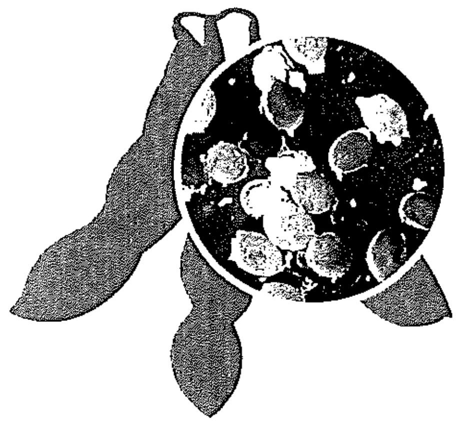
2016

Soybean Cyst Nematode Conference

December 13–15, 2016 • Coral Gables, Florida

Adapting to an Evolving Pathogen



ABSTRACT BOOK

Presentations and Posters

Presented by

The American Phytopathological Society

3340 Pilot Knob Road, St. Paul, MN 55121 U.S.A.

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SESSION ABSTRACTS

PLENARY I

Soybean cyst nematode: current status, challenges and opportunities.
Gregory Tylka, *Iowa State University*

The soybean cyst nematode (SCN), Heterodera glycines, was first discovered in North America in 1954. Although a great deal has been learned about SCN biology, ecology, and management, it continues to be a serious soybean pest. Challenges in managing SCN include high levels of reproduction, long-term persistence in the absence of hosts, strong influence of edaphic factors on population dynamics, synergistic interactions with other pests and pathogens, and lack of genetically diverse resistance in commercially available soybean varieties. There is a unique opportunity in soybean-producing areas where SCN is not yet widespread to discover infested fields while nematode population densities are relatively low and to implement management practices to prevent population densities from increasing unchecked. In areas where SCN populations are prevalent and have developed elevated levels of reproduction on SCN-resistant varieties, there is great potential for widespread adoption of varieties with sources of resistance other than PI88788 as well as varieties with engineered SCN resistance, when such varieties become available. Also, opportunities for using nematode-protectant seed treatments for more integrated management of SCN are great. Continued research is needed to understand the nematode's basic biology, to discover the molecular basis of feeding-site development, to determine the mechanisms of interactions of SCN with other pathogens and pests, and to understand the basis of the effects of soil pH and moisture on SCN reproduction. The future of sustained, profitable soybean production in North America will be determined, in part, by how well the aforementioned challenges are overcome and the opportunities embraced.

Future challenges in breeding for resistance to soybean cyst nematode
J. Grover Shannon, *University of Missouri*

Intense production of soybeans as a high protein, high oil, crop for human and animal consumption continues to grow annually. However, soybean cyst nematode (SCN) remains the number one, soybean yield limiting pest, worldwide. Cost effective nematicides are unavailable therefore, crop rotation and resistant varieties are best management strategies to reduce losses. A primary tactic to manage SCN losses is host resistance. Of the 100 plus resistant sources, SCN cultivars or germplasm trace to a few sources with Peking, PI 88788 and PI 437654 being the major resistance sources. Most resistant varieties today trace to PI 88788. Development of resistant varieties is challenging because of variability of the pathogen and the multigenic inheritance of resistance. Although PI 88788 still provides protection, continued use of this source has allowed a shift to other HG types rendering it less effective in reducing losses. The rhg1 locus on chromosome 18 is the primary locus associated with resistance from sources studied to date. The rhg1 allele from Peking via Hartwig, different from the rhg1 gene from PI 88788, is associated with moderate resistance to HG types 1.2-, 0, 2-, and 1.3 (races 2, 3, 5 and 14). In addition, the Peking gene renders resistance to reniform nematode and is associated with an allele for resistance to root knot nematode. Monitoring nematode populations, the need for quicker SCN testing methods, deployment of genes with broader resistance and using combinations of resistance and chemical or biological control methods will likely become more important in managing resistance.

HOST

Copy number variation mediated resistance to nematode.

Tong Geon Lee, *University of Florida*

Since the release of the soybean reference genome assembly, genomic technologies allowed significant advances in the study of important soybean traits. This presentation will cover an analysis of copy number variation in a major soybean nematode resistance locus Rhg1. The implications of sequence information of this locus for both soybean population genetics and future breeding approaches will be discussed.

