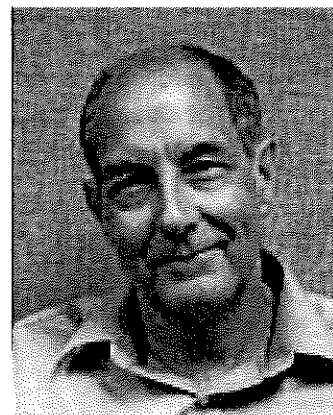
Nematologists and others with SCN responsibilities need to hash out a unified message for stakeholders. Sure, there will always be differences in opinion, and some aspects of SCN management will always be different from state to state (even region to region); however, it is possible, even necessary, to come up with a more unified SCN message to stakeholders.

Find a better way to give stakeholders the “take home” message. Perhaps, there needs to be a national SCN website to serve as the point of contact for SCN information? Existing state websites could still exist, but a national website, putting out a national level message, could have great impact.

Assuming funds are available, periodically conduct cooperative, statistically-valid regional SCN surveys to characterize SCN populations.

SCN – The Checkoff Strikes Back

Mark Halsey
Research Director
United Soybean Board
MHalsey@smithbucklin.com



As farmer-directed organizations, national, regional and state soybean checkoff boards have long recognized the importance of SCN to soybean yields and farmer profitability. The checkoff has a tradition of investing in diverse kinds of research to help farmers to manage this disease, and to seek long-term, fundamental solutions to SCN.

At the national level, the Production Program of the United Soybean Board (USB) has identified SCN as a major research focus, for a number of very good reasons. Foremost, SCN fits the mission of USB – it is national in scope, affecting crops from the Deep South to the far North. It also has a very dramatic impact on our national soybean productivity and grower profitability – SCN causes the loss of over 120 million bushels of soybeans each year. This amounts to well over \$1 billion lost to U.S. soybean farmers due to this one problem. SCN also presents some challenging technical issues that are amenable to a national approach. While different sources of resistance to SCN have long been identified, many useful resistance genes have proven difficult to manipulate in breeding programs leading to an unhealthy reliance on a single source of resistance, PI88788. We are now seeing that SCN is capable of adapting over time and that nematode populations are arising that can overcome this limited base of resistance. Disruption of root

tissues caused by the nematode may also increase susceptibility of the plant to other root diseases, leading to further yield loss.

In order to combat SCN, the Production Committee of USB has supported an approach that you might call a ‘balanced portfolio of research investments’. This portfolio includes a number of projects, some focused on practical solutions, such as improving the efficiency and effectiveness of resistance breeding to provide farmers with good near-term fixes for the problem. Other projects are looking at longer-term, technology driven solutions that we hope will give durable, broad – spectrum control of the disease in the future. The research portfolio also spans ‘worm-based’ and ‘plant-based’ projects.

One good example of the technology-driven, long-term approach is one of the ‘worm’ projects, lead by Dr Thomas Baum of Iowa State University. His team is studying proteins and isolating genes that are critical to the nematode in establishing an infection in the root of the soybean plant. The group has isolated over 50 genes that may be implicated in SCN parasitism. If they can understand these genes, and knock out critical ones using RNA interference (RNAi) or gene-silencing, it could lead to sustainable control of SCN through biotechnology. They are working closely with the

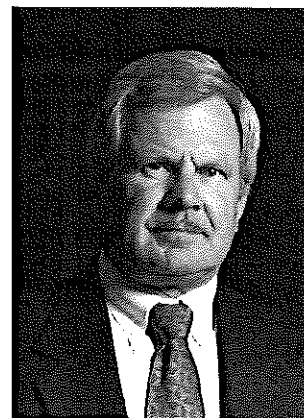
USB-funded Soybean Tissue Culture and Genetic Engineering Center led by Dr Wayne Parrott of the University of Georgia, who are engineering soybean plants with gene-silencing constructs to provide nematode resistance. Already, the groups have demonstrated proof-of-concept by effectively controlling root-knot nematodes with this

approach, and are now extending their findings to SCN.

Another worm-based project, lead by Dr Kris Lambert of the University of Illinois, is the genomic analysis of different HG types (races) of SCN for specific virulence factors.

SCN – Perspectives of Soybean Growers

Ken Dalenberg
Soybean producer
Director, United Soybean Board
dberg@prairie.net



The annual yield loss from soybean cyst nematode is approximately 100-150 million bushels in the United States. This is equivalent to all of the production in Arkansas 100,440,000 bushels or North Dakota 104,650,000 bushels or South Dakota 133,560,000 bushels. If you look at a year of extreme losses it can be as much as the production of Missouri 168,350,000 bushels. Economically this at today's prices is \$1 billion to \$1.5 billion dollar loss for producers.

We have seen the SCN populations changing and becoming more aggressive in areas of the country. Iowa is the latest to have producers looking for answers. Their yields are going down in some areas even though they are using SCN resistant varieties. The reproduction of SCN on roots of SCN resistant soybeans is not suppose to happen. There are hundreds of commercial varieties that this same problem is occurring on.

Farmer management strategies have been evolving also. You can not simply choose a high yielding variety with so called SCN resistance. It is important to monitor by sampling what HG type your farm has. In Illinois we use to primarily have a Type 0 or old Race 3, now we have more commonly SCN type 2. Field history is also important to know so if the Type is changing so will need to look at different varieties. Another question of farmers is if they change rotations to corn will this solve the problem. Will aggressive SCN populations decrease with more years of corn or will corn nematodes mold themselves into being aggressive on soybeans.

Making choices is the answer in most cases for todays solutions. We as farmers need to use the resources at the seed companies and Universities to help get answers. Using the VIPS database (www.vipsoybean.org) is one source with answers to the questions. This database will give company data as to resistance and also the source of resistance if known. Since all sources of resistance are not created equal, the VIPS database varieties are screened in the greenhouse to determine what HG Type they are and what the level of resistance is HR, R, MR, LR, NR and ND. One also has to look at the other disease screens as there are linkages with SCN and other soybean diseases. For instance SDS is much more prevalent on SCN susceptible soybeans. This disease comes on sooner in the environment of SCN. The VIPS database has rating for these other diseases and is very useful to pick high yielding disease resistant soybeans.

SCN is the number one pest in terms of yield reduction and is still spreading after all these years of focusing on research to solve the problem. It has been shown that there is linkages to other diseases. Growers need to look at different sources of resistance used in the breeding programs of seed companies. It could be PI88788, Peking or CystX. Growers need a better education of the importance to use different sources or even to use varieties with different levels of resistance of PI88788. Seed companies need to more frank in their marketing schemes as to solutions to SCN problems within their seed lineup. Labels should do more than say resistant to SCN. They should give level of resistance and type of resistance. Don't just sell seed. Make sure the grower gets what he pays for.

Assessment of Resistance to SCN

Terry Niblack
Department of Crop Sciences
University of Illinois
tniblack@uiuc.edu



Assessment of soybean resistance to SCN has been going on for more than half a century. The first SCN-resistant variety was released in 1966 (Brim & Ross), but it was not until 1992 that resistance was defined by Schmitt & Shannon. They used the same definition for host resistance that Golden et al. (1970) had used to define SCN races: a "cyst index." In other words, both sides of the SCN-soybean interaction (resistance and virulence) were assessed based on the identical measurement: the percentage of SCN females that were able to develop on a soybean line compared with the number that developed on a susceptible line. Thus was the beginning of the complete confounding of the concepts of resistance and virulence in SCN-soybean interactions. This confounding would not be a problem if SCN resistance in soybean and SCN virulence were controlled by single major genes, resulting in easily identifiable, qualitative "+" or "-" categories. Unfortunately, however, both soybean resistance and SCN virulence are quantitative.

