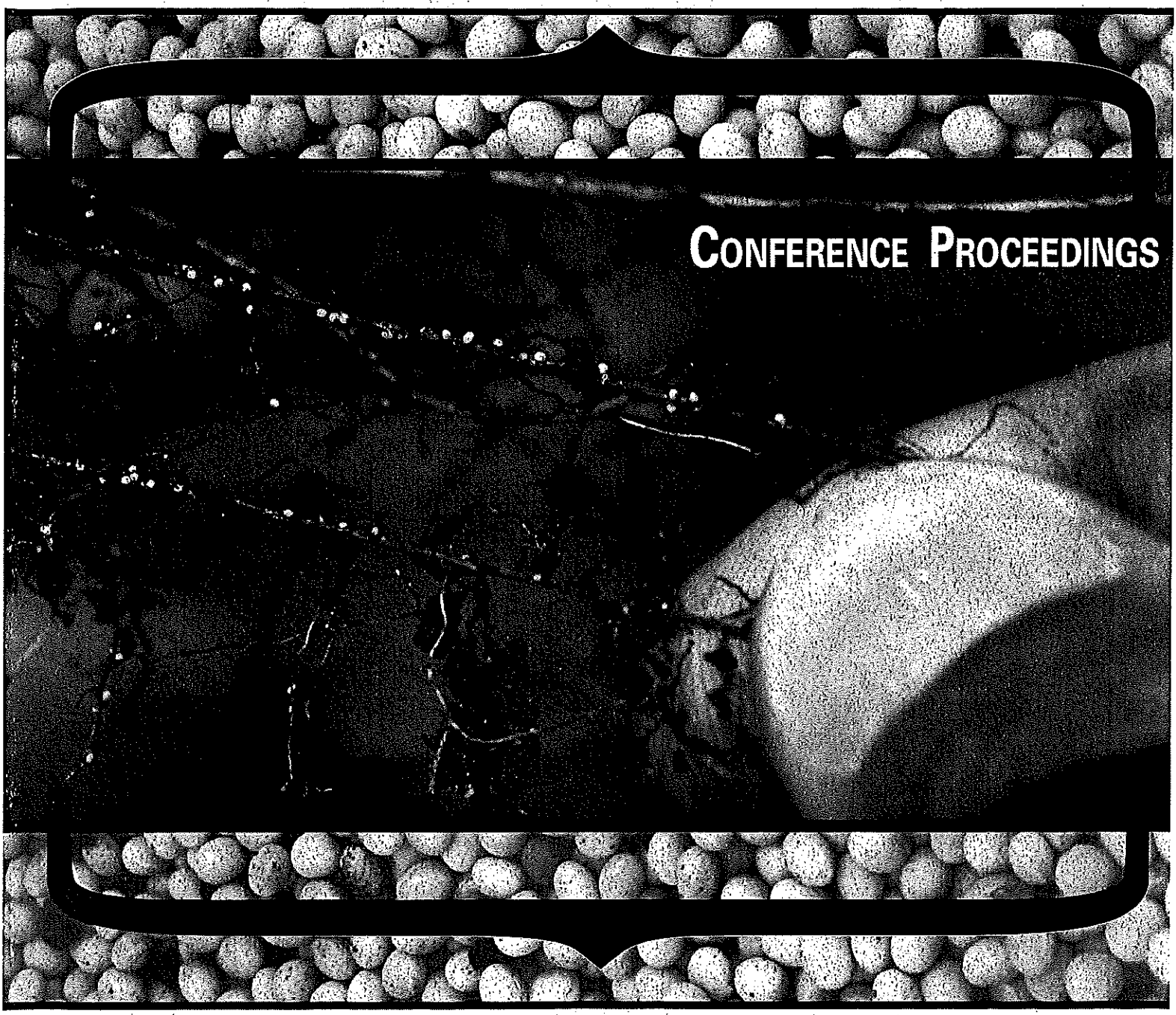
CONFERENCE ON

SOYBEAN CYST NEMATODE IN THE NORTH CENTRAL REGION

MARCH 8-9, 1995 — HOLIDAY INN GATEWAY CENTER ◉ AMES, IOWA



CONFERENCE PROCEEDINGS

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Conference on SOYBEAN CYST NEMATODE in the North Central Region

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Conference on SOYBEAN CYST NEMATODE in the North Central Region

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Conference Sponsors: **Iowa Soybean Promotion Board**
Illinois Soybean Checkoff Board
Minnesota Soybean Research & Promotion Council
Iowa State University Department of Plant Pathology

Soybean Cyst Nematode (SCN) Distribution and History

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Since its discovery in Japan in 1915, soybean cyst nematode (*Heterodera glycines*) has been reported in Asia in China, Indonesia, Japan, Korea, Russia (Amur Oblast), and Taiwan. In North America, *H. glycines* occurs in Canada and the U.S. In South America, the nematode has been reported in the literature as occurring in Brazil and Colombia. However, researchers have indicated that *H. glycines* also occurs in Argentina, Bolivia, and Paraguay. These reports need confirmation. Presence of the nematode in Africa has not been confirmed. *Heterodera glycines* was reported in Egypt, but *H. cajani* apparently was misidentified as *H. glycines*. The nematode has not been found in Europe.

The history of soybean cyst nematode in the U.S. is interesting as an example of how intensive farming of a crop leads to development of a pest problem. In the early 1900's soybean was grown as hay and as a green manure crop. Only 100,000 acres produced soybean for seed in 1919. With the possibility of war and loss of vegetable oils from Asia, soybean production for seed increased to 4.2 million acres in 1939. By 1942 soybean acreage for production of seed had increased to 8 million acres. In 1995 approximately 50 million acres of soybean were planted. Coupled with the increase in soybean acreage was a decrease in oat acreage in the Midwest and cotton acreage in the South. This change in cropping systems and rotations lead to the discovery of *H. glycines* in North Carolina in 1954 and to the current problem experienced by many soybean producers throughout the country.

Of interest to many who are involved with various aspects of controlling soybean cyst nematode are the questions, "How did the nematode get to the U.S.?" and "How long has it been here?" Some believe the recent "spread" of the nematode is due to indigenous populations derived from a common ancient ancestor found in Asia and North America. One study used 2-D electrophoresis to evaluate homogenates of 30 *H. glycines* females from one Japanese and six U.S. populations and concluded that the data support the ancient indigenous hypothesis. Another hypothesis is that the nematode was introduced during the early years of soybean production in the U.S.

References indicate that the soybean was domesticated in northeastern China between 3000 and 2000 B.C. and was introduced into Japan about 200 B.C. to 300 A.D. During the mid 1700's to the late 1800's, soybean seed was imported from the Orient into the U.S. by various individuals. Importation of seed during the 150 years prior to 1900 could have provided opportunity to move cysts in soil peds. There are many "Old Domestic" soybeans in the USDA Germplasm Collection which were imported from China, Japan, and Korea in the early 1900's and also may have provided opportunities to import the nematode. However, importation of soil from the rhizosphere of soybean would provide significant opportunity to introduce soybean cyst nematode into the U.S. In the 1880's Hellriegel elucidated the role of bacteria in forming nitrogen-fixing nodules on legumenous plants. Soybean did not grow well in early experiments in the U.S. because *Bradyrhizobium japonicum* is not native to the U.S. The bacterium was imported in soil to infest plots in the U.S. One researcher, D. Fairchild, "Wrote out to Japan and imported several pounds of soil from a soybean field." Another researcher imported plants growing in Japanese soil. Although difficult to document directly, one can infer that importation of soil was common. In an article published in 1930, the authors reported that "naturalized" *B. japonicum* in the U.S. consisted of 156 strains and 6 serotypes common to China, Japan, and the U.S.

Once the benefit of "nodulating" soil was established, researchers began sending soil to various experiment stations. Growers also began to infest their land with soil from other farms. An Illinois Agricultural Experiment Station Bulletin published in 1904 recommended that growers spread 500 lbs of infested soil/acre to ensure that the soybean crop would be nodulated. As late as 1919 prior to the acceptance of commercial inoculant, researchers were still studying methods of spreading soil to nodulate soybean. An interesting statement concerning "soil inoculation" was published in 1916 in the Georgia Experiment Station Bulletin. The passage was, "Naturally, as

soon as it was learned that these bacteria were essential to the successful growing of legumes, men made efforts to ensure their presence, and this has led to the practice of seed and soil inoculation. This was first practiced by transferring soil from a field on which the particular legume to be inoculated had been successfully grown, and scattering it over the field to be planted...this method gave excellent results, but it had serious drawbacks...it was heavy to apply, and was liable to carry noxious weed seeds, and troublesome plant maladies, such as cotton wilt, pea wilt, melon wilt, and nematodes (Root-knot nematode, author's note)."

Research done with my colleague D.I. Edwards has shown that at least 10 years are required following the introduction of *H. glycines* for the nematode to reach population levels that will damage soybean. Several more years are required on typical midwestern soils to attain population levels that will cause stunting and yellowing. The soybean field in North Carolina in which *H. glycines* was first found in the US in 1954 had apparently been planted to gladiolus imported from Japan many years earlier and subsequently planted to soybean for several years. These two examples plus others involving infested equipment to install electrical towers and natural gas lines across farms show that many years are required for *H. glycines* populations to increase to damaging levels following introduction of the nematode.

Recently, I have collaborated with Z. L. Liu to study genetic diversity of esterase polymorphism of individual females from 20 *H. glycines* populations from China, Japan, and the U.S. Eight esterase phenotypes were resolved. Three loci, *est*-1, *est*-2, and *est*-3 were identified and had two, three, and one allele, respectively. The three loci and six alleles expressed six genotypes in the Chinese populations. In Japanese populations, *est*-1 and *est*-2 and three alleles were identified and expressed four genotypes, but in U.S. populations only *est*-2 and two alleles were expressed in three genotypes. Genetic composition at each locus was used as a character for data analysis. Phylogenetic (phylogenetic analysis using parsimony [PAUP]) and phenetic (unweighted pair-group method with arithmetic mean [UPGMA]) analyses demonstrated consistent relationships. Larger numbers of loci and alleles in populations from China and close relationships among Japanese and US populations of *H. glycines* support the hypotheses that the ancestral *H. glycines* originated in China and the Japanese and US populations are the result of recent migration to Japan and to the US.

Reference

Noel, G. R. 1992. History, distribution, and economics. Pp. 1-13 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of the soybean cyst nematode. St. Paul: American Phytopathogical Society Press.

Soybean Cyst Nematode in the North Central Region: Existing Management Options

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Abstract

Soybeans can be produced profitably in the presence of the soybean cyst nematode (SCN), as researchers and many careful farmers have proven. The important first step in minimizing losses due to SCN is to confirm its presence through soil samples submitted to qualified laboratories. SCN is not easy to diagnose and is overlooked in the field because it causes no specific symptoms and may intensify the expression of symptoms due to other stresses. Once SCN is confirmed, an integrated rotation plan consisting of non-host crops, SCN-resistant cultivars, and susceptible or tolerant soybean cultivars, should be combined with various other practices designed to enhance overall plant health. SCN levels should be monitored periodically to determine the effects of the management plan, but soybean yields will tell the story of a successful plan.

Introduction

Don't be deceived by the pictures you see of stunted, yellow soybean plants infected with the *Heterodera glycines*, the soybean cyst nematode: you may never see such sickly soybean plants in your own infested fields. SCN can cause up to 38% yield losses in high-yield environments without causing obvious symptoms.

In the years 1989 through 1991, SCN cost soybean producers in the North Central Region over $250 million dollars, more than any other soybean disease (1). We know that SCN infests a large percentage of the soybean acreage in the region, and is found in every one of the North Central states except North Dakota (so far). We know how to manage soybean production to reduce losses due to SCN, yet it continues to reduce soybean profits in our region. We know the reason: it's because we tend to discount diseases that don't cause symptoms. Like high blood pressure in humans, the disease caused by SCN usually does not advertise itself except on the bottom line. Like high blood pressure, the disease due to SCN develops over time, and a negative test one year can turn into a serious problem in a subsequent year. To prevent losses due to either disease, specific actions must be taken to identify and control them.

The purpose of this paper is to summarize the most important recommendations for dealing with SCN (2,3). For specific recommendations in infested fields, farmers have valuable resources in local Cooperative Extension Service publications and personnel. In most states, variations on the general recommendations have been or are being thoroughly tested under local conditions. This information is extremely important, because while SCN is always damaging to soybean, the magnitude of the damage is highly dependent on location. Likewise, the most effective tactics to reduce the damage are also highly dependent on the individual farmer's circumstances.

Identification of SCN

The most important step in reducing losses due to SCN is to confirm its presence in a soybean field. This nematode can reduce soybean yields 5-35% in individual infested fields *without* causing dramatic symptoms, and up to 90% in heavily infested fields. Extremely high SCN infection can even kill seedling soybean plants, especially if the plants are stressed by some other factor. By itself, SCN can cause stunting and perhaps chlorosis (yellowing), but these symptoms may be the result of many different factors and are not *specific* to SCN. In fact, the lack of specific symptoms is one of the reasons that yield losses due to SCN continue to be so high year after year: disease symptoms and low yields caused by the nematode are easily attributed to other causes such as low fertility, improper herbicide application, low moisture, or even other diseases. In other words, SCN infestations can be overlooked because SCN cannot be diagnosed on the basis of symptoms.

There is only one good way to confirm the presence of SCN in a field, and that is to submit a good, representative soil sample to a qualified laboratory for analysis. Such laboratories provide confirmation of SCN as well as an indication

of the level of infestation, or the numbers of SCN in the field. The analyses vary from lab to lab, but the level of infestation will ordinarily be in terms of cysts or eggs in a certain volume of soil, and a comparative term (e.g., high, moderate, low). The comparative term is important to help determine whether chosen management practices are reducing SCN levels over time. Keep in mind that the information obtained from a soil sample analysis is only as good as the sample. Instructions on proper sampling are available from laboratories that analyze soil samples for nematodes.

Some laboratories can also provide SCN race determinations. There are 16 SCN races, and most SCN-resistant soybean cultivars are labeled as resistant to only one or two of them. New races arise in a field when the same resistant cultivar is grown for more than one year without benefit of rotation. A race determination test can detect race shifts, but with a good rotation plan, a SCN race determination is rarely necessary.

Rotate, rotate, rotate

In a SCN-infested field, there are three goals of soybean management: 1) to improve soybean health and yield; 2) to reduce SCN infestation levels; and 3) to preserve the yield potential of SCN-resistant cultivars. No single management tactic will achieve all three goals, therefore integration of rotation approaches is required. The four general rotation recommendations are summarized below:

1. Rotate with non-host crops

SCN is not able to reproduce on certain crop plants, including all the grasses (corn, sorghum, rice, etc.) and others such as alfalfa, cotton, and peanut. Thus, rotation with these crops causes the population of SCN to decline in a field. The rate of decline is highly variable, however, and depends on location (slower in the northern states than in the south) and other factors. In general, two years of a non-host crop are recommended when SCN levels are high enough to damage a susceptible cultivar. After two years, the SCN level in the soil should be checked before soybeans are planted in the field again. Exhaustive lists of non-host crops suitable for different geographic areas are available through Cooperative Extension.

2. Rotate with SCN-resistant soybean cultivars

Soybean farmers used to avoid planting SCN-resistant cultivars whenever possible because they were reputed to have lower yield potential than susceptible cultivars. Chemical control with nematicides was a viable option, and allowed farmers to grow their high-yield, susceptible cultivars. Today, soybean breeders have developed many SCN-resistant cultivars with high yield potential, equivalent to that of susceptible cultivars. Resistant cultivars are highly preferable (economically and environmentally) to chemicals as tools to minimize losses due to SCN. In infested fields, depending on the level of infestation, resistant cultivars yield 5-90% more than susceptibles. Dozens of SCN-resistant cultivars are available in each maturity group, and many have multiple pest resistance or have been bred for a specific production niche, such as double-cropping.

There is only one problem with the use of SCN-resistant cultivars: continued use of the same cultivar on the same field will result in development of a new race of SCN that is not affected by the resistance. This is because most resistant cultivars allow some SCN reproduction, although not enough to affect yield until the new race builds up to damaging levels. This is true for all resistant cultivars except for some of the newest ones developed from a plant introduction, PI 437654, which is highly resistant to all SCN races. Even these new cultivars should not be grown continuously in the same fields, however, to avoid developing other problems associated with continuous soybean production. The following two recommendations, combined with non-host and resistant cultivar rotations, will help farmers avoid these problems.

3. Rotate with tolerant or susceptible soybean cultivars when SCN numbers are low

The purpose of using tolerant or susceptible cultivars in the rotation is to avoid selection of a new SCN race. When SCN levels are low, they will build up on tolerant or susceptible cultivars, but not in time to reduce soybean yields because yield loss is dependent on SCN levels present at planting. Of course, this recommendation requires that SCN infestation levels be monitored through soil sampling.

4. Rotate resistant cultivars with different sources of resistance

If SCN levels are not low, then planting a tolerant or susceptible cultivar may not be an option. If soybeans must be planted in a field with moderate or high SCN levels, and a SCN-resistant cultivar has previously been planted in the same field, then a resistant cultivar with a different source of resistance (resistant parent) should be chosen. For example, resistant cultivars labeled "resistant to SCN race 3" has a different source of resistance than one labeled "resistant to race 5" or "resistant to races 3 and 14". Sometimes, the actual name of the source of resistance is available from seed dealers or Cooperative Extension. For example, PI 88788, Pickett, and PI 437654 are three different sources of SCN resistance. The purpose of choosing resistant cultivars with different sources of resistance for rotation in an infested field is, again, to avoid selection of a new SCN race and other problems due to continuously planting the same cultivar.

Cultural and chemical management of SCN

Rotation is, by far, the most effective tool for minimizing SCN damage, primarily by reducing SCN levels. But the goal of improving soybean health and yield can be enhanced by other beneficial practices. The value of these practices may be highly dependent on local conditions.

1. Relieve stress

Any other factor that increases soybean crop stress will compound the damage due to SCN. Good management of weeds, water, and fertility will increase soybean *tolerance* of SCN infection.

2. Reduced tillage

Some researchers have found that reduced tillage caused reductions in SCN levels compared with conventional tillage, but in other cases SCN levels did not go down under no-till. The beneficial effects of reduced tillage probably require several years to become measurable. At the very least, switching to a reduced tillage production system will not increase SCN damage.

3. Other practices

Practices such as late planting, or planting into standing wheat stubble in double-crop fields, have been beneficial in some locations. Some rotation crops have a greater than normal detrimental effect on SCN populations. Under certain conditions, chemical control with a nematicide/insecticide may be recommended. Local recommendations should be checked for these and other practices.

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2. Niblack, T. L., ed. 1993. Protect your soybean profits: manage soybean cyst nematode. St. Louis, MO; American Soybean Association.
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The North Central Soybean Cyst Nematode Project

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Abstract

A regional effort was initiated in 1993 to determine the effects of susceptible and resistant soybean varieties and nonhost crops on population densities of soybean cyst nematode (SCN), to assess yield loss due to SCN, and to increase awareness of the nematode and its management among growers in the North Central Region of the USA. This project is being conducted by cooperating scientists from universities in Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, Ohio, and Wisconsin. The project is funded by a grant from the North Central Soybean Research Program. Research and education objectives were collectively developed by the cooperating scientists. Experiments are conducted in three to five locations per state each year with as much uniformity as possible to facilitate combined analyses of data from across the region. The project coordinator visits all participating states each year, works to promote uniformity among the experimental sites, and participates in field days to promote education about SCN. Results obtained to date indicate differences in effects of standard management practices among locations throughout the North Central Region.

Introduction

Scientists from throughout the midwestern United States met in St. Paul, Minnesota in July 1992 to exchange information and discuss needs and priorities for research and extension efforts related to SCN, *Heterodera glycines*. At the meeting, it was decided that a coordinated regional effort was needed to standardize research methods and management recommendations and to make more efficient use of research funds. A grant proposal for a regional SCN research and education project was submitted in October 1992. In January 1993, funding for the project was approved by the North Central Soybean Research Program, an organization of eight midwestern states which coordinates and supports regional soybean research.

Once funding for the project was secured, research and extension faculty from universities in the 10 midwestern states infested with SCN were invited to participate in the project, and an organization meeting of project participants was held in St. Paul, Minnesota in March 1993. Experimental and financial details of the project were discussed and agreed upon at the organizational meeting. Detailed experimental protocols were prepared and distributed to all cooperating scientists in May 1993. A full-time project coordinator, Dr. Peggy Thorson, was hired in June 1993 to coordinate the efforts of scientists in the 10 states, to participate in extension education efforts in all participating states, to determine SCN population densities from soil samples collected at all experimental sites, and to compile, analyze, and summarize all experimental data. Following is a description of the specific project objectives and selected research results from the 1993 and 1994 growing seasons.

Project Objectives

1. To determine the effects of susceptible and resistant soybean varieties and nonhost crops on SCN population densities in SCN-infested fields in the North Central Region, and to determine whether effects of these practices vary by geographic location.
2. To develop a method to accurately assess soybean yield losses due to SCN throughout the north central United States.

3. To identify regional testing sites and develop standard operating procedures for unbiased evaluation of SCN-resistant soybean varieties.
4. To increase awareness of SCN and its management among farmers throughout the north central United States.
5. To determine distribution of SCN races throughout the north central United States.

Cooperative Research

Research is being conducted by university faculty in 10 midwestern states. Although a list of cooperating scientists is presented in Table 1, the scope of this project involves many others including plant breeders, extension personnel, and growers. To maintain cohesion among cooperating scientists across this large geographical area, the project coordinator visits all states each growing season, frequently participating in field demonstration days.

Soybean Variety Experiments

To determine the effects of susceptible and resistant soybean varieties on yield and SCN population densities, experiments are conducted in two SCN infested fields and one noninfested field in most participating states (Fig. 1). Data were collected from 27 experimental sites in 1993, and 32 sites were established in 1994. Four replicate plots of several SCN-resistant and susceptible soybean varieties (maturity groups I, II, III, and IV) are planted in a randomized complete block design at each location. Individual plots usually consist of four rows, each 20 feet or 6.1 meters long. Soil samples are taken from the center 16 feet or 4.9 meters of the middle two rows per plot at planting and at harvest. All soil samples are sent to Iowa State University, where they are processed to determine SCN egg densities. At the end of the growing season, the center two rows of the plots are harvested. Data are statistically analyzed collectively and by soybean maturity group zones (Fig. 1). To adjust for differences in experimental sites and to facilitate comparison of results among sites, relative yields of varieties at each site are calculated by adjusting each plot yield to a percentage of the highest-yielding variety at that particular site. Relative yields subsequently are converted to standardized yields, expressed in bushels per acre, by multiplying the relative yields by the average soybean yield for the appropriate maturity group zone. Average standardized yields for varieties in each soybean maturity group zone in 1993 are presented in Table 2, and unadjusted average yields for varieties in each zone in 1994 are presented in Table 3.

In 1993, differences in unadjusted and standardized yields of the resistant and susceptible soybean varieties were evident in many, but not all, experimental sites. Also, differences in yields of all varieties in SCN-infested and noninfested fields were detected. However, the magnitude of the aforementioned differences varied greatly between the western and the eastern experimental sites of the project. Initial SCN egg densities averaged 3,585 eggs/100cc soil in infested sites in maturity group zone I; egg densities averaged 4,913 and 3,034 eggs/100cc soil in zones II and III, respectively, in 1993. Final SCN egg densities were less than initial densities in most plots in several locations, even in plots planted with susceptible soybeans. Such results were typical of experiments located in the western areas of the experimental region, where excess moisture and cooler temperatures were common throughout the 1993 growing season. Generally, SCN egg densities increased on susceptible soybean varieties and decreased or stayed the same on resistant soybean varieties in the eastern experimental sites, which had average or below-average rainfall in 1993.

Results obtained in 1994 were more consistent across the experimental region than in 1993. Differences in average unadjusted yields between SCN-resistant and susceptible soybean varieties and between SCN-infested and noninfested fields were much greater in the second year of experimentation. Average initial SCN egg densities for infested sites in maturity group zones I, II, III, and IV were 1,973, 3,321, 2,217, and 1,482 eggs/100 cc soil, respectively, in 1994. SCN egg densities decreased in most plots where SCN-resistant varieties were grown, whereas egg densities increased in plots planted with SCN-susceptible varieties. Additional statistical analyses are being performed on the 1994 data.

Nonhost Crop Experiments

Experiments are conducted in several states each year to determine and compare the effects of growing corn, a SCN nonhost crop, on SCN population densities. Soil samples are collected at the beginning and end of each growing season, and SCN population densities are determined. Changes in SCN densities in soils cropped to corn are calculated and statistically compared. For example, average percent decreases in SCN egg densities in plots planted with corn in 1993 were 77%, 56%, and 39% in Iowa, Missouri, and Ohio, respectively.

Race Survey

To determine the distribution of SCN races throughout the North Central Region, SCN populations isolated from soil collected from infested soybean variety experiment locations are being evaluated. Race identification has been completed for many of the SCN isolates, but many more have yet to be tested. To date, race 3 is the most prevalent race among the experimental field sites, although races 4, 6, 9, and 14 also have been identified (Table 4).

Extension Activities

Cooperators in each state are encouraged to organize field day demonstrations to educate farmers about SCN biology and management practices. Field days have been conducted in Iowa, Kansas, Michigan, Missouri, Minnesota, Nebraska, Ohio, and Wisconsin, and the project coordinator has participated in numerous demonstrations throughout most of these states. Field days have increased growers' awareness about SCN. Some growers have detected SCN in their fields after learning how to check for the presence of the nematode at a field demonstration, whereas others have collected soil samples and sent them to cooperating universities for testing. Since the project's inception, SCN has been discovered in counties in Iowa, Kansas, Michigan, Minnesota, Nebraska, and Ohio that had not previously been known to be infested with the nematode. Slides of the project logo, maps of the known distribution of SCN in the region (Figure 2), maps of experimental locations, and results are sent to cooperating scientists in participating states for use in educational efforts.

Summary

The North Central Soybean Cyst Nematode Project has been met with great interest among researchers, extension personnel, agribusiness, and growers in all participating states. The coordinated efforts of nematologists and plant pathologists from universities across the North Central Region have been instrumental to the success of the project. Grower awareness of SCN has increased dramatically as a direct result of the efforts of the project coordinator and the university scientists cooperating in the project. Results of research conducted in 1993 and 1994 reveal that management strategies may have very different effects on SCN population densities and soybean yields, depending on weather, soil type, and, perhaps, other factors. Results of 1995 research will be combined with existing data and analyzed in its entirety to attempt to better understand, and perhaps predict, the effects of standard SCN management strategies on nematode densities and crop yields across the region.

Table 1. Cooperating scientists of the North Central Region Soybean Cyst Nematode Project.

State	Affiliation	Cooperator(s)
Project Coordinator	Iowa State University	Peggy R. Thorson
Illinois	University of Illinois	Gregory R. Noel
Indiana	Purdue University	John M. Ferris Jamal Faghihi
Iowa	Iowa State University	Gregory L. Tylka
Kansas	Kansas State University	Douglas J. Jardine Tim C. Todd
Michigan	Michigan State University	Haddish Melakeberhan George W. Bird
Minnesota	University of Minnesota	Ward C. Stienstra
Missouri	University of Missouri	Terry L. Niblack Harry L. Minor
Nebraska	University of Nebraska	David S. Wysong
Ohio	Ohio State University	Patrick E. Lipps Paulette Pierson Richard M. Riedel A. F. Schmitthenner Terry A. Wheeler
Wisconsin	University of Wisconsin	Ann E. MacGuidwin Craig Grau Ed Oplinger

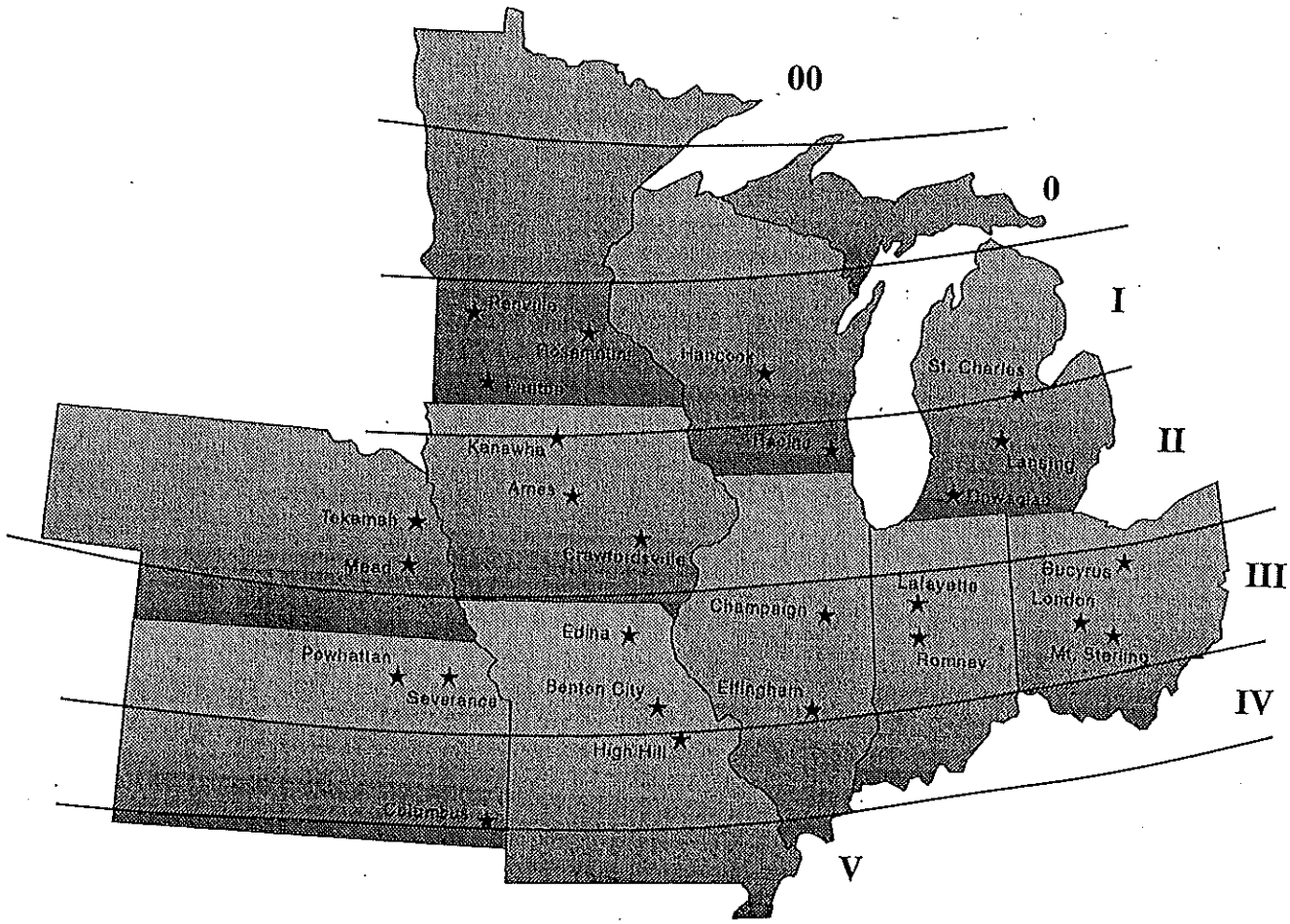

Figure 1. Location of soybean variety experiments among the soybean maturity group zones in 10 states in the North Central Region in 1994.

Table 2. Standardized yield (Bu/A) of SCN-resistant (R) and SCN-susceptible (S) soybean varieties grown in infested and noninfested sites in soybean maturity group zones I, II, and III in 1993.

Variety	Maturity Group	Maturity Group I Zone		Maturity Group II Zone		Maturity Group III Zone	
		Infested	Noninfested	Infested	Noninfested	Infested	Noninfested
Alpha (R)	I	27.2	28.6	18.2	29.7	25.8	30.8
Bell (R)	I	30.9	29.9	28.6	37.8	32.5	37.8
Hardin (S)	I	19.2	24.6	16.7	32.4	24.2	27.4
Parker (S)	I	24.7	36.0	20.8	34.0	23.9	28.6
Sturdy (S)	I	25.9	31.4	21.4	37.7	27.8	20.7
Jack (R)	II	34.9	30.3	29.3	43.8	41.9	40.6
Newton (R)	II	26.4	28.2	20.4	29.8	35.1	33.3
Pioneer 9221 (R)	II	28.3	25.6	20.0	25.7	33.1	32.3
Corsoy 79 (S)	II	25.3	26.8	16.7	40.4	27.0	31.7
Kenwood (S)	II	23.7	37.8	22.4	35.9	33.3	38.8
Linford (R)	III	30.3	32.0	25.8	32.9	43.9	41.5
Resnik (S)	III	22.4	35.7	21.7	37.8	36.1	43.3
No. of sites		5	5	4	4	6	2
Overall mean		26.6	30.6	21.7	36.1	32.0	33.7
Resistant mean		29.7	29.1	25.7	33.3	35.4	35.6
Susceptible mean		23.5	32.1	19.8	39.0	28.7	31.7

Table 3. Unadjusted yield (Bu/A) of SCN-resistant (R) and SCN-susceptible (S) soybean varieties grown in infested and noninfested sites in soybean maturity group zones I, II, III, and IV in 1994.

Variety	Maturity Group	Maturity Group I Zone		Maturity Group II Zone		Maturity Group III Zone		Maturity Group IV Zone	
		Infested	Noninfested	Infested	Noninfested	Infested	Noninfested	Infested	Noninfested
AP1991 (R)	I	35.0	51.7	42.7	43.4	31.2	34.0	15.8	18.6
Bell (R)	I	48.2	53.1	49.0	48.1	37.4	38.9	16.2	20.1
Parker (S)	I	32.3	58.2	34.1	45.5	29.1	37.9	10.8	19.7
Sturdy (S)	I	34.6	53.2	35.1	49.8	33.6	40.6	9.3	22.3
Jack (R)	II	58.3	55.3	49.1	48.6	45.4	47.4	25.3	26.4
Newton (R)	II	42.5	45.3	39.8	44.3	39.6	37.9	21.2	22.5
Corsoy 79 (S)	II	35.1	54.5	31.7	44.0	35.0	44.6	19.4	25.0
Kenwood (S)	II	44.1	59.5	37.9	52.6	40.1	39.9	15.0	28.0
Linford (R)	III	-----	-----	47.3	49.2	45.7	47.5	31.6	27.0
MFA 9043 (R)	III	-----	-----	36.8	44.3	39.5	38.5	26.8	23.6
Resnik (S)	III	-----	-----	40.1	48.9	44.8	50.9	19.4	24.2
Williams 82 (S)	III	-----	-----	34.6	41.7	41.1	48.2	23.3	22.1
Delsoy 4210 (R)	IV	-----	-----	40.8	44.0	47.3	45.5	37.2	21.7
Pharaoh (R)	IV	-----	-----	30.2	25.8	38.2	34.5	39.9	33.7
Flyer (S)	IV	-----	-----	36.4	47.8	41.9	47.9	26.5	23.9
Spencer (S)	IV	-----	-----	41.5	51.8	42.7	49.6	25.6	27.8
No. of sites		3	2	7	4	8	4	1	1
Overall mean		41.3	53.9	39.2	45.7	39.5	42.8	22.8	24.1
Resistant mean		46.0	51.4	42.0	43.5	40.5	40.5	26.8	24.2
Susceptible mean		36.5	56.4	36.4	47.8	38.5	45.0	18.7	24.1

Table 4. Soybean cyst nematode race tests of bulk soil samples from 1993 and 1994 variety trial experimental site locations.

Site[c]	Mean No. Cysts on 'Lee'	Female Index[a]				Race Designation[b]
		'Peking'	'Pickett'	PI 88788	PI 90763	
IL-I1	395	38.8	111.4	7.0	31.7	14
IL-2	254	24.0	88.9	1.3	1.0	9
IL-3	162	0.0	0.7	0.5	0.0	3
IA-I1	537	2.6	2.1	3.3	0.1	3
IA-I2	1038	2.0	18.6	3.3	0.5	6
IA-3	825	0.0	0.1	1.4	0.0	3
KS-1	455	0.0	1.7	1.4	0.0	3
KS-2	143	0.1	0.6	4.2	0.6	3
KS-I2	152	20.7	56.5	21.7	27.7	4
MI-I1	406	0.1	0.3	5.7	0.4	3
MI-I2	361	7.4	1.7	6.3	0.0	3
MO-I1	92	0.3	0.0	0.2	0.0	3
MO-I2	527	0.0	4.3	3.3	0.0	3
MO-N	351	7.7	0.9	5.1	0.0	3
MN-1	95	0.0	2.6	3.0	0.0	3
MN-I1	573	0.4	15.8	1.0	0.1	6
MN-I2	65	13.9	13.7	8.0	2.9	9
MN-I3	83	2.3	17.3	6.2	0.0	6
NE-1	918	0.0	1.1	1.3	0.8	3
NE-2	770	0.0	0.4	3.2	1.8	3
NE-I2	109	0.0	0.6	0.0	0.0	3
OH-I1	120	5.2	0.4	2.7	0.0	3
OH-I2	221	2.8	0.2	1.2	0.0	3
OH-N	121	4.9	0.1	2.3	0.0	3
OH-1	231	44.0	21.2	6.1	15.1	14
OH-2	573	38.2	90.5	9.3	10.2	14
WI-I1	86	0.0	0.2	0.8	0.0	3
WI-I2	381	0.9	12.4	0.7	0.0	6
WI-2	312	0.3	0.2	8.5	0.1	3

[a] Female index = (average # females on differential/ average # females on 'Lee') X 100

[b] Based on 16-race scheme of Riggs and Schmitt

[c] First two letters of field location designation indicates the state in which the field is located; numbers represent field numbers.

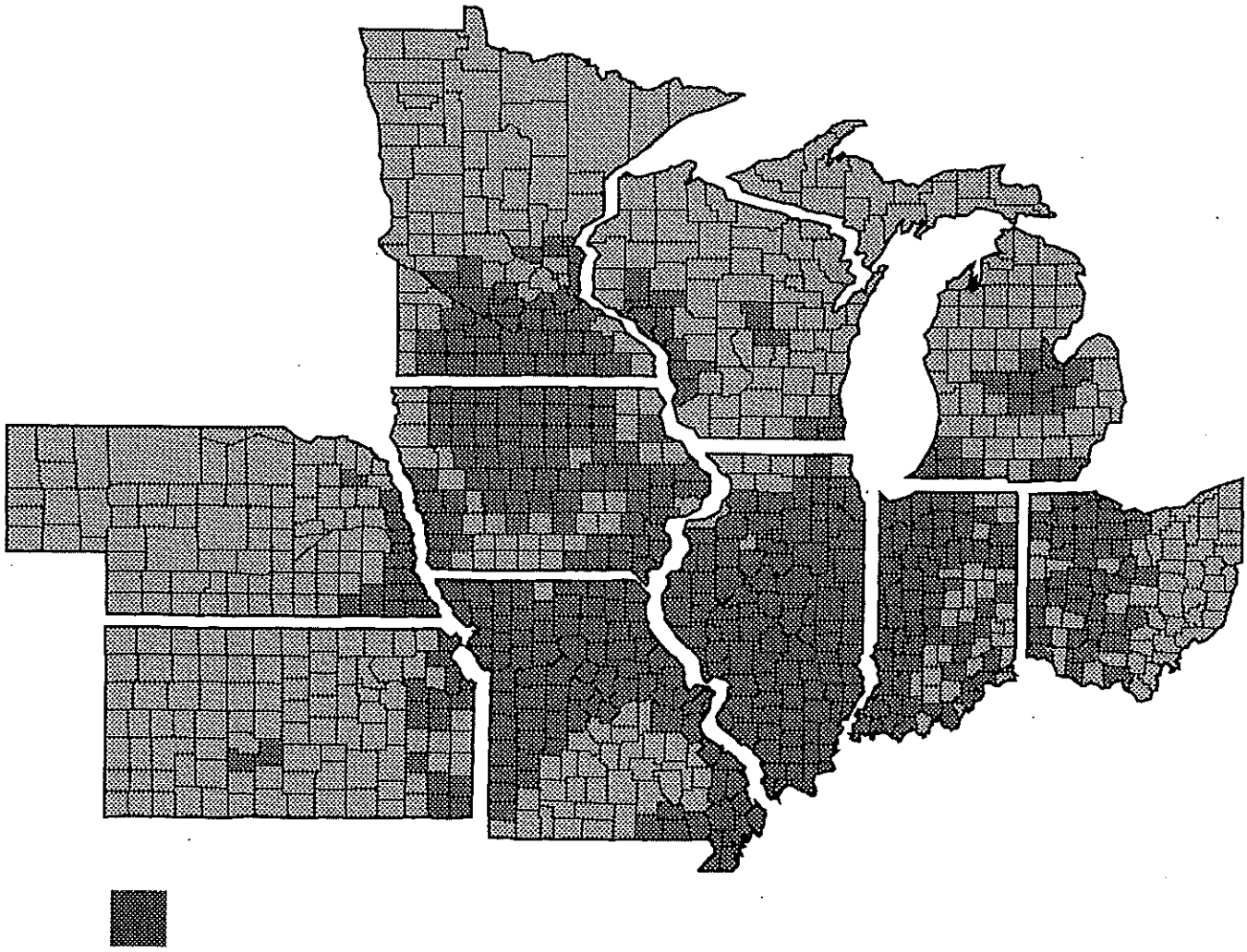

Figure 2. Known distribution of soybean cyst nematode in the 10 states participating in the North Central Soybean Cyst Nematode Project.

Coordination of Regional Soybean Cyst Nematode Tests

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Introduction:

The soybean cyst nematode (SCN), *Heterodera glycines*, is a serious problem in the soybean production area. The initial search for genetic resistance to SCN identified the cultivar, Peking, with resistance to SCN populations designated races 1 and 3. Further evaluation of the soybean germplasm identified PI 88.788 and PI 89.772 with high levels of resistance to SCN population designated race 4. As additional sources (PI 437.654) of resistance to the soybean cyst nematode are identified in the greenhouse and laboratory, they are incorporated into adapted breeding lines. The critical evaluation of soybean cyst resistant breeding lines throughout the soybean production area from Canada to Tennessee, and Maryland to Nebraska, facilitates the release of soybean cyst nematode resistant germplasm and varieties. These new releases are essential to protect against yield losses and increase soybean farmer profits in the United States and Canada.

As part of the annual Soybean Breeder's Workshop effort to coordinate Soybean Cyst Nematode research, the Northern Regional Soybean Cyst Nematode Test was initiated on a limited basis in 1979 by R. L. Bernard. Upon R. L. Bernard's retirement, C. D. Nickell continues to compile and publish Regional Soybean Cyst Nematode Reports (1988 through 1995). Since 1993, the United Soybean Board has partially funded the Coordination of the Northern Regional Soybean Cyst Nematode Test. The goals of this test are to evaluate soybean cyst nematode resistant breeding lines in cyst infested soils in the United States and Canada, to compile data from these cyst nematode infested test locations, and publish this information for use in releasing new soybean cyst resistant germplasm and varieties. Also, this soybean cyst nematode cooperative test provides information on the interaction of the genes for resistance to SCN in the plant and the soybean cyst nematode population in the soil. With this information, long range plans for variety development can be coordinated to reduce the future losses to new races of the soybean cyst nematode.

Procedures:

In January, researchers in public breeding programs submit a list of soybean cyst nematode resistant breeding lines for inclusion in the Northern Regional Soybean Cyst Nematode Test. Entries are grouped by maturity into four tests (I, II, III, and IV). Cooperators, that include soybean breeders, geneticists, nematologist, and pathologist, in 13 states (Delaware, Iowa, Illinois, Indiana, Kansas, Kentucky, Maryland, Minnesota, Missouri, Nebraska, Ohio, Tennessee, and Virginia) and Canada indicate the number of locations and tests that they will plant. In February, the cooperators meet in St. Louis, Missouri to review the procedures used in the tests

and discuss new potential variety releases. In March, seed for each entry is sent to one location (C. D. Nickell, Illinois) for packaging and distribution. In September, data forms are prepared by C. D. Nickell and sent to the participants. Information requested from multiple row replicated tests include yield, maturity, height, lodging, seed quality, seed protein and oil, and cyst data (cyst counts, plant vigor ratings, cyst reproduction etc.). In December, data forms are returned by cooperators to C. D. Nickell. Information is compiled by test and location; and summarized for each year and over several years. The Northern Soybean Cyst Nematode Report is published and distributed to test participants and non-participants.

Results:

The SCN Regional Test has expanded from two locations in 1979 to 40 locations in 14 states and Canada in 1994. Since 1979, over 700 soybean breeding lines have been evaluated. The 1994 entry list includes 96 different SCN resistant soybean lines from 10 soybean research programs. From these cooperative tests, over twenty soybean varieties and germplasm lines have been released (Bronson; Purdue), (Alpha, Freeborn, and Faribault; Minnesota), (Avery, Delsoy 4210, Delsoy 4710, and Delsoy 4500; Missouri-Portageville), (Saline; Missouri-Columbia), (Nile and Pharaoh; Southern Illinois University) (A20 and Newton; Iowa) (Fayette, Cartter, and Linford; Illinois-USDA) (Jack, Bell, and Yale; Illinois). Also, a number of soybean cyst nematode resistant breeding lines (unreleased) evaluated in this test are being used by many soybean breeders in their research programs.

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DNA Marker Analysis and Linkage Mapping of Soybean Cyst Nematode Resistance

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Abstract

To identify genes for soybean cyst nematode resistance, we analyzed four segregating soybean F2 populations ('Evans' x PI 209332, Evans X PI 90763, Evans X PI 88788 and Evans X 'Peking') and a F5:6 nearly-recombinant inbred population derived from the Evans X PI 209332 cross. Among all populations, two to four independent partial resistant loci were found to be significantly associated with SCN resistance. One of these loci, located on the top of linkage group G, behaved as a major partial resistance gene and was common in all the populations studied. This locus explained up to 48.6% of total phenotypic variation based on r-square estimates in PI 209332, 44.8% in PI 90763, 30% in PI 88788 and 22.5% in Peking populations.

To uncover DNA markers tightly linked to SCN resistance, comparative genome mapping with soybean relatives was performed. This analysis indicated that several Vigna and Phaseolus markers were tightly linked to SCN resistance on linkage group G, leading to an increase in marker density from one marker every 10 centimorgans to one marker every 2 centimorgans. Conservation at the physical level may be especially helpful in positional gene cloning in soybean since the genome size of V. radiata is approximately 40% that of G. max.

Introduction

Genome mapping and positional cloning are revolutionizing the study of plant disease genetics. Recently, we have begun to focus on soybean cyst nematode (SCN) resistance based on DNA marker mapping of the most important resistance loci . In the long run, these experiments will have a major impact on marker-assisted breeding for SCN resistance, development of durable resistance strategies to control the nematode, and eventually, positional cloning of the underlying resistance genes.

Materials and Methods

The mapping populations were constructed by crossing 'Evans' with the following sources of SCN resistance: PI 209332, PI 90763, PI 88788, and 'Peking'. Seventy-six to 113 F2 individuals, together with the parents, were either grown in the greenhouse or in the field and used as source of leaf tissue for DNA extraction and RFLP analysis. Plants were allowed to recover and set F3 seeds, which were saved for SCN disease assay. The PI 209332 population was studied in detail and advanced to the F5 generation

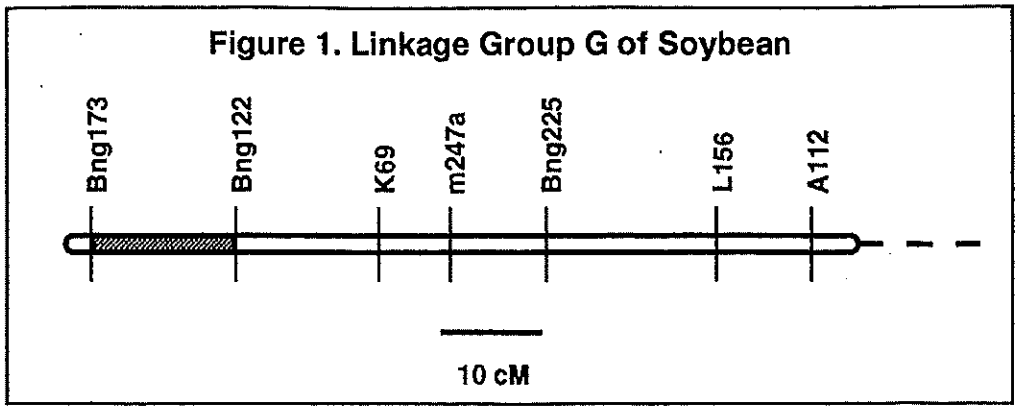
by single seed descent to generate a nearly-recombinant inbred population. For the recombinant inbred population, F5 plants were row-planted and used as source of leaf material for DNA analysis. F6 seeds were bulked from each F5 line for SCN analysis. DNA extraction, restriction digests, electrophoresis, Southern blots, hybridization, and autoradiography were performed with methods previously described (Young *et al,* 1992). A total of 10-12 progeny seedlings for each line were assayed for SCN resistance using the waterbath method (Concibido *et al,* 1994). Each plant was inoculated three days after germination with 2,000 SCN eggs of a field isolate from Minnesota that behaved as Race 3. Soil temperatures were maintained at 28 °C at 16-hour daylength for 28 days. On the 28th day, individual plants were uprooted and cysts were collected by blasting the roots with pressurized water. The total number of cysts from individual plants were counted under a dissecting microscope and converted to an index by dividing this number by the total number of cysts on the susceptible parent.

The mapping strategy used in locating potential partial resistance loci in this study was to detect significant associations between DNA marker genotypes and corresponding SCN disease responses using regression analysis and analysis of variances. A level of significance of $P < 0.002$ (Lander and Botstein, 1989) was chosen to minimize the chances of false positives experiment-wide.

Comparative mapping was accomplished by analyzing a common set of DNA markers in *V. radiata, P. vulgaris, and G. max.* Methods for probe preparation, Southern hybridization, and autoradiography were the same as described previously for mungbean (Young *et al,* 1992), common bean (Valleijos *et al,* 1992), and soybean (Keim *et al,* 1988). Three mapping populations were used to analyze a common set of RFLP markers. The mungbean F2 population (58 individuals) was derived from a cross between *V. radiata* cv (VC3890A) and subspecies, *sublobata* (TC1966). The common bean population of 68 individuals was derived from the first backcross of *P. vulgaris* line XR-235-1-1 (Mesoamerican) and 'Calima' (Andean), (the two major gene pools). Line XR-235-1-1 was the recurrent parent. The soybean F2 population (60 individuals) was derived from a cross between *G. max* (A81-356022) and a *G. soja* accession (PI 468916). These experiments were carried out in collaboration with Dr. Randy Shoemaker and associates at USDA-ARS / Iowa State University and Dr. Eduardo Valleijos and associates at the University of Florida.

Results and Discussion

Analysis of genetic relationships using classical methods has revealed that resistance genes to SCN Race 3 may be shared among sources of resistance (Rao Arelli and Anand, 1988). We have demonstrated that a major partial resistance gene for SCN Race 3 on linkage group G (Figure 1) is common between PI 209332, PI 88788, PI 90763 and Peking using restriction fragment length polymorphism (RFLP) analysis. This same region has been found to be significant in PI 437654 (D. Webb, Pioneer Hi-Bred, Johnston, IA, personal communication) and PI 88287 (S. Mackenzie, Purdue University, W. Lafayette, IN, personal communication). This information now provides a basis for our gene deployment strategies in breeding SCN resistant soybeans. Table 1 shows the estimated phenotypic effect on SCN disease response that can be explained by the partial resistance locus on linkage group G in the different populations studied. In the Evans x PI 90763 population for instance, homozygotes for the resistant allele on average had a cyst index that was more than 0.50 (2 x 0.25) cyst index units less than that of homozygous susceptible individuals.



Now that we have identified this major partial SCN resistance locus, efforts are being directed to further characterize and isolate this region. Among the mapping populations, the PI209332 F2 and RIL populations were the most characterized. Using comparative mapping between *G. max*, *V. radiata* and *P. vulgaris*, we uncovered several *Vigna* and *Phaseolus* markers that are tightly linked with SCN resistance in soybean (the region around Bng173 and Bng122). This led to an increase in marker density near the SCN resistance gene from one marker every 10 centimorgans to one marker every two centimorgans. Specifically, the public RFLP map of soybean contained only two markers within 10 cM of the putative SCN gene at the initiative of these experiments (1993). Though the use of comparative genome analysis, eight additional markers that originally came from *Phaseolus* have been mapped to the target interval in soybean. Experiments are now underway using pulse field gel electrophoresis to construct a high resolution map of the G region as a starting point to cloning this major partial resistance gene based on map position.

Table 1. Partial SCN Resistance Locus on Linkage Group G in Various Mapping Populations

Mapping Population (x Evans)	Percent Variation	Phenotypic Effect	d/a†	Probability
209332 (F2)	44.6	-0.20	-0.04	<0.0001
209332 (RIL)	48.6	-0.18	-0.03	<0.0001
90763	44.8	-0.25	-0.08	<0.0001
88788	30.0	-0.17	-0.23	<0.0001
'Peking'	22.5	-0.16	-0.58	<0.0001

†-The ratio of dominance to additivity, 0 indicates complete additivity, 1 indicates completely dominant, -1 indicates completely recessive.

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GENETIC ANALYSIS OF THE SOYBEAN - *HETERODERA GLYCINES* INTERACTION

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INTRODUCTION

An understanding of the genetics of nematode-plant interaction is essential to development of both classically and biotechnologically derived resistant host cultivars. Unfortunately, the study of this interaction has been very one-sided, focusing primarily on the genetics of plant resistance and almost not at all on the genetics of nematode parasitism. The small size and obligately parasitic life habit of phytophagous nematodes has hindered genetic analysis of nematode parasitism. In addition, many of the most important sedentary endoparasitic forms exhibit modified reproductive strategies (e.g., mitotic or meiotic parthenogenesis in *Meloidogyne* spp.) that preclude classical genetic approaches to analysis. The cyst nematodes, however, are primarily amphimictic and are amenable to genetic analyses.

Nematode-host interactions are complex and poorly understood. The relationship between cyst nematodes and their hosts appears to have co-evolved, and as a result there are numerous genes for host resistance that are complemented by nematode parasitism genes (Triantaphyllou, 1987). Different alleles of these genes may interact in various combinations to give a range of host-nematode interactions. Because there may be numerous genes for resistance in a given host species, the interpretation of these interactions is complicated. A complete lack of knowledge regarding the functions of either resistance or parasitism genes further confuses this picture.

The interaction of soybean cyst nematode (*H. glycines*) (SCN) with soybean (*Glycine max*) has been extensively studied in the United States. This system has been chosen as a model to dissect the genetics of nematode parasitism due to both the extensive characterization of soybean resistance genes and the tractability of classical genetic manipulation of the nematode. The remainder of this review will focus on the interaction of SCN with its host and the progress that has been made in unraveling the complex genetic systems controlling parasitic behavior.

GENETIC RESISTANCE TO SOYBEAN CYST NEMATODE

Within two years of the discovery of SCN in the United States, screening of existing soybean germplasm resulted in the identification of several lines carrying resistance to the known field isolate of SCN (now known to be race 1) (Ross and Brim, 1957). These lines, Ilsoy, Peking, PI 90763, and PI 84751, were used in breeding programs to develop resistant cultivars of soybean. The first resistant cultivar released was Pickett, which resulted from a cross involving Peking

(Brim and Ross, 1966). As variability in SCN parasitic ability was characterized, further germplasm screening identified lines resistant to some of the new nematode races, including PI 88788, PI 89772, PI 87631-1, Cloud, Columbia, Peking, PI 84751, and PI 90763 (Epps and Hartwig, 1972; Caviness, 1992). Many new cultivars carrying resistance incorporated from some of these lines, particularly PI88788, were subsequently released. New sources of resistance to various SCN races have been identified since that time, including PI 209332, PI 437654, and others (Anand and Brar, 1983; Anand and Gallo, 1984). Recently, the cultivar Hartwig has been released and carries resistance to all characterized races of SCN (Anand, 1992). However, this cultivar is not adapted to all soybean growing regions.

Resistance to SCN is oligogenic and inheritance patterns may be complex. Resistance to SCN has been demonstrated to be controlled by at least four recessive and one or more dominant major genes (Ross and Brim, 1957; Caldwell et al., 1960; Matson and Williams, 1965; Thomas et al., 1975; Rao-Arelli et al., 1992). Each of these genes may have multiple allelic states. Resistance to race 5 carried by PI 437654 is controlled by 2 dominant and 1 recessive genes, and at least some of these genes are also present in Peking and PI 90763 (Anand et al., 1988). Analysis of resistance to race 3 in Peking and PI 90763 indicates that 1 dominant and 2 recessive genes confer resistance (Rao-Arelli et al., 1992). Other experiments have shown that some resistance genes may be shared among the various lines, and that some genes may be linked or have multiple alleles involved (Hartwig, 1985; Hancock et al., 1987; Rao-Arelli and Anand, 1988; Anand and Rao-Arelli, 1989). The complex genetic nature of soybean resistance to SCN combined with the variability observed in nematode populations has impeded progress in the development of improved varieties.

SOYBEAN CYST NEMATODE GENETICS

Soybean Cyst Nematode Races

Genetic variability in *H. glycines* was detected almost as soon as host resistance was identified (Ross, 1962; Miller, 1970). Initially, four races of *H. glycines* were designated based on reproduction on four resistant host differentials: Pickett, Peking, PI 88788, and PI 90763 (Golden et al., 1970). A fifth race was soon added to this system, but field populations did not always fit into one of these races (Riggs et al., 1981). As a result, 16 races of *H. glycines* are now recognized in the fully expanded scheme (Riggs and Schmitt, 1988). Nematode reproduction on one of the host differentials is considered positive if it exceeds 10% of that observed on the fully susceptible cultivar, Lee. In essence, races of *H. glycines* are field populations that possess a number of genotypes (Triantaphyllou, 1975; Leudders, 1983). Selection pressure by cropping resistant cultivars is likely to alter the frequency of alleles for parasitism, and therefore the race designation. The race concept as it is applied to *H. glycines* is not based on genotype, but rather on the predominant phenotype encountered at a particular time (Niblack, 1992). This has made it extremely difficult to compare populations of nematodes for diagnostic purposes and has obscured the understanding of host-parasite interaction. The genetics of the soybean cyst nematode-soybean interaction do not conform to a conventional pattern of gene-for-gene interaction (i.e., dominant resistance genes/recessive parasitism genes). Nevertheless, it has been possible to make certain observations regarding SCN genetics.

Early genetic studies on SCN variability were mainly by directional selection experiments on the various host differentials. In these studies, selection on a resistant host resulted in a gradual increase in the ability of the nematode population to reproduce on that host (Triantaphyllou, 1975; Young, 1982; McCann et al., 1982). However, selection on one resistant differential had no effect on the nematode population's ability to parasitize a different host. From these studies, it was supposed that multiple genes in the nematode were involved in parasitism of resistant cultivars, and that these genes could be separated into three relatively independent groups (Triantaphyllou, 1975; Young, 1982; McCann et al., 1982). These groups corresponded to the ability to parasitize PI 88788, PI 90763, and Pickett, respectively. Soybean lines carrying genes derived from one of these differentials responded to the selected nematode populations in the same manner as the

original differential, although some lines actually carry genes derived from several sources. These lines can often be attacked with equal intensity by the selected nematode populations (Young, 1984). Later tests combining a primary directional selection on a resistant cultivar with a secondary selection on a cultivar with different resistance genes have further complicated the issue. In these experiments, secondary selection resulted in increased parasitic ability on the secondary host, but a loss of parasitism on the primary host (Leudders and Dropkin, 1983; Leudders, 1985). Although it may be argued that these data support the idea that different alleles at the same locus are responsible for parasitic ability (Leudders and Dropkin, 1983; Leudders, 1985), the lack of fixation of parasitism genes during primary selection casts doubts on that conclusion (Triantaphyllou, 1987). More recent experiments support the shift in reproductive abilities as being due to secondary selection, but still do not provide genetic evidence as to the mechanism (Young, 1984; Anand and Shumway, 1985).

Controlled crosses between various races of SCN have suggested that parasitic ability is inherited in a dominant fashion, but no specific ratios were observed (Triantaphyllou, 1975; Price et al., 1978). This was most likely because the populations used for these experiments represented a mixture of genotypes rather than pure strains. Inbred lines of SCN have been developed that have been selected over a seven year period for parasitic ability on a given soybean host (Dropkin and Halbrendt, 1986). Experiments with these lines revealed that nematode genes for parasitism on one soybean genotype may have a dominant effect over genes for parasitism on a different host genotype. Unfortunately, these studies ended at the F_1, and segregation patterns were not observed, but this type of data points out the need to utilize pure genetic lines for the study of SCN parasitic ability.

GENETIC MAPPING OF THE SCN GENOME

All of the previously described experiments have relied upon phenotypic analysis of genotypes. There are many factors that can mitigate expression of a particular genetic trait, including the environment, quantitative inheritance, or partial and complete dominance. These types of factors can have a significant impact on interpretation of genetic analyses based on phenotype. In the past 10 years, direct assay of genotype through use of DNA-based markers has greatly enhanced our abilities to perform genetic analysis. For most of that time, the technology of choice has been the use of restriction fragment length polymorphisms (RFLP) (Botstein et al., 1980; Burr et al., 1983). RFLPs reveal DNA polymorphisms by restriction endonuclease digestions combined with hybridizations. These assays are generally time consuming and labor intensive, not to mention costly. The use of the polymerase chain reaction (PCR) has greatly facilitated genetic analysis, and several assays based on selective amplifications have been developed (Innes et al., 1990). Although PCR assays for genetic analysis have greatly increased the numbers of individuals that can be analyzed compared to RFLP technology, the requirement for sequence information for primer synthesis limits applications. Even so, there are now a number of nematode labs around the world applying PCR-based DNA diagnostics to the study of population variation.

A significant advance in PCR technology for genetic analysis was the development of RAPD assays (random amplified polymorphic DNA) (Welsh and McClelland, 1990; Williams et al., 1990). The RAPD procedure obviates the need for prior sequence information. Instead, polymorphic DNA sequences are detected by amplification with a single random 10 base primer. If the primer binds to complementary DNA strands within an amplifiable distance, a discrete product is formed. Generally, RAPD primers may direct the amplification of multiple discrete products from a genomic DNA sample, making this an efficient way to screen for polymorphisms between individuals (Welsh and McClelland, 1990; Williams et al., 1990).

Our laboratory maintains a substantial collection of soybean cyst nematode populations from multiple locations in the United States. In addition to our field populations, we have over 70 populations that have been selected repeatedly on certain resistant soybean hosts. Although many of these lines still maintain a degree of heterogeneity due to the limited number of selection cycles that have been imposed, we have several highly inbred lines.

These lines have been repeatedly selected for several traits of interest, including enzyme phenotype and parasitism. In particular, we are working with three lines that have been carried forward by single cyst selection and are highly homozygous. The three lines, OP20, OP25, and OP50, have been inbred for a minimum of 22 generations. Isozyme analysis has demonstrated that these three lines are homozygous for the esterase and glucose phosphate isomerase (GPI) loci. Genetic analysis of esterase pattern indicates that the three observable phenotypes correspond to three codominant alleles at a single locus (Esbenshade and Triantaphyllou, 1988). No maternal effects were detected in these studies. Host range testing using the standard soybean differential genotypes reveals that these lines are highly specific in their parasitic abilities. Unlike the standard race concept, parasitism of a differential by one of the inbred lines is either positive or negative. No cysts develop on resistant soybean lines. This is an extremely important factor for genetic analysis using phenotypic selection.

Controlled crosses have been performed between these strains. Preliminary analysis has revealed several important factors. Segregation patterns in the F_2 progeny of the crosses suggest that inheritance of parasitic ability is Mendelian in nature. The genes controlling parasitic ability appear to be unlinked loci. Additionally, none of these loci appear to be linked to esterase or GPI phenotype. The progeny lines carrying genes conferring parasitic abilities on both resistant soybean genotypes did not appear to have altered interactions compared to the parents, suggesting that these loci do not interact, at least for parasitism of the soybean genotypes tested. No additional host range was detected. Finally, reciprocal crosses revealed no pattern of maternal or sex-linked inheritance for parasitic abilities.

Given these factors, it should be possible to obtain RAPD markers linked to the parasitism loci through bulked segregant analysis (Michelmore et al., 1991). In this protocol, segregating F_2 populations can be screened for host preference and the individuals pooled into groups of compatible and incompatible lines. RAPD screening of these pools should identify markers linked to parasitism. In theory, this procedure is a quick and straightforward method to generate markers linked to parasitism genes. In practice, the necessity of screening individual progeny (i.e., single nematodes) introduces a level of variability that is unacceptable for genetic mapping. Therefore, we have chosen the alternative strategy of making recombinant inbred lines (RIL). The lines are being developed by full sib mating and single cyst descent through the F_8 generation. Large amounts of clean DNA can be obtained from each of these lines, markers linked to the parasitism loci can be quickly identified, and the necessary host range tests may be performed on a large population. This approach provides a great deal more certainty regarding data collected from the individual lines.

The most efficient way to identify markers linked to a particular area of the genome is to pool samples of DNA into two separate bulks composed of DNA from individual lines that are segregating for one of the parental parasitism phenotypes. All RAPD markers in these bulks should appear in linkage equilibrium except for those linked to the parasitism loci. Markers linked to these loci will appear polymorphic between the two pools of DNA. The use of a large number of segregating recombinant inbred lines should minimize the chances of identifying polymorphic markers that are unlinked.

Because of the large number of primers that can be screened, RAPDs are very useful for obtaining markers tightly linked to genes for which no mapping has previously been performed (Williams et al., 1990), such as is the case for *H. glycines*. The OP20 x OP50 cross was performed to generate recombinant inbred lines that would provide information regarding segregation of two independent parasitism loci, namely, those for parasitism of Peking and of PI 90763. More than 300 random 10-mer primers have been screened against the parental lines from the cross. Numerous polymorphic bands have been identified between the parental lines, and the stability of these bands is being verified by repeated assay from independent DNA extractions. Markers identified in this procedure will be analyzed for cosegregation with host preference phenotype and linkage groups assigned by a χ^2 analysis. Computer map construction software will be utilized to assign order of markers within the identified linkage group (Michelmore et al., 1991).

The investigations discussed above should lead to the identification of putative virulence genes. However, the only unambiguous way to determine whether or not a given sequence is related to parasitism is through nematode transformation. In this way, SCN strains unable to infect a particular host genotype could be transformed with genomic clones containing the putative gene. Because the parasitism genes appear to be dominant in nature, a screen of the R1 generation would provide definitive evidence for involvement of a particular sequence in parasitism. Phenotypic rescue of *C. elegans* mutant strains by cosmid rescue is now a standard procedure. It may be possible to develop an SCN transformation system based on microinjection of the male gonad (Fire, 1986). One alternative strategy is microprojectile bombardment of developing females. A hydroponic or root explant culture system would be ideally suited to adaptation for this type of approach. This is necessary because the female gonad is not visible through the opaque nematode body, making injection of the ovary impossible. A second alternative approach is the direct injection of the genital primordia in second-stage juveniles. This approach is fraught with problems of its own and would certainly yield very low numbers of transformed animals. This strategy seems viable only as a last resort if the other two strategies fail.

FUTURE CONSIDERATIONS

Within the next year, a linkage map should be generated for parasitism loci. The biggest hurdle is how tightly linked the markers obtained will be. We believe that the approach of using RAPD combined with expressed sequence tags will provide markers that map close to the target gene. The small size of the nematode genome and previous success with map based cloning in *C. elegans* (Emmons, 1988) provides confidence in this phase of the project. Due to the low level of repetitive DNA sequences in nematodes, the isolation of cosmid clones and chromosome walking should be very straightforward and no particular difficulties are anticipated with this portion of the project. Sequencing of an entire 50 kb cosmid may also be readily accomplished through automated sequencers, therefore cloning and sequencing of an SCN parasitism gene should be attainable in the near future.

Once a transformation protocol is in place, analysis can begin of cosmid clones isolated in the mapping part of the project. Clones containing markers linked to parasitism can be microinjected into males, and resulting transformed second-stage juveniles will be inoculated to resistant soybean cultivars to assay for parasitic abilities. Although this seems to be a relatively straightforward approach, numerous things could confound the analysis. For example, position effects may alter expression patterns of the parasitism gene or poor integration may cause the generation of mosaic animals that vary in their ability to parasitize the resistant host. For this reason, a large number of independent transformants must be evaluated. Although isolation of a cosmid clone containing putative parasitism genes is possible based on the mapping strategies, unambiguous identification of a gene requires the transformation of a non-parasitic strain and its subsequent alteration of parasitism. In addition, the development of a plant parasitic nematode transformation system will be of tremendous significance to future studies on a large number of traits.

In the short term, once a parasitism gene has been isolated its expression patterns and control elements may be characterized. Of particular interest is the function of the gene product in the interaction between SCN and soybean. Localization of the peptide product and its structure may provide important clues as to how it functions in the nematode. Finally, we are optimistic that new ideas for developing host resistance and nematode control strategies will be devised once an understanding of the interaction at the molecular level is achieved.

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Analysis of Soybean Cyst Nematode Secretions Involved in Parasitism

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Abstract

The soybean cyst nematode (SCN), Heterodera glycines, *is considered to be the primary pathogen of soybean in the United States and is a threat to global soybean production. The growth, development, and reproduction of SCN is absolutely dependent on the induction of an elaborate feeding site (syncytium) in soybean roots by the nematode. Secretions that originate in the esophageal glands of the soybean cyst nematode (SCN) are exuded through its stylet into plant tissue to induce and maintain a syncytium in soybean roots. We have developed a panel of monoclonal antibodies (MAbs) that bind to subventral and dorsal esophageal gland secretions of SCN and have demonstrated binding of these MAbs to stylet secretions from juveniles of SCN. Western blots of SCN proteins probed with the esophageal gland MAbs have produced bands of approximately 140, 120, 50, and 40 kDa. A MAb specific to a SCN dorsal esophageal gland protein has been used to isolate and initially characterize a cDNA from a SCN expression library. Sequence analysis of the isolated cDNA suggests that it encodes a basic protein, but no significant homology to genes in the database has been detected. This cDNA clone may represent the first isolated dorsal gland secretory protein gene directly involved in plant parasitism by a nematode. Present research efforts are designed to identify esophageal gland secretory proteins by characterizing both the purified secretory proteins and their corresponding genes, to evaluate the expression and localization of these secretions during parasitism of soybean by SCN, and to conduct bioassays to assess the function of isolated secretory proteins and gene products* in planta *The long-term objective of this research is to develop novel resistance in transgenic plants that specifically inhibit the expression or function of stylet secretions from SCN.*

Background

The soybean cyst nematode (SCN), *Heterodera glycines*, is a sedentary endoparasite of soybean that has become one of the major limiting factors in soybean production in the United States and other major soybean producing areas (Riggs & Wrather, 1992). Like other species of cyst nematodes, SCN has a limited host range and is dependent upon its ability to establish a complex feeding site (syncytium) within soybean roots for its development and reproduction. Infective second-stage juveniles (J2) hatch from eggs within the cyst, migrate in the soil, and penetrate plant roots generally near the tip. The nematode moves intracellularly towards the vascular tissue of the root and induces localized necrosis along its migratory path (Endo, 1992; Wyss & Zunke, 1986,1992). Within 18 hours of root penetration, the nematode has located the appropriate root pericycle cells and secreted compounds from its esophageal glands through its stylet (feeding apparatus) to modify an initial syncytial cell (Endo, 1992; Hussey, 1989a). Cells adjacent to the initial syncytial cell are incorporated into the syncytium by cell wall dissolution, resulting in a multinucleate feeding site (Endo, 1992; Jones, 1981; Jones & Dropkin, 1975). The enzymes responsible for cell wall dissolution appear to originate from the plant cell (Jones & Dropkin, 1975). Hypertrophy and hyperplasia of syncytial tissues in the pericycle and hypertrophy of the nucleus within the initial syncytial cell are evident within 2 days after penetration (Endo, 1992). Cell wall perforations increase in size between adjacent syncytial cells, vacuoles decrease in size, and the cytoplasm becomes dense with an increase in plastids and reticular material within 4 days after inoculation. During this time the J2 has become swollen, sedentary, and is approaching the molt to third-stage juvenile. By day 5 syncytial nuclei are greatly enlarged, with prominent nucleoli and lobed nuclear membranes. Thickening and numerous invaginations of the peripheral cell wall occur and the syncytium continues to develop almost to the culmination of nematode feeding. All life stages of SCN feed on the syncytia with males developing in about 9 days and females developing in about 20 days.

The elaborate modifications induced in syncytial cells suggest that this interaction is under significant, specific control by SCN. In plant-parasitic nematodes, secretions from the stylet that originate in the nematode esophageal gland cells appear to play the major role in nematode feeding and modification of plant cells (Hussey, 1989a). The secretions are synthesized and packaged into secretory granules within the single dorsal and two subventral esophageal gland cells in SCN . The dorsal esophageal gland cell extends anteriorly to an ampulla (collecting reservoir) that opens through elaborate valves into the esophageal lumen at the base of the stylet . The position of this opening is in the immediate proximity necessary to favor flow of dorsal gland secretions out

through the stylet. The two subventral gland cells extend anteriorly to ampullae that empty through elaborate valves into the esophageal lumen just posterior to the metacorpal pump chamber. Researchers have suggested that the position of the subventral gland valves would preclude anterior flow of subventral gland secretions out through the stylet, and hence, these secretions probably function in digestion (Doncaster, 1971; Wyss & Zunke, 1986, 1992). When J2 of SCN hatch, the subventral glands are packed with secretory granules while relatively few granules are apparent within the dorsal gland (Endo, 1987, 1993). Dorsal gland secretory granules are small and electron-dense and subventral gland granules are large with irregular central cores. During host penetration and induction of syncytia, secretory granules in the dorsal gland enlarge and become electron-translucent and subventral gland granules become small and electron-dense (Endo, 1987, 1993). Secretions from the stylet of SCN, including some that may form feeding tubes, have been observed in the initial syncytial cell within 18-72 hours of infection (Endo, 1992; Rumpenhorst, 1984). Video-enhanced microscopy of the feeding cycle of *Heterodera schachtii* has demonstrated that secretions from the dorsal gland are secreted through the stylet tip inserted into the plant cell (Wyss & Zunke, 1986, 1992). Ingestion of plant cell nutrients later ensues through pumping of the metacorpus and concomitant restriction in forward flow of gland secretions. When ingestion ceases, the valves of the subventral glands open and the contents of the ampullae are released. The size of the gland and number of secretory granules greatly increases in the dorsal gland throughout the SCN life cycle, while the opposite occurs with the subventral glands. The reduced size and contents of the subventral glands during the active feeding in later developmental stages appears in contrast to the purported digestive function of the subventral gland secretions.

Investigations concerning the nature of esophageal gland secretions have been hampered by the nematode's obligate parasitic relationship with its host and the minute quantities of material that can be obtained for analysis. Histochemical analyses have been conducted on the esophageal gland secretions of *Meloidogyne* (Bird, 1968; Bird & Sauer, 1967; Cardin & Dalmasso, 1985; Sundermann & Hussey, 1988) that indicate the presence of proteins but do not confirm the presence of nucleic acids. SDS-PAGE of secretions from the stylets of *Meloidogyne incognita* females produced nine major bands, three of which appeared to be glycoproteins (Veech, *et al.*, 1987). The development of monoclonal antibodies (MAbs) that bind specifically to nematode esophageal gland antigens and secretions from the nematode stylet represents significant progress toward the isolation of biologically-active secretions (Atkinson, *et al.*, 1988; Davis, *et al.*, 1992; Goverse, *et al.*, 1994; Hussey 1989b). The specificity of monoclonal antibody binding allows the identification, localization, and affinity-purification of esophageal gland antigens. Monoclonal antibodies have been used to isolate a high molecular weight secretory glycoprotein and a gene encoding a subventral gland secretory antigen (a putative myosin) from *M. incognita* (Hussey, *et al.*, 1990; Ray, *et al.*, 1994). Techniques to produce and collect secretions from the style of *M. incognita, in vitro* (Bird, 1968; McClure & Von Mende, 1987), have been utilized to develop MAbs that bind to esophageal gland secretions and to demonstrate that antigens from the subventral glands can be secreted through the stylet (Davis, *et al.*, 1992, 1994). This latter finding suggests that both dorsal and subventral gland secretions may be important in feeding site formation. Immunofluorescence microscopy has been used to monitor the change in expression of several dorsal and subventral esophageal gland antigens in several developmental stages of *M. incognita* (Davis,*et al.*, 1994). SCN J2 stylet secretions and homogenates of J2 and females of SCN have been used to develop MAbs that bind to antigens within SCN esophageal glands (Atkinson *et al.*, 1988; Goverse, *et al.*, 1994). Monoclonal antibodies have been used in enzyme-linked immunosorbent assay (ELISA) to detect differential expression of esophageal gland antigens during the first 120 hours of infection by SCN (Atkinson & Harris, 1989). Attempts to isolate and identify esophageal gland secretory molecules involved in parasitism by SCN, however, have only recently been initiated. Until recently, the obligate parasitic nature of SCN has made these types of investigations extremely difficult.

Preliminary Results

A system has been developed to produce and collect relatively large (µg) quantities of esophageal gland secretions from the stylets of SCN J2, *in vitro* (Goverse, *et al.*, 1994). Freshly hatched J2 of SCN that are incubated for 4 hours in a solution of 5-methoxy DMT oxalate (Research Biochemicals, Inc., MA) thrust their stylets and produce viscous stylet secretions that stain with the general protein stain, Coomassie Brilliant Blue. DMT is a tryptamine analogue that is a potent agonist of serotonin (5-hydroxytryptamine), a neuroactive compound that stimulates esophageal activity and egg-laying in *Caenorhabditis elegans* (Chalfie and White, 1988). In DMT, the SCN J2 subventral esophageal gland extensions are packed with secretory granules and some accumulation of secretory granules is also apparent in the dorsal gland ampulla. Little effect on SCN viability or infectivity of soybean is observed after treatment of J2 with DMT. Stylet secretions from millions of SCN J2 can now be solubilized in alkaline buffer or a few select biological detergents and collected daily. Collected secretions are concentrated using filters with 10,000 NMWL membranes, and the concentrated secretions greater than 10,000 kDa are the focus of this research. Secretions smaller than 10,000 kDa are collected and stored at -80 C to be analyzed when an efficient bioassay for SCN secretory molecule activity has been established.

Concentrated stylet secretions and homogenates of SCN J2 have been used in an intrasplenic immunization technique (Davis, *et al.*, 1992) to develop a panel of MAbs (Table 1) that bind specifically to esophageal gland antigens in SCN (Goverse, *et al.*, 1994). These MAbs differ in their esophageal gland binding patterns and specificities. All the MAbs bind to only subventral gland antigens in SCN J2 except for one MAb, $5B_9$, which was generated using SCN stylet secretions as immunogen and binds to only a dorsal gland antigen. Binding of all the MAbs to stylet secretions from J2 of SCN has been observed by immunofluorescence microscopy, but low concentrations of several antigens in stylet secretions results in variable binding of some MAbs. The binding of MAbs to stylet secretions has indicated that antigens in the subventral esophageal glands of SCN can be secreted through the nematode's stylet (Goverse, *et al.*, 1994). All the MAbs bind to esophageal gland antigens within hatched, preinfective SCN J2, but only two of the MAbs bind to esophageal gland antigens in adult females of SCN. These two MAbs,$9C_2$ and $6A_3$, bind to antigens in both the subventral and dorsal glands of the adult developmental stage of SCN. Immunofluorescence assays with *H. schachtii*, *Globodera tabacum*, *M. incognita*, and *C. elegans* suggest that the esophageal gland antigens appear to be specific to cyst nematodes. Additional MAbs raised to esophageal gland antigens in the potato cyst nematode, *Globodera rostochiensis*, bind to SCN esophageal glands and have been received for use from Dr. A. F. Schots of the Agricultural University, Wageningen, the Netherlands (Table 1). Concentrated SCN stylet secretions are also being used to generate polyclonal sera in rabbits.

Concentrated SCN J2 stylet secretions, as well as esophageal gland secretions within preparations of homogenized SCN J2, are currently being used in various analyses as one approach to determine the identity and function of SCN secretions. Preliminary Western blots have been conducted to analyze several of the protein antigens that are bound by the esophageal gland MAbs (Fig. 1). Two subventral gland MAbs, $9C_2$and $6A_3$, bind to apparently the same broad protein band of approximately 120 kDa, and another subventral gland MAb, MGR 48, binds to a strong band of about 50 kDa. The dorsal gland MAb, $5B_9$, binds to a strong band of approximately 140 kDa and a weaker band of about 40 kDa. Periodate oxidation of carbohydrate epitopes of proteins on Western blots (Woodward, *et al.*, 1985) eliminated the binding of MAbs $9C_2$and $6A_3$, but did not effect binding of $5B_9$ or MGR 48 to their respective protein bands. Crosslinking of the MAbs to magnetic beads (Karlsson and Platt, 1991) is now be conducted to immunoaffinity purify SCN secretory molecules for structural and functional analyses.

The MAbs are also being used to screen expression libraries to isolate and identify SCN genes that encode esophageal gland secretory proteins. This is a second approach that has been initiated to determine the identity and function of SCN secretory molecules involved in parasitism. Degenerate oligonucleotides designed from the amino acid sequence of purified secretory proteins may also be used to probe for SCN genes in future experiments. A cDNA library established from SCN J2 was kindly provided by Dr. C. H. Opperman of N.C. State University and colony lifts of this expression library have been initially screened with the MAbs specific to SCN esophageal glands. A cDNA clone that is recognized by the dorsal gland MAb, $5B_9$, has recently been isolated that contains an insert of 585 bp. The cDNA insert has been sequenced and subjected to database searches for nucleic acid and amino acid homology to reported genes. Nucleotide sequence analysis and comparisons with the 140 kDa protein isolated on Western blots indicate that the isolated cDNA is not full-length . The cDNA appears to contain a 3' untranslated sequence and an open reading frame that encodes a putative basic protein. No significant homologies (BLAST scores < 100) to known genes have been obtained in database searches of the derived amino acid sequence, but similarities to basic proteins such as protamines, histones, and some nucleic acid-binding proteins are observed. The putative basic residues are consistent with earlier histochemical observations of basic secretory proteins in *Meloidogyne* (Bird, 1968), and this knowledge may help overcome some of the difficulties thus far experienced with the isolation of esophageal gland secretory proteins. The cDNA clone hybridizes to SCN genomic DNA on preliminary Southern blots, but does not hybridize to genomic DNA from *G. tabacum* or *M. incognita* (Fig. 2). Our initial evidence suggests that this cDNA may represent the first isolated dorsal gland secretory protein gene involved in plant parasitism by a nematode.

The long-term objective of this research is to use information generated from this project to develop novel resistance in transgenic plants that specifically inhibit the expression or function of stylet secretions from SCN. Syncytium formation represents a fundamental and vulnerable target for inhibition of parasitism by all races of SCN. Experiments to isolate and identify several cyst nematode secretory antigens and their corresponding genes are ongoing in this laboratory. Subsequent immunocytological research will evaluate the expression and localization of these secretions during parasitism of soybean by SCN to assess their role in syncytium formation by SCN. Microinjection of purified SCN secretions into soybean tissue and expression of SCN secretion genes in transformed plant tissue will be used for more direct analyses of the function of SCN secretions. Development of transgenic plants that express antibodies (Hiatt, *et al.*, 1989) that bind to secretions or other transgenes that neutralize the function of SCN secretions may provide novel and durable resistance to SCN.

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Table 1. Binding specificity of monoclonal antibodies to the esophageal glands[a] in second-stage juveniles (J2) and young females of the soybean cyst nematode (*Heterodera glycines*), J2 of *Heterodera schachtii* , *Globodera tabacum*, and *Meloidogyne incognita*, mixed life stages of *Caenorhabditis elegans*, and to stylet secretions[b] of J2 of *H. glycines*.

Monoclonal Antibody	Antibody Class	*H. glycines* J2	*H. glycines* Female	Secretions (FITC)	*H. schachtii* J2	*G. tabacum* J2	*M. incognita* J2
From Goverse, *et al.*, 1994							
$9C_2$	IgM	SvG	SvG, DG	+	SvG	ND	ND
$6A_3$	IgM	SvG	SvG, DG	+	SvG	ND	ND
$1D_9$	$IgG2_b$	SvG	ND	+/-	SvG	ND	ND
$3H_5$	$IgG2_a$	SvG	ND	+	SvG	ND	ND
$9H_{12}$	IgM	SvG	ND	+/-	SvG	SvG	ND
$5B_9$	IgG_3	DG	ND	+/-	ND	ND	ND
From A. Schots (Netherlands)							
MGR 22	IgM	SvG	NT	NT	SvG	SvG	SvG
MGR 48	IgG_1	SvG	NT	NT	ND	SvG	ND

[a] Antibody binding was specific to secretory granules within the dorsal (DG) or subventral esophageal glands (SvG), was not detected (ND), or was not tested (NT) by indirect immunofluorescence microcopy using fluoroscein isothiocyanate (FITC)-conjugated anti-mouse second antibody. No MAb binding to *C. elegans* was observed.

[b] Positive (+), negative (-), or variable (+/-) detection of antibody binding to stylet secretions collected, *in vitro*, from J2 of SCN that were incubated in 5-methoxy DMT oxalate. Antibody binding was confirmed by indirect immunofluorescence (FITC) of intact J2 secretions.

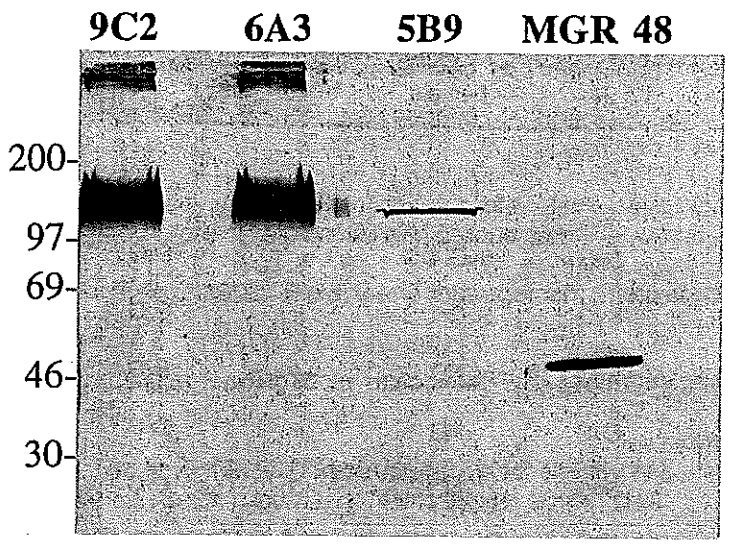

Fig. 1. Western blot of proteins from homogenates of soybean cyst nematode second-stage juveniles (J2) probed with several esophageal gland-specific monoclonal antibodies listed in Table 1.

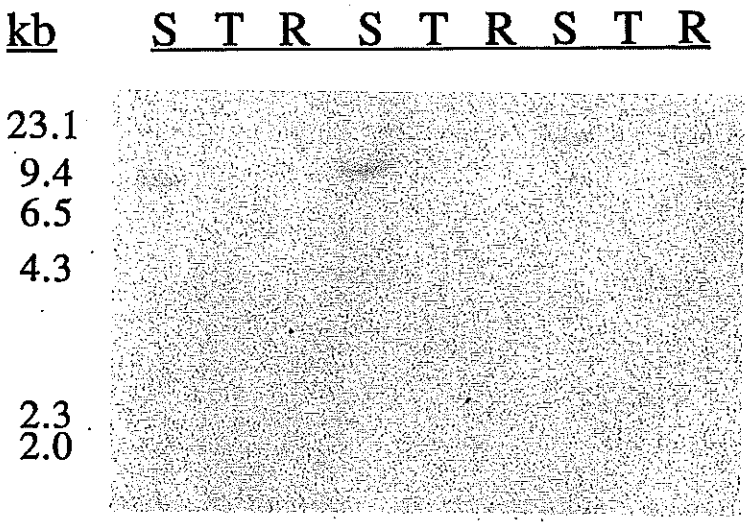

Fig. 2. Southern blot probed with a cDNA that encodes a putative soybean cyst nematode dorsal esophageal gland protein recognized by monoclonal antibody 5Bg. Lanes contain genomic DNA isolated from the soybean cyst nematode (S), tobacco cyst nematode (T), and southern root-knot nematode (R) digested with restriction endonucleases *Cla* 1 (lanes 1-3), *Sal* 1 (lanes 4-6), and *Xba* 1 (lanes 7-9).

SOYBEAN CYST NEMATODE INTERACTIONS WITH OTHER PATHOGENS OF SOYBEAN

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Abstract

The majority of putatively important soybean pathogens are soilborne, and thus, are physically in a position to interact with the soybean cyst nematode. No report was found of an example where the presence of the soybean cyst nematode was required for the invasion of soybean roots by a plant parasitic fungus or bacterium However, increased disease incidence and severity are reported for several soilborne pathogens in soybean cyst nematode-infected soybean. In contrast to sudden death syndrome, the relationship between the soybean cyst nematode and the brown stem rot pathogen, has not been explored extensively. The interrelationships have been described between the soybean cyst nematode and several root rotting pathogens of soybean. In most cases the combination of pathogens has an additive effect on yield loss. However, this greater pathogenic effect frequently results in less reproduction by the cyst nematode. Soybean production is entering the era of multiple disease resistant cultivars. Although crop rotation and other cultural practices are sound disease control tactics, the soybean genotype is the foundation of a soybean management system designed to improve crop health. Research should be focused on the effect of the soybean cyst nematode on the "interaction phenotype" of soybean genotypes. Tactics are in place to control individual soybean pathogens. However, future advancements to improve the efficiency of soybean production will require an integration of management practices into a system which is environmentally and economically sound; and with economic thresholds for individual pests which take into account the interactive relationships among pests.

Introduction

The soybean cyst nematode and plant pathogenic fungi, bacteria and viruses are common deterrents to soybean production. Tactics are in place to control individual pathogens. However, it will be necessary to integrate management practices into a system which is environmentally and economically sound to further advance the efficiency of soybean production; and with economic thresholds for individual pests which take into account the interactive relationships among pests. Some may argue that more information is needed on individual pathogens and the diseases they cause before attention can be turned to studying the relationship of soybean pathogens to one another. We contend that more knowledge will be acquired on individual pathogens by investigating their relationship with each other in agroecosystems.

McGawley (1992) recently reviewed the relationships of soybean cyst nematode with other organisms that influence soybean health and productivity. Our intent is to build on McGawley's review and introduce ideas and concepts that may provide guidance for future research to discover the means to refine integrated soybean management systems.

General Review of Nematode Complexes

Plant parasitic nematodes and other soilborne plant pathogens are in close proximity on or in roots so there is often potential for an interaction to occur. Numerous studies describing the outcomes of joint infection by nematode and microorganisms have been reviewed and will not be discussed in-depth (Powell, 1971 & 1979). Some of the assumptions and conceptual framework developed to describe concomitant relationships of nematodes and pathogens do need to be mentioned, however, to understand our current models of disease caused entirely, or in part, by the soybean cyst nematode.

Types of Complexes

Interactions are generally defined with the host plant as the reference point. Ample research has demonstrated, including many studies on the soybean cyst nematode, cases where the effects (i.e., growth, yield, etc.) of coinfection of plants by a nematode and another microorganism are equivalent to the sum of the effects that occur when each organism is present alone. This type of relationship is described as an "additive interaction". A "synergistic interaction" occurs when the effects of concomitant infection are greater than that predicted from the

effects of each pathogen alone. The most thoroughly documented synergistic interaction between a nematode and a fungus is that of *Verticillium dahliae* and *Pratylenchus penetrans* for the early potato dying disease. Levels of the fungus and nematode which alone had no effect on disease symptomology together reduced yield in Ohio, Wisconsin and Washington . Similar studies pairing comparable levels of *V. dahliae* with another nematode, *Meloidogyne hapla*, demonstrated an additive rather than synergistic interaction.

Interactions can also be viewed from the perspective of the participating organisms, with alteration of the life cycle and/or symptoms typical of the nematode or microorganism as the reference point. There are numerous accounts of enhanced symptomology of fungal diseases in nematode-infected plants (Powell, 1971 & 1979; Jeffers & Roberts, 1993; McGawley, 1992), but the data on the role of other pests on the reproductive activity of nematodes is equivocal. Being obligate parasites, plant health is vital to the reproduction of plant-parasitic nematodes. Reproduction of the soybean cyst nematode may be reduced if root health is severely compromised by root-infecting microorganisms. Some soybean pathogens are also capable of parasitizing cysts and eggs of the soybean cyst nematode (Carris, et al., 1989). The implications of this discovery are not known, but warrant investigation to determine if parasitism of cysts and eggs may influence population dynamics of the soybean cyst nematode and its interactive relationships with other pathogens of soybean.

Role of Nematodes in Complexes

The role of nematodes in disease complexes has not been elucidated for any system. The interpretation of early studies (Powell, 1979) was that nematodes initiate a sequence of events by providing wounds which facilitates invasion by other pathogens. Subsequent work showed that the relationship between nematodes and microorganisms is generally more complex than simply coinhabiting a site on a root. The term "biopredisposition" (Powell, 1979) was coined to describe enhanced disease severity and has been explained as a "breakdown" of host resistance to, and/or increased inoculum potential of the pathogen in complex with the nematode. Also, nematodes have been suggested to predispose plants to invasion by normally "weak" pathogens, thus, enhancing their pathogenic effects on plants. The mere act of feeding injury may not explain greater disease severity resulting from nematode-fungal complexes. Nematode activity in roots may alter physiological functions which stimulate root production, accelerate root senescence, or weaken host defense systems resulting in greater damage by other pathogens. The latter example also has implications related to leaf or stem infecting pathogens.

Summary of Major Microbes Associated with Soybean

The majority of putatively important soybean pathogens are soilborne, thus, are physically in position to interact with the soybean cyst nematode. A summary of these pathogens is presented in Table 1. *Bradyrhizobium japonicum* and vesicular arbuscular mycorrhizae fungi (Glomus spp.) also are common inhabitants of soybean roots and should be considered in a discussion of the soybean cyst nematode interactions with other microbes.

Table 1. Microorganisms commonly associated with soybean in the North Central States.

Microorganism	Common Name	Plant Parts Infected
Heterodera glycines	Soybean cyst nematode	Roots
Phytophthora sojae	Phytophthora root rot	Roots, Lower Stems
Macrophomina phaseolina	Charcoal rot	Roots, Lower Stems
Fusarium solani (Form A)	Sudden death syndrome	Roots, Lower Stems
Fusarium oxysporum	Fusarium wilt	Roots, Lower Stems
Thielaviopsis basicola	Black root rot	Roots
Phialophora gregata	Brown stem rot	Roots, Stem/Vascular
Sclerotinia sclerotiorum	Sclerotinia stem rot	Stems
Diaporthe phaseolorum var. *caulivora*	Stem canker	Stems
Septoria glycines	Septoria brown spot	Leaves
Glomus spp.	Mycorrhizal fungi	Roots
Bradyrhizobium japonicum	Nitrogen-fixing bacteria	Roots

Microbe/Soybean Cyst Nematode Relationships

A comprehensive understanding of pathogen survival, inoculum thresholds, epidemiology and interaction among soybean pathogens is needed to refine disease/crop management systems. Although not totally perfected, population density and yield relationships have been established for *H. glycines* and soybean. Unfortunately, information is limited on the relationship of fungal pathogens to disease severity and yield. Some data is available for *M. phaseolina* (charcoal rot; Todd et al., 1987) and *Phialophora gregata* (brown stem rot; Adee, et al., 1995).

Effects on Disease Incidence/Severity and Yield

Juveniles of *H. glycines* invade mature roots and migrate intracellularly, thereby destroying cortical cells (Endo, 1964). Therefore, penetration of roots by *H. glycines* could conceivably modify soybean roots to facilitate invasion by plant parasitic microorganisms. Penetration of roots by *H. glycines* could also expand the influence of the rhizosphere by enhancing root exudates that stimulate germination of fungal propagules. However, the cohabitation of a plant root does not guarantee an interaction will develop between *H. glycines* and a necrotrophic plant pathogen. Soil conditions that influence the activity of individual root invaders will dictate if and when root pathogens form disease complexes. Root penetration by the soybean cyst nematode is favored in drier soils and at higher O_2 concentrations than preferred by many soilborne pathogens (Johnson, et al., 1993). Yield loss caused by the soybean cyst nematode can be greater if plants are subjected to a water deficit stress during the pod-fill stage (Johnson, et al., 1993; Young and Heatherly, 1988). Thus, it is unlikely that H. *glycines* will interact with other root invaders which infect during periods of water saturated soils.

No report was found of an example where the presence of the soybean cyst nematode was required for the invasion of soybean roots by a plant parasitic fungus or bacterium. However, increased disease incidence and severity are reported for *Phytophthora sojae* (Adeniji, et al., 1975), foliar symptoms of *Fusarium solani* (form A), (Roy, et al., 1989), *F. oxysporum* (Ross, 1965), *Rhizoctonia solani* (Schenck and Kinloch, 1974) and *Macrophomina phaseolina* (Todd, et al., 1987).

Sudden death syndrome of soybean has been observed for 20 years, but the causal agent was recently determined to be a specialized form of *F. solani* (Form A). The soybean cyst nematode was frequently found in association with symptoms of sudden death syndrome and for a time was believed to be necessary for the disease to occur. Roy et al. (1989) and Rupe (1989) showed that the soybean cyst nematode is not required for the symptoms of sudden death syndrome to develop. However, symptoms of sudden death syndrome occurred earlier and more severely if *H. glycines* was present with *F. solani* (Form A) (Melgar, et al., 1994). Irrigation resulted in greater disease incidence and severity of sudden death syndrome. Maturity group of cultivars did not effect the severity of sudden death syndrome regardless of the presence of the soybean cyst nematode. (Rupe & Gbur, 1995).

In contrast to sudden death syndrome, the relationship between the soybean cyst nematode and *Phialophora gregata*, the cause of brown stem rot, has not been explored extensively. Niblack et al. (1992) determined that the soybean cyst nematode was the dominant pathogen in a mixed infestation with *P. gregata*. However, disease severity caused by *P. gregata* appeared to be below thresholds to reduce soybean yield. More recent studies in Iowa suggest that the incidence of brown stem rot is greater in the presence of the soybean cyst nematode (Tubajika, et al., 1994). Preliminary studies in Wisconsin suggest that the soybean cyst nematode and *P. gregata* have an additive effect on yield (unpublished data). However, more extensive investigations are needed before definitive conclusions can be made on their relationship. The common occurrence of each pathogen necessitates further research.

Lesions caused by the stem canker fungus were reduced when roots were colonized by the soybean cyst nematode (Russin, et al., 1989). In a separate study, *H. glycines* and the stem canker pathogen combined to cause a negative additive effect on soybean yield (Pacumbaba, 1992)

Effect of Interactions on Pathogen Population Density

An enumeration of plant pathogen propagules in host roots is frequently restricted to root tissues even though many pathogens progress from roots to lower stems or beyond. Propagules in stems may relate better to seed yield (Adee, et al., 1995) than propagules in root tissues. Although not always predictive of current year yield, root phenotypes are important in terms of future inoculum densities. Soybean pathologists have not explored

pathogen reproduction as a measure of host reaction, inoculum generation and prediction of crop productivity. The study of disease complexes may change this situation. The influence of *H. glycines* on the reproduction of fungal pathogens must become a priority if soybean disease management systems are to be refined and improved.

Populations of sedentary nematodes generally are suppressed as a result of interaction with fungi (Powell, 1971). Plant parasitic nematodes are obligate parasites with a long life cycle. If root tissues die or decline in physiological activity, so does the nematode. The pathogenic effects of the fungal pathogen may influence assimilate partitioning to roots, thus reducing substrates needed for reproduction by plant parasitic nematodes.

Root infecting vascular pathogens generally do not cause extensive root rot, and foliar symptoms are typically delayed until pod development. Like vascular diseases, stem diseases such as stem canker and Sclerotinia stem rot also do not result in "obviously" impaired root systems until stems and leaves express symptoms. In spite of delayed symptoms, lower populations of the soybean cyst nematode were associated with Fusarium wilt (Ross, 1965), stem canker (Russin et al., 1990), and brown stem rot (unpublished data). At the end of the growing season, soil populations of the soybean cyst nematode were higher for a resistant compared to a soybean cultivar susceptible to *P. gregata*. Although only two cultivars were compared, this discovery raises the concern that the use of resistance to *P. gregata* may lead to a faster buildup of the soybean cyst nematode. This example illustrates the importance of early detection of the soybean cyst nematode and selecting soybean cultivars to match disease potentials. Stress caused by foliar applied herbicides and insect feeding can also result in lower populations of the soybean cyst nematode (Browde et al., 1994a & 1994b).

Studies indicate that *H. glycines* infection can increase colonization of soybean roots by *M. phaseolina* resulting in greater yield loss due to charcoal rot (Todd, et al., 1987). Root densities of *M. phaseolina* were positively correlated with densities of *H. glycines* and negatively correlated with yield. These investigators also found soybean cultivars resistant to *H. glycines* supported fewer nematodes and propagules of *M. phaseolina*. The use of a nematicide, carbofuran, resulted in significantly fewer propagules of *M. phaseolina* in roots, even for cultivars resistant to H. glycines. Although resistant cultivars support less reproduction of *H. glycines*, the nematode still invades roots, thus possibly altering a host plant's response to *M. phaseolina*. Although *H. glycines* populations were reduced by carbofuran, it is interesting to note that the population of *M. phaseolina* was not reduced in roots of Pella soybean. Pella may represent a genotype highly susceptible to *M. phaseolina* that is not influenced by *H. glycines*. The data of Todd et al. (1987) suggests that *H. glycines* facilitates infection of soybean roots by *M. phaseolina*, but charcoal rot susceptibility is host genotype dependent. However, an increase in propagules of *M. phaseolina* may be the result of saprophytic rather than parasitic reproduction.

Microbes Associated with Cysts and Eggs

Microorganisms are implicated as a cause of nematode mortality. Nematode trapping fungi commonly occur in agricultural soils and have a potential as biocontrol agents. Niblack and Hussey (1986) evaluated *Arthrobotrys amerospora*, but determined it was not effective as a biocontrol agent of *H. glycines*. Although many fungi are reported to colonize cysts of *H. glycines* (Carris, et al., 1989), Kim et al. (1992) report the only naturally occurring fungus to exert biocontrol of *H. glycines*. A crop monoculture may be necessary to enhance the effectiveness of naturally occurring soil fungi to cause a decline of cyst nematode species (Kerry, et al., 1982). *Fusarium solani*, *Corynespora cassiicola* and *Phialophora gregata* are reported to colonize cyst of *H. glycines* (Carris, et al., 1986; Carris, et al., 1989). Although several soybean pathogens were isolated from cysts, the role of cysts in the etiology and epidemiology was not determined for these plant pathogenic fungi.

Management of SCN/Microorganism Interactions

Soybean is an integral component of the feed grain based cropping system of the North Central States. Soybean productivity has increased through changes in management practices, such as earlier planting, narrowed row width and increased seeding rate, nitrogen fertilizer and balanced soil fertility (Meese, et al., 1991; Oplinger & Philbrook, 1992). These advances in crop management are jeopardized by the soybean cyst nematode and other plant pathogens (Doupnik, 1993).

Influence of Host Resistance

Soybean production is entering the era of multiple disease resistant cultivars. Although crop rotation and other cultural practices are sound disease control tactics, the soybean genotype is the foundation of a soybean

management system designed to improve crop health. Soybean genotypes are important to reduce yield loss in the current year, but also are a tactic to influence pathogen population densities and frequencies of virulence phenoytypes.

Research should be focused on the effect of the soybean cyst nematode on the "interaction phenotype" of a soybean genotype. Only one report was found that suggests that the soybean cyst nematode "breaks down" resistance to other soybean pathogens. Fusarium wilt resistant soybean cultivars died early in the season if the soybean cyst nematode was present compared to plant performance in the absence of the nematode (Ross, 1965).

Few soybean cultivars on the market today are fully susceptible to *Phytophthora sojae*. Greater disease severity caused by *P. sojae* in association with the soybean cyst nematode was observed for a susceptible, but not a soybean genotype resistant (Rps1 gene) *P. sojae* (Adeniji, et al., 1975). The stability of other race-specific genes is not known. Interactive effects are likely to be associated with field resistant genotypes rather than cultivars with race-specific genes. Today's cultivars generally have a race-specific gene(s) which confers resistance to multiple races of *P. sojae*. These cultivars also may possess non-race specific resistance which also are described as field resistant or tolerant. The latter form of resistance offers protection against a broad range of races.

Resistance to the soybean cyst nematode is reported to influence disease severity caused by *F. solani* (Form A), the cause of sudden death syndrome. High degrees of resistance to sudden death syndrome are not available, thus, the ability of the soybean cyst nematode to "breakdown" resistance to sudden death syndrome cannot be determined at this time. Several researchers (Hershman et al., 1990; Rupe et al., 1991; Rupe & Gbur, 1995) reported soybean cultivars resistant to *H. glycines* are less affected by sudden death syndrome than are cultivars susceptible to *H. glycines* in field trials. However, yields were not correlated to populations of *H. glycines* or severity of sudden death syndrome. There was no mention of correlations between sudden death syndrome severity and soil populations of *H. glycines*. Cultivars susceptible to *H. glycines*, race 6, developed symptoms 10 days earlier and had significantly higher severity of sudden death syndrome than cultivars resistant to race 6. Rupe et al. (1991) suggest that *H. glycines* may cause biopredisposition, but not all soybean cultivars react in this fashion. In contrast, Abney et al. (1994) reported PI 437654, resistant to all current races of *H. glycines*, to be highly susceptible to sudden death syndrome. Thus, it appears prudent to evaluate soybean germplasm resistant to the soybean cyst nematode for reaction to sudden death syndrome before extensive use in cultivar development.

Several sources of resistance to *P. gregata* (Mengistu, et al., 1989) and the soybean cyst nematode (Wrather, et al., 1984) are available for genetic improvement of soybean germplasm. Resistance to each pathogen is independently inherited. Thus, segregating families must be subjected to selection pressure from each pathogen if both traits are desired in a single soybean genotype. The wide geographic distribution of each pathogen suggests the need for soybean cultivars with each trait. A20 soybean germplasm (Cianzio, et al., 1991) is an example of a successful integration of resistance genes to each pathogen in a common germplasm. Soybean germplasm evaluated in Wisconsin with 'Fayette' (Bernard, et al., 1988) as a source of resistance to the soybean cyst nematode has also expressed resistance to brown stem rot (MacGuidwin, et al., 1995; MacGuidwin, et al., unpublished data). Resistance to *P. gregata* appears to remain stable in the presence of the soybean cyst nematode (MacGuidwin, et al., 1995). In contrast, 'Newton', which is a cross between CN210 germplasm (soybean cyst nematode resistant) and BSR 101 soybean (brown stem rot resistant), is susceptible to brown stem rot.

Influence of Cultural Practices

Corn alternated annually with soybean has become a popular rotation sequence in the North Central Region. Unfortunately, soybean does not benefit as greatly from this crop sequence, especially cultivars that are susceptible to diseases favored by shortened rotation intervals. An integrated approach to studying a broad spectrum of pests within the corn\soybean ecosystem is necessary to successfully implement crop rotation plus other changes in soybean management practices. Experiments should be designed to determine the interactive effects among the targeted pests on soybean productivity and population dynamics of the pests.

Pesticides have been the primary tactic to control weed and arthropod pests of soybean. In contrast, cultural practices, primarily crop rotation, and disease resistant soybean cultivars are standard tactics to control plant pathogens (Adee, et al., 1994; Francl, et al., 1988; Hussey and Boerma, 1983; Todd, 1993; Wrather, et al., 1984; Young and Hartwig, 1992) or reduce their effects on yield. However, the yield enhancing effects of early

planting date and narrow row spacing are subject to modification depending on pest and pathogen pressure (Grau, et al., 1994). In anticipation of pending Federal regulations, the relationship between pathogens and tillage systems has been studied in several states. Results of early studies on tillage effects indicate an increase in brown stem rot severity (Adee, et al., 1994), but a decline in population density of the soybean cyst nematode (Tyler, et al., 1983). Although corn is an excellent nonhost crop to manage soybean pathogens, additional crops are needed to lengthen crop rotations to more effectively manage population densities of soybean pathogens. However, the susceptibility of candidate crops to soybean pathogens needs to be evaluated carefully before they are put in place. Similarly, weeds must be examined for host suitability to soybean pathogens. If these losses are not properly evaluated, then unrealistic weed interference threshold values may be used in decision making models. The presence of unknown hosts in a cropping system could negate the benefits of disease suppression anticipated by rotating soybean with corn, wheat and other nonhosts.

Summary

This summary of soybean management and pests relationships suggests that a complex situation exists which needs more investigation. Efforts to improve the efficiency of soybean production will require an integration of management components into a system which accounts for interactions between management components and soybean pests, and interactive dynamics among soybean pests. Interrelationships have been described between the soybean cyst nematode and several root rotting pathogens of soybean. In most cases the combination of pathogens has an additive effect on yield loss. However, this greater pathogenic effect frequently results in less reproduction by the cyst nematode.

Soybean health starts with an assessment of stress factors in specific fields. Fields should be sampled for the soybean cyst nematode and plants inspected for symptoms and signs related to other plant pathogens. Soybean farmers need to select cultivars that match the pathogen complex in a specific field. In addition, cultural practices should be integrated into a total crop management plan that augments desirable cultivar traits.

In summary, plant pathologists should try viewing the world more from the plant's point of view. They should think about pathogen reproduction and not just disease severity in regard to host x pathogen interactions. Nematologists should remember there is a world out there that includes more than the soybean cyst nematode. And finally, soybean breeders need to realize that the soybean cyst nematode is altering plant phenotypes more than we realize. A concerted effort should be given to breeding for multiple pathogen complexes. Genes for resistance to soybean pathogens should be treated as natural resources and deployed on the basis of scientific experimentation (Young, 1992).

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USE OF RESISTANCE GENES FOR MANAGING SCN

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Soybean cyst nematode (SCN) (Heterodera glycines Ichinohe) is a disease that has become an increasing problem in the North Central region of the U.S. in recent years. Since it is virtually impossible to eliminate SCN once it is present in a field, producers must learn how to manage SCN populations. There are several components to the current recommended SCN management programs. One of the keys to the successful management of SCN is the use of soybean [Glycine max (L.) Merr.] varieties which are resistant to SCN.

Resistance to SCN is the result of the interaction between two different organisms, the soybean plant and the soybean cyst nematode. A resistant variety then is a soybean variety where few if any soybean cyst nematodes of a given race can grow and reproduce (form a cyst with viable eggs) on it. A virulent SCN race is one which can reproduce freely on a given soybean variety. Thus to determine if there is a resistant reaction the researcher needs to describe the soybean variety (genotype) as well as the race (genotype) of the soybean cyst nematode used in the test.

For the purposes of this discussion the term resistance will refer to the plants ability to restrict or inhibit reproduction of the nematode. A susceptible plant allows a high level of nematode reproduction. Researchers recognize that there is wide physiological variation among soybean cyst nematode populations. While there are considerable differences of opinions on how to classify this variation in SCN populations a race classification for SCN using four selected soybean lines as differentials was developed (Table 1). This discussion is not intended to address SCN races, however as noted above resistance or a resistant reaction of a soybean variety occurs as a result of the interaction of that variety with a population of soybean cyst nematodes (race).

Soon after SCN was identified in the U.S. soybean researchers decided that in order for a variety to be considered resistant it had to have less than 10% of the number of white females (cysts) that a fully susceptible check variety had on it after a defined period of time. One of the reasons the level of 10% of a fully susceptible variety was chosen was because researchers felt that was the maximum level of reproduction that could occur without the population of nematodes reaching such high numbers that they would be a continuing major problem yet was high enough so that immediate shifts in nematode races would not occur. Ideally researchers would like to have varieties where there are no cysts (0% reproduction) but in most cases that has not been observed with most sources of SCN resistance.

Although a female index of 10% or less was used to define resistance from a genetic standpoint, breeders realized that female indices greater than 10% but less than 100% would be useful in some situations in producers fields and more importantly would indicate that some genes for partial resistance were present. A scheme prepared a few years ago divided the resistance reaction of soybean genotypes into four categories (Table 2).

After screening the soybean germplasm collection for SCN resistance to several races of SCN researchers found there were a few plant introductions that were resistant and a number of others that were partially resistant. When crosses were made between the resistant plant introductions and the best susceptible cultivars, researchers observed that the distribution of the female index (the number of white females on a given plant compared to the number of white females on the fully susceptible parent) of the F_2 generation approximated a normal curve. Only a small proportion of the population could actually be considered resistant (using the 10% or less index as a truly resistant phenotype). This observation suggested that several genes were required for complete resistance with each gene contributing some part or percentage to the resistant reaction. Using crosses involving several different plant introductions researchers determined there were at least four genes involved in resistance to various races of SCN. Not all the genes are necessarily present in each plant introduction. Other genes for resistance to other races of SCN have been identified in additional plant introductions. Currently there is one source of resistance, PI 437654, that has a high level of resistance to all known races of SCN presently described. The use of this source of resistance could be very beneficial in managing SCN.

Researchers using classical genetic studies of varieties were able to infer that different plant introductions carried different genes for resistance based on their reactions to difference races of the cyst nematode. This was the basis for the suggestion that the source of SCN resistance be used for designing the potential for varieties carrying different genes for resistance. Thus, for example, resistance to race 3 of SCN in a variety derived from PI 88788 was inferred to be different than resistance to race 3 of SCN in a variety derived from Peking or a variety derived from PI 209332. For a soybean producer, knowing the source of SCN resistance was an important consideration in selecting a resistant variety if he/she wanted to manage SCN in the most effective way. Until just recently there were few if any choices of sources of resistance. For race 3 of SCN almost all commercial varieties available had PI 88788 as the source of SCN resistance. There were a few much less productive varieties with Peking as the source of resistance. Last year the Minnesota Agricultural Experiment Station released a variety, 'Faribault,' that had PI 209332 as its source of resistance to race 3 of SCN. This now affords producers an opportunity to rotate sources of SCN resistance as part of their management scheme.

Research still underway at the University of Minnesota using molecular markers has confirmed that different plant introductions have different genes for resistance. Without going into the details of the techniques used for determining molecular markers, this new tool provides researchers with a more precise way to determine that the genes from different sources of SCN resistance are different. This is especially important since during the breeding process of incorporating resistance into improved varieties some of the genes for resistance can be lost. Also, if two different sources of resistance to SCN are crossed, molecular markers can help to determine which source of resistance contributed the genes that actually ended up in the variety. Again this knowledge is critical if producers want to be able to rotate genes for resistance to SCN as part of their management scheme to deal with SCN on their farm.

More genes probably condition resistance or partial resistance then have currently been described. This is partially due to the use of heterogenous SCN populations in genetic studies. Researchers also report that results from different genetic studies indicate that genes for resistance to some races of SCN may be linked or may be multiple alleles at a single locus. The complete situation with regard to resistance genes is needed since knowledge of resistance genes is critical for the deployment of genes based on the race or races of SCN present in individual fields. Use of varieties with the correct complement of genes will be important to producers in their SCN management plan. Knowing which SCN race(s) and/or the virulence genes in the cyst nematode will allow the producer to plant the appropriate varieties to help manage the SCN population and numbers of nematodes.

As we learn more about the nematode or as new races of SCN develop it may be necessary to pyramid genes for resistance from the different resistant sources. Pyramiding genes means putting together genes from several sources of resistance into new combinations that will be effective against the nematode. The pyramiding of genes requires that we have a good idea of the genes in each source of resistance so we can breed varieties with the proper combination of genes to manage SCN.

The use of known resistance genes can be used by soybean producers presently to help manage SCN problems. It is important to keep in mind that a resistant reaction involves both the soybean variety (the plant) and the nematode population (race). Thus as much information as possible should be obtained about each of these components of the interaction so the very best decisions can be made. As our knowledge of both the nematode populations and soybean varieties improves we should be able to use resistance genes even more effectively in the future to manage SCN.

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Table 1. Race classification for soybean cyst nematode using the host differentials as described by Riggs and Schmitt (1988).

Race	Reaction on differential			
	Pickett	Peking	PI 88788	PI 90763
1	-†	-	+	-
2	+‡	+	+	-
3	-	-	-	-
4	+	+	+	+
5	+	-	+	-
6	+	-	-	-
7	-	-	+	+
8	-	-	-	+
9	+	+	-	-
10	+	-	-	+
11	-	+	+	-
12	-	+	-	+
13	-	+	-	-
14	+	+	-	+
15	+	-	+	+
16	-	+	+	+

†- = Number of females and cysts recovered was <10% of the number on Lee soybean.
‡+ = Number of females and cysts recovered was ≥ 10% of the number on Lee soybean.

Table 2. Proposed ranking of resistance for soybean genotypes based on reproduction of soybean cyst nematode on host differentials evaluated for resistance as proposed by Schmitt and Shannon (1992).

Reproduction on test plant compared with susceptible host	Rating
%	
0-9	Resistant
10-30	Moderately resistant
31-60	Moderately susceptible
>60	Susceptible

Private Industry Perspective on Soybean Cyst Nematode

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Abstract

Commercial soybean breeders have been involved in the development of varieties resistant to the Soybean Cyst Nematode (SCN), Hederodera glycines Ichinohe, since the early 1970's. During that period, a large number of successful commercial SCN-resistant cultivars have been developed and released for use in the North Central United States, primarily utilizing traditional resistance sources derived from 'Peking' and PI 88788. Development of superior lines possessing SCN resistance has been hampered by the complex nature of SCN populations, the multigenic nature of SCN resistance, and the laborious of nature of traditional SCN breeding and screening techniques. As we move into the future, commercial breeding companies on the leading edge of technology may harness the power of genetic marker assisted selection to accelerate the development of superior SCN-resistant cultivars.

Introduction

My goal is to address Soybean Cyst Nematode (SCN) issues from the perspective of private industry, in particular, that of a commercial soybean breeder.

The Soybean Cyst Nematode is a leading cause of soybean yield reduction across the United States. Since its discovery in the USA in 1954, SCN has been detected in nearly all key soybean production states. This includes all states in the North Central region with the possible exception of North and South Dakota. According to a survey recently completed by the North Central Regional Committee on Soybeans Diseases, SCN was the leading cause of soybean production losses (among diseases and pests) in the North Central Region between 1989 and 1991, estimated at $1.3 billion (NSRL, 1994). This loss estimate surpassed that caused by key soybean diseases such as Phytophthora root rot, *Phytophthora megasperma* Drechs. f. sp. *glycinia* Kuan & Erwin., and brown stem rot, *Phialophora gregata* (Allington and Chamberlain) W. Gams, which have received substantial attention over the past 20 years by commercial soybean breeders in the North Central region.

As the incidence of SCN detection and damage escalate in the North Central region, one would speculate that the research investment in the private sector to combat SCN would escalate proportionally. This is certainly the case at Pioneer Hi-Bred International, where traditional breeding efforts aimed at incorporating SCN resistance into cultivars suitable for northern production regions has increased substantially over the past few years, and where teams of scientists are working together to develop strategies to deploy the tools of biotechnology to assist in the selection and development of improved SCN-resistant cultivars.

Management Strategies to Control SCN

While opinions vary somewhat amongst commercial breeders and companies as to the optimum strategies for controlling SCN, most advocate conventional approaches which are supported by solid research data and personal experience.

Generally accepted practices include proper sanitation to prevent movement of SCN from infected fields to SCN-free fields, and the maintenance of general plant health through proper fertilization, irrigation, and

control of weeds and insect pests. There also is general agreement, at least among commercial soybean breeders, that the utility of nematicides for control of SCN is currently limited by economic considerations under most circumstances. Use of non-host crops in rotation with soybeans is viewed as highly beneficial, especially when SCN population levels rise above economic thresholds.

Of course, from a plant breeder's perspective, inclusion of SCN-resistant soybean cultivars in rotation systems is highly desirable and allows growers to continue producing soybeans without breaking up traditional soybean-corn rotation patterns. The rotation scheme proposed by Melton et al. (1985) is probably referred to most frequently in discussions between commercial agronomists and their customers regarding SCN control strategies. This strategy employs a 4-year rotation, including an SCN-resistant cultivar one year in four (Figure 1.). The goal of this strategy is to reduce SCN field populations while minimizing the pressure for SCN race population shifts. An alternate proposal by Tylka (1994) suggests a six year cycle which deploys SCN-resistant cultivars in two years of a 6-year rotation, utilizing cultivars with different sources of resistance. Tylka's proposal seems to have some merit on a theoretical basis for minimizing unidirectional selection pressure towards a specific race shift. One difficulty with implementing Tylka's rotation scheme at the present time is the paucity of cultivars with Peking-type resistance available for use by soybean producers in the North Central region.

Factors that need to be considered in any crop rotation system include economic thresholds, comparative yields of SCN-resistant and susceptible lines in the target environment, and the long term implications of SCN population increase or decline. Several commercial and public breeding programs have initiated breeding strategies involving new sources of resistance such as PI 437654. If these breeding efforts produce SCN-resistant varieties with yields comparable to susceptible products, new rotation schemes which exclude the use of susceptible soybean cultivars may become feasible.

Figure 1. Proposed Crop Rotations for controlling Soybean Cyst Nematode populations.

Four Year Rotation (Melton et al., 1985)		Six Year Rotation (Tylka, 1994)	
Year 1	Nonhost crop	Year 1	Nonhost crop
Year 2	SCN-resistant soybean cultivar	Year 2	"PI 88788" SCN-resistant Soybeans
Year 3	Non-host crop	Year 3	Nonhost crop
Year 4	High yielding susceptible soybean cultivar	Year 4	"Peking" SCN-resistant Soybeans
		Year 5	Nonhost crop
		Year 6	Susceptible soybean cultivar

Another issue worth investigating is the rate at which race shifts occur when producers continuously plant cultivars with a single source of resistance. Our pathology training and experience with fungal diseases suggests a high probability of such shifts occurring at a relatively rapid rate when selection pressure is intense. However, the biology of SCN differs from that of many fungal pathogens in that SCN cysts may persist in the soils for many years, and while laying dormant, are not exposed to extreme selection pressure. This reservoir of inactive cysts may actually provide a buffer against rapid race shifts. Research by Hartwig et al. (1987) indicated no detectable race shifts in a Mississippi experiment where the Race 3 resistant cultivar 'Centennial' was grown continuously over a 10 year period. The same experiment suggested no advantage for including a susceptible soybean cultivar in the rotation. It is important for further research to be conducted in this area to supply useful data for optimizing rotation recommendations for the North Central region.

One final potential control method that should not be excluded from consideration is that of biological control, using natural parasites and predators of SCN. While no economically feasible alternatives are available at the present time, I believe that further investigations into biological control of SCN is warranted.

Commercial Variety Development

Traditional

Developing varieties resistant to SCN has been a primary target of commercial soybean breeders since the early 1970's when soybean breeding expanded into the private sector due to the passage of the Plant Variety Protection Act of 1970. To appreciate the impact of commercial breeding efforts on the development of SCN-resistant varieties, one may scan one of many surveys listing available SCN-resistant lines from public and private sector breeding programs. Although somewhat misleading due to the impact of foundation seed distribution systems within the private sector, a recent survey by the University of Illinois Cooperative Extension Service listed 22 public varieties and 240 private brand/varieties available possessing SCN resistance in maturity Groups I through IV (Shier, 1995).

Most commercial breeders, for whom improved yield performance is imperative, have focused their SCN resistance breeding efforts on the utilization of PI 88788, 'Peking', and their offspring. Peking was used first by soybean breeders to provide resistance to SCN due to its superior agronomic type compared to other available resistance sources. The use of PI 88788 followed as new races emerged which had the ability to colonize cultivars with Peking-type resistance. PI 88788 provided resistance to that newly emergent race, know characterized as race 14 (Thomas et al., 1975). Commercial breeders capitalized on SCN-resistant cultivars derived from these two sources and today, all, or nearly all commercial varieties with SCN resistance have ancestries tracing back to either Peking or PI 88788. A recent entrant from the public sector is 'Hartwig', a cultivar released in 1990 by the Missouri Agricultural Experiment station which is reported to possess resistance to all common races of SCN (1, 2, 3, 4, 5, 6, 9, and 14) and possesses a unique parentage including SCN-resistant PI43765 (Anand et al., 1991). Many commercial breeding programs are trying to incorporate the higher level of SCN resistance provided by Hartwig and PI 437654 into high yielding commercial cultivars.

Traditional approaches to breeding for SCN-resistance are hampered by several factors. First, SCN resistance is multigenic. This makes traditional breeding approaches employed to incorporate simply inherited resistance characteristics, such as backcrossing, quite difficult. Second, SCN-resistance is rarely expressed in an absolute manner. A low level of SCN reproduction is a usually noted on "resistant" cultivars. Third, traditional screening techniques employed to test for SCN resistance are tedious, time-consuming, subject to unpredictability. Breeders typically screen for SCN resistance in large field tests having a known race makeup. The procedure is to gently remove (dig) plants from the ground and count the number of female cysts on the roots, comparing the cyst counts to known susceptibles. The efficacy of field screening is often limited by highly variable SCN populations across the field.
Of all the duties I have encountered as a soybean breeder, digging SCN-infested plants ranks near the bottom of the "pleasure scale", particularly on a hot, sultry day. Confirmation screening is usually conducted in a greenhouse setting, where attempts are made to standardize and optimize environmental growing conditions, soil makeup, and cyst or egg levels in the potting medium. Even in the greenhouse, results are often variable.

New Approaches

SCN resistance appears to be an excellent candidate for trying out emerging technologies such as "marker assisted selection", which would allow breeders to track the genetic alleles associated with SCN resistance. The attraction is based on the potential of marker assisted selection to track the multitude of alleles required for wide spectrum race resistance, and the potential to eliminate or at least reduce the breeders need to rely on unpredictable field and laboratory screening. This is a very complex and resource intensive undertaking, but the end result could be quite rewarding.

Pioneer has been actively involved in mapping the SCN race 3 resistance genes of PI 437654 using RFLP's and RAPDs in order to support the development of a marker assisted selection system. Use of *Mapmaker*

and *SAS General Linear Models* procedures allow us to estimate of genetic distances between linked markers and the association of these markers with alleles responsible for SCN resistance.

Effective marker assisted selection has several prerequisites, including accurate mapping, one or two (flanking) markers near each SCN-resistance QTL, polymorphisms in breeding populations, production capacity, and breeder/lab cooperation to carry out the interactive tasks of plant propagation, DNA collection, genetic screening, data analysis, and breeding.

Preliminary results are quite promising and we believe that marker assisted selection will soon allow us to vastly improve the efficiency with which our breeders are able to develop SCN-resistant cultivars.

Future Trends

As one who has been asked for a perspective, I feel free to speculate on the future of SCN in the North Central Region. The one things that seems clear, at least for the foreseeable future, is that SCN is here to stay and will continue to present an challenge to soybean producers and researchers in the North Central Region. However, we are fortunate to live in an era where emerging technologies offer potential solutions to many of our dilemmas.

Biotechnology will provide unique tools to assist plant breeders with the development of superior SCN-resistant soybean cultivars. Our ability to map SCN resistance genes and develop genetically-based marker assisted selection systems may allow breeders to incorporate resistance alleles with improved efficiency from a wide array of germplasm sources. Likewise, soybean transformation capabilities and the ability to create "designer" gene constructs may provide an opportunity to deploy unique genetic resistance strategies, heretofore, not possible.

The challenge is still great, but we have exciting opportunities to manage SCN in ways we only dreamed of in the not-so-distant past.

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