Role of Rhg4-encoded serine hydroxymethyltransferase in resistance to SCN.

Pramod Kandoth, Division of Plant Sciences and Bond Life Sciences Center, *University of Missouri*

Rhg4 is a major genetic locus that contributes to soybean cyst nematode (SCN) resistance in Peking-type resistance of soybean along with Rhg1. We previously showed that the Rhg4 gene encodes a predicted cytoplasmic serine hydroxymethyltransferase (SHMT) by map-based cloning and functional genomic approaches. Despite the ubiquitous and essential role of this enzyme in 1-C metabolism, the novel gain-of-function of Rhg4-SHMT in SCN resistance among the 14 gene family members in soybean remains to be characterized. Using a forward genetic screen, we have identified an allelic series of Rhg4-SHMT mutants, including 16 additional mutations that shed new light on the mechanistic aspects of SHMT-mediated resistance. Mapping of these mutations on the SHMT structural model has uncovered key residues for structural stability, ligand binding, enzyme activity, and protein interactions. Moreover, the new mutants identified provide compelling genetic evidence that this gene is essential to confer effective SCN resistance in Peking-type resistant cultivars irrespective of carrying a resistant Rhg1 allele. In a multifaceted approach to understand the mechanistic aspects of Rhg4-mediated resistance, we are combining RNAseq and metabolite analysis of these SHMT mutants with biochemical approaches to identify protein interactors. We also validated an efficient CRISPR-CAS9 system for targeted gene knock-outs to elucidate the unique function of this enzyme in nematode resistance.

The GmSNAP18 is the Peking-type rhg1-a gene for resistance to soybean cyst nematode.

Khalid Meksem Department of Plant, Soil and Agricultural Systems, *Southern Illinois University*

Two types of resistant soybeans [Glycine max (L.) Merr.] sources are widely used against soybean cyst nematode (SCN, Heterodera glycine Ichinohe): The Peking-type soybean resistance requires both the rhg1-a and Rhg4 alleles, and the PI 88788-type soybean resistance requires only the rhg1-b allele for resistance. While the multiple copy number of PI 88788-type GmSNAP18 (Glyma18g02590), Glyma18g02580, and Glyma18g02610 in one genomic segment has been shown to simultaneously contribute to the rhg1-b resistance (Cook et al., 2012), we identified, by map based cloning, targeted genome sequencing, haplotyping, and genetic complementation, that the Peking-type GmSNAP18 alone is the rhg1-a gene conferring resistance to SCN at the rhg1-a locus. In

GmSNAP18, there are nine amino acid differences between resistant (Peking-type soybean cv. Forrest and PI 88788) and susceptible (Essex) soybeans and five amino acid differences between two resistant soybeans: Forrest and PI 88788. Our findings reveal that Peking-type GmSNAP18 is most likely performing a different role in SCN resistance than PI 88788-type GmSNAP18. To our best knowledge, this is the first report of a gene that evolved to possibly use two mechanisms to ensure the same function within the same species, in this case, resistance to a pathogen. The knowledge gained from this study can be readily used to improve nematode resistance of soybeans.

Stacking alleles from multiple sources to increase broad-spectrum genetic resistance to highly virulent soybean cyst nematode isolates.

Lillian Brzostowski, *University of Illinois at Urbana-Champaign*

Soybean cyst nematode (SCN), Heterodera glycines (HG) Ichinohe, is estimated to be the pathogen that causes the greatest economic loss to soybean [Glycine max (L.) Merrill] in the USA. Genetic resistance is an effective way to manage SCN. Resistance sources have been identified, and resistance QTL from these sources have been mapped. However, there is a need to diversify SCN resistance genes in cultivars as most grown in the northern USA have resistance tracing only to the resistance source PI 88788. The objective of this study was to determine the effectiveness of combinations of SCN resistance alleles from different sources in two populations formed via backcrossing. Population 1 segregates for a resistance quantitative trait locus (QTL) from both PI567516C and PI88788 while Population 2 segregates for the same QTL as Population 1 and two QTL from PI468916. Lines from both populations were evaluated with two virulent nematode isolates. Furthermore, a subset of lines from Population 2 (Population 2 Subset) was evaluated with two additional nematode isolates. The SCN resistance alleles from each source significantly increased SCN resistance compared to the alternative alleles. The effect of resistance alleles varied depending on SCN isolate and population, and there was generally an increase in resistance as more resistance alleles were stacked together. These results indicate stacking multiple sources of resistance can be an effective means to increase broad-spectrum SCN resistance.