Because the focus of this session is on assessment of resistance, the issue of SCN virulence will mostly be left aside except for the following example, to illustrate why labeling a soybean variety "resistant to race 3" is often misleading or completely untrue. In Figs 1, 2, and 3 are the results of three different trials of the same 657 soybean varieties, all labeled "resistant to race 3." Three different labs in Illinois conducted the trials concurrently, each using a separate "race 3" SCN isolate. Given the standard definition of resistance (a Female Index of <10%), Fig. 1

shows that about 2/3 of the varieties were not resistant to that particular "race 3" isolate. Figs. 2

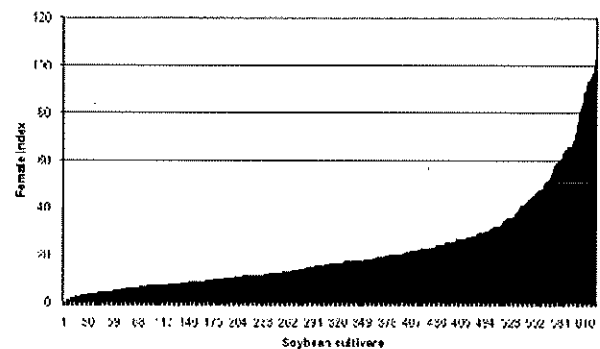


Figure 1. Reactions of >650 soybean varieties to SCN "race 3" (GN-USDA HG Type 7).

and 3 show somewhat greater agreement and larger numbers of varieties that were resistant, but also show that many of the "resistant" varieties had little or no resistance. Were they mislabeled, or would they have shown resistance to a different "race 3"?

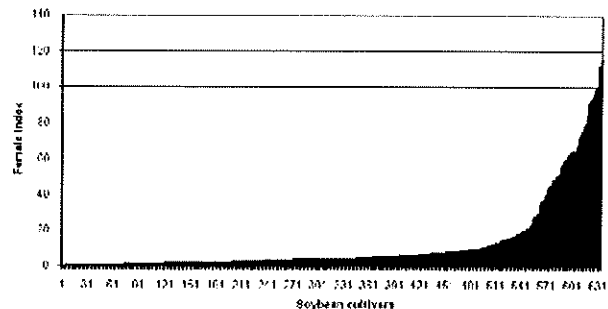


Figure 2. Reactions of >650 soybean varieties to SCN "race 3" (UIUC0, HG Type 0).

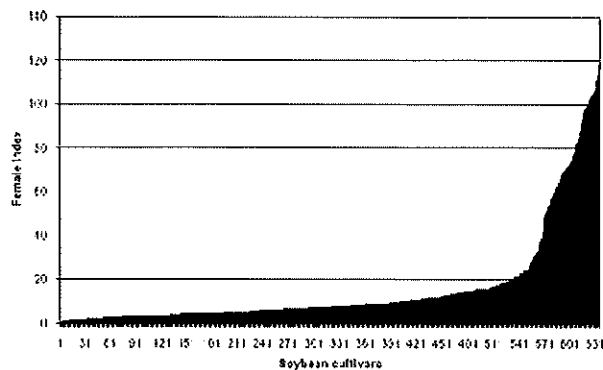


Figure 3. Reactions of >650 soybean varieties to SCN "race 3" (SIU0, HG Type 0).

The details of how to assess and label SCN-resistant soybean varieties will be the subject of discussion in this session. Improving the consistency of how we assess resistance will be of benefit to researchers, but soybean farmers will be the ones who will benefit most from any improvements. Once again, our experience in Illinois illustrates the benefit. In 2001, we screened 343 varieties labeled "resistant to race 3," and found that only 184 (48%) were resistant to any of the three of our "race 3" isolates. In 2007, we screened over 500 varieties (varying slightly by lab), and found that about 80% were resistant by the classic definition. The change is dramatic: most of the varieties labeled as SCN-resistant in Illinois are now actually resistant.

Acknowledgments: SCN resistance screening from 2001-2006 was conducted by J. Bond at Southern Illinois University-Carbondale, G. Noel, USDA-ARS at the University of Illinois Urbana-Champaign, and T. Niblack, University of Illinois Urbana-Champaign. Bond and Niblack conducted similar screening in 2007. Support for the SCN screening program is provided by the Illinois Soybean Association.

Evaluating Phenotypic SCN Screening Methods

Jennifer Hicks
Monsanto, Inc.
jennifer.r.hicks@monsanto.com



The effect of soybean cyst nematode (SCN), *Heterodera glycines*, on soybean production in the United States has been well documented. The estimated loss in 2006 was approximately 124 million bushels (Wrather and Koennig, 2006).

Previous research has shown that the use of resistant soybean varieties is effective at decreasing yield loss due to SCN and is somewhat effective at preventing the increase of SCN population numbers in the soil (Tylka et al., 2003). Accurate phenotypic screening is essential in the identification and development of resistant varieties.

A preliminary study at Monsanto Company in 2002 illustrated significant differences in screening results among five greenhouses and among replications within each facility. Factors affecting screening results were inoculum source, type, and age; temperature and moisture regimes; resistance classification; and differences in greenhouse procedures.

In 2004, a cooperative study was conducted by thirteen participants from public and private institutions to investigate the consistency of screening results for SCN resistance. Each institution screened three replications of 100 entries, in a completely randomized design, with a race 3 nematode population (Hg type 0).

Participants used their current lab procedures and nematode cultures. Final results were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) for ease in analysis.

Significant differences were noted between participating institutions, among replications within a lab, and between labs and the known resistance of the entries to SCN race 3. Similarities in screening procedures were noted among those institutions with data exhibiting less variability from the known resistance. Concurrent replications and seeds per replication had significant effect on the variation of the SCN score from the actual rating.

In a separate study, differentials and other checks used by ten of the participating labs were typed using eight SSR markers covering the genomic regions containing *rhg1* and *Rhg4* to determine whether there was variation in the checks compared to those obtained from the USDA. Markers indicated that variances were seen in the checks at five labs.

Hg type and race differentials were included as blind checks in the screening. Data from labs utilizing correctly classified nematode populations in the screening were analyzed. Significant

differences were noted between participating institutions and among replications within labs.

Several greenhouse procedures have been cited in literature (Niblack et al., 2002; Riggs et al., 1988; Riggs and Schmitt, 1991). Data from the cooperative study and differential tests indicate the importance of maintaining a temperature of 27C, utilizing several replications, growing one plant per container, using all differentials in screening, and obtaining differentials from the USDA Soybean Germplasm Collection.

References

Niblack, T.L., P.R. Arelli, G.R. Noel, C.H. Opperman, J.H. Orf, D.P. Schmitt, J.G. Shannon, and G.L. Tylka. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of Nematology* 34:279-288.

Riggs, R.D., D.P. Schmitt, and G.R. Noel. 1988. Variability in race tests with *Heterodera glycines*. *Journal of Nematology* 20:565-572.

Riggs, R.D. and D.P. Schmitt. 1991. Optimization of the *Heterodera glycines* race test procedure. *Journal of Nematology* 23:149-154.

Tylka, G.L., G. Gebhart, and C. Marett. 2003. Evaluation of soybean varieties resistant to soybean cyst nematode in Iowa in 2003. Iowa State University Extension, IPM 52, Revised January 2004. Online.
<http://www.isuscentrials.info>

Wrather, J.A. and S. Koennig. 2006. Soybean disease loss estimates for the United States, 1996-2006. Online. Missouri Agricultural Experiment Station.
<http://aes.missouri.edu/delta/research/soyloss.stm>

Proposal for a standard method of evaluating soybean varieties for SCN resistance – SCE07

Ralph Von Qualen
Agricultural Consulting and Testing Services (ACTS), Inc.
Carroll, IA
ralph@phytopath.com



When producers purchase a SCN resistant soybean variety they need to know whether that variety's resistance will be effective in their field. Universities have reported that varieties labeled as resistant to Race 3 have allowed reproduction of SCN populations that could be described as Race 3 (Niblack 2002, Tylka et al 2008). These varieties had likely been assessed for resistance to SCN and gave results indicating they had resistance to SCN race 3 (a.k.a. HG Type 0). However, third party testing gave different results. Such differences in results could be caused by differences in the nematode population used for the screening, differences in temperature at which the test was conducted, or the susceptible variety used for comparison (Colgrove 2002). Differences in field conditions could also give different results. This has serious consequences for the producer. Rather than limiting the increase in SCN populations in his fields he may actually be increasing them. He may suffer concurrent and subsequent yield loss due to SCN reproduction on the supposedly resistant variety. Disappointing yields and apparent SCN reproduction on a variety purchased as being resistant to the SCN population in that particular field may ultimately cost the seed company a customer.

With these things in mind, twenty one scientists and researchers from the USDA, Agriculture and Agri-food Canada, universities and industry

gathered at the Hilton Garden Inn in Champaign, Illinois March 23 and 24, 2007 to discuss screening techniques for the evaluation of soybean varieties and their interaction with soybean cyst nematode (SCN) (*Heterodera glycines*). A separate focus group worked to develop standardized protocol that could be used to evaluate soybean varieties before labeling them as SCN resistant. This group included: Karen Gallo (Syngenta, Inc.), Brian Diers (University of Illinois), Tom Welacky (Agriculture and Agri-Food Canada), Jason Bond (Southern Illinois University), Keith Smith (North Central Soybean Research Program), Prakash Arelli (USDA-ARS), Aaron Coburn (Monsanto), Mark Halsey (United Soybean Board), Edward Bazos (Hornbeck Seed, Inc.), and Ralph von Qualen (ACTS, Inc.).

Our focus group shared a concern that varieties labeled as having resistance to SCN should perform as expected in fields or when evaluated by third party testing programs like those conducted by universities. We felt that a more rigorous and standardized procedure for evaluating soybean varieties' SCN resistance should be adopted by the industry. We outlined a method of evaluation that we felt would provide more credible information to the producer and results in less variability. We named this method the *Standardized Cyst Evaluation 2007* or SCE07 for short.

We recommend this method be used as a standard for public labeling of any released variety. Companies will continue to use their own methods to screen germplasm for resistance but we recommend that before seed is labeled for SCN resistance it should be evaluated using the following protocol and that after this the label simply state the variety was evaluated 'following SCE07 protocol'.

Evaluation:

Use three different SCN populations. Nematode populations can vary in their virulence and by screening with three different populations the seed company and producers will be more certain the results of an assessment can be reproduced by a third party lab and the indicated type of resistance will hold up in the field. The company may maintain these populations or some or all of the testing could be sent to independent laboratories. In order for the assessment to be meaningful, the populations should have similar HG types. The populations should be increased for at least five generations on a susceptible cultivar such as Lee 74, Hutcheson, or Essex. Increasing the population on a susceptible variety will provide ample nematodes for conducting multiple tests with the populations and will help prevent a shift in the gene frequency for virulence. Sometimes field populations of SCN provide inconsistent data.

Three single plant replications should be used as a minimum. Three populations with three replications each will result in nine data points for each variety thus assessed.

Standard environmental conditions as described in HG Type recommendations. (Niblack et al 2002) shall be used.

- Surface-disinfest the seed
- Germinate in germination paper 3 days
- Transplant uniform seedlings into pasteurized sandy soil mix
- Container size should be 100 to 500 cm³

- Use randomized complete block or completely randomized design
- After one day extract eggs from cysts of a population maintained as above to liberate eggs and J2 larvae. Eggs can be disinfested with sodium hypochlorite or other chemicals to improve egg hatch (Charleson 2003).
- After liberating, deliver suspension of eggs and J2 larvae to containers at the rate of 20 eggs/J2 larva per cm³ of container volume
- maintain soil temperature at 27° C to 28° C
- maintain light under 16 hour day
- maintain 28 to 30 days
- at take down, dislodge females from roots over nested sieves (20 over 60 mesh) sieves using water spray
- count females using appropriate magnification

Appropriate HG Type indicator lines shall be included in the test. The seed for these shall be procured from the USDA soybean germplasm collection (rlnelson@uiuc.edu). For susceptible check use Lee 74 or other recommended susceptible check (Essex, Hutcheson, Macon, Williams 82 etc.)

100 cysts per susceptible check are required for the test to be valid, there must be at least on the susceptible check. Female Index is used for the final scoring and will be based on a mean Female Index across populations.

Labeling:

1) label product according to the following scale:

Label	Female Index*
Resistant	0 – 9
Moderately Resistant	10 – 30
Moderately Susceptible	31 – 60
No Effective Resistance	>60

*Female Index = (average number of cysts per plant / average number cysts per plant on the susceptible check) times 100.

- 2) Identify the race and HG Type of the SCN population used in evaluating variety. For example: “Resistant to SCN race3 or HG Type 0, following SCE07 standard protocol,” or “Moderately Resistant to SCN race1 or HG Type 2.5.7 following SCE07 standard protocol.”

References

Charleston, D. V. and G. L. Tylka. 2003. *Heterodera glycines* cyst components and surface disinfectants affect *H. glycines* egg hatching. *Journal of Nematology* 35:458-464.

Colgrove, A. L., G. S. Smith, , J. A. Wrather, R. D. Heinz, and T. L. Niblack. 2002. Lack of predictable race shift in *Heterodera glycines* – infested fields. *Plant Disease* 86:1101-1108.

Niblack, T. L. 2005. Soybean Cyst management Reconsidered. *Plants Disease* 89:1020-1026.

Niblack, T. L., P. R. Arelli, G. R. Noel, C. H. Opperman, J. H. Orf, D. P. Schmitt, J. G. Shannon, and G. L. Tylka. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of Nematology* 34: 279-288.

Tylka, G.L., G. D. Gebhart, C.C. Marrett. 2008. Evaluation of Soybean Varieties Resistant to Soybean Cyst Nematode in Iowa in 2007. Iowa State University IPM 52 Revised January 2008.

Abstracts of posters

- 1 Afzal, Ahmed J., Navinder Saini, Ali Srour, and David A. Lightfoot . **The molecular basis of SCN resistance conferred by the rhg1 locus.** Genomics Core Facility and Illinois Soybean Center of Excellence, Department of Plant, Soil and Agricultural Systems, Southern Illinois University at Carbondale, Carbondale, IL 62901.

The genes underlying rhg1 lie at a co-dominant locus, necessary for resistance to all Hg types of the soybean (*Glycine max* (L.) Merr.) cyst nematode (*Heterodera glycines*). Genomic research identified nucleotide changes within a candidate gene encoding a receptor like kinase (RLK) that were capable of altering root development and thereby resistance to Hg type 0 (race 3). Root development is slowed in the resistant seedling and results in end of season yield loss when SCN is not present. However, in the presence of SCN resistant seedling roots grow just as vigorously as susceptible roots and have little loss to SCN parasitism. Reverse genetic approaches have failed to show this gene acts alone to confer resistance. Further, functional paralogs has been found on other linkage groups including A1, G, and O. Therefore, confirmation of the activity is awaited from stable soybean transgenics. Following the effect on development by precise genetic mapping with massive RIL and NIL populations a quantitative trait nucleotide (QTN) in the RLK at rhg1 was inferred that alters A47 to V47 in the context of H297 rather than N297. The allele differences change the structure, interacting partners and activity of the RHG1 protein. The two amino acid changes between the alleles result in 53 other proteins (judged by 2 D gels), 112 metabolites (by FTICRMS) and 8 metabolites (by GCMS) to increase in abundance in roots during SCN infection in the resistant NILs. To examine the mechanism of action further a method for protein expression in *E.coli* was developed that allowed refolding of the LRR domain of the RLK to the normal structure as measured by circular dichroism. The refolded protein was effective in identifying interacting partners, among proteins (cyclophilin), protein domains (CLE) and small molecules. A molecular basis for recessive and co-dominant resistance that involves interactions among paralogous disease resistance genes that are also involved in plant development was inferred. Further analysis of the interactome during the root cells responses to SCN will lead to discoveries in cell sensing. Understanding the basis of root stunting by resistance alleles will be used to improve methods for developing new nematode resistant soybean cultivars that do not suffer from the yield suppression and low seed germination rates of existing cultivars. Ruben et al. *Molec Genet & Genom* 276: 320-330. Afzal and Lightfoot *Prot Exp and Purif* 53: 346-355.

- 2 Cirrinicione, Peter A. and Paul M. Tefft. **Comparison of egg hatching in two populations of the soybean cyst nematode.** Department of Biology, Saint Joseph's University, Philadelphia, PA.

Heterodera glycines, the soybean cyst nematode (SCN), is a common pest of soybeans that causes an estimated annual loss of 1.5 billion dollars in the United States. Eggs of this pest may remain viable for over ten years in the soil making control difficult. We compared egg hatching in a recently collected population of SCN from a farm in Lancaster, PA to egg hatching in a population of SCN that has been reared in the laboratory for 25 years. Eggs used in this study were collected from soybean plants infected with cysts from the two populations. The infected soybean plants were grown in an environmental chamber kept in a 14 hour photoperiod and fed a modified Hoagland's solution once a week. To compare hatching in the two populations eggs were exposed to four different concentrations (0 to 3.0 mM) of zinc chloride, a known hatching stimulant. Eggs hatched in double distilled water served as controls. Percent egg hatching was determined following incubation for 14 days at 28 °C. In order for

hatching to take place in SCN, the eggshells undergo a permeability change allowing an influx of water, causing the nematode to become metabolically active leading to eclosion. Since hatching is dependent on this permeability change we looked at the uptake of the histological stain Nile blue as an indicator of eggshell permeability. Eggs were pretreated in zinc chloride (0 to 3.0 mM) for ten days prior to staining and the percentage of stained eggs was determined. Finally, we studied the effects of incubation time on egg hatching and permeability. Eggs were pre-treated in zinc chloride from 0 to 7 days prior to assessment of hatching percentage and uptake of stain. Results showed that hatching in the Lancaster population occurred at a significantly higher percentage in concentrations of zinc chloride above 0.5 mM. However, the permeability change as indicated by the uptake of Nile blue stain in eggs in these two populations was similar throughout the range of treatment. There were also differences in hatching but not permeability when eggs were pre-treated in zinc chloride for different times.

- 3 Colgrove, A. L., U. Reuter-Carlson, and T. L. Niblack. **An investigation of interspecific associations of cyst nematode species in controlled and natural crossings towards identification of potential hybridizations.** Department of Crop Sciences, University of Illinois at Urbana and Champaign, Urbana, IL 61801.

Although differentiated by morphology and host range, species of cyst nematodes are considered closely related and incidences of interbreeding have been reported. Interspecific reproduction between economically important cyst nematode species could have significance in field situations and laboratory research. Identification of common hosts and molecular markers for species identification would enable investigation of hybridizations. The potential for interspecies hybridizations existed in fields infested with both *Heterodera glycines*, the soybean cyst nematode, and *H. schachtii*, the sugar beet cyst nematode. Soil samples were collected from these fields (George Bird, Appold, MI) and mass-selected on soybean and sugar beet. A PCR-RFLP protocol that distinguishes *H. glycines* and *H. schachtii* with FokI-digested ITS1 fragments (Szalanski, et al., 1997, JON 29: 255-267) was used to investigate the banding patterns of individuals from these isolates. Fragments unique to each species included a 252-bp fragment for *H. glycines* and a 181-bp one for *H. schachtii*. Towards development of *H. glycines-schachtii* hybrids and observation of the hybrid PCR-RFLP banding pattern, controlled crosses were performed. At 10 days after infestation (DAI), *H. glycines*-infected soybeans (*Glycine max*) and *H. schachtii*-infected sugar beets (*Beta vulgaris*) were placed in hydroponic culture to separate males and females and eliminate intraspecific matings. Virgin females were harvested from roots at 25 DAI and transferred to agar plates; males of the opposite species were added, and mating was observed. Egg-filled females were transferred to pots containing a common host, *Lespedeza striata* 'Kobe'. Mature cysts were harvested and subjected to PCR-RFLP. For all progeny tested, both diagnostic bands were present, confirming the occurrence of hybridization between the two species. Hybrid banding patterns were also observed among cysts from field isolates reared on both soybean and sugar beet, prompting an investigation of hybrid persistence. The frequency of hybrid banding patterns among individual virgin females from field isolates reared on soybean or sugar beet for 15 generations ranged from 16 to 38%. The frequency of hybrid banding patterns among cysts from controlled-cross hybrid lines maintained on Kobe lespedeza for 10 generations then transferred to soybean and sugar beet for 3 generations was 100% except for 3 cases where it was 90%. In a separate test, the *H. glycines* and *H. schachtii* populations used for the controlled crosses were reared hydroponically on sugar beet and soybean, respectively, to observe male and female development on the opposite hosts. In both cases, males developed in the absence of females indicating the mode of hybrid development in natural populations. A wider range of species is being investigated for male development on hosts without female development and potential hybridizations when common hosts are identified.

- 4 Faghihi, J.¹, V. Ferris¹, P. Donald², G. Noel³, and T. Welacky⁴. **Changes in resistance of PI 88788 to field populations of soybean cyst nematode (SCN).** ¹Department of Entomology, Purdue University,

West Lafayette, IN; ²USDA-ARS, Jackson, TN; ³USDA-ARS, University of Illinois, Urbana, IL; ⁴Agriculture and Agri-Food Canada, Harrow, ON.

PI 88788 has been the major source of resistance to SCN for about three decades, and is estimated to be present in about 97% of varieties said to be resistant to SCN in the United States. In recent years researchers in the Midwest have observed that varieties with PI 88788 do not seem to be as resistant as they were formerly. Either the resistant soybean varieties have changed or the field populations of SCN have themselves changed in response to long exposure to PI 88788. Goals of this NCSRP funded research were to: 1) determine the current effectiveness of PI 88788 as a source of resistance to SCN in TN, IL, IN, and Ontario, Canada; and 2) determine the reaction to other sources of SCN resistance in areas where PI 88788 may no longer be as effective. The areas were chosen on the basis of length of time that SCN varieties with PI 88788 resistance have been widely used. In TN, the area where PI 88788 has been used the longest, every SCN field population recovered with a high SCN population density was able to reproduce on PI 88788, and no Hg-Type 0 populations were found. In Ontario, where varieties with PI 88788 have been used for a shorter period of time, PI 88788 proved to be resistant to many field populations; but a larger than expected number of Ontario populations were found to develop on PI 548402 (Peking) and also on PI 90763, two sources of resistance not generally present in varieties grown in Ontario. While many field populations in IL and IN were unable to develop on PI 88788, other field populations were capable of reproducing on PI 88788. PI 548402, PI 90763, and PI 437654 continued to be effective sources of resistance to such populations tested. One SCN population from Ontario had a low level of reproduction on PI 437654, while none of the SCN populations from IN or IL were able to reproduce on PI 437654. The discoveries from this research will have important implications for future management options in all of these soybean-growing areas.

- 5 Gao, X., C. Pavon, A. L. Colgrove, and T. L. Niblack. **The use of the DNA quantities of *Heterodera glycines* in soybean roots as a parameter for testing the effectiveness of nematicidal compounds for seed treatments.** Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Growers' interest has increased in the use of seed treatments to enhance the root health of crops. Effective and high throughput techniques for quantification of nematodes in root tissues are essential for large scale screening of nematicidal compounds. Microscopic observation of nematodes has been the primary tool to quantify nematodes in root samples from different nematicide treatments. In this study, we explored the application of an optimized real-time quantitative polymerase chain reaction (QPCR) assay for testing the efficacy of novel nematicidal compounds. A greenhouse experiment was conducted to determine the effects of different seed treatments on *Heterodera glycines*, the soybean cyst nematode (SCN). Two identical tests were prepared, one for harvest at 7 days after infestation (DAI), the other at 31 DAI. The treatments totaled fourteen, including different rates and combinations of nematicidal compounds. Pots were arranged in a randomized complete block design with seven replications for each treatment. Experimental units were PVC tubes (3-cm-diameter × 20 cm) filled with steam-pasteurized soil contained within soil-filled plastic crocks, and were infested with 1,500 *H. glycines* eggs of Hg type 2.5.7 and planted with one seedling per tube, previously germinated in moist germination paper for 48 hours at 27°C. Soybean seedlings (cultivar S30-04RR) were susceptible to *H. glycines*. Prepared crocks were suspended in a water bath maintained at 27°C under a 14-hour light regimen. On the seventh DAP, roots were harvested and stored at -80°C. The DNA quantities of SCN were determined with a QPCR protocol performed on fresh soybean root tissues. At 31 DAI, nematode counts (cysts and eggs) and root weights of soybean plants were determined. Two treatments (5 and 14) had lower DNA quantities 7 DAP, which suggested they best suppressed SCN early infection. Three other treatments (4, 11 and 12) also significantly suppressed SCN early infection in roots. DNA quantities per mg of roots are highly correlated with those per plant ($r = 0.91$). At 31 DAI, in treatments with combined chemicals, when either two or three chemicals combined were tested, a greater suppression of nematode development was

found with three chemicals combined, and all combinations had lower infection levels than single chemicals. Between the two kinds of combinations with three chemicals, treatments 11 through 14 resulted in about 65,000 more SCN eggs per plant than the ones with treatments 7 through 10 ($P < 0.01$), indicating that the compound in treatments 7 through 10 is more effective in suppressing SCN at 7 DAP. SCN DNA quantity in root tissues determined by the optimized QPCR assay for SCN is a powerful tool for screening novel nematicidal compounds, especially at the early growth stages of plants.

- 6 Hewlett, T. E., S. T. Griswold, J. Waters, and K. S. Smith. **The potential for economic biological control of SCN using the bacterial parasite *Pasteuria* spp.** Pasteuria Bioscience Inc. 12085 Research Drive Suite 132, Alachua, FL.

Pasteuria spp. are endospore forming bacteria parasitic to a large number of nematode species. Nine species of *Heterodera* in eight different countries have been reported to be parasitized by *Pasteuria* spp. Continued observations will undoubtedly demonstrate that it is a ubiquitous parasite of cyst nematodes worldwide. Suppression of *Heterodera* spp. by *Pasteuria* spp. has been observed on rice (*H. elachista*) and on soybean (*H. glycines*) and *Pasteuria* spp., parasitizing *H. glycines* on soybean, has been found in the United States. Initial research has demonstrated that the introduction of spore infested soil into *H. glycines* infested areas significantly reduces the nematode population on soybean. Until recently the mass production of *Pasteuria* spp. was not available and therefore this bacterium was not considered to be useful for large scale nematode control. Pasteuria Bioscience Inc. has developed a liquid culture medium suitable for growing *Pasteuria* spp.. Initial laboratory, greenhouse, and field applications of in-vitro-produced *Pasteuria penetrans* and *P. usgae* have demonstrated control of root-knot and sting nematodes. Recently *Pasteuria* spp. parasitizing cyst nematodes on turf was successfully cultured in this medium. Higher cell counts recently achieved in liquid culture will allow for even higher spore production and lower cost per acre. Large scale fermentation would allow reductions in cost below that of the remaining nematicides and allow for treatment of high acreage, relatively low value crops like corn and soybean. Initial field trials have demonstrated that *Pasteuria* endospores can be applied as a liquid or granular formulation to the soil surface or mixed in the soil. In the future seed coating with *Pasteuria* endospores may also be possible, for efficient application in the root zone.

- 7 Holshouser, D., K. Balderson, W. Lawrence, D. Moore, J. Eisenback, and P. Phipps. **Control of soybean cyst nematode race 1 and 4 with variety selection and nematicides.** Virginia Cooperative Extension.

Soybean cyst nematode (SCN) is an economically important pest throughout the United States. SCN races 1, 3, and 4 have been identified in Virginia. Most of the SCN-resistant varieties adapted to Virginia are only resistant to race 3 and/or 14. Our objective was to determine the effect of variety selection and nematicides on SCN control in fields infested primarily with race 1 or 4. Pioneer brands 95M50 (SCN race 3 and RKN resistance) and 95M60 (SCN race 1, 2, 3, 4, 14) were planted in large replicated strips in fields infested with SCN races 3 and 4 (Chesapeake) or SCN races 1 and 3 (Essex County). The Essex site was irrigated. Within these same fields, a small plot experiment was conducted to evaluate Temik® 15G (aldicarb) soil insecticide, AERIS® (thiodicarb + imidacloprid) seed treatment, and Avicta® Complete Pak [Avicta™ (abamectin) + Cruiser® (thiamethoxam) + Dynasty CST® (azoxystrobin, fludioxonil, mefenoxam)] seed treatment as nematicides on Asgrow brand AG4801 (SCN race 3, 14 resistance) and AG4903 (no SCN resistance) soybean varieties. Temik was applied at 4.5 oz per 100 foot of row and seed treatments were applied at rates similar to those labeled for cotton. At the Chesapeake site, 95M60 yielded 10.2 bushels per acre greater than 95M50. In the small-plot experiment, variety had no effect on soybean yield nor did it influence the nematicide treatments. Soybean receiving Temik® 15G yielded 7.6 bushels per acre greater than the control. Yields of soybean treated with AERIS® or

Avicta® seed treatments were equal to the control. At the Essex site, 95M60 yielded 19.2 bushels greater than 95M50. In the small-plot experiment, AG4801 and AG4903 yielded 2.6 and 1.3 bushels per acre without a nematicide. Soybean yields were not different from the control when seed were treated with Aeris® or Avicta™. Yields of Temik®-treated AG4801 and AG4903 were higher than the control, yielding 8.7 and 3.5 bushels per acre, respectively. These experiments indicate variety selection is most important when growing soybean in fields infested with non-race 3 nematodes. Temik® increased yields at both sites, but did not provide sufficient protection at 4.5 oz per 1000 feet to prevent yield loss.

- 8 Kazi, S.¹, N. Saini¹, A. J. Afzal¹, P. Arelli², J. Bond¹, and D. A. Lightfoot¹. **Loci underlying resistance to SCN HG Type 1.3.5 (race 14) cluster with loci for SDS susceptibility and decreased seed yield.** ¹Illinois Soybean Center for Excellence, Department of Plant, Soil and General Agriculture, Southern Illinois University, Carbondale, IL; ²USDA-ARS Soybean Genetics, Jackson, TN.

Genes and loci cluster in the soybean genome. A recombinant inbred line (RIL) population Flyer x Hartwig (F_xH; n=92) and a BAC library from 'Forrest' provided genetic resources for a new map that will play a key role in the soybean comparative cultivar genomics. Four loci for resistance to soybean cyst nematode (SCN) Hg types 0; 1.2.3.5.6.7 and 1.3.5.7 (races 2, 3 and 14) were identified on linkage group (LG) G, identified by Satt275 (P = 0.001), Satt163 (P = 0.005), Satt309 (P = 0.0001) and TMD1 (P = 0.008). All derived the beneficial allele from Hartwig. Resistance to SCN Hg types 0; and 1.2.3.5.6.7 (race 2) was studied further in two near-isogenic line (NIL) populations, derived from F_xH19 and F_xH33, to prepare for fine map construction. Mean SCN FI was significantly lower (P = 0.046, R² = 19%) for genotypes carrying the Hartwig allele for both Satt543 (D2) and Satt237 (N) in F_xH19-derived NIL population. Mean SCN female index was also lower (P < 0.0001, R² = 27%) for genotypes carrying the Hartwig allele for Satt309 (G), Satt610 (G) and minimum tile-derived microsatellite marker SIUC-SattB02K20 (F) in F_xH33-derived NIL population. Bulk segregant analysis was used to detect additional SCN QTL. To look at the basis of yield depression in SCN resistant lines yield QTL were mapped in the *Fusarium virguliforme* same lines. Three linkage groups contain regions that are positively associated with seed yield. Two were on linkage group K identified by (Satt539; P = 0.0005) and (Satt337; P = 0.001). One each on linkage group (LG) D2 and G, detected by Satt514 (P = 0.0006) and TMD1 (P = 0.0007) respectively. Flyer provided the yield beneficial alleles showing the negative effect of SCN on SDS as predicted in 1994 by P. Gibson. To further look at clustering loci underlying SDS were mapped. One new QTL for resistance to sudden death syndrome (SDS) associated leaf scorch measured by disease index was discovered on LG C2 (Satt277) and two QTL for the severity of root infection by the causal organism of SDS, one QTL each on LG D2 (Satt574) and G (Satt115). Therefore, resistance to SCN and SDS were linked, clustered or pleiotropic on two linkage groups, in attraction on G and in repulsion on D2.

- 9 MacGuidwin, A.¹, and K. Thalacker². **Developmental versus reproductive measures for comparing the host status of diverse genotypes of snap bean for SCN.** ¹University of Wisconsin, Madison, WI; ²Seminis Seeds, Inc.

Seventy-three snap bean cultivars (*Phaseolus vulgaris*) were evaluated for their ability to support infection, development, and reproduction of the soybean cyst nematode (SCN) in pots maintained in growth chambers. Using the soybean cv. BSR101 as the susceptible control, four experiments were conducted with each cultivar evaluated twice. The duration of the experiments was 34 to 49 days and based on the time required for SCN to complete one generation on soybean under the same growing conditions. Reproduction and developmental criteria were discrepant for estimating the host suitability of snap bean for SCN relative to SCN-susceptible soybean, with reproduction as the more conservative measure. More than 90% of the snap bean cultivars were comparable or better than soybean for

sustaining SCN to the adult stage. Only 63% of the cultivars were equal or superior to soybean for supporting egg production. The latter figure was close to the percentage of cultivars capable of supporting sufficient reproduction to replace the eggs added as inoculum.

- 10 Maier, Tom. **RNAi silencing of cyst nematode parasitism genes.** Department of Plant Pathology, Iowa State University, Ames, IA.

Agronomically, the interaction between cyst nematodes and their host crops can be devastating. In the U.S., the soybean cyst nematode alone causes yield losses of approximately \$1 billion annually. Cyst nematodes are highly evolved pests that use secretions of parasitism proteins, which they injected through their stylet into host tissues, to successfully parasitize host plants. These parasitism proteins are encoded by parasitism genes expressed in the esophageal gland cells of cyst nematodes and are thought to control the complex process of plant parasitism. With the advent of RNAi technology and the demonstration of host-induced gene silencing in parasites, a new strategy to control plant pathogens has become available. Plant host-induced silencing of cyst nematode parasitism genes, therefore, should provide some disruption of the parasitic cycle and render a host plant less susceptible or resistant to the cyst nematode. Our initial strategy focused on five RNAi constructs, representing four parasitism genes in our host-induced RNAi gene silencing experiments. Transgenic *Arabidopsis* plants expressing these various RNAi constructs were inoculated with approximately 250 second-stage juveniles of *H. schachtii* per plant. At 14 days post inoculation, the number of fourth-stage developing females per plant root were counted and used as a measure of plant susceptibility. In our experiments, host-induced silencing of all four nematode parasitism genes led to a highly significant reduction of developing female numbers, giving up to 64% reduction as compared with wild type controls, with certain constructs showing more effective reduction than others. Additional parasitism genes, different promoters, and varying RNAi construct sizes, domains and combinations are currently being investigated.

- 11 Matthews, Benjamin F., Kyung-Huan Kim, So-Hyeon Baek, Seong-Kon Lee, Soo-Chul Park, Nadim Alkharouf, Margaret MacDonald, Veronica Martins, and Vincent P. Klink. **Identification of gene constructs lethal to the soybean cyst nematode.** USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD, and Rural Development Administration, Suweon, Korea.

The soybean cyst nematode (*Heterodera glycines*) is the major pathogen of soybean (*Glycine max*), and causes an estimated \$0.5-\$0.8 billion in losses per annum in the U.S. We identified genes expressed in soybean roots and by SCN before and during infection using microarrays containing 37,500 soybean and 7,500 SCN gene probes. We used laser capture microdissection (LCM) to isolate syncytia from roots to study gene expression specifically at the feeding site. This provided a group of soybean genes that may influence soybean resistance to SCN. Then we identified cyst nematode genes that may be useful in developing soybean resistant to nematodes using genetic engineering. The genes were selected by comparing the SCN EST database with genes from *Caenorhabditis elegans*. Genes were identified that would cause *C. elegans* death if mutated or silenced. We developed a system to rapidly transform soybean roots and screen DNA constructs to determine their effect on SCN survival. A series of transformation vectors, designated as pRAP, was constructed using Gateway (Invitrogen) technology to rapidly clone DNA without restriction digestion. Gene over-expression, gene silencing (RNAi), and promoter analysis can be studied using these vectors. The vectors contain the tetracycline resistance gene for easy selection of transformed *Agrobacterium rhizogenes* K599 and contain the gene encoding enhanced green fluorescent protein for easy selection of transformed roots. We transformed soybean roots with a series of vector constructs. The transformed soybean roots were challenged with soybean cyst nematodes and analyzed to determine if there were changes in resistance compared to control roots. This system also can be used for general studies in functional genomics using plant roots.

- 12 Moore, D. M., T. K. Balderson, W. E. Thomason, D. L. Holshouser, P. M. Phipps, and J. Eisenback. **Survey of nematode populations and prevalence in Virginia corn and soybean fields.** Virginia Cooperative Extension.

Reports of nematode damage in eastern Virginia corn and soybean fields have increased in recent years. Shifts in nematode populations are suspected with changes in farming practices that include: conversion to continuous no-till; repeated planting of varieties with single-gene resistance to soybean cyst nematode (SCN); less wheat in the crop rotation; changes in corn and soybean genetics; and conversion from in-furrow insecticide/nematicide treatments to seed-applied treatments in corn. In 2007, 174 samples (107 soybean/67 corn) were taken from the root zone during the growing season (corn: early July, soybean: mid-August to mid-September) in fields with historically poor growth. Production information along with GPS coordinates was gathered and recorded with each sample. Populations high enough to adversely affect yields and warrant control measures were found in over 47% of the soybean samples and in nearly 35% of the corn samples. Another 24% of the soybean and 37% of corn samples were identified with borderline-damaging populations. The most common nematodes found in soybeans were lesion, SCN, stunt, and root-knot (RKN). The most common found in corn were stubby root, lance, and sting. Twenty-seven percent of the soybean samples were found to have SCN. Race determination is in progress and results will be presented at the conference. In corn, 54% of the samples had stubby root nematode numbers and 40% had lance nematode numbers at levels high enough to be considered problematic. This survey suggests that research is needed to determine the level of susceptibility and tolerance of currently grown soybean varieties and corn hybrids to various nematodes. Of particular concern is the lack of RKN resistance in corn hybrids and the possible shift from race 3 SCN to other races that cannot be controlled with current resistant varieties. The survey will be repeated in 2008.

- 13 Niblack, T. L., A. Colgrove, and K. Colgrove. **SCN management in the real world: is knowing that it's there enough?** Department of Crop Sciences, University of Illinois, Urbana, IL.

In a recently published survey of Illinois, we found that more than 80% of soybean fields are infested with *Heterodera glycines*, the soybean cyst nematode (SCN) (Niblack et al., 2008, *Plant Health Progress* doi:10.1094/PHP-2008-0118-01-RS). This result was not surprising, as Workneh et al. (1999, *Phytopathol.* 89:204-211) had reported a similar percentage previously. The densities of these populations indicated infestation levels capable of causing significant yield loss. Further investigation of the Illinois SCN populations revealed that 70% of them were virulent (able to reproduce) on PI 88788, the source of resistance in more than 90% of SCN-resistant soybean cultivars available from year to year in Illinois. The Female Indices (FI) of the virulent populations on PI 88788 ranged from 10 to 98, and were more frequent in southern than in northern Illinois. Comparison of our results with those obtained more than a decade earlier by Sikora & Noel (1991, *J. Nematol.* 23:624-628) showed a distinct shift in SCN populations from avirulence to virulence on PI 88788. Conversely, SCN populations showed little or no virulence on PIs 548402 and 437654, sources of resistance that are available but not deployed widely in Illinois. A reasonable recommendation to farmers with PI 88788-virulent SCN infestations is to use resistant varieties carrying one of the other sources of resistance. In order to make efficient use of this recommendation, a farmer should know both the population density and virulence of SCN in a particular field. However, there is little evidence that the threat of resistance-breaking SCN populations is of concern. For example, in surveys of farmers and other agricultural professionals conducted in January 2007 and 2008 during SCN-related extension programs, over half (54%) of the respondents said that more than 75% of their fields were infested with SCN, and nearly two-thirds (61%) said that more than 75% of the soybeans they plant are resistant to SCN. When asked whether they sampled their fields for SCN population densities, however, more than half (53%) said "no." Although there is a high level of awareness of SCN as a yield-reducing factor among farmers and agricultural professionals in Illinois,

most farmers appear to believe that simply planting a soybean variety labeled "SCN-resistant" sufficiently reduces their risk of yield loss. Addressing this problem will require new approaches in applied research and educational programs.

- 14 Peng, Deliang¹, Shiqi OU^{1,2}, Maurice Moens^{3,4}, and S. Subbotin⁵. **Genetic variation and identification of *Heterodera glycines* using PCR with sequence characterized amplified region (SCAR) primers.** ¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; ²Jilin Agricultural University, Changchun, Jilin, China; ³Institute for Agricultural and Fisheries Research, Melle, Belgium; ⁴Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; ⁵Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA.

Genomic variation within 28 populations of *Heterodera glycines* from China and USA were estimated by a random amplified polymorphic DNA (RAPD) technique. Out of 28 primers, 12 primers produced bands in each population tested. A total 169 bands were scored. RAPD revealed ten common bands for all populations examined, which may be used to design a specific primer for the species. This RAPD analyses did not reveal any specific bands for race or population from different host plants or geographical origin. An unrooted tree obtained by the UPGMA method illustrated the divergence among 28 populations of *H. glycines* from China and USA. The range of similarities within Chinese populations varied from 87.5 to 96.5%, and between two American ones, 89.7%. The two USA populations (HaAr and HgNc) stood apart from Chinese populations; however, bootstrap analyses revealed relatively low support for this American clade (36%). Only four Chinese populations formed two separate clusters with high and moderate (>70%) statistical support. A single RAPD marker, OPA06477, species-specific to *Heterodera glycines*, was identified. After sequencing the RAPD-PCR products, primers of 24 nucleotides were designed to complement the terminal DNA sequence of the DNA fragments. This resulted in one pair of species-specific primers that were used to amplify the sequence characterized amplified regions (SCAR). The developed sets of SCAR primers were successfully used in straightforward, fast and reliable PCR assays to identify *H. glycines*. The SCAR markers can be amplified from DNA from single second-stage juveniles and females. This is the first time SCAR primers have been combined with universal primers D2A and D3B in order to avoid false negative results.

- 15 Robbins, Robert T., and Terry Kirkpatrick. **Rotation of three soybean cultivars with different cyst nematode resistance reactions.** University of Arkansas, Department of Plant Pathology, Fayetteville, AR.

The cytological reaction of resistant soybean are different for Forrest and Peking than for Bedford (derived from Peking x PI 88788) according to Kim and Riggs (1992). A third resistance reaction reported for PI 437654 by Mahalingam and Skorupska (1996) is different than the reactions of Forrest-Peking or Bedford. All three are different than that of the susceptible cultivar Essex. In our study cultivars derived for Peking (Manokin = M), PI 88788 (AG5501 = AG), and PI 437654 (Anand = AN) are rotated in all six possible combinations (AG-MI-AN; AG-AN-MI; MI-AN-AG; MI-AG-AN; AN-AG-MI; AN-MI-AG). Checks are these three resistant cultivars in succession (AG-AG-AG; MI-MI-MI; AN-AN-AN) and the SCN susceptible cultivar Hutcheson (H-H-H). The field studied was naturally infested with SCN race 6 at the beginning of the study. Each year samples are taken at planting and at harvest and the SCN egg counts are determined. For the study four replications of each treatment (40 plots) are used. Plots are 100 ft long and eight rows in width (30 inch centers = 20 feet). The 2008 season will be the sixth year of this study. A similar study of Roundup Ready soybean varieties was started in 2006 with plots 50 ft long. The varieties used include resistance types derived from PI-88788 (AG5501), and PI-437654 (one from Anand (SO2-3934-RR), one from CystX (HBK R 4946 CX)). No

Peking resistance type cultivars are available in Roundup Ready cultivars. The rotation is as above except with Cystex (HBKR4946 CX) substituting for Peking (Manokin); SO2-3934-RR for Anand; and HBK 4924 (RR) for Hutcheson. At the end of the 2008 growing season we should have data showing how effective these rotations are in preventing SCN population number buildup and in the prevention of formation of, or shift to, different SCN races.

- 16 Schroeder, N. E., and A. E. MacGuidwin. **Mortality and behavioral effects in J2 *Heterodera glycines* due to exposure to isothiocyanate compounds.** University of Wisconsin, Madison, WI.

We examined the toxic effects of the plant-derived compounds, allyl-, benzyl- and phenyl isothiocyanate, on J2 *Heterodera glycines* using both mortality and behavioral measurements. Our behavioral assay consisted of examining tracks on agarose produced by nematodes exposed to isothiocyanates. We examined five behavioral parameters using the tracks, including the ability to move, the distance from start to stop location, the total distance traveled measured by a quadrat method, and the average wavelength and amplitude of track waveforms. We found significant differences among compounds in the concentration required to effect nematodes, using both lethality and behavioral measurements. The concentration for each compound required to effect behavior was significantly lower than for lethality. For a given compound, each of the five behavioral parameters studied resulted in similar estimates of isothiocyanate concentration required to inhibit the measured behavior. Both lethality and behavioral measurements were used to show whether nematodes in a quiescent state displayed decreased sensitivity to isothiocyanates compared with actively moving nematodes. Lethality measurements revealed that quiescent nematodes were significantly less sensitive to the isothiocyanates than active nematodes. All behavioral measurements for exposure to benzyl- and phenyl isothiocyanate showed significant differences in sensitivity between quiescent and active nematodes. However, significant differences between quiescent and active nematodes were only found using one of the five behavioral measurements for allyl isothiocyanate. These results expand our knowledge of the effects of plant-derived compounds on an important plant-parasitic nematode and illustrate the effects of behavioral quiescence on nematode sensitivity to exogenous toxins.

- 17 Tylka, Gregory L., Gregory D. Gebhart, and Christopher C. Marett. **Field evaluation of SCN-resistant soybean varieties in Iowa.** Department of Plant Pathology, Iowa State University, Ames, IA.

Soybean varieties that are resistant to the soybean cyst nematode (SCN) are an important management tool, and there are hundreds of resistant varieties available. The varieties can vary considerably in yield and their effects on SCN population densities. The overall objective of the Iowa State University SCN-resistant Soybean Variety Trial Program is to assess and compare the agronomic performance and control of SCN population densities provided by SCN-resistant soybean varieties in SCN-infested fields throughout Iowa. Each variety is grown in four replicate plots at each experimental location (there are 9 to 11 sites statewide annually). Plots are four rows wide, spaced 30 inches apart, and 17 feet long. Experiments are organized in three geographic districts (northern Iowa, central Iowa, and southern Iowa), and there are at least 3 experiments located in each district every year. In 2007, 45 SCN-resistant soybean varieties were evaluated in the northern Iowa district, 34 were evaluated in the central Iowa district, and 27 were evaluated in the southern Iowa district. Soil samples are collected from each four-row plot at planting and analyzed to verify the presence of SCN in every plot and to determine the initial SCN population density. An HG type test is conducted on the SCN population at each location using the soil samples collected at planting to determine the parasitic capabilities of the SCN population present in each field. At harvest, another soil sample is collected from each plot, and SCN population densities are determined to assess the SCN reproduction that occurred during the season. Commonly grown SCN-susceptible varieties are included in each of the variety trials. All plots are end trimmed to 14 feet long

during September. The date on which each variety becomes mature is noted, and just prior to harvest, average plant height and lodging (1=all plants fully erect, 5=all plants flat) are assessed for each plot. The center two rows of each four-row plot are harvested with a plot combine, total seed weight per plot and seed moisture are determined, and total plot seed weights are converted to bushels per acre. The agronomic data and final SCN population densities are averaged for each variety and compiled into tables for an annual report. Additionally, yield and SCN reproduction results for each variety are presented in a bar graph for each location. Yield is represented by the length of the bars, and the least-significant-difference value ($P=0.05$) for yield.

- 18 Tylka, Gregory L. **Distribution of the soybean cyst nematode in Iowa: 1995-96 versus 2007.** Department of Plant Pathology, Iowa State University, Ames, IA.

The soybean cyst nematode (SCN) is widely distributed throughout Iowa. SCN was first discovered in Iowa in a field in Winnebago County in 1978. Currently, the nematode has been found in 96 of 99 Iowa counties (all except Allamakee, Ida, and Sac Counties). In 1995 and 1996, a survey of Iowa was conducted in collaboration with the USDA National Agricultural Statistics Service (NASS) and supported by soybean checkoff funds. Fields were randomly selected for sampling by NASS personnel. In August and September of each year, NASS field personnel collected soil samples in a zig-zag pattern from a randomly selected area of each chosen field. Samples were shipped via overnight delivery to Iowa State University, where cysts were extracted from a 100-cc subsample of each soil sample, and then eggs were extracted from cysts and observed microscopically. There were 399 Iowa fields sampled in 1995-96, and 74% of the sampled fields were found to be infested with SCN. Also, growers provided information about tillage practices that had been used in the sampled fields, so the data were examined for effects of tillage on SCN infestations. Significantly ($P=0.02$) fewer no-till fields (64%) were infested with SCN in the 1995-96 survey than tilled fields (77%). In 2007, a follow-up, three-year survey of Iowa was initiated using the same random field selection procedure in collaboration with NASS and supported by soybean checkoff funds. Preliminary results of analysis of samples obtained in 2007 indicate that SCN was present in 71% of the 205 randomly selected fields sampled. The percentage of tilled and no-till Iowa fields sampled in 2007 that were infested with SCN were nearly identical (72% for no-till, 73% for tilled fields). Based on the preliminary results obtained from samples collected in 2007, it appears that the distribution of SCN in Iowa has not changed much but significant differences in the incidence of SCN infestations between tilled and no-till fields appear to have disappeared in the past decade.

- 19 Wei, Zhongmin, and Ned M. French. **Harp-N-Tek[®], a new technology for plant health and crop production.** Plant Health Care Inc., Bothell, WA, and Little Rock, AR.

Harp-N-Tek[®] products are based on a proprietary harpin protein technology and manufacturing process. Harpin proteins are a class of nontoxic proteins derived from naturally-occurring microorganisms. Harpin proteins activate signaling receptors present in all plants designed to specifically detect the presence of harpins. This warning signal is transmitted throughout the plant and turns on the plant's intrinsic ability to protect itself by deploying both growth and defense responses. The outcome of this signaling is expression of at least 124 plant genes involved in the hypersensitive response, plant growth enhancement, and activation of an induced systemic defense response. When applied to crops, Harp-N-Tek products provide these harmless yet potent signal-inducing harpin proteins, which trigger beneficial responses designed to protect plants, to help plants grow through stress, and to enhance overall level of plant health.

Harpin-induced plant responses start with a hypersensitive response followed by increased ion exchange and oxidative burst through cell membranes which induces production of active oxygen and K/Cl effluxes and Ca/H influxes. Subsequent plant responses include induction of salicylic acid accumulation

locally and systemically, activation of multiple plant defense pathways including the salicylic acid pathway, jasmonic acid pathway, phenylalanine ammonia-lyase (PAL) mediated pathways; and each of these responses leads to expression of a substantial number of genes. Genes up or down-regulated are generally associated with signal transduction pathways related to specific functions including protein, sugar, and starch transport, cell growth, plant development, and flower induction and fruit set as well as defense and stress resistance. Harpin-induced responses are initiated through the binding process between harpin and HrBP1, a putative harpin receptor protein known to exist in all crops tested. Through numerous laboratory, greenhouse, and field trials conducted across multiple crops, benefits from the use of Harp-N-Tek based products have been found to include nematode egg suppression, increased root biomass, improved nutrient utilization, and increased yield and quality.

Harp-N-Tek has been advanced to the marketplace in the form of various products such as N-Hibit® Gold CST seed treatment and ProAct™ foliar spray, which have been granted registration by the Environmental Protection Agency. For more EPA-related information, refer to "*Harpin $\alpha\beta$ protein (006506) Fact sheet.*" The mode of action of Harp-N-Tek products is unique among plant health and protection products. Standard plant protection products are designed to directly attack pests in the plant's external environment using either an outside only (non-systemic) or an outside-in (systemic) mode of action. A natural "inside-out" growth and defense response is activated by Harp-N-Tek products. Harp-N-Tek products complement these outside-only and outside-in modes of action and can be used in conjunction with most standard plant protection products to gain the added advantage of the inside-out mode of action. The products not only suppress various diseases including nematode infection but also significantly enhance growth and increase the marketable yield.

- 20 Welacky, T.W.¹, S. J. Park¹, and A. Tenuta². **Soybean cyst nematode adaptation to Ontario pulse crops.** ¹Agriculture & Agri-Food Canada, Harrow, ON; ²Ontario Ministry of Agriculture and Rural Affairs, Ridgetown, ON.

In southwestern Ontario, soybean cyst nematode (SCN), *Heterodera glycines*, has been a major pathogen of soybeans since the late 1980's. Evaluations of edible bean types and varieties rotated with soybeans in the mid 1990's indicated very little cyst reproduction on roots. In the past 4-5 years, extension and agribusiness agronomists anecdotally reported increased root populations of SCN on various pulse crops. Evaluation of field SCN cyst reproduction on roots of pulse crops, indicated that white beans and pinto beans had 20-35%, and dark and cranberry kidneys 35-60%, as many SCN cysts as susceptible soybeans during 2005 to 2007. The 3 year average count of eggs per susceptible soybean root was 4,790. Additional, greenhouse bio-assays of the commercial varieties demonstrated higher root infestations. For example, numbers of cysts on white beans were 40-70%, pinto beans were 25-50%, and dark and cranberry kidneys were 90-100%, of the number of cysts on susceptible soybean roots. This suggests that changes in the virulence of local populations of SCN have occurred over the past 10 years and that integrated management practices to reduce reproduction of SCN damage in edible beans are warranted in Southwestern Ontario.