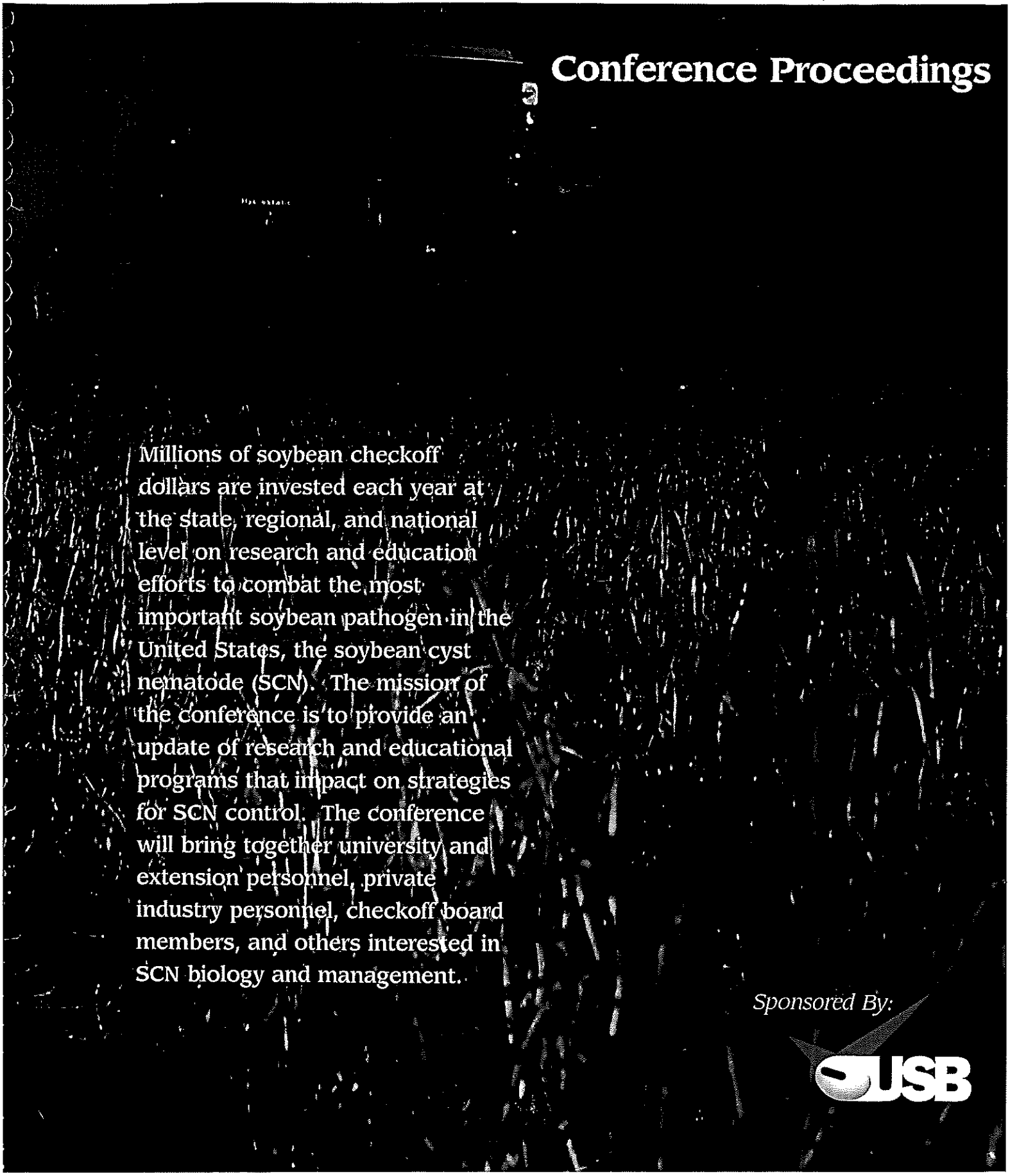
National Soybean Cyst Nematode Conference

JANUARY 7-8, 1999 SHERATON WORLD RESORT • ORLANDO, FLORIDA

Conference Proceedings



Millions of soybean checkoff dollars are invested each year at the state, regional, and national level on research and education efforts to combat the most important soybean pathogen in the United States, the soybean cyst nematode (SCN). The mission of the conference is to provide an update of research and educational programs that impact on strategies for SCN control. The conference will bring together university and extension personnel, private industry personnel, checkoff board members, and others interested in SCN biology and management.

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NATIONAL SOYBEAN CYST NEMATODE CONFERENCE

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Soybean Disease Loss Estimates for the United States in 1996-1997

Allen Wrather, University of Missouri-Delta Center, Portageville,
and Ward Stienstra, University of Minnesota, St. Paul

Diseases have reduced soybean production in the United States, and they will continue to reduce production unless research and education efforts are expanded to provide more effective controls for the diseases causing the greatest yield losses. Scientists must focus on the management of soybean diseases that cause extensive losses, especially when funds for research are limited. In order to focus funding the research efforts on the most insidious diseases, a clear picture of the magnitude of losses each disease has caused is necessary. A time-lapse picture, covering several years, is need because the importance of individual diseases can vary over time. The objective of this project was to determine the major soybean disease problems in the United States during 1996-1998. The specific goal was to estimate the soybean yield losses caused by individual diseases in each state for each of these years. Data for 1996-1997 have been collected and are being collected for 1998. The percent yield loss estimates due to individual diseases were solicited each year from scientists within each state. Methods used to develop the estimates in each state include field surveys, plant disease diagnostic clinic samples, variety trial data, information from field workers and university extension staff, research plots, grower demonstration plots, and private crop consultant reports. Diseases reduced soybean production in both the Southern and Northern United States during 1996 and 1997. The greatest losses across the United States in 1996 and 1997 were caused by soybean cyst nematode. This pest reduced soybean production in the United States 213.8 million bushels in 1996 and 218.9 million bushels in 1997. Yield losses due to this nematode were greater in the Northern than Southern United States. Other diseases that caused massive reductions in soybean production were Phytophthora root and stem rot, 40.5 million bushels in 1996 and 53.6 million bushels in 1997, Sclerotinia stem rot, 22.6 million bushels in 1996 and 35.2 million bushels in 1997, seedling diseases, 31.3 million bushels in 1996 and 24.5 million bushels in 1997, and brown stem rot, 30.8 million bushels in 1996 and 24.0 million bushels in 1997. All of these data are on the World Wide Web at *http://aes.missouri.edu/delta/research/soyloss.htm.*

Management of Soybean Cyst Nematode with Cultural Practices

Terry L. Niblack
Department of Plant Pathology, University of Missouri-Columbia

Researchers and many soybean producers have proven that soybeans can be produced profitably in fields infested with the soybean cyst nematode. Because the nematode does not always cause symptoms, even when yields are reduced 30%, infestations are easy to overlook. Even when symptoms are visible, they are frequently misdiagnosed because they resemble symptoms of other problems. The most important first step in minimizing losses due to the soybean cyst nematode is to confirm its presence through soil samples submitted to qualified laboratories. Once the diagnosis is confirmed, the three best strategies to reduce losses due to soybean cyst nematode are: 1) rotate with non-host crops; 2) rotate with resistant cultivars; and 3) rotate with susceptible or tolerant cultivars when nematode populations are reduced. The length of rotations must be determined by their effects on population densities–a site-specific characteristic. Nematode levels should be monitored periodically to determine the effects of the management plan, but soybean yields will tell the story of a successful plan. Any practices that relieve stress in soybean cyst nematode-infested fields will lessen the detrimental effects of the nematode, such as good weed, fertility, and water management, for example. Cultural strategies have been tested for use in integrated crop management programs, such as delayed planting, use of early-maturing cultivars, no-till, planting in standing wheat stubble, intercropping, and others. The effects of these strategies tend to be site-specific; therefore, they cannot be generally recommended for management of soybean in infested fields. Nonetheless, many specific practices have been tested in locations relevant to most soybean farmers. For this reason, local advisors should always be consulted so that the most effective strategies can be chosen to fit an individual farm management plan.

Biological Control of Soybean Cyst Nematode

Robert D. Riggs
Department of Plant Pathology, University of Arkansas, Fayetteville

Soybean cyst nematode causes serious damage to soybean throughout the soybean-growing area of the United States of America and in most other areas where soybean is grown. Attempts to eliminate this pest from the soil have been unsuccessful, therefore management strategies must be designed to maintain population levels below the damage threshold to minimize yield losses to this pest. Biological control, management through the use of naturally-occurring parasites or predators of soybean cyst nematode, has shown some promise but much work remains to be done to make it a reality. Observations of apparent suppression of soybean cyst nematode populations by a fungus in infested fields in Arkansas encouraged research on this tactic. A fungus called ARF18, because it does not produce spores and cannot be identified, was isolated from soybean cyst nematode eggs and second-stage juveniles from an Arkansas soybean field. AFR18 parasitizes the eggs, second-stage juveniles, and immature and mature females and can penetrate directly through the cyst wall. In greenhouse tests, nematode population increases were reduced by as much as 98%. When the fungus was added to outdoor microplots about 7.5 in. x 13 in. x 24 in deep, soybean cyst nematode population levels have been suppressed with as little as 25 lbs. of the fungus mycelium per acre. Some success has been attained in field plots but not at levels that would be economical. Other studies at Arkansas involve bacteria isolated from cyst and from soybean root surfaces or the soil surrounding the roots. No field studies have been done but laboratory and greenhouse studies indicate that some of these bacteria can aid reproduction of soybean cyst nematode whereas others suppress nematode egg hatch and juvenile development. The bacteria that are most effective in reducing cyst nematode egg production are chitin-degrading. In two greenhouse tests five of the isolates significantly reduced egg production. Additional research is being conducted at the Universities of Illinois, Minnesota, and Missouri in the northern USA and in Georgia in the southern USA. In a Minnesota survey, about 55% of the soil samples checked contained cysts (29-89%) that were colonized by fungi. However, fungi were isolated from only about 3.5% of the cysts and 1.2%of the eggs from those samples. About 70 species of fungi were isolated, and of 40 that were tested in the lab, only three parasitized eggs at a significant level.

Chemical Control of the Soybean Cyst Nematode

Gregory L. Tylka
Department of Plant Pathology, Iowa State University, Ames

Numerous effective and relatively inexpensive nematicides were available for management of plant-parasitic nematodes, including the soybean cyst nematode (SCN), in the 1960s, 1970s, and 1980s. Unfortunately, many of these nematicides are no longer produced and sold in the United States, and several other currently available nematicides are not labeled for use on SCN. The three nematicides that currently are labeled for management of SCN in the United States are aldicarb, oxamyl, and 1,3-dichloropropene. Despite the availability of these nematicides, chemical control is not a widely used management strategy for SCN, primarily because of the relatively high cost of the nematicides in comparison to the per-acre value of the soybean crop. Research funded by the soybean checkoff is underway to develop new compounds for management of SCN. In general, these compounds can be grouped into two classes – natural plant compounds and synthetic analogs of natural plant compounds.

In 1982, Japanese researchers reported extracting from the roots of kidney bean a complex, natural compound, called glycinoeclepin A, that stimulated hatching of SCN eggs at extremely low concentrations (parts per trillion). Hatch-stimulating compounds like glycinoeclepin A could be effective in decreasing SCN numbers in soil if applied to infested fields during years when a nonhost crop, such as corn, is planted. SCN juveniles that hatch from eggs in the absence of a host crop will die from starvation, parasitism, or predation. Conversely, inhibiting SCN egg hatching in SCN-infested fields planted with soybeans could reduce the build-up in SCN numbers, and likely would increase soybean yields, by decreasing early season infection of soybean roots by SCN. It is impractical and cost prohibitive to obtain sufficient quantities of natural glycinoeclepin A for field-scale use. Several researchers have synthesized glycinoeclepin A or structurally similar analogs, but elaborate procedures and expensive materials were needed to produce small quantities of the compounds. Research has been ongoing at Iowa State University since 1990 to synthesize glycinoeclepin A and related compounds using inexpensive materials and reactions that can be industrialized. To date, no consistent hatch-stimulating compounds have been synthesized, but numerous glycinoeclepin A analogs have been discovered that inhibit SCN egg hatching at concentrations feasible for field application. Work to develop these hatch-inhibiting compounds into commercial products for management of SCN in ongoing.

Plants produce defense chemicals called secondary metabolites. Some of the secondary plant metabolites are effective insecticides, and one class of these compounds, the glucosinolates, holds particular promise for control of nematodes. Glucosinolates occur in crambe, rape seed, canola, mustards, and other plants. Research was initiated in 1995 at Iowa State University to explore the development of glucosinolates into economical, effective nematicides for SCN. A glucosinolate-like compound, cyanohydroxypropene or CHP, and several closely related compounds have been discovered to be extremely effective and irreversible inhibitors of SCN egg hatching. Also, the inhibitory effect of CHP and related compounds is volatile; eggs incubated in water but placed near containers of CHP or related compounds still are irreversibly inhibited from hatching. The volatile and irreversible nature of the SCN egg hatch inhibition indicates that the compounds may be effective for nematode control in soil environments.

Management of SCN through Conventional Breeding for Resistance – Midwest Perspective

Brian W. Diers, Prakash Arelli, and Silvia R. Cianzio
University of Illinois, University of Missouri, and Iowa State University

Soybean cyst nematode (SCN) is the most important soybean disease in the midwest. Because of the importance of SCN, most midwestern soybean breeders in the public sector are actively developing cultivars with resistance to the pathogen. These efforts have led to the successful development of SCN resistant varieties that have helped reduce losses from the disease. Of the 16 SCN resistant cultivars released by the public sector in the midwest during the 1990's, 14 have SCN resistance from PI 88788. Most of these public breeders are continuing to use PI 88788 as the main source of resistance in their cultivar development programs because the highest yielding lines have been developed from this source. The heavy dependence on resistance from PI 88788 poses a risk that population shifts in SCN may result in this resistance breaking down. In addition, genetic mapping studies indicate that other sources of resistance being used by breeders (PI 207332, Peking and PI 437654) may have major resistance genes in common with PI 88788. Because of a concern about the reliance on few SCN resistance genes, research is ongoing to determine if new plant introductions (PIs) have new SCN resistance genes. Populations developed from crossing these PIs are being evaluated for both genetic markers and SCN resistance in the greenhouse. Progress to date shows that even these new PIs have many of the same resistance genes as our current resistance sources suggesting that the search for new sources of resistance should be extended to other related species such as *Glycine soja*.

Management of SCN through Conventional Breeding for Resistance - Southern Perspective

Lawrence D. Young, USDA Agricultural Research Service, Jackson, TN

Soybean cyst nematode races change over time when resistant varieties are planted frequently. Therefore, the genes for resistance deployed in soybean varieties must change to effectively combat the nematode. Race 3 was common throughout the southern United States when the nematode was first discovered. Peking was the first source of resistance used in varieties such as Pickett and Forrest to control the nematode. After these resistant varieties were planted, other races such as races 6, 9, and 14 became common. Plant Introduction (PI) 88,788 was used to develop new resistant varieties such as Bedford. In recent years, race 2 has become a frequently encountered race after varieties resistant to race 14 have been planted for several years. Farmers need varieties with resistance derived from PI 437654, such as Hartwig and Delsoy 5710, to plant in fields infested with race 2 or race 5. Although Hartwig has only been planted in a limited number of fields and Delsoy 5710 was just released, we have selected nematode populations that parasitize these varieties. Several of these selected populations were derived from farmers' fields. Thus, it is reasonable to expect nematode populations to develop in farmers' fields that will parasitize varieties such as Hartwig after they have been planted for several years. More genes for resistance must be found to combat these nematode populations of the future. Because resistance genes are rare, cultural practices to slow the change in nematode race should be utilized to lengthen the time a set of resistance genes are effective. Rotations involving resistant varieties with nonhost crops, such as corn, cotton, and grain sorghum, can increase the time required for a change in nematode race and also increase yield. Susceptible soybean can also be included in these rotations. Blends of resistant and susceptible soybeans may also be effective in increasing the longevity of resistance genes. Other methods of controlling the nematode should also be evaluated and used when practical in order to preserve the effectiveness of resistance genes. Although there are limitations on the use of resistance to manage the soybean cyst nematode, planting resistant varieties have given large economic returns. During a six-year period, planting Forrest soybeans, resistant to race 3, increased farmers' income by more than $400 million due to control of the nematode. The experience of southern farmers with the nematode can be valuable to others in their use of resistance to combat this nematode.

Breeding For Soybean Cyst Nematode Resistance at Novartis Seeds, Inc.

Roger L. McBroom
Novartis Seeds, Inc., St. Joseph, IL

Novartis Seeds, Inc. has had a long-term traditional breeding program for cyst nematode resistance. The southern program traces its roots back to the work started by Josh Stanton at Coker Pedigreed Seeds, one of the earliest private soybean research programs. We are working with several sources of resistance: Peking, through numerous southern lines, PI 88.788, mostly through Fayette or Bedford, PI 90.763, through a few southern experimental lines, PI 89.772, through LN89-5717, and PI 437.654, mostly through Hartwig. Of the lines being sold for 1999, three southern lines are pure Peking type, one northern line is pure 88.788, and all the rest are mixed for Peking and 88.788 resistance. Our traditional programs have consisted of making crosses, carrying the populations to the F5 or F6 by single seed descent, pulling single plants, and screening for resistance in greenhouse or field, either as they go into a progeny row or concurrently with the first yield trial. The amount of total breeding effort going into cyst nematode resistance varies from nearly 100% in the south to 20% in the north. This emphasis is based both on severity of the problem and demand for cyst resistant products, both of which are moving targets. The addition of molecular markers and marker-assisted selection should allow us to make faster progress in improving yield of cyst resistant material in the north. Unless we stop making susceptible by susceptible crosses or cyst nematode pressure becomes the yield-limiting factor, I wouldn't expect the resistant material to fully catch up with susceptible material. I say that because as yet there is no reason to believe we will make a faster rate of gain within cyst resistant populations than within cyst susceptible populations. I think it is much more likely that future novel genes for cyst nematode resistance will be discovered and incorporated into higher yielding soybeans faster than we can make progress with markers and native resistance.

Developing Soybean Cyst Nematode Resistant Cultivars– One Company's Strategy

Tom Corbin
Pioneer Hi-Bred International, Inc.

Soybean cyst nematodes (SCN) are receiving more attention than any other soybean pest. Resistant cultivars are being developed from several sources of resistance. PI88.788 (Fayette) is the predominant source of resistance in our breeding programs. Peking and PI437.654 (Hartwig) are also being used as sources of resistance but the effort is smaller than the Fayette source. The source of resistance needs to be matched with the races endemic to an area for successful cultivar development. A race survey can help determine the proper source of resistance to be working for an area. Rotating sources of resistance is not an option in some maturity groups. MG III cultivars are almost exclusively from the Fayette source of resistance. Developing new products with resistance to SCN has changed in the last few years. The breeding process involved digging up the roots of a line and scoring it for the presence of the cyst nematodes. Molecular markers are beginning to replace digging as a screen for resistance. A population of plants can be screened in the lab for the resistant genes and then resistance is verified in the field, greenhouse, or growth chamber by challenging the roots with SCN in the soil. Currently all of the MG III SCN breeding lines are being screened in the lab for the presence of the linkage group G marker. This marker is tightly linked to the resistance gene in this particular region of the chromosome. The goal is to develop cultivars for all of the growing areas Pioneer services.

Breeding for SCN Resistance: Two Decades of Progress

Cecil Nickell[1], Greg Noel[2], and Richard Bernard[1]
[1]Department of Crop Sciences and [2]USDA,ARS, University of Illinois, Urbana

The research to develop soybean cyst nematode (SCN), *Heterodera glycines*, resistant soybeans at the University of Illinois began in the 1970's. Initially, the research was a USDA, ARS effort with greenhouse evaluations done at Portageville, Missouri. G. R. Noel was transferred by the USDA to Urbana in 1978 and the University of Illinois hired C. D. Nickell in 1979 to complement the program and to maintain the continuity of the soybean breeding effort upon the retirement of R. L. Bernard (former USDA breeder). Cooperative effort between the soybean breeding programs and the USDA nematology program at the University of Illinois has resulted in the release of 17 SCN-resistant varieties and four SCN-resistant germplasm lines. The releases have ranged from maturity Group IV to maturity Group I and have utilized resistant germplasm from the plant introductions (PI), PI88.788, PI89.772, PI90.763, PI209.332, and PI437.654 and the varieties 'Cloud' and 'Peking'. The value of these releases to soybean producers has been in the hundreds of millions of dollars as evidenced by production of certified seed by the Illinois Crop Improvement Association. For example in 1996, 46% of the certified seed acreage in Illinois was planted to varieties developed by the program. The variety 'Fayette', which was released in 1981, has been the most widely used source of resistance in both public and private breeding programs in the midwest. The year of release, maturity group, source of resistance, and reaction to various races of SCN of each variety and germplasm line are provided.

Chinese Origin of Soybean Cyst Nematode

Greg Noel[1] and Zonglin Liu[2]
[1]USDA, ARS, and [2]Department of Veterinary Pathobiology,
University of Illinois, Urbana

The soybean cyst nematode (SCN), *Heterodera glycines*, was first observed in China ca. 1880 and at approximately the same time in Japan. In 1955 SCN was reported for the first time in the United States when it was found in a soybean field in North Carolina. In 1965 infestations were identified in several counties in Arkansas, Illinois, Kentucky, Mississippi, Missouri,and Tennessee in the central Mississippi Valley and in North Carolina and Virginia. Research has shown that SCN could not have spread so widely and increased to produce visible symptoms within 10 years. The infestatons were due to populations that had been in the fields for many years. Did these populations result from recent introductions into the United States or is SCN native to North America? Experiments were done to determine if this question could be answered. The founder effect is a well established evolutionary phenomenon in which colonizing populations that arise from progenitor populations exhibit reduced heterozygosity and reduced genetic variability, including loss of rare alleles. The founder effect has been demonstrated in a wide range of plants and animals. Much of the research has utilized analysis of enzymes. Allozymes are enzymes that are gene products that provide molecular markers for analyzing single locus variation in populations. Individual females from 19 populations of *H. glycines* from China, Japan, and the United States were analyzed for esterase allozyme polymorphism. Eight esterase electrophoretic phenotypes were resolved. Four putative loci, *est-1*, *est-2*, *est-3*, and *est-4*, were identified, having one, one, two, and one allele, respectively. The four loci expressed six genotypes in the four Chinese populations. Loci *est-2, est-3,* and *est-4* were identified in five Japanese populations and expressed five genotypes, whereas only loci *est-2* and *est-3* were identified in 10 populations from the United States and expressed four genotypes. Putative alleles at each locus were defined as characters for data analysis. Phylogenetic analysis using parsimony (PAUP) was utilized to determine relationships among the 19 populations. More loci and alleles in populations from China and phylogenetic similarities among populations from Japan and the United States are consistent with a founder effect resulting from dissemination of progenitor *H. glycines* from China to Japan and subsequent introductions of founder populations from Japan to the United States. The similarities between populations from Japan and United States indicate that infestations in the United States probably originated from several colonizations from Japanese sources. The founding of multiple colonies is consistent with known importation of soil from Japan to obtain *Bradyrhizobium japonicm* to nodulate soybean in the United States. The bacterium is not native to North America, but originated in Asia . Soil was imported from Japan in the late 1800's to obtain the bacterium. In order to conduct experiments, researchers shipped soil to various experiment stations and spread soil in research plots. Soybean growers would spread "inoculated" soil obtained from neighbors in order to nodulate their soybeans. Thus, both researchers and growers may have inadvertently spread SCN for many years before it was discovered in the United States.

EFFECT OF RESISTANCE SOURCE ON SCN POPULATIONS

B. Holmes, Asgrow Seed Co., Oxford, IN;
T.C. Todd, W.T. Schapaugh, Depts. of Plant Pathology and Agronomy,
Kansas State University, Manhattan, KS

Differential soybean response to SCN race and concern over selection pressure are the reasons for the prosposal to rotate soybean cultivars derived from different sources of resistance as a means of managing SCN population density and stabilizing race. Research was conducted to evaluate rotations of resistance source for management of SCN race structure and field population density. Yield performance of soybean cultivars with different sources of resistance (grown continuously and in rotation) in the presence of genetically diverse SCN populations was evaluated also. Treatments were applied in 1993-1998 and consisted of 9 rotations of 3 soybean cultivars (Delsoy 4210, Delsoy 4500, and Hartwig), each with a different source of resistance, plus a continuous susceptible cultivar (Flyer) at 3 locations, each infested with a different race of SCN. Nematode race structure (female index), nematode population density (Pf/Pi), and soybean yield (bu/a) were measured. Female indices on Peking remained stable or declined; female indices on PI 88788 steadily increased across all locations in the presence of Delsoy 4210 (PI 88788), resulting in a race shift at the race 3 location after only 2-3 years; the effect of resistance source rotations were not consistent across locations, despite lower overall indices for the Delsoy 4210/Delsoy 4500 rotations. Rotations with Hartwig resulted in population densities that were barely detectable in the years that Hartwig was grown. Egg densities increased on Delsoy 4210 within 1 year of the observed race shift; Pf/Pi values were greater for Delsoy 4210 in rotation vs. continuous planting. Yield loss in Delsoy 4210 was observed for the first time at the race 3 location in 1998; rotations did not affect soybean yield performance. Short-term (2 year) rotations of resistance sources are ineffective for managing SCN race structure and yield loss. Longer term rotations may be effective. The benefit of less frequent selection pressure from alternating cultivars with different sources of resistance is offset by density-dependent population increases. Race shifts can occur rapidly on at least some PI 88788 sources of resistance but subsequent yield loss may be delayed for several years until damaging population levels are reached.

Effect of Row Spacing on the Eclosion of SCN Eggs

A. E. MacGuidwin[1], C. G. Grau[1], E. S. Oplinger[2]
Departments of Plant Pathology[1] & Agronomy[2], University of Wisconsin, Madison

Increasing soybean plant populations in fields by narrowing row spacing has been demonstrated to increase yields in Wisconsin if foliar disease pressure is low. The response of soybeans to different row spacings in SCN-infested fields is one of the research objectives in the current SCN research project funded by the North Central Soybean Research Program. In 1997 soybeans grown in narrow (7.5-inch) rows supported higher ($P = 0.05$) SCN population densities than beans grown in wide (30-inch) rows at our research site in East Troy, Wisconsin, even though plant populations were essentially the same for both row spacings. These data suggest that the population dynamics of SCN can be influenced at the field level by the spatial and temporal attributes of root growth. We tested the hypothesis that SCN egg hatch is affected by row spacing by comparing the abundance of hatched second-stage juvenile (J2) SCN from samples collected in- and between- rows of 'Hardin' soybean grown in 7.5 versus 30-inch rows. For the first month after planting there were more ($P = 0.05$) hatched J2s "in" versus "between" the rows, regardless of row spacing. During June new egg production was negligible, but by July 8th egg production had commenced and there were more ($P = 0.01$) SCN in the 7.5-inch plots. There was a significant ($P = 0.05$) yield advantage for planting soybeans in narrow rows, but our data suggest that increasing the number of rows in a soybean field contributes to greater egg hatch in the first month after planting, which in turn contributes to increased SCN population densities over the course of the season.

Effect of Various Crop Sequences on SCN Population Densities and Soybean Yield

Donald Hershman, Paul Bachi and Debbie Morgan
Department of Plant Pathology, University of Kentucky, Princeton

SCN-resistant soybean varieties are widely deployed in SCN management programs in Kentucky. However, few soybean farmers follow the University's recommended crop sequence of corn (year 1); SCN-resistant soybean (year two); corn (year three); SCN-susceptible soybean (year 4). Instead, most farmers plant a resistant soybean variety every other year in rotation with corn. Producers follow this sequence because: 1) newer SCN-resistant soybean varieties in maturity groups IV and V are high-yielding; and 2) farmers find the risk of planting a SCN-susceptible variety unacceptable. We feared that cropping a SCN-resistant variety in alternate years may lead to a breakdown in host resistance and, thus, may not be sustainable in the long term. However, data were not available to either support or allay our concerns. Thus, a six-year study was established in Christian County, Kentucky in 1994 to determine the effects of this and three other crop sequences on SCN egg densities and associated soybean yields. Five years of the six-year study have been completed. Results, thus far, indicate that SCN resistance begins to break down the third time the same resistant variety is cropped : 1) continuously; 2) alternately with corn; or 3) alternately with a different SCN-resistant variety. However, even after five years of continuous cropping, SCN egg densities associated with the resistant varieties Manokin and Pioneer 9551, were significantly lower than the egg density associated with the susceptible variety, Essex. Yields of plots did not follow the same pattern. When compared with the yield of Essex, yields of the two resistant varieties were superior, except in the fifth year under continuous cropping. In that instance, neither resistant variety yielded significantly more than Essex. Regarding the crop sequence recommended by the University of Kentucky, data clearly show that SCN populations can be effectively managed, and that a SCN-susceptible variety grown once every four years can produce yields which compare favorably with yields of SCN-resistant soybean varieties.

Effects of Gibberellin on the Soybean Cyst Nematode

Louis Casta, William Anderson, Colby Shirk and Paul M. Tefft
Department of Biology, St. Joseph's University. Philadelphia, PA

Egg hatching and development of the soybean cyst nematode is influenced by many factors including the physiological status of the host plant. Because plant growth regulators have profound effects on plant physiology we studied the development of the soybean cyst nematode grown on plants treated with the hormone gibberellin. In one experiment three groups of plants (24 plants per group) were treated with either 10^{-6}M/week and 10^{-4}M/week gibberellin or distilled water as controls (foliage sprayed). These plants were inoculate with equal volume of a nematode eggs in suspension (30 cyst/plant). Nematode development was assessed by sampling infected plants at weekly intervals for four weeks. Plant roots were cleared and stain with acid fuchsin. Worms were counted and developmentally staged. Morphogenic effects of the hormone on soybean plants was monitored by determining plant height, number of nodes and root weight. In all trials the gibberellin treated plants yielded fewer total worms compared to the untreated controls. In another experiment one group of soybean plants were treated with gibberellin (10^{-4}M/week) and a control group was sprayed with distilled water. These plants were used to collect root diffusates. Surface disinfested nematode eggs were exposed to diffuates calibrated to 1 RGH from the roots of treated and untreated plants. Hatching response was monitored after two weeks and compared to hatching in water and zinc chloride (3 mM). Hatching in the diffusate derived from plants treated with gibberellin were significantly less than hatching in diffusates from untreated controls.

Effects of New Soybean Production Practices on Soybean Cyst Nematode and Associated Soybean Yield Losses

P. Donald[1], G. Noel[2], H. Melakeberhan[3], A. Ndeem[2], G. Tylka[4], S. Chen[5], R. Riggs[6], T Niblack[1], D. Hershman[7], J. Faghihi[8], A. MacGuidwin[9], T. Welackey[10], T. Anderson[10]. [1]University of Missouri, [2]University of Illinois, [3]Michigan State University,[4] Iowa State University, [5]University of Minnesota, [6]University of Arkansas, [7]University of Kentucky, [8]Purdue University, [9]University of Wisconsin, [10]Ag Canada

Research was initiated to provide additional options for more economical production of soybeans in the presence of soybean cyst nematode. Soybean cyst nematode (SCN) egg population density and yield were measured in conventional tillage and no-tillage plots and under narrow (7 to 8'), medium (14-15"), and wide (28-30") row spacing within each of the tillage regimes and in the presence of SCN resistant or susceptible soybean germplasm. Additional data collected were soil nutrients (macro and micronutrients), plant tissue nutrient levels (at R4), and incidence and severity of other diseases present. The data were analyzed over all locations and also by location. Square root transformation was used to normalize combined yield data and the yield data from the individual states except for Wisconsin and Illinois where no transformation successfully reduced the variability. Log-transformation was used with the soybean cyst nematode egg population data.

Overall, soybean yields responded to the genotype (resistance or susceptibility) and row spacing. Data were collected from the tillage plots but this was the first year of no-tillage at the sites with the exception of one location. Responses varied with location. For example, although the resistant cultivars outperformed the susceptible ones in all locations, the magnitude of the difference varied from location to location.

Optimum soil pH for soybean is believed to be 5.5 to 7.0 and deviations from the norm can have significant effect on plant growth as well as confounding interpretation of the data. Minnesota, Wisconsin, Michigan and Iowa sites were above pH of 7.0 at the study sites. Soils in Michigan, Wisconsin and Iowa appear to have higher levels of calcium than other states; whereas, magnesium seems to be much more uniform at sites across the study.

Brown stem rot was the other significant disease found at most of the northern sites. Plant samples were analyzed quantitatively and qualitatively for the presence of the fungus in plant tissue.

Effects Of Swine Manure On Soybean Cyst Nematode.

Dean A. Reynolds, Gregory L. Tylka, and Charlie A. Martinson
Department of Plant Pathology, Iowa State University, Ames.

The recent increase and concentration in confined hog production systems in the U.S. has created a need for an environmentally sound way to dispose of large amounts of hog manure. Traditionally, swine manure has been applied to corn production fields but some manure now is being applied to soybean production fields. In fact, one-third of Iowa livestock farmers apply swine manure to soybean fields. Little is known about the effect of swine manure on soybean cyst nematode (SCN). Preliminary experiments in the greenhouse showed that swine manure may reduce SCN infection of soybean roots. Swine manure is composed of many simple and complex organic acids and volatile aromatics and contains high levels of mineral nutrients, as well. The objectives of this research were to determine the effect of swine manure application in the field on SCN and the effects of the various components of swine manure on SCN egg hatch and juvenile behavior.

In a field experiment conducted near Ames, Iowa, in 1997, swine manure at 6000 gallons per acre and inorganic fertilizer with nitrogen, phosphorus, and potassium analysis similar to that of the swine manure was applied broadcast and incorporated in the row and between the row prior to planting soybeans. A treatment with the standard farmer practice, which is no nutrient application, was included as an untreated control. Experimental plots were 4-rows wide by 25 ft long. Ten soil cores were collected from each row of the two middle rows. A separate set of 10 cores were collected from the soil between the rows on either side of and between the two middle rows of each plot. SCN eggs and second-stage juveniles (J2) were collected and extracted from the soil samples and counted. Soybean yield was increased by broadcasting and incorporating swine manure compared to the standard farmer practice. SCN egg numbers at harvest were greater when swine manure was applied prior to planting in the furrow or between the rows than when no manure or fertilizer was applied.

Based on preliminary laboratory studies that showed that contact with and vapors from swine manure inhibited egg hatch of SCN, several selected swine manure components and breakdown products were evaluated for effects on eggs and J2. The six aromatic compounds evaluated were indole, 4-ethyl phenol, butylated hydroxytoluene, 4-amino acetophenone, 3-methyl indole and 4-methyl phenol. In airtight containers, free eggs were immersed in trays containing a selected compound; eggs also were incubated in deionized water in a separate adjacent tray to assess volatile effects. Hatch was inhibited by contact with 3-methyl phenol and indole, but was stimulated by 4-ethyl phenol and 4-methyl phenol. Volatiles from indole, 4-ethyl phenol, and 4-methyl phenol stimulated egg hatch and J2 movement after hatch; both 4-ethyl phenol and 4-methyl phenol stimulated stylet thrusting of J2. Other compounds had no effect.

In summary, the effect of applying swine manure to a soybean field infested with SCN is complex. Initially the high concentrations of nutrient salts and organic chemicals in swine manure can kill the SCN eggs or at least inhibit them from hatching, as evidenced in the laboratory results of this study. However, swine manure may actually increase SCN egg numbers at harvest. This research was funded by the Iowa Soybean Promotion Board.

Effects of Tillage and Date of Planting on Soybean Yields and Soybean Cyst Nematode Populations

T.L. Niblack, G. S. Smith, H. C. Minor, J. A. Wrather
Department of Plant Pathology, University of Missouri, Columbia, MO

Field trials were conducted from 1991 through 1997 at three locations (southeastern, central, and northern) Missouri in soybean cyst nematode-infested fields. The split-split plot experiment was replicated four times at each location. Main plots were planting dates: early, middle, and late, at ca. 1-month intervals beginning in May. Subplots were tillage treatments: no-till, conventional-till, and ridge-till. Ridge-till treatments were not separated from no-till after 1994 because the effects were not distinguishable from those of no-till. Sub-subplots were soybean cultivars differing in host suitability for soybean cyst nematode: one susceptible and three resistant cultivars with different sources of resistance. In overall analyses, there were heterogeneous variances in soybean yield, population densities of nematodes at harvest, and nematode reproduction; therefore, data for each location was analyzed separately. Because the location accounted for a majority of the variation in yield, general recommendations for cultural control of soybean cyst nematode may not apply to specific fields. Planting date and cultivar had significant impacts on soybean yield in 15 of 17 and 14 of 17 environments (year by location), respectively; however, only cultivar consistently affected nematode population densities at harvest and reproduction rate (15 of 17 and 12 of 16 environments, respectively). Resistant cultivars yielded more than susceptible cultivars regardless of population density, year, or race of soybean cyst nematode. Tillage had no consistent effect on soybean cyst nematode at these locations, thus, tillage practices should not be altered solely to manage soybean cyst nematode.

Evaluation of Soybean Varieties Resistant to Soybean Cyst Nematode in Iowa

Gregory L. Tylka and Susan K. Souhrada
Department of Plant Pathology, Iowa State University, Ames

Resistant soybean cultivars are used by Iowa soybean growers to manage the soybean cyst nematode (SCN). Often, these SCN-resistant varieties are planted in SCN-infested fields that have high nematode population densities. Experiments are conducted each year to evaluate the agronomic performance of maturity group (MG) I and II, SCN-resistant soybean cultivars in SCN-infested and nearby noninfested fields in north central Iowa. Several susceptible cultivars of each MG also are included in the experiments.

In 1997, there were significant differences in stand, height, maturity date, and lodging among varieties, but stands and maturity dates in the infested and nearby noninfested field were similar. All varieties were taller in the noninfested field than in the nearby infested field. Also, varieties generally had greater lodging scores in the noninfested field than in the infested field, most likely due to greater plant height in the noninfested field. Most resistant varieties produced greater yields than the susceptible varieties in the SCN-infested field, and many resistant varieties produced greater yields than susceptible varieties in the noninfested field. Yields of resistant varieties of both MG were consistently greater in the noninfested field than the nearby SCN-infested field. Individual resistant varieties yielded 9 to 21 bushels per acre (21.4% to 65.0%) greater in the noninfested field than in the nearby infested field. There were no differences in SCN population densities at the beginning of the experiment, but densities averaged four to five times (407% to 540%) greater in plots with susceptible varieties than resistant varieties at the end of the season.

SCN-resistant soybean cultivars consistently have yielded less in SCN-infested fields than in noninfested fields in experiments such as these conducted in Iowa for the past four years. These differences in yield represent yield loss of the SCN-resistant varieties caused by SCN parasitism. Such yield losses illustrate that SCN-resistant varieties are not a "cure", but only a management tool that must be used in an integrated management program with nonhost crops and scouting for early detection in order to maintain profitable soybean production in SCN-infested fields. The research described above was funded, in part, with soybean checkoff from the Iowa Soybean Promotion Board.

Impact of Fungal Antagonists on SCN — A Minnesota Perspective

Senyu Chen, Fajun Chen, Xingzhong Liu, and Curtis D. Reese
University of Minnesota Southern Experiment Station

Management of the soybean cyst nematode (SCN) has largely relied on the use of resistant varieties and crop rotation. A sustainable soybean production system may only be obtainable through an integrated management program. Biological control appears to be a promising strategy that can be integrated into a management program to reduce the nematode population and minimize yield loss. During 1996-1998, research was done to determine the impact of fungal antagonists on the SCN populations in Minnesota. Soil samples were collected from 47 fields in 26 counties across southern Minnesota and about 50,000 eggs, 6,000 females, and 9,000 cysts from the samples were examined for fungal colonization. About 7,000 isolates belonging to more than 80 species of fungi were isolated and identified. Fungi that were frequently encountered in cysts, females, and/or eggs were tested for their pathogenicity (ability to cause disease) to the nematode eggs. Fungal parasites of the second-stage juveniles (J2) of the nematode were also investigated in southern Minnesota soybean fields. A total of 435 soil samples, representing about 270 fields in 27 counties in southern Minnesota, were examined for parasites of J2. Two fungal species, *Hirsutella rhossiliensis* and *Hirsutella* sp., were frequently isolated from the SCN J2. About 39% of soil samples were infested with *H. rhossiliensis* and/or *Hirsutella* sp. High percentages (about 60%) of J2 parasitized by the *Hirsutella* species were observed in a few fields. A total of 22 isolates, belonging to 16 species of egg parasites and 18 isolates of J2-parasitic fungi, *Hirsutella* species, were evaluated in the greenhouse. A few fungal species significantly reduced the nematode population density. An egg-parasitic fungus, ARF18, reduced egg density by 98.4% and one isolate of *H. rhossiliensis* reduced egg density by 97.4% in the greenhouse soil. Five fungal species were evaluated in a field as biological control agents in 1998. The J2-parasitic fungi, *H. rhossiliensis* and *Hirsutella* sp. significantly reduced the nematode density. The efficacy of *Hirsutella* sp. in reducing egg density (58% reduction) at the end of season was similar to the efficacy of the nematicide aldicarb (Temik) (64% reduction). Parasitism of J2 by *H. rhossiliensis* was also investigated in a field with various crop sequences. Crop sequence had a significant influence on parasitism of the J2 by the fungus. Fungal parasitism of the J2 was higher in plots of soybean following soybean than following corn. The significance of the fungus in controlling the nematode in soybean fields annually rotated with corn may be reduced in comparison with continuous soybean. Nevertheless, presence of the fungus in soybean fields annually rotated with corn may increase in midseason and reduce the nematode population density and its associated soybean yield loss. **(This research was supported by grants from the Minnesota Soybean Research and Promotion Council and the Minnesota Agricultural Experiment Station.)**

Management of SCN through the Use of Resistant Varieties in Minnesota

S. Y. Chen, J. H. Orf, C. D. Reese, P. M. Porter, W. C. Stienstra,
N. D. Young, D. D. Walgenbach, P. J. Schaus,
T. J. Arlt, and F. R. Breitenbach
University of Minnesota

The soybean cyst nematode (SCN) is one of the most significant yield-limiting factors of soybean production in Minnesota. In 1998, the nematode was found in six additional counties in the state with a total of 44 counties confirmed with the nematode infestation. Use of resistant varieties and crop rotation has been successful in reducing the nematode population and limiting yield losses in many other soybean growing regions. During the past two decades, especially in recent years, a number of SCN-resistant varieties in Maturity Groups I and II were developed by the researchers in universities and private companies. In 1996-1998, a total of 50 resistant varieties and eight susceptible varieties were evaluated at 15 field sites infested with the SCN. Nematode egg densities were determined at planting and harvest. Susceptible varieties maintained the same egg density or increased egg density through the growing season. Average nematode density in plots planted to resistant varieties decreased at all sites. The level of resistance, however, varied among the varieties. Resistant varieties produced an average of 44 bushels per acre that was compared to 38 bushels per acre produced by the susceptible varieties in the infested fields. A variety with a high level of resistance and good agronomic characteristics may be able to increase yield by 10 bushels per acre when compared to susceptible varieties in the infested fields. High nematode densities, however, can cause significant yield losses even to resistant varieties. Resistant varieties should be used in an integrated management program with non-host crop rotation. **(This research was supported by grants from the Minnesota Soybean Research and Promotion Council and the Minnesota Agricultural Experiment Station.)**

Mapping Changes in Soybean Cyst Nematode, *Heterodera glycines* Population Density in Continuous Soybeans (*Glycines max*) over Four Years

P.A. Donald[1], W. W. Donald[2], A.J. Keaster[3], R.J. Kremer[2], B.S. Sims[5], J.A. Kendig[4], and Jeanne Mihail [1],[1] Department of Plant Pathology, [2] USDA-ARS Department of Natural Resources, [3]Department of Entomology, [4]Department of Agronomy, Plant Science Unit, University of Missouri, Columbia, Portageville, [5]FMC, University of Tennessee, Experiment Station, TN.

Geostatistical methods were used to describe and map the nonrandom distribution and egg population density of the soybean cyst nematode, *Heterodera glycines*, and soybean (*Glycine max*) yield over four years in a soybean field in southeastern Missouri. Soil samples were collected at 100 grid locations twice a year in a 3-acre field for four consecutive years. Soybean yield was collected from the center of a 20 x 22 m area surrounding each sampling site. Semivariogram functions and kriging, an interpolation method, were used to prepare isoarithmic contour maps and associated error maps. Spatial variation existed for soybean cyst nematode egg population density and soybean yield in the field over four years at different measurement times. There was a 10-fold decrease in soybean cyst nematode egg population numbers when spring egg population counts were compared with the preceding fall egg population counts. Egg population was higher in samples collected in the spring (Pi) of 1993 than at the beginning of the research in the spring of 1990. Soybean cyst nematode eggs at the fall sampling (Pf) were detected at all of the 100 sample points in the field at the end of the research in 1993 while no eggs were detected at several sample points at the beginning of the experiment in 1990. Before this research began there was no information on geographical or temporal stability of nematode population density within a field over time. Information such as this is needed if assumptions relating to precision farming can be implemented. Understanding of soybean cyst nematode distribution changes over time throughout a field is needed for management recommendations and the reduction of yield loss due to this nematode.

Michigan Field Crop Ecology Education Program

George W. Bird, Richard Harwood
Department of Entomology and Department of Crop and Soil Sciences
Michigan State University, East Lansing, MI

Michigan agriculture is diverse, and reflects a wide variety of crops, soils, microclimates and nematode problems. For example, the following seven species of cyst nematodes are encountered in various ecosystems throughout the state: *Heterodera glycines* (soybean), *H. schachtii* (sugarbeet), *H. avenae* (oat), *H. trifolii* (clover), *H. carotae* (carrot), *H. iri* (turf), and *Cactodera milleri* (lambsquarter). Michigan agriculture must be competitive in the production of commodities such as soybeans, corn, wheat, dry beans and sugarbeets that are moved great distances at low costs. It must also be competitive in relation to local food systems. In 1996-97, Michigan State University faculty, in direct cooperation with the agriculture community, developed, a book, associated slide set, and education program entitled, *Michigan Field Crop Ecology.* This initiative is designed to assist in meeting the long-term goals described above. The purpose of this poster is to provide an overview of these materials and how they are being used.

Michigan Field Crop Ecology consists of 10 chapters: *Introduction: Why This Book?, Field Crop Ecosystems, Soil Ecology, Carbon, Nitrogen, Cover Crops, Pest Ecology and Management, The Insect Community, Nematodes, and Directions for Farm Change: Bringing It All Together.* Special reference is given to soybean cyst nematode management. In the winter of 1996, members of the agriculture community interacted with Michigan State University faculty and specialists to identify appropriate topics for future agricultural educational initiatives. These individuals, including farmers, were identified through the Michigan Agricultural Stewardship Association, Michigan Organic Food and Farm Alliance, Organic Growers of Michigan and the United States Department of Agriculture Natural Resources Conservation Service. During the next year the faculty and specialists produced a first draft of the book, and sent it to selected farmers and consultants for their review. The material was presented at a public meeting in the winter of 1997. This was followed by presentations by the reviewers, and meaningful and extensive dialogue. As a result of the process, many aspects of the book were revised. The book was published in January of 1998.

Funding for the publication was obtained from the W. K. Kellogg Foundation, National Science Foundation Long-Term Ecological Research, Michigan Department of Agriculture, and Michigan State University. The educational materials were used successfully in the winter of 1998 in several prototype education programs. They will serve as the basis for a major state-wide educational imitative on field crop ecology during the winter of 1999. Nematode ecology and nematode management, including information abou the the soybean cyst nematode, are integral components of this educational initiative. It is anticipated that both the process and the product described in this poster will facilitate development of both biologically-based and sustainable food and farming systems throughout Michigan. Copies of *Michigan Field Crop Ecology*, can be purchased for $12.00 from Michigan State University-Extension, Agriculture Hall, Michigan State University, E. Lansing, MI 48824. Kindly request Bulletin E-2646 published in 1998.

Precision IPM and Soybean Cyst Nematode

Albert U. Tenuta[1], Tom W. Welacky[2] and Terry R. Anderson[2]
[1]Ontario Ministry of Agriculture, Food and Rural Affairs;
and [2] Agriculture and Agri-Food Canada

The soybean cyst nematode, *Heterodera glycines* Ichinohoe (SCN), has been a major pest of soybean for almost a century. It was first reported in Japan in 1915 and has since been found in Egypt, Korea, China, Taiwan, Indonesia, the former Soviet Union, Colombia, Argentina, Brazil, the United States and Canada. In 1954, the nematode was found for the first time in North America in Hanover County, North Carolina. Since its initial discovery, SCN has progressed along the eastern seaboard into the southern United States and upwards through the mid-west into Canada. All of the main soybean producing states within the U.S. have confirmed SCN infestations. In the United States, SCN has become the major economically significant pest of soybeans with annual production losses estimated at over 215,000,000 bushels which is three times Ontario's yearly soybean production. The pest typically reduces yield by 30 to 60 percent, but can result in complete crop loss. We have observed yield losses of 5 to 30 percent in fields in Ontario that have no apparent visible symptoms. To illustrate the importance and threat to soybean producers a number of GPS demonstration sites were established to field map SCN infestations and corresponding yield losses. From these sites, we can accurately (1) identify the areas of the field that are infested and the levels of infestation; (2) determine the economic significance of the pest and verify economic thresholds for SCN; (3) determine if and where chemical or non-chemical (biological, resistant seed, seed treatments) treatments are needed; and (4) locate SCN race populations within a field and better target resistant varieties with different sources of resistance to those areas of the field where they will be effective. The first report of SCN in Canada occurred in two fields in Kent County in 1987 and, since that time, it has been identified in six other counties: Essex, Lambton, Elgin, Perth, Haldimand-Norfolk and most recently Middlesex. As population levels continue to increase in Ontario, the number of acres infested with SCN will also expand. During the past two years, a study was initiated whose primary objective was to survey counties that surround known soybean cyst nematode-infected areas and determine the extent of infestation within these counties. Counties that were surveyed include Middlesex, Huron, Oxford, Brant, Hamilton-Wentworth and Wellington. Over 1500 soil samples have been collected from more than 350 soybean fields. Sample locations were taken in the field with a GPS either through grid samples (2.5 acre grids) or according to the OMAFRA recommended soil sampling techniques for SCN. Field histories and a questionnaire was also taken at time of sampling. Processing and nematode identification of the soil samples is being performed by Agriculture and Agri-Food Canada. SCN distribution maps for Ontario were updated to include Middlesex and Huron counties. This technology allows extension pathologists an opportunity to keep abreast of the spread and distribution of disease causing pathogens on a field, county, regional (province/state) or country level.

Relationship of Soil pH and Population Density Of the Soybean Cyst Nematode

C.R. Grau, A.E. MacGuidwin, E.S. Oplinger, N.C. Kurtzweil
Departments of Plant Pathology and Agronomy; University of Wisconsin, Madison, WI

The soybean cyst nematode (SCN) is a relatively new and serious pest of soybean in Wisconsin. As SCN becomes more prevalent throughout Wisconsin, soybean growers will need to effectively manage infested fields to maximize yield. Soils in Wisconsin are diverse for several chemical and physical characteristics: pH, percentage of organic matter, cation exchange capacity, and soil type. The SCN is found across this range of chemical characteristics and soil type. While resistant varieties are the best control strategy, it is unclear how these varieties perform across the various soil factors found in Wisconsin. Our objectives are: 1) to examine the relationship of SCN population density and these soil factors; and 2) to ascertain the performance of resistant soybean varieties relative to susceptible varieties in regard to soil factors and SCN population density. A population of SCN located in a cooperator's field near East Troy, Wisconsin (Sebewa silt loam and Matherton silt loam) provided an ideal situation to determine interactions between SCN egg density/ reproduction and various soil factors. In 1997 initial egg population densities ranged from 50 to 7000 eggs per 100 cc of soil (avg. 1800 eggs) within each plot (20 x 25ft) after two years of corn. Soil pH varied from 5.5 to 8.2. In 1998, following three years of corn, initial egg populations of 0 to 10,000 eggs per 100cc of soil (avg. 1800 eggs) were detected. The soil pH ranged from 5.8 to 8.4. Population density of SCN increased as soil pH increased in 1997 (r=0. 84) and 1998 (r= 0.76). Organic matter in this study ranged from 1.9% to 8.6% in 1997 and 2.3% to 11.4% in 1998 and was correlated with egg density in 1997 (r=0.54) and 1998 (r=0.64) and pH (r=0.64 in 1997 and r=0.82 in 1998) as well. Estimated cation exchange capacity was highly correlated with egg density and pH in 1998. As phosphorous levels decreased, egg density increased (r= -0. 61) in 1997 and 1998. The relationship between soil potassium and SCN egg density was variable and inconclusive in the two years studied. Yield difference between resistant and susceptible varieties increase as nematode population density and pH increases (r = 0.62). A susceptible variety grown in a high soil pH/ high SCN situation yield 11- 30 bushels per acre less than its resistant counterpart grown in the same soil pH/ SCN situation. A susceptible variety grown in a low soil pH/ low SCN situation averaged 3 bushel per acre less than the resistant counterpart in 1997 and essentially no difference in bushels per acre in 1998. Lower SCN population densities on resistant varieties were associated with lower soil pH at harvest, but degrees of decreased reproduction differed by variety. At harvest, susceptible varieties were associated with higher SCN population densities across all soil pH levels and initial SCN population densities. In 1997, an STS herbicide resistant/ SCN resistant variety (PI88788) supported less SCN reproduction than a Roundup Ready/ SCN resistant (PI88788 and Peking) or conventional SCN resistant (PI8878) variety. Future studies will determine whether soil pH affects SCN egg survival and how biological antagonists influence survival. This project was supported by the Soybean Research and Development Council and the Wisconsin Soybean Marketing Board.

Relationships Among Soybean Cyst Nematode Population Densities, Soybean Yields, and Soil pH

Gregory L. Tylka, Carmen Sanogo, and Susan K. Souhrada
Department of Plant Pathology, Iowa State University, Ames

Global positioning system (GPS) technology is relatively new, satellite-based equipment that can be used to create accurate, geo-referenced maps of large areas, including agricultural fields. GPS units and electronic yield monitors are being purchased by increasing numbers of corn and soybean growers in the Midwest to manage crop production on a more site-specific basis. Research was conducted to utilize GPS technology to relate population densities of the soybean cyst nematode to soybean yield and to other soil variables. A study area of 50 acres was established with GPS technology in 1997 in an Iowa field infested with the soybean cyst nematode. The study area was divided up into square, half-acre cells. A twenty-core soil sample was collected from each cell at a randomly selected site that was located using a hand-held GPS unit prior to planting the field with a soybean cyst nematode-susceptible soybean variety. For each soil sample, pH was determined, cysts of soybean cyst nematode were extracted and counted, then eggs were extracted from cysts and counted. At the end of the growing season, the average yield of each half-acre cell also was determined. Cyst population densities ranged from 2 to 463 per 100 cm^3 (approximately half-cup) of soil; egg densities ranged from 200 to 35,800 per 100 cm^3 of soil. Soil pH ranged from 5.5 to 8.0; soybean yields of 14 to 55.6 bushels per acre were obtained. There were significant negative linear correlations between soybean yield and cyst and egg population densities. Also, significant positive linear correlations between soil pH and cyst and egg densities were detected. At this time, it is not known whether there is a direct relationship between soil pH and population densities of the soybean cyst nematode or if the effect is indirect and plant mediated. This research was funded, in part, by soybean checkoff funds administered through the Iowa Soybean Promotion Board.

Rotation of Varieties That Have Different Resistant Parents Can Reduce SCN Below the Detection Level

Greg Noel[1] and Dale Edwards[2]
[1]USDA, ARS, and [2]Department of Crop Sciences,University of Illinois, Urbana

An 11-yr field study examined population development of soybean cyst nematode (SCN), *Heterodera glycines*, after introduction of 50 cysts/plot into a field that had no history of soybean production. Soybean cultivars either susceptible or resistant to SCN were grown either in monoculture or rotated with corn in 2-year rotations. During the first 5 years, resistant cultivars with the 'Peking' source of resistance were planted. By year 5, monoculture of 'Peking' resistance resulted in a population increase to 18 cysts/250 cm^3 of soil, whereas populations in the continuous cropping of susceptible soybean increased to 45/250 cm^3. During years 6-11, SCN-resistant 'Fayette' (PI88.788 source of resistance) was planted. In year 6, numbers of cysts declined to 1/250 cm^3 of soil when 'Fayette' was planted in plots that had been in the monoculture of 'Peking' resistance. In years 10 and 11 no cysts were found in any plots planted to 'Fayette'. Numbers of cysts in plots planted to continuous susceptible soybean remained relatively constant during years 6-11.. In years 1, 3, and 4 SCN populations were low in plots planted to susceptible soybean. In spite of low SCN numbers, susceptible soybean yielded more than the resistant varieties that had Peking as the source of resistance. In year 5, Phytophthora root rot severely affected SCN-resistant 'CN290' but not SCN-susceptible 'Beeson 80'. In years 6-9 and 11 yield of 'Fayette' averaged across rotations was greater than 'Williams 82'. Plots planted to SCN-susceptible soybean never expressed symptoms of stunting or chlorosis during the experiment or during 6 additional years of planting 'Williams 82'after the experiment was terminated. This experiment showed that rotation of varieties that have different sources of resistance to SCN can reduce SCN below detection levels provided that genes for virulence against the rotated source of resistance do not occur in the particular population of SCN. This research provided the field research basis for Objective 2c, "Rotation of resistance genes to maintain *Heterodera.glycines* populations below the damage threshold" of the NCSRP project "Effects of new soybean production practices on soybean cyst nematode and associated soybean yield losses".

Soil Texture and Tillage Affect *Phytophthora sojae* and Soybean Cyst Nematode

Fekede Workneh, XB Yang, and Gregory L. Tylka
Department of Plant Pathology, Iowa State University, Ames

The effects of soil texture and tillage on the prevalence of soybean cyst nematode (SCN) and *Phytophthora sojae*, the fungus that causes Phytophthora root rot of soybean, in the northcentral United States was investigated in the fall of 1995 and 1996. In collaboration with the National Agricultural Statistics Service (NASS), 1,462 fields in Illinois, Iowa, Minnesota, Missouri, and Ohio were randomly selected using an area-frame sampling design. NASS field staff were trained to collect soil and tillage information from the selected fields in the targeted states. The prevalence and population densities of *P. sojae* and SCN were determined from the soil samples.

In general, both *P. sojae* and SCN were widespread throughout the region. *Phytophthora sojae* was detected in two-thirds of the soybean fields in Ohio and Minnesota, 63% of Iowa fields, 55% of Missouri fields, and 41% of fields in Illinois. SCN was detected in 83% of Illinois fields, 74% of Iowa fields, 71% of Missouri fields, 60% of Ohio fields, and 54% of the fields sampled in Minnesota.

Tillage practices and soil texture affected the two pathogens differently. Overall, the recovery of *P. sojae* was significantly greater in fields managed with conservation tillage (no-till and minimum till) than in fields with conventional tillage in all states except Iowa. However, the prevalence and relative population densities of the fungus were greater in heavy soils than in lighter soils in conservation-till but not conventional-till fields. In contrast to results obtained with *P. sojae*, SCN prevalence and population densities both were consistently affected by tillage and by soil texture. Significantly fewer no-till fields were infested with SCN relative to tilled fields (minimum till and conventional till). Furthermore, of all fields that were infested with SCN, the population densities were significantly lower in no-till fields than in fields that received some type of tillage. In no-till fields, the prevalence and population densities were greater in light soils than in heavy soils, but this trend was not detected in tilled fields.

Results of this research indicate that certain tillage practices and soil textures may favor *P. sojae* and SCN. Such information may be useful in targeting scouting activities and implementing integrated management programs for these two important soil-borne soybean pathogens. This research was supported by soybean checkoff funds from the North Central Soybean Research Program and the Iowa Soybean Promotion Board.

Soybean Cyst Nematode: History and Distribution in South Dakota

Martin A. Draper, James D. Smolik, and Mary E. Thompson
Plant Science Department, South Dakota State University

Limited surveys were conducted for soybean cyst nematode (SCN) annually from 1985-94. In 1995 a more extensive survey was undertaken. More than 250 fields were surveyed and SCN was detected in Union County. In 1996 a similar number of samples were processed and additional fields in Turner County were detected. In 1997, nearly 270 soil samples were processed and SCN infested fields were identified in Lincoln, Clay, Moody, Brookings, Hamlin, Grant and Day Counties. So far in 1998, about 900 samples have been processed and infested fields have been identified in Minnehaha, Deuel, Brown, Yankton, and Roberts Counties. Currently, there are 14 counties in the state where SCN has been identified in fields. Populations detected in samples have ranged from 50 to 38,000 eggs plus second stage juveniles per 100 cm^3 of soil. The only race that has been identified in South Dakota is race 3. SCN was a featured plant disease problem at 32 county meetings and 5 field tours during 1998, as well as media interviews, newspaper features and soybean association newsletters. The increased number of samples received for SCN analysis in 1998 is apparently a result of improved awareness of SCN among growers, consultants, Extension educators and others in agribusiness.

Soybean Cyst Nematode Management in Minnesota by Tillage, Rotation & Resistant Varieties in a Soybean/Corn Production System.

W. C. Stienstra, S. Chen, W. E. Lueschen & T. Hoverstad.

The influence of tillage and row spacing on Soybean Cyst Nematode (SCN) populations and yield of resistant and susceptible soybean cultivars was investigated in a soybean/corn production system in Minnesota in 1993 to 1996. The experiment was a factorial including five tillage treatments and four combinations of cropping sequence and row spacing. Tillage treatments included 1) annual moldboard plows, 2) moldboard plow after corn and chisel plow after soybeans, 3) annual chisel plow, 4) annual ridge tillage and 5) no-tillage. Cropping sequences in 75 cm row spacing (30 inch) were 1) two years of susceptible soybeans in a four year corn/soybean rotation, 2) two years of resistant soybeans in a four year corn/soybean rotation, 3) one year of resistant soybeans followed by one year of susceptible soybeans in a corn/soybean rotation and 4) one year of resistant soybeans in 25 cm row spacing (15 inch) followed by one year of susceptible soybean in 75 cm (30 inch) in the corn/soybean rotation. No effect of tillage was observed on the nematode population density. The effect of row spacing on nematode population was inconclusive. The susceptible cultivar consistently supported higher nematode density than did the resistant cultivar. Nematode reproduction varied among years, apparently due to weather conditions and root health. Response of soybean yield to tillage and row spacing was inconsistent. The resistant cultivar produced higher yields in 1994, 1995 and 1996, but not in 1993. Planting a resistant cultivar increased yield of the following susceptible soybean compared to planting susceptible cultivar. The sequence with two resistant cultivars produced a higher overall yield and lower nematode density at the end of the four-year rotation cycles than the sequence, which included both a resistant and susceptible cultivar. Soybean yield of resistant and susceptible cultivars was negatively correlated with nematode population density.

Soybean Cyst Nematode Race Shift is Not Predictable Based on Soybean Variety Choice in 7-Year Corn-Soybean Rotations in Missouri

T.L. Niblack, G. S. Smith, J. A. Wrather, R. D. Heinz, A. Colgrove
Department of Plant Pathology, University of Missouri, Columbia, MO

Soybean cultivars with different sources of resistance to the soybean cyst nematode were grown at three locations in Missouri initially infested with races 6, 3, and 3. The locations were in northern, central, and southeastern Missouri. Plots were paired field plots rotated annually with corn from 1991 through 1997. Plots were sampled each spring and fall beginning in 1994 to determine cultivar effects on race. Each of four cultivars was planted in a 4-row by 20-foot plot, replicated four times at each location, and always in the same plot in each year in which soybeans were planted. Cultivars at the northern and central sites were in Maturity Group III: Williams 82 (susceptible to soybean cyst nematode); Jackson (Plant Introduction [PI] 88788 + Peking sources of resistance); Linford (PI 88788); and a Morsoy cultivar (PI 90763). Jackson and Morsoy were replaced by cultivars with similar pedigrees during the study. Cultivars at the southern site were in Maturity Group V: Essex or Hutcheson (susceptible); Hartwig (PI 437654); Forrest (Peking); and Rhodes (PI 88788). Resistant cultivars consistently had lower reproduction and population densities of nematodes at harvest than susceptible cultivars, but race determination tests did not reflect the consistent use of the same cultivar, i.e., there was no increase in nematode development on the soybean differential line that corresponded to the source of resistance of the cultivar. Race determinations were highly influenced by time of sampling in spring (pre- or post-plant). Because "race shifts" were not predictable based on the source of resistance of the soybean cultivar, the utility of race testing in cultivar recommendations is limited.

Soybean Cyst Nematode Regional Education Project

Gregory L. Tylka
Department of Plant Pathology, Iowa State University, Ames

The Soybean Cyst Nematode (SCN) Coalition is a ground-breaking regional education project that is a partnership of state soybean checkoff boards and land grant universities from 12 North Central states, plus selected soybean industry leaders, including seed companies and grower cooperatives. The coalition's goal is to get producers to test their fields for SCN, and, if they discover that they have it, to take steps to manage the problem. The American Soybean Association, Asgrow Seed Company, Cargill Seeds, CENEX / Land O'Lakes, DEKALB Genetics, Growmark, Mycogen Seeds, Novartis Seeds, Pioneer Hi-Bred International, Soybean Digest, and the United Soybean Board are the Coalition's industry partners. Additional members of the coalition are:

State Soybean Checkoff Boards:
Illinois Soybean Checkoff Board
Indiana Soybean Board
Iowa Soybean Promotion Board
Kansas Commodity Commissions
Michigan Soybean Promotion Committee
Minnesota Soybean Research and Promotion Council
Missouri Soybean Merchandising Council
Nebraska Soybean Board
North Dakota Soybean Promotion Board
Ohio Soybean Board
South Dakota Soybean Reseach & Promotion Council
Wisconsin Soybean Marketing Board

Universities & Extension Services:
Iowa State University
Kansas State University
Michigan State University
North Dakota State University
Ohio State University
Purdue University
South Dakota State Univeraity
University of Illinois
University of Minnesota
University of Missouri
University of Nebraska
University of Wisconsin

The SCN Coalition is funded by the North Central Soybean Research Program, an alliance of 12 state soybean checkoff boards that finances research projects related to Midwest soybean production. The 12-member farmer board designated SCN as a priority and approved the creation of an education and awareness program in 1997. The goal is to get producers to test their fields for SCN and, if they have it, to act on managing the pest. The need for delivering these messages is supported by the findings of a recent United Soybean Board survey which revealed that SCN is the most important soybean pest in the region and by market research conducted by the North Central Soybean Research Program in 1997 that indicated that nearly two-thirds of the producers surveyed DO NOT test their fields for SCN.

Why does SCN warrant an effort like this? SCN is a silent thief that often goes unnoticed. Unlike diseases caused by other pathogens, SCN does most of its damage underground. By the time visible symptoms are apparent, the damage to yield and plant health already has been done. A perfectly healthy looking field of soybeans could be performing significantly under its potential, and a grower might never know. The real tragedy is that SCN can be managed and growers wouldn't have to suffer needless losses to yield and profit. And the first step to recovering those yield losses is a simple soil test.

Spatial Patterns of Soybean Cyst Nematode: Relationships with Tillage Systems and Soybean Yield

Walber L. Gavassoni, Gary P. Munkvold, and Gregory L. Tylka
Department of Plant Pathology, Iowa State University, Ames

Soybean cyst nematode (SCN) has a limited ability to move on its own power. Consequently, movement of soil is an especially important mechanism for the dissemination of this pathogen. Previous research has not elucidated the effects of tillage on SCN dissemination, spatial pattern, and population density. The objectives of our research were to: determine influence of tillage on SCN spatial patterns and dissemination, and evaluate the relationship between spatial patterns and yield loss caused by the nematode.

Fields naturally infested with SCN at the Iowa State University Kanawha and Bruner Farms were divided into 50x100 ft plots and four tillage treatments were implemented. The tillage treatments were conventional tillage, reduced tillage, ridge tillage, and no tillage. Soil samples were taken from a 7 x 14 grid in each plot with sampling points 7.5 ft apart in the fall of 1994, before any tillage was performed, and in the spring of the following three years shortly after planting. Results of geostatistical analyses indicated that the spatial patterns of cysts and eggs initially were aggregated and that aggregation decreased over time in the plots under conventional tillage, but not in the absence of tillage.

Two additional experiments were conducted in an area of a field initially free of SCN at the Iowa State University Crossley Farm. The first experiment, established in the spring of 1995, was designed to evaluate the effects of different tillage practices on the dissemination and population density of SCN. The five treatments were no-tillage infested, no tillage non-infested, ridge tillage infested, conventional tillage infested, and reduced tillage infested. Prior to planting, the 20 x 40 ft plots were infested with SCN in a 10.8 ft^2 area in the east side of the plot. Tillage was always performed from the east to the west direction. Plots were sampled in 3.75 ft grid (66 samples/plot) in the spring of 1996 and 1997 shortly after planting. Soybean cyst nematode was disseminated 22.6 ft away from the infestation site by the spring of 1997. Furthermore, population densities of the nematode decreased in plots under no-tillage and increased in the plots under conventional tillage. The second experiment was initiated in the spring of 1996 and was designed to evaluate the effects of spatial patterns of SCN on soybean yield loss. Plots were either non-infested or infested with SCN in a uniform or aggregated pattern. In the aggregated treatment, the nematode inoculum was distributed over a 10-ft-diameter circle located in the central part of the plot. Plots were kept under no tillage. Plots were sampled in the spring of 1996 and 1997 shortly after planting, and also in the fall after harvest. There were no detectable differences in yield between the aggregated and uniform infestation treatments in 1996, 1997, and 1998.

This research was funded by the Iowa Soybean Promotion Board. The first author's financial support provided by the Brazilian Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal de Mato Grosso do Sul.

Yield Challenges by SCN in Southern Ontario

[1]T.W. Welacky, [1]T. R. Anderson, [2]A.U. Tenuta,[1]Greenhouse & Processing Crops Research Centre, Agriculture & Agri-Food Canada, Harrow, Ontario, [2]Ontario Ministry of Agriculture & Rural Affairs, Ridgetown, Ontario

Yield loss estimates were obtained over 4 years from 18 farm fields and from microplot studies of controlled population levels of SCN. High populations of 5,000 or more eggs/100g can result in serious yield loss. Populations greater than 10,000 eggs/100g are High Risk fields and can result in serious yield loss even for SCN resistant varieties. Producers on sand type soils should plant resistant varieties as soon as egg populations are detected as low as 0-250 eggs/100g since yield loss determined on small plots indicated that egg populations of 100-500 per 100g of dry soil range between 20-30% and egg populations of 1,000 to 2,000 had yield losses of 35- 40%. Farm field sampling of SCN over the four year period indicated a yield loss relationship of susceptible soybeans to egg populations illustrated in the following table.

Eggs/100g	**Potential Yield Loss (%)**
0	0
500-3,000	2-8
3,000-5,000	10-15
5,000-10,000	15-25
10,000-20,000	25-55
20,000 +	55-85

Yield Data for Soybean Lines with Hartwig Resistance to Soybean Cyst Nematode

J. Faghihi, R.A. Vierling, V.R. Ferris & J.M. Ferris
Department of Entomology, Purdue University, West Lafayette, IN
Indiana Crop Improvement Association and Department of Agronomy, Purdue University, West Lafayette, IN

A determinate, SCN resistant F_8 line derived from a Williams 82 X Hartwig cross was backcrossed to Williams 82. BC_1F_2 progenies were screened with a virulent race 4 phenotype true SCN inbred. In addition to SCN resistance, the population also segregated to determinate and indeterminate growth types. In 1997, seeds from an indeterminate, SCN resistant BC_1F_3 plant were sown in a non-SCN infested field. Each plant was harvested individually and screened with the same race 4 phenotype SCN inbred, producing a range of resistant and susceptible reactions. Most of the indeterminate plants had the Williams 82 growth type with Hartwig resistance. The yields from the backcross plants were significantly higher than Williams 82 in the non-infested field. In 1998, seeds from resistant indeterminate backcross plants were planted in a highly virulent SCN infested field. The highest yield was from one of these plants (62 bushels per acre) while the lowest was from the susceptible cultivar Williams 82 (23 bushels per acre). This research was funded by the Indiana Soybean Board.

The Soybean Cyst Nematode Life Cycle: A Pathway to Management: Hatching and Finding the Root

Robert I. Bolla, Robert Harms, Gary Brown, and Terry Niblick*
Department of Biology, Saint Louis University, St. Louis, MO and *Department of Plant Pathology, University of Missouri-Columbia

Soybean cyst nematode affects soybean production world-wide. Several approaches can be taken to management SCN. These include application of nematicides, crop rotation, planting resistant varieties, biological controls with bacteria or fungi, genetic manipulation of resistance genes or a combination. Each of these is effective in some way, but each has deficiencies as well. The efficacy management could be improved, by targeting a particular segment of the life cycle with a selected mechanism of control. Such a focus can developed only if we understand the life cycle of SCN and it is controlled under different conditions, how the nematode recognizes and finds the host, how the nematode survives adverse conditions, and how the nematode interacts with the host plant to establish a feeding site. Therefore we established a multi-investigator project to learn more about the life cycle of SCN and to find a point or points where biological or safe chemical control might be used for management. Embryogenesis, hatching, and host location might be considered the initial phases of the SCN life cycle, however, little known about the process, control, and mechanism of these critical events. Hatching is induced in vitro by divalent cations such as Zn^{++}, by heterocyclic compounds such as carbofuran, and by glycinoeclepin A. Compounds with ring structures similar to carbofuran have positive effects on hatching. The ionic charge on the side chain of these molecules also might play an important role in inducing hatching. Understanding the molecular structure involved in induction of hatching will help to define the nature of molecules that might be developed as environmentally save management tools. As it stimulates eggs to hatch, Zn^{++} depolarizes the egg shell. That is it opens a channel for exchange with the environment. This depolarization is permanent; it does not reverse when the ion is removed. Ca^{++} also induces depolarization of the egg shell membrane, but it does not induce hatching and the depolarization is reversed when the ion is removed. When eggs are treated with Zn^{++}, activity of a gene with sequence homology to genes from the Tolloid/BMP gene family is induced. This gene has a high degree of homology with *hch-1* from *C. elegans*. In *C. elegans hch-1* encodes a protein with three functional domains, one a zinc protease and another an epidermal growth factor like domain. Homology between *hch-1* and the gene from SCN is in "functional domain" regions. The SCN gene product may be key to solubilization of the egg shell and associated membranes so the juvenile can escape from the egg. HCH-1 directs movement of a major neuronal cell to its functional location during embryogenesis in *C. elegans*. The SCN gene product may have a similar role. Another important part of the hatching phenomenon relates to the protein composition of the egg shell membrane. Two proteins have been sequenced in part. These proteins have no homologues in published data bases and may be characteristic or unique to SCN. It is important to understand these proteins, the induction of their synthesis and their role in hatching. Once hatching occurs, a gradient of leachate from the host root or a CO_2 gradient may be involved in the attraction of the nematode to the host. Since the nematode invades the root just anterior to the growing root cap, plant hormones involved in root elongation may serve as attractants to the nematode. This area requires further exploration.

Penetration, Migration, and Feeding: the Roles of SCN Secretions

Thomas J. Baum
Department of Plant Pathology, Iowa State University, Ames

The soybean cyst nematode (SCN), *Heterodera glycines*, spends a significant part of its life inside the soybean root feeding on modified tissues. Generally, this *in planta* period is dominated by three distinct events. First, *H. glycines* needs to gain entry into the host by penetrating the root surface. Second, once inside, the nematode needs to migrate intracellularly towards the vascular cylinder to find a cell suitable for feeding site (syncytium) formation. Third, *H. glycines* must transform the cells into feeding cells, which are the sole source of nutrients for the developing nematode for the remainder of its sedentary life. Secretions produced in the esophageal glands of SCN are regarded as playing fundamental roles in mediating all of these events. As such, it has been hypothesized that *H. glycines* softens root cell walls to enable root penetration and migration by secreting cell wall-digesting enzymes. Furthermore, *H. glycines* secretions appear to be the molecular signals that induce the formation and mediate the maintenance of the feeding sites that are essential for SCN survival. Several approaches to identify these *H. glycines* secretions have been initiated. Monoclonal antibodies with high specificities to different esophageal gland secretions have been generated. Their use for the screening of cDNA expression libraries has led to the discovery of potential soybean cyst nematode secretion genes. A significant breakthrough was achieved when a monoclonal antibody was used to purify one secretion from SCN homogenates. Obtaining the amino acid sequence of this protein led to the cloning of the first nematode parasitism genes, which coded for cell wall-digesting cellulase enzymes. In-depth analyses of cellulase expression throughout the *H. glycines* life cycle and assessment of *in planta* cellulase secretion documented that these enzymes are, in fact, involved in facilitating root penetration and migration by the SCN. In the latest and, to date, most comprehensive approach to identify *H. glycines* disease-inducing secretions, the secretion-producing nematode glands were microaspirated for the cloning of cDNAs of expressed genes. A yeast-based selection for cDNA clones coding for secreted proteins revealed candidate secretion genes of *H. glycines*. Construction of additional gland-specific cDNA libraries, large-scale sequencing of these cDNAs (ESTs), and further characterization of candidate clones is ongoing and is expected to lead to the discovery of additional parasitism genes. Results of these experiments will revolutionize our understanding of the roles of SCN secretions in parasitism of soybean roots. In the future, inhibition of the activities of *H. glycines* secretions in roots of transgenic soybean plants has great promise for developing soybean cultivars with durable resistance to the soybean cyst nematode.

The presented work is the result of cooperative efforts of the laboratories of Drs. R. S. Hussey, E. L. Davis, T. J. Baum, and the nematology group at Wageningen Agricultural University. These projects were made possible through the funding by state soybean checkoff boards, the United Soybean Board, USDA, NATO, the European Community, and other agencies.

The Soybean Cyst Nematode Life Cycle: A Pathway to Management: Parasitism, Reproduction, and Survival

Charles H. Opperman

Departments of Plant Pathology and Genetics, North Carolina State University

The development of new management strategies and tactics relies upon a basic understanding of both parasite and host biology. Our goal is to identify the weak points in the parasitic interaction that will permit intervention for parasite control. There are several key things that all parasites must do to successfully complete their life cycles. These include hatching at the appropriate time to find a host, location and penetration of a host, migration to an appropriate feeding site, development to maturity, reproduction, and survival in the absence of a host. In addition, all parasites must evade host defense responses. Because all parasites must function in a similar manner, we believe that the basic biological mechanisms used will be conserved between plant parasitic nematodes, although the direct signals and functions may be somewhat different.

The most effective approach to understanding biology of living organisms is through genetics. Genetic approaches are extremely powerful because they do not rely upon preconceived assumptions about how something might work. Instead, they make use of observations regarding the alteration of important traits or behaviors. During the past ten years, we have developed a genetic system using the obligate plant-parasitic nematode, soybean cyst nematode (SCN), as a model. We have devised an extremely powerful system to study and understand the basic biological mechanisms necessary for both successful parasitism of the host (soybean) and overcoming host defense responses. The previous talks have discussed the events leading up to nematode reproduction, the climactic event in the parasite life cycle. Little is understood about reproductive mechanisms in SCN, however. We are beginning to study sex determination in SCN. This is an important trait because male nematodes feed very little and leave the root upon maturity. We are primarily interested in genes in the nematode controlling masculinization. SCN also exhibits remarkable survival abilities in the absence of a host plant. In fact, hatching of a large percentage of SCN eggs is tied to the presence of the host root. This host-mediated diapause is another potential target for biological intervention. To this end, SCN lines that have either high or low diapause states have been developed. These lines are being used in a crossing strategy to determine the genetic basis of diapause. Our primary focus has been studying nematode genes responsible for overcoming or eluding host defense responses. We have characterized a number of genes involved in overcoming host resistance and will soon have them isolated and identified. We have developed all the tools necessary to fully characterize and manipulate these genes. Findings from these studies will certainly lead to new, specific, and environmentally safe methods for controlling plant-parasitic nematodes. Use of the nematode's own biological mechanisms against it will result in durable and stable methods that should be applicable across the entire soybean-producing region in the United States.

Global Gene Expression and Discovery in Soybean

Paul Keim, Dept. of Biological Sciences, Northern Arizona University, Flagstaff AZ 86011-5640

The soybean genome contains tens of thousands of genes that are yet to be described and discovered. In fact, only a very small fraction (<1%) of the expected total soybean gene composition can be found in public databases. This is a consequence of the prevalent scientific approaches that have focused on single gene discovery and characterization. In this report, I will present a new paradigm for soybean where thousands of genes will be identified and characterized in a relatively small number of studies. I will also present how this exponential expansion in described genes will facilitate the discovery of genes for SCN resistance. Foremost in this approach is the development of an EST (expressed sequence tag) database. This work is based upon the DNA sequencing of a large number of cDNA clones that represent gene-coding regions of the genome. These are referred to as expressed sequence tags because the cDNA clones are derived from mRNA, which represents the direct expression product from genes. If enough of these cDNA clones are sequenced, a very large percentage of soybean genes will be described. The EST sequences can be physically "spotted" onto microscope slides (microarrays) and used as an analytical tool for examining the patterns of gene expression in different tissues, or in the same tissue under different physiological or environmental conditions. One of the most relevant to this group, is the examination of SCN infections in resistant and susceptible cultivars. Not only can qualitative differences in gene expression be identified, but quantitative differences with be observed as well. A second approach to the global expression characterization is referred to as SAGE (serial analysis of gene expression). While more tedious than fully developed microarray analysis, SAGE also offers the opportunity to identify novel genes missed in the EST project as it requires no prior knowledge of particular genes (microarrays do). The goal of both microarrays and SAGE is to examine whole batteries of genes and how the respond in concert to changing conditions (e.g. SCN infection). Initial studies using these three approaches will not identify the function of all the genes described. However, they will greatly enhance other studies by providing a large number of genes for examination by the soybean research community.

Positional Cloning of Resistance Genes on the G Segment and Applications of Cloning Research to Marker-Assisted Breeding for Resistance to SCN

D.A. Lightfoot[1], K. Meksem[1], K. Zobrist[1], E. Ruben[1], K. Chancharoenchai[1], P. Panzatopoulos[1], H.B. Zhang[2], P. Arelli[3],[1] Department of Plant Soil and General Agriculture, Southern Illinois University, Carbondale, IL [2]Department of Soil and Crop Sciences, Crop Biotechnology Center, Texas A&M University, College Station, TX [3]Department of Agronomy, University of Missouri-Columbia

Inheritance of field resistance to soybean cyst nematode (SCN) race 3 can be conditioned by two genes: rhg1 on linkage group G and Rhg4 on linkage group A2. Markers very close to the genes were obtained from bulked segregant analysis, AFLP's and microsatellite markers Of 10,000 AFLP polymorphic bands, 20 map to G in coupling with rhg1 and 9 of which map to A2 in coupling with Rhg4. Two AFLP markers place rhg1 within a 1 cM interval and three AFLP markers place Rhg4 within a 0.5 cM interval. We have constructed a Forrest Bacterial Artificial Chromosome (BAC) library in the V41 vector that is capable of direct transformation into soybean. Candidate clones containing target genes have been directly used to transform plants for genetic complementation tests via Agrobacterium-mediated methods. We are developing new techniques for recreating soybean chromosomes in a test tube based on BAC fingerprinting (Tao and Zhang). We isolated a contiguous 950 Kb region from overlapping BAC DNAs spanning the 5 cM interval carrying rhg1 (and rfs1) and a 450 Kb region in the 3 cM interval around Rhg4. Sequencing of subclones of A109-4 has identified candidate rhg1 resistance gene. The development of the soybean chromosome physical map will provide a "highway" for isolation of large number of genes and markers for marker assisted breeding. For example two AFLP markers that place rhg1 within a 1 cM interval (127 Kb BAC) were cloned sequenced and converted to breeder friendly SCAR markers. Using these along with two microsatelite markers : rhg1 and rfs1 could be separated. SCARs were also developed from the three AFLP markers that place Rhg4 within 0.5 cM. The new SCAR markers have been used successfully in our Marker assisted selection (MAS) program. They are more effective than the existing microsatellite markers across a range of breeders material because the resistance allele is constant. They have been adapted for use with TaqMan technology that cuts time and cost by half. To reduce the time, money and the greenhouse space needed for DNA preparations in MAS we developed new method for DNA isolation from the seed. A single person can process 1,000 DNA extraction per day. The same single seed used for DNA extraction can be germinated and grown normally for seed production. Costs of MAS for SCN ($1.50) is now one tenth that for a greenhouse assay ($15) and falling. Further we did 200,000 selections last year by MAS and could do 500,000 in a small laboratory (1000 sq ft) but only had space to do about 10,000 SCN tests in our large greenhouse (5,000 sq ft).

Positional Cloning of SCN Resistance Genes on the A Segment

Lila O. Vodkin[1], Rose Gregoire[1], Laura F. Marek[2], and Randy C. Shoemaker[3]

[1]Department of Crop Sciences, University of Illinois,[2]Department of Agronomy, Iowa State University, [3] USDA/ARS, Department of Agronomy, Iowa State Univeristy.

The cyst nematode (*Heterodera glycines*) is the number one disease of soybean in many states based on yield loss and economic impact. Although a greater problem in southern Illinois, a larger than expected number of nematodes were found in the recent growing seasons in central and northern Illinois. Current control of the cyst nematode relies on resistant varieties and crop rotation as use of nematacides is not a practical option economically or environmentally. The physical isolation of disease resistance genes as DNA segments has been achieved only recently for a few plant species such as Arabidopsis and tomato. No genetically-defined disease resistance genes have been isolated from soybean. A molecular analysis of nematode resistance genes will point the way to new control strategies.

From classical genetic studies conducted over thirty years ago, three resessive genes (*rhg1*, *rhg2*, and *rhg3*) were required for resistance to the nematode (Caldwell et al., 1960). In addition, a fourth dominant gene (*Rhg4*) from Peking was shown to be very closely linked at less than 0.35 centimorgans to the *I* locus that controls seed coat color (Matson and Williams, 1965). Other RFLP markers exist on linkage group A (Weisemann et al., 1992) but none are as close to *Rhg4* as the *I* locus. We have recently shown that the molecular basic of the *I* locus is a cluster of genes that encode chalcone synthase (CHS), an enzyme that regulates flavonoid and pigment production in the seed coat (Todd and Vodkin, 1996).

Our approach towards physical isolation of the *Rhg4* locus has been to isolate and characterize BAC (bacterial artificial chromosome) clones containing the closely linked *I* locus. Five BAC clones were isolated from a library of the cultivar Williams 82 (*rhg4* allele) using gene specific or open reading frame primers to identify the BAC clones. One of the BACs contains 3 CHS genes and two of them contain 5 CHS genes. Most likely all three BACs represent the same genomic region as slightly overlapping contiguous clones.

One BAC clone is being completely sequenced in order to gain information on the organization of genes within this region. BAC DNA digests with three enzymes (EcoRI, HindIII, and MunI) have been made to generate fragments of various sizes that were subcloned into a plasmid vector. Thirty-seven unique subclones containing inserts of 1.5 to 9 kilobases have been identified and are being sequenced. To date a total 110 kilobases of sequence information has been obtained. It is being assembled and analyzed by comparison to the sequences of DNA from other organisms (comparative genomics). Approaches to examine the expression of the genes on the BAC clones and determine whether any are possible candidates for the resistance gene locus will be discussed. The sequence information from the BACs will allow development of additional markers that can be used to introgress the *Rhg4* locus into newly developed cultivars. Chromosome walking and high throughput sequencing to examine a larger region of this genomic segment will continue and mesh with biological tests to determine the exact location of candidate resistance genes within the region.

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Automated Marker Assisted Selection for Dual Resistance: The Soybean Cyst Nematode and *Fusarium solani*

K. Meksem, D. Hyten, K. Chancharoenchai, E. Ruben, V.N. Njiti, D.A. Lightfoot, Department of Plant, Soil and General Agriculture, Southern Illinois University, Carbondale, IL

Inheritance of field resistance in soybean [Glycine max (L.) Merr. In "Forrest" cultivar (Peking source) to soybean cyst nematode (SCN) race 3(Heterodera glycines i.) is conditioned by two loci: Rhg1 on linkage group G and Rhg4 on linkage group A2. We have constructed a physical genetic map for the 5 cM interval carrying Rhg1 and rfs1 as well as the 3 cM interval carrying Rhg4. Two AFLP markers that place Rhg1 within a 1 cM interval (127 Kb BAC) were cloned sequenced and converted to SCAR markers. Along with two microsatellite markers: rhg1 and rfs1 could be separated. SCARs were also developed from the three AFLP markers that place Rhg4 within 0.5 cM. The new SCAR markers have been used successfully in our Marker assisted selection (MAS) program. They are more effective than the existing microsatellite markers across a range of breeders material because the resistance allele is constant. They have been adapted for use with TaqMan technology. Matrix mill DNA preparation from the leaves was used in our lab. To overcome the problem: time, money and the greenhouse space needed to get leaves for DNA preparations in MAS we developed new method for DNA isolation directly from the seed. A single person can process 1,000 DNA extraction per day. The same single seed used for DNA extraction can be germinated and grown normally for seed production.

cDNA Array Representing Genes Expressed by Soybean Two Days After Invasion by the Soybean Cyst Nematode

B.F. Matthews, USDA ARS PSI, Soybean and Alfalfa Research Laboratory, Beltsville, MD bmatthew@asrr.arsusda.gov

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe is a major pest of the soybean worldwide and is responsible for millions of dollars worth of damage in the USA. There are numerous races of SCN and control of damage can be difficult. Genes involved in the soybean defense response to SCN can be identified and studied using ordered arrays. An ordered, replicated array allows the scientist to examine quantitated expression of many genes at once rather than only one gene at a time. Thus, scientists can obtain a more comprehensive picture of what defense response the soybean mounts at the molecular level during nematode invasion. A cDNA represents the coding region of a gene. Many cDNAs were constructed from soybean leaf and root tissue of the SCN-resistant variety Peking two days after invasion by SCN, race 3. Each cDNA was placed in a unique well of a microtiter dish. This gave each cDNA a unique address so it could be found and examined whenever necessary. An array of 384 cDNAs was isolated and DNA sequenced at one end to provide information on the identity of each clone. Four cDNAs were most abundant in the array. The most abundant cDNAs were similar to β-galactosidase (72% identity at the amino acid level), soybean hydroxyproline rich (HPR) glycoprotein (76%) and a protein kinase (68%), respectively, while the fourth cDNA was not represented in Gen Bank. Other clones of lower abundance had moderate homology at the amino acid level to genes encoding enzymes involved in the phenylpropanoid pathway leading to the defense compounds glyceollin and lignin, numerous protein kinases, and peroxidases. This array is being used to identify relationships, functions and identities of soybean genes important in defense against nematodes. This information is useful to scientists studying genes involved in pest resistance.

Cloning a gene conferring resistance to soybean cyst nematode

K. Lewers, R. Heinz, H. Beard, L. Marek, R. Shoemaker, B. Matthews
USDA-ARS, Agriculture and Agri-Food Canada, Iowa State University

The geographic distribution of and the economic damage caused by the soybean cyst nematode (SCN) make it the most serious pest of soybeans in the United States. Because rotation schedules and chemical soil fumigation control methods are not economically feasible, the most desirable control method is the use of soybean cultivars having SCN resistance genes. The first documented SCN resistance gene, *Rhg4*, was located near a gene called the *I* gene controlling black *vs* yellow seed coat color. Therefore, this color gene was a marker for *Rhg4*. We identified and made available to soybean breeders a useful molecular marker, pBLT65, also located very close to *Rhg4*. To develop a marker directly from the resistance gene instead of a nearby gene, and to understand and better utilize the resistance mechanism, we will clone *Rhg4*, insert it into a susceptible cultivar through transformation, and study its DNA sequence. To isolate *Rhg4*, the flanking markers have been used to screen collections (libraries) of cloned pieces of the soybean genome. A pre-existing Bacterial Artificial Chromosome (BAC) library made from the genome of a cultivar susceptible to SCN was screened. A second library was made from a resistant cultivar. This second library (a lambda library) contains cloned soybean genome pieces about a tenth the size of a BAC. One BAC clone was found containing both flanking markers and the resistance gene. This BAC clone was cut into smaller pieces, and these pieces were cloned (sub-clones) for DNA sequencing. The sub-clones were sequenced to determine what kinds of genes might be on them. Some of the sub-clones were placed in order along the BAC clone. Markers were developed from the sub-clones for comparing the susceptible and resistant genomes around the *Rhg4* gene. Comparison between susceptible and resistant genomes in this region will help identify cloned pieces of the resistant library likely to contain the *Rhg4* gene. These library clones will be inserted into susceptible seedlings through root transformation. The transformed seedlings will be exposed to SCN to determine which transformant is resistant and, therefore, which library clone contains the *Rhg4* gene.

Comparison Between Marker Assisted Selection for *rhg1* and Greenhouse Screening

[1]E.G. Boehlke, [1]J. Mudge, [1]R.L. Denny, [2]J. H. Orf,
[3]P.B. Cregan, [1]N.D. Young*
[1]Department of Plant Pathology, University of Minnesota, St. Paul
[2]Department of Agronomy, University of Minnesota, St. Paul
[3]USDA-ARS, Soybean and Alfalfa Research Lab., Beltsville, MD

Accurate and rapid methods for screening soybean lines for resistance to the cyst nematode (SCN) are critical in assisting breeders to reduce the number of lines in resistance breeding programs. In the past, scientists have relied on results obtained by testing lines inoculated with SCN in the greenhouse (GH). Resistance has been based on the number of cysts recovered from the soybean's roots as a percentage of the susceptible check. Lines with cyst counts less than 30% of the check are defined as resistant or moderately resistant; counts greater than 30% are considered susceptible. Still, the GH screening method has several deficiencies. It is slow, requiring more than 30 days before results are available; it is labor intensive, typically requiring 3-4 people at either end of the process; finally, it is difficult to control the many variables present in a GH environment, leading to wide fluctuations in cyst counts. Because of these constraints, as well as GH method logistics, the number of replications tend to be limited, reducing the confidence in results. An alternative method, marker assisted selection (MAS), circumvents many of these problems. Soybean genome mapping research has recently located several genes involved in SCN resistance. One gene, *rhg1*, located on molecular linkage group 'G', has been strongly correlated with resistance. MAS involves testing soybean DNA with an informative molecular marker, such as BARC-Satt 309, that is near *rhg1* on the chromosome. The presence or absence of resistance at *rhg1* can then be determined based on the presence or absence of resistant parent's DNA band at the nearby molecular marker. MAS is faster, more accurate and requires less labor and space than does GH testing, making it possible to screen large numbers of soybean lines within the lab setting. In order to determine how well BARC-Satt 309 predicts resistance, we analyzed data from lines screened using both GH and molecular marker methods. Lines that would be discarded based on a marker prediction of susceptible were almost always (93%) susceptible in the GH whereas lines to be advanced based on a marker prediction of resistant showed only modest correlation with resistance in the GH. We conclude that MAS can be used to efficiently predict susceptibility, thereby reducing the number of lines that must be carried in the breeding program. However, those lines that are retained should be further tested to determine if indeed they are resistant. This research was supported by grants from the United States Soybean Board, the Minnesota Soybean Research and Promotion Council, and the Minnesota Agricultural Experiment Station.

Development of Nematode-Resistant Soybean Cultivars for the Southern U.S.A.

E. R. Shipe, H. T. Knap, S. A. Lewis, and J. D. Mueller
Department of Crop and Soil Environmental Science, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC

A survey (1987-1989) to determine the distribution of plant-parasitic nematodes in soybean production areas of South Carolina found 14% of the fields sampled contained soybean cyst nematode (SCN). Of these , 47% were race 14 and 32% were race 3. Races 6, 9, and 10 were also observed. Breeding for resistance to SCN, as well as other nematode species, has been an objective of the Clemson soybean breeding program for several years. Sources of SCN resistance are productive, SCN-resistant cultivars and breeding lines. Resistance to race 3 traces to Peking and resistance to race 14 generally traces to PI 88,788. In recent years, Hartwig (PI 437,654) has also been used as a source of resistance to multiple SCN races. We combine preliminary greenhouse screening procedures with follow-up field evaluations to evaluate breeding populations and experimental lines. Primary emphasis is given to screening for resistance to race 3 with secondary emphasis given to race 14 and other races. Initial screening efforts begin with F_2 progenies and continue with F_4 progenies and experimental breeding lines. White plastic laundry tubs serve as microplots for rearing large numbers of nematodes in the greenhouse. Generally, five plants of each genotype are grown in cone-tainers and inoculated with 1500 eggs. Race appropriate resistant and susceptible checks are grown at regular intervals on the greenhouse bench. Plants are grown for 30 to 35 days and then cysts on roots are counted. A rating scale is used to make the root evaluation phase more expeditious. Experimental lines advanced to USDA Regional Tests are also screened by Dr. Lawrence Young (USDA-ARS) at Jackson, TN for races 3 and 14. Field screening is accomplished at the Edisto Research and Education Center near Blackville, SC. South Carolina lines are evaluated for seed yield and standard agronomic traits in SCN-infested fields. Breeding lines are evaluated initially in a preliminary field nursery and the second year in an advanced nursery. Recent cultivar releases include Motte, a maturity group (MG) VIII cultivar resistant to race 3, and Musen, a MG VI cultivar resistant to races 3 and 14. Musen, released in 1997, is 3-5 days later in maturity and similar in seed yield to Brim, which is susceptible to SCN, races 3 and 14. In USDA tests across the Southeast U.S., mean seed yield of Musen (43.5 bu/A) exceeded that of Brim (41.9 bu/A) by 4%. Musen is also resistant to southern root-knot nematode and stem canker disease. Seed yields of Motte, released in 1998, are comparable to Cook and have exceeded Maxcy in USDA Southern Regional Tests. Motte is also resistant to Southern root-knot nematode, reniform nematode, and stem canker disease. It is tolerant to Columbia lance nematode.

Differential Expression of Genes During the Susceptible Interaction Between Soybean and SCN

Zarir Vaghchhipawala*, Ronald Bassuner#, Kui Lin*, Kim Lewers**, Randy Shoemaker** and Sally MacKenzie*
*Dept. of Agronomy, Purdue University, West Lafayette, IN 47907
#Agracetus, Madison, Wisconsin
**Dept. of Agronomy, Iowa State University, Ames IA 50011

Infection of the soybean root by the soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) induces the development of a differentiated feeding structure called a syncytium within the root. We have attempted to understand the plant-nematode interactions at the molecular level using a genetically simplified system involving a single dominant soybean gene conferring susceptibility (susceptible line PI89008) to an inbred nematode strain VL1. We have been able to replicate the nematode life cycle in *in vitro* culture as well. Several genes have been identified as being induced upon nematode infection using differential display and subtractive hybridization techniques. These candidates have also been confirmed by RT-PCR analysis. Several of these clones share similarities to sequences in the database while others are novel sequences. Many of these candidate genes have been genetically positioned on the public linkage map of soybean by the Shoemaker lab and one of these, designated 'gene F', has been shown to map to the interval between RFLP markers pB053T and Bng122E at the top of Linkage Group G. This interval corresponds to the region encompassing a major SCN resistance gene.The promoter and structural components of this gene have been sub-cloned from a BAC library and are being analyzed.

Induction of Hairy Roots with High Transformation Efficiency on Soybean Genotypes and Propagation of the Soybean Cyst Nematode

Hyeon-Je Cho, Stephen K.Farrand, Greg R.Noel* and Jack M.Widholm
Department of Crop Sciences and USDA-ARS*, University of Illinois, Urbana, IL 61801

Soybean (*Glycine max* (L.)Merr.) is grown widely in the United States as a source of oil and high-protein meal. Annually, the soybean crop is valued at an estimated 11 billion dollars. *Heterodara glycine* Ichinohe, the soybean cyst nematode, occurs in Canada, the People' Republic of China, Colombia, Indonesia, Japan, Korea, the Soviet Union, and throught the soybean production areas of the United States. This obligate root parasite is a major yield-limiting pest of soybean in the United States. The soybean cyst nematode can be propagated gnotobiotically on normal soybean root explants. However, this technique requires the continual establishment of root explants because these organs have a determinant period of growth in culture. Soybean hairy roots, which should exhibit indeterminate growth in tissue culture, could provide an alternative to normal root explants for monoxenic propagation and study of obligate soybean root parasites such as the soybean cyst nematode.

In this study, cotyledon explants of 10 soybean cultivars, Cartter, Fayette, Hartwig, Jack, Lee 68, Mandarin, Maple Arrow, Peking, PI 437654 and Williams 82, were inoculated with *Agrobacterium rhizogenes* K599 without and with binary vectors pBI121 or pBIN-mGFP5-ER possessing both *npt*II and *gus* or *npt*II and *gfp* genes, respectively. Hairy roots were produced from the wounding surface of cotyledon explants of 10 cultivars at rates of 54-95 % on MXB selective medium containing 200 μg/ml kanamycin and 500 μg/ml carbenicillin. Putative individual transformed hairy roots were screened for transgene incorporation using a polymerase chain reaction (PCR) and cucumopine analysis. Based on PCR and cucumopine screenings, all of the tested roots were co-transformed with T-DNAs from Ri-plasmid and binary vector. Southern blot analysis confirmed the presence of 35S-*gus* and 35S-*gfp* genes in the plant genomes. Transgene expressions were also confirmed by histochemical GUS assay and Western blot analysis for GFP. Furthermore, infection of soybean nematode susceptible cultivars, Williams 82 and Lee 68 hairy root cultures expressing GFP with eggs of the soybean cyst nematode, *Heterodera glycines* race1, led to the appearance of mature cycsts about 4-5 weeks after inoculation and could complete its entire life cycle in transformed hairy root cultures expressing GFP. The *A.rhizogenes*-mediated efficient transformation and expression of *gfp* and *gus* genes in various soybean cultivars and its succesful propagation of the SCN described here will provides a way to insert new genes into differentiated roots, novel genes conferring nematode resistance or the biosynthesis of potential control compounds could be engineered into the soybean genome and directly tested for their efficacy in conferring resistance to *H. glycines*.

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A Method for in situ Hybridization to Esophageal Gland mRNA in *Heterodera glycines*

De Boer, Jan M.,[1] Y. Yan,[2] E. L. Davis,[2] and T. J. Baum.[1]
[1]Department of Plant Pathology, Iowa State University, Ames, and
[2]Department of Plant Pathology, North Carolina State University, Raleigh

A digoxigenin-labeled RNA probe transcribed from the subventral gland cellulase gene *Hg-eng-2* (G. Smant *et al.* 1998, Proc. Natl. Acad. Sci. U.S.A. 95:4906-4911) was used to develop a procedure for in situ hybridization to esophageal gland mRNA in *Heterodera glycines*. Nematodes were fixed in buffered 2% paraformaldehyde and cut into sections. After permeabilization with proteinase-K, methanol, and acetone, the nematode sections were incubated with the RNA probe at 55°C in hybridization buffer (50% formamide, 4x SSC, 2% SDS, 1% Boehringer blocking reagent, 1 mM EDTA, 1x Denhardt's, 200 µg/ml sperm DNA, 166 µg/ml tRNA), washed in 4x SSC, treated with RNAse A, and washed in 0.1x SSC, 0.1% SDS. The RNA probe was detected with an alkaline phosphatase-conjugated antibody to digoxigenin. This procedure resulted in a strong and highly specific staining of the cytoplasm of the subventral gland cells. This method will be useful for the characterization of cyst nematode secretion genes.

Molecular Analysis of Soybean Cyst Nematode Races

David Sui[1], Greg Noel[2], Les Domier[2]
[1]Department of Crop Sciences and [2]USDA, ARS, University of Illinois, Urbana

Identification of the parasitic ability (race) of soybean cyst nematode (SCN), *Heterodera glycines,* populations relies on the use of soybean differentials. The results of race tests often are variable within and among laboratories. In many cases sufficient numbers of nematodes must be produced from a grower's sample by increasing the numbers of nematodes in the sample for one to two generations in a greenhouse prior to conducting the test. Thus, a race determination often requires more than 3 months to complete. The need for about 4 sq. ft of greenhouse bench space per test places additional constraints on the ability to identify races. For example, the entire multimillion dollar greenhouse complex at the University of Illinois could accommodate only 1,000 tests per year. Even before the initial race scheme based on soybean differentials was published in 1970, nematologists recognized the need to develop an alternative method of SCN race determination. Various nematologists have studied morphology of juveniles and adults with light and electron microscopy, native proteins and enzymes separated by electrophoresis, and immunology. None of this research has been able to replace soybean differentials as a method of identifying the parasitic ability of different populations of SCN. Analysis of nucleotide sequence variation in ribosomal DNA (RDNA) coding and spacer regions has been used to study phylogenetic relationships among many types of organisms, including animals, bacteria, fungi, and plants. We examined the level of polymorphism among the internal transcribed spacer (ITS) regions of *H. glycines* races 1-6, 9, and 14, *H. lespedezae* (Lespedeza cyst nematode), *H. schachtii* (Sugarbeet cyst nematode), and *H. trifolii* (Clover cyst nematode) by restriction enzyme digestion of polymerase-chain-reaction-amplified DNA fragments. Second-stage juveniles from all *Heterodera* populations analyzed produced RDNA fragments of approximately 1.28 kb. The amplified RDNA fragments were digested with 14 restriction endonucleases. We were able to differentiate *H. schachtii* from the other *Heterodera* species with three restriction enzymes. All *H. glycines* races and the *H. lespedezae* and *H. trifolii* isolates produced very similar banding patterns with all 14 restriction enzymes. Some variation was observed in the relative intensities of the DNA bands among *H. glycines* races. We are attempting to determine whether these differences were due to incomplete digestion of the PCR products or the result of race-specific sequence variation in the ITS regions of *H. glycines*.

Pasteuria sp.: a Promising Biological Control Agent of the Soybean Cyst Nematode

N. Atibalentja[1], Greg Noel[2]
[1]Department of Crop Sciences, University of Illinois, [2]USDA-ARS, Urbana

Pasteuria spp. are obligate parasites that can only grow and reproduce in their hosts, and that have characteristics of both bacteria and fungi. An undescribed species of *Pasteuria* that infects the soybean cyst nematode (SCN), *Heterodera glycines*, was reported for the first time when it was found in Illinois in 1993. The objectives of the present studies were i) to investigate the population dynamics of both the SCN and *Pasteuria* sp., ii) to determine the potential of *Pasteuria* sp. as a biological control agent of the SCN, iii) to elucidate the life-cycle and ultrastructure of *Pasteuria* sp., and iv) to determine its phylogenetic position. The first and second objectives were achieved through a 2-year study conducted in naturally infested microplots. The life-cycle of *Pasteuria* was based on microscopic examinations of SCN adults and cysts extracted from the rhizosphere of soybean plants, and of juvenile stages excised from the roots of the same plants. The ultrastucture was described from transmission electron microscopic observations of *Pasteuria*-infected cysts. The phylogeny of the *Pasteuria* from Illinois was derived from the analysis of the 16S rDNA sequence. During the study a significant declining trend was observed in the numbers of cysts per 250 cm^3 soil, which fell from 20 at the beginning to 9 at the end of the study. Several years before *Pasteuria* was discovered in the microplots, SCN cysts averaged 300/250 cm^3 soil. Numbers of J2 also decreased exponentially with increasing numbers of endospores per J2 from 288 J2/250 cm^3 to an equilibrium density of 67 J2/250 cm^3 soil, consistent with the predictions of the Lotka-Volterra model of population dynamics. The endospores that adhered to the cuticle of J2 did not germinate until the nematodes invaded soybean roots. Then, germ tubes differentiated from the endospores and penetrated into the body of late J2. The life-cycle is completed only in females. The stages of endosporogenesis were typical of *Pasteuria* spp. Transmission electron microscopy revealed that the Illinois isolate of *Pasteuria* and *P. nishizawae*, the only other *Pasteuria* known to attack SCN and which is native to Japan, have both similarities and differences. The mature endospores had similar ultrastructure, but the endospores of *Pasteuria* from Illinois were larger. Also, laminated mesosome-like bodies were observed in the earlier stages of the sporogenesis of the Illinois isolate of *Pasteuria*, which differed in both their nature and function from the vesicular mesosomes involved in the forespore septum formation in *P. nishizawae*. The 16S rDNA was sequenced and phylogenetic analyses using maximum likelihood recovered the Illinois isolate of *Pasteuria* and its sister species, *Pasteuria ramosa*, at the base of a clade that contained *Alicyclobacillus* spp. Molecular studies are being done to determine if the Japanese and Illinois *Pasteuria* are different species. The microplot study indicates that, given sufficient time following introduction into a field, *Pasteuria* might increase to levels that would be effective as one component in an integrated pest management program to control SCN.

Soybean Cyst Nematode Research at the Nematology Laboratory of the U.S. Department of Agriculture at Beltsville, Maryland

David J. Chitwood, Susan L.F. Meyer, Edward P. Masler, Lynn K. Carta,
Andrea M. Skantar and William P. Wergin,
Nematology Laboratory, USDA, ARS, Beltsville, MD 20705

The Nematology Laboratory at the Beltsville Agricultural Research Center of the USDA Agricultural Research Service is a federally funded research laboratory with a mission of developing environmentally safe management tools for plant-parasitic nematodes. Because the Laboratory's purpose is to address national problems caused by nematodes, much of the laboratory's research is designed to minimize damage caused by the soybean cyst nematode (SCN). The laboratory is focusing its efforts on basic research designed to achieve control of SCN through approaches based upon use of biocontrol organisms, development of natural bioregulators as alternatives to chemical nematicides, and investigation of the biochemistry and molecular biology of feeding, growth, and development of the SCN.

Biocontrol: Through classical genetic methods, we developed a mutant of a biocontrol fungus with enhanced antagonism against SCN. The mutant was demonstrated to be effective at providing control of SCN in field situations. Current studies are elucidating the interactions among the fungus, the nematode, and the soil, in order to maximize nematode control. In addition, because soybean and SCN are indigenous to China, fungi with potential use as biocontrol agents were collected from China. Many of these fungi were found to have biological activity against SCN. We are isolating and identifying compounds secreted by these fungi. We are also investigating the potential of bacteria to be antagonists of SCN.

Bioregulators: Two types of bioregulators are receiving study. First, chemical analogs of the sex attractant of the SCN have been applied in field experiments; the compounds significantly increased soybean yields in one year of a two-year study. Second, specialized types of fats are involved in the transfer of cell-to-cell messages in all organisms, and our research has shown that nematodes possess unique kinds of these fats. Analogs of these compounds are being evaluated in laboratory experiments for toxicity to nematodes.

Biochemistry and Molecular Biology of Feeding, Growth and Development: We are identifying proteins and other bioactive molecules involved with 1) the control of muscular activity and feeding and 2) regulation of female maturation and egg development. We have discovered peptides (i.e., very small proteins) associated with feeding activity and oral muscle pumping in females. Exploitation of these molecules will clearly inhibit feeding and growth. We have also identified two proteins that are critical for reproduction in SCN. They are female-specific and appear to be necessary for juvenile development within the egg. Inhibition of production and/or use of these proteins by the female will halt reproduction and population growth. The endogenous enzymes that are responsible for regulating levels of the above molecules are also being investigated.

Soybean Gene Golfing: Positional Cloning of the Cyst Nematode Resistance Loci in Soybean

K. Meksem[1], K. Zobrist[1], E. Ruben[1], K. Chancharoenchai[1], P. Panzatopoulos[1], H.B. Zhang[2], P. Arelli[3], D.A. Lightfoot[1], [1]Department of Plant Soil and General Agriculture, Southern Illinois University, Carbondale, IL [2]Department of Soil and Crop Sciences, Crop Biotechnology Center, Texas A & M University, College Station, TX [3] Department of Agronomy, University of Missouri-Columbia

Inheritance of field resistance in soybean [Glycine max (L.) Merr. In"Forrest" cultivar (Peking source) to soybean cyst nematode (SCN) race 3(Heterodera glycines i.) is conditioned by two loci: rhg1 on linkage group G and Rhg4 on linkage group A2. Bulked segregant analysis, AFLP's and microsatellite were used to select molecular markers closely linked to rhg1 and Rhg4. We have screened 1024 (EcoRI/MseI) primer combinations against two pools of DNA (a resistant and a susceptible pool). This revealed about 10,000 AFLP polymorphic bands, 20 of which map to G in coupling with rhg1 and 9 of which map to A2 in coupling with Rhg4. Two AFLP markers place rhg1 within a 1 cM interval and three AFLP markers place Rhg4 within a 0.5 cM interval. We have constructed a Forrest Bacterial Artificial Chromosome (BAC) library in the Binary V41 vector. The library provides clones for physical mapping of the soybean genome and for chromosome walking or landing. Candidate clones containing target genes can be directly used to transform plants for genetic complementation tests via Agrobacterium-mediated methods. We are developing new techniques for BAC contig assembly based on BAC fingerprinting (Tao and Zhang). We isolated a contiguous 950 Kb region from overlapping BAC DNAs spaning the 5 cM interval carrying rhg1 (and rfs1) and a 450 Kb region in the 3 cM interval around Rhg4. Within the 950 kb region on one clone, A109-4 (insert size of 127 Kb) contains the two AFLP markers flanking either side of rhg1 in Forrest. Sequencing of subclones of A109-4 has identified candidate resistance genes. Transformation with candidate genes from the A109-4 clone is in process. A physical map integrated with the developed DNA marker genetic maps will provide a new strategy to clone genes known only by their phenotypes by gene golfing (Zhang and Wing 1997). The development of the soybean integrated physical map will provide a "highway" for isolation of large number of genes. Methods developed will aid many other genetic and biological studies of plant and animal genomes. Currently, the integrated physical map of the soybean is under development by a multi-disciplinary group using the BAC fingerprinting and contigs assembly technologies.

Stop That Juvenile! Targeting Developmental Events for the Control of SCN

Andrea M. Skantar, Nematology Lab, USDA-ARS, Beltsville, MD 20705

A major impediment to the durability of nematode control agents has been a lack of understanding of basic nematode biology. Identification of potential nematode control targets is a slow process, often requiring painstaking isolation and characterization of important but rare molecules from small quantities of nematodes. Fortunately, recent advancements in nematode genetics have resulted from the application of powerful new strategies for identifying and characterizing important nematode genes. Research in the Nematology Lab at Beltsville Agricultural Research Center is dedicated to the adaptation of these technologies to soybean cyst nematode. One of the most vulnerable points in the life cycle of soybean cyst nematodes is the pre-infective juvenile (J2) stage. Nematodes at this stage remain in a developmentally arrested state until they locate and penetrate a host in which to mature and reproduce. Increasing our understanding of how SCN senses and responds to the environmental signals that influence development will benefit the initial design of control measures and our ability to efficiently adapt these agents to changes in target pest populations. Molecular biology-based methods are being applied for the identification of developmental arrest genes from SCN. In addition, methods for mechanically introducing DNA into plant-parasitic nematodes are being examined. This technology will facilitate examination of gene function inside the worm, the discovery of modulatory agents, and the development of ways to disrupt gene activity.