Molecular breeding and novel QTL discovery for soybean cyst nematode resistance.

Zenglu Li, *University of Georgia*

Soybean cyst nematode (SCN) is one of the most destructive pests in soybean production in the world. Development of nematode resistance cultivars is the most effective way to controlling nematodes in soybean production. Two resistantance loci, Rhg1 and Rhg4 have been the main sources of resistance to SCN. Over 95% of SCN resistant cultivars used in the U.S. soybean production have derived their resistance from two genetic sources: PI 88788 and Peking. Based on the published and our own genomic DNA sequences of soybean lines with known SCN phenotypes, we have identified one SNP that can be used to select the rhg1 resistance allele and another SNP that can be employed to differentiate PI 88788 and Peking-type resistance. We also developed two SNP assays for the selection of the Rhg4 resistance allele. A strong accordance was observed between these SNP haplotypes and reactions to SCN race 3. The SNP marker assays could be applied for the high throughput marker-assisted selection. To discover new sources of resistance, we have genotyped number of accessions from various origins using the marker assays that we developed at Rhg1 and Rhg4 loci. These lines were also evaluated for SCN resistance using greenhouse bioassays. Five lines rated as SCN resistance in greenhouse phenotyping do not carry known resistance alleles at Rhg1 and Rhg4 loci. Haplotype analysis at the Rhg1 and Rhg4 loci assembled with Soy50kSNP Infinium Chip data grouped them separately. The results suggested that these lines might possess novel SCN resistance genes.

The confrontation: Soybean cyst nematode and common bean.
Berlin Nelson Jr, *North Dakota State University*

Common bean is a major crop in North Dakota and northern Minnesota where 35% of the US bean production occurs with a production value of 370 million dollars. In 2003 soybean cyst nematode (SCN) was found in North Dakota and since then it has spread throughout the eastern part of ND and into parts of northwestern Minnesota. SCN infestations are now found in counties with high hectares of bean production. SCN reproduces on dry bean and can decrease yield. Most important classes of dry bean grown in ND such as pinto, navy and kidney are susceptible to SCN with black beans having a moderate level of resistance. An intensive research program at North Dakota State University has been initiated to study the biology and management of SCN on dry bean. The reproduction of SCN on various bean classes grown in the area, yield losses at different egg densities and the resistance/susceptibility of commercial bean varieties and plant introductions have been investigated. Sources of resistance to multiple HG types of SCN were identified and efforts to incorporate resistance into adapted germplasm for the development of resistant dry bean varieties are currently underway. In addition, a strong outreach program is in progress to inform growers of SCN biology, the potential damage to dry bean, and management options to prevent yield reductions. Interaction of SCN with soil borne fungal patHogens is being studied in order to evaluate other potential problems with SCN.

Expression of a receptor-like protein enhances resistance of soybean to multiple pathogen and pests including soybean cyst nematodes.
Madan K. Bhattacharyya, *Iowa State University*

In a transcriptomic study, we identified a novel soybean gene, GmDS1, transcript levels of which declined immediately following infection of roots with the fungal pathogen, Fusarium virguliforme that causes sudden death syndrome (SDS) in soybean. We hypothesized that the pathogen suppresses the expression of this gene to overcome a defense mechanism controlled by this gene. To test the hypothesis, we expressed the gene during F. virguliforme infection by replacing its promoter with three infection-inducible promoters, two of which are also expressed strongly in roots. We were able to demonstrate that under both growth chamber and field conditions, most of the transgenic lines carrying the GmDS1 fusion genes were SDS resistant. The SDS resistant transgenic plants were also resistant to spider mites, soybean aphids and soybean cyst nematodes. The gene encodes a small plasma membrane protein (7.9 kDa) containing two membrane-spanning domains. The protein was localized to plasma membrane using a GFP-tagged protein in Nicotiana benthamiana. Our data suggest that GmDS1 is most likely a receptor-like protein that recognizes pathogen and pest-associated molecular patterns (PAMP) to induce PAMP-triggered immunity against multiple pests and a fungal pathogen.

The nematode is talking, but are we listening?

Melissa Goellner Mitchum, Division of Plant Sciences and Bond Life Sciences Center, *University of Missouri*

Planting of resistant soybean cultivars is the primary management strategy used to reduce crop damage by the soybean cyst nematode (SCN). However, reliance on a single source of resistance has driven a widespread shift toward virulence in SCN populations, leaving farmers with few alternatives to effectively manage this nematode. As multiple mechanisms of resistance come to light from the plant side, it is no surprise that the nematode side of the interaction is equally complex and involves multiple genes for virulence. Undoubtedly, we have much to learn about how SCN adapts to overcome or evade soybean resistance. As a fully annotated SCN genome soon will be available, comparative transcriptomics across populations that differ in their virulence profiles is facilitating our understanding of SCN effectors in nematode parasitism and adaptation to resistant cultivars. Elucidating the molecular details of nematode virulence will not only aid researchers in understanding soybean resistance mechanisms, but will accelerate strategies to engineer novel resistance and provide better diagnostic tools to predict population virulence in the field. Likewise, the recent knowledge gained in our understanding of soybean resistance genes provides an opportunity to breed with greater efficiency and precision while making use of the nematode as a tool to evaluate adaptation to different types and combinations of resistance. Consequently, community efforts are underway to develop a long-term strategic plan for diversification and deployment of resistant cultivars.

Genomic resources for the soybean cyst nematode.

Thomas Baum Department of Plant Pathology and Microbiology, *Iowa State University*

Like all nematodes, the soybean cyst nematode is a multicellular animal complete with differentiated tissues and organs like digestive tracts, nervous systems, and reproductive organs. In addition, the soybean cyst nematode is a sophisticated obligate parasite that uses a multitude of morphological, chemical and biochemical tools to infect soybean roots in a long-term host-parasite relationship. It can thus be expected that this parasite has a complex eukaryotic genome and a high number of genes, regulatory sequences, repetitive DNA stretches, and a high level of allelic as well as genic variation among individuals. It is imperative for a long-term perspective towards cyst nematode control, to have complete, fully annotated and readily available genome and transcriptome sequences deposited in public databases. Concerted research community efforts have now produced a number of such genomic resources, several of which are on the verge of completion and public release. Maybe most importantly, we have assembled a fully annotated and high-quality genome sequence using PacBio long-read sequencing technology, overcoming assembly challenges stemming from high levels of heterozygosity and repeat content. This assembly contains approximately 20,000 genes in 738 scaffolds with a total of 124.5 Mb of nucleotide content, 33.6% of which are repeat sequences. In addition, we sequenced the genomes of 15 additional inbred and cross-bred soybean cyst nematode populations using Illumina technology. To consolidate all data into a single knowledge repository, we are creating the community-driven web resource SCNbase.org, which will store and visualize all genomic and gene function data for the soybean cyst nematode.

Struggling to understand soybean cyst nematode HG types? Are you lactose intolerant??

Gregory Tylka, *Iowa State University*

"The race test system for the soybean cyst nematode (SCN), Heterodera glycines, was created in 1969 to describe populations of SCN that reproduce on resistant soybean varieties. And the HG type test system was established as an updated approach to describing such SCN populations in 2002. But confusion still exists about SCN race and HG type. Common questions include: Is there a molecular test for HG type? What HG type is this soybean variety resistant to? What is the predominant HG type in my field? These seemingly logical questions of practical significance are unanswerable, and unreasoned, if one fully understands the concept of SCN HG type. An individual soybean cyst nematode does not have a specific HG type or race because HG types and races are not homogeneous strains of SCN that co-infest a field. HG types and races are descriptions of SCN populations with characteristics defined by the HG type and race systems. And a population is defined as "a group of individuals of the same species occupying a particular geographic area." All of the soybean cyst nematodes in a field are part of, or comprise, the SCN population in that field, and each field has a single HG type or race, with parasitic abilities described by the HG type test and race test results. A hypothetical test for human food sensitivities, including lactose intolerance, is an analogy that may provide a new perspective and may make HG types more easily understood and used to characterize populations of SCN."

Characterizing virulence phenotypes of soybean cyst nematode (Heterodera glycines) in infested fields of North Dakota.

Intiaz Chowdhury, *North Dakota State University*

Soybean cyst nematode (SCN) is responsible for the greatest yield loss to soybean production in the U.S. To assess the prevalence of SCN in North Dakota, soil samples were collected in 2015 and 2016 from soybean fields or fields with a history of SCN across 14 counties. To characterize the virulence phenotypes of SCN in infested fields, HG type bioassays were performed with seven soybean Plant Introduction lines, PI 548402 (#1), PI 88788 (#2), PI 90763 (#3), PI 437654 (#4), PI 209332 (#5), PI 89772 (#6) and PI 548316 (#7), used as test lines and the local cultivar (Barnes) as a susceptible check. HG refers to Heterodera glycines and the type refers to the seven PI lines with various forms of resistance. For example, HG type 1.2 refers to a SCN population that is capable of reproducing on #1 PI line and #2 PI line. In 2015, forty-five SCN populations from ND fields were subjected to HG type bioassays. Among the successful assays, the most common HG types were HG type 0 (frequency rate: 43%) and HG type 7 (22%). Other HG types included 2.7 (9%), 2.5 (9%), 5 (9%), and 2.5.7 (8%). In 2016, to date 16 fields were assayed for the SCN population diversity. The HG types of these populations were 7 (40%), 0 (33%), 5.7 (20%), and 2.5.7 (7%). Repetitions of the bioassays proved that there are SCN populations in ND that can successfully reproduce on PI 88788, the most widely used source of resistance.

The loss of virulence genes may aid the soybean cyst nematode in parasitizing resistant soybean.
Kris Lambert, *University of Illinois*

Soybean cyst nematodes (SCN) that are able to reproduce on resistant soybean varieties are referred to as virulent. Due to the overuse of one source of SCN resistance, virulent SCN are increasingly more common in soybean fields. A better understanding of how SCN evades host plant resistance will be useful for devising SCN control strategies. To gain a better understanding of the molecular nature of SCN virulence, we employed a genetic strategy to associate single nucleotide polymorphisms (SNPs) with SCN virulence genes. Two candidate SCN virulence genes that were tightly linked to the SNPs were identified. One SCN gene encoded biotin synthase (HgBioB), and the other encoded a bacterial-like protein containing a putative SNARE domain (HgSLP-1). The gene encoding HgSLP-1 had reduced copy number in a number of different virulent nematode populations, suggesting the lack of this gene aided the nematode in parasitizing resistant plants. Interestingly, the HgSLP-1 is an esophageal gland protein and is secreted by the nematode during plant parasitism. Furthermore, in bacterial co-expression experiments, HgSLP-1 co-purified with the SCN resistance protein Rhg1 α-SNAP, suggesting that these two proteins physically interact. The SCN HgBioB contained sequence polymorphisms between avirulent and virulent nematodes. When the two allelic forms were measured for enzymatic activity, the virulent form was shown to be inactive. This data suggests that for both putative virulence genes identified in SCN, the virulent form of the gene is missing or inactive, suggesting a loss of function aids the nematode in parasitizing resistant soybean.

Molecular characterization and functional analysis of the ran binding protein genes from soybean cyst nematodes Heterodra glycines.
Deliang Peng, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, *Chinese Academy of Agricultural Sciences*

Soybean cyst nematode (SCN) Heterodera glycines is the most destructive pest for soybean product. Three novel ran binding proteins (rbp) genes(Hg-rbp-2, Hg-rbp-4 and Hg-rbp-6 were cloned and characterized from H. glycines. Sequences analysis indicated that the similarity between Hg-rbp-2、Hg-rbp-4 and Hg-rbp-6 was only 29-31% and three RBP putative proteins all contains signal peptides at N terminal and B30.2 and SPRY domain at C terminal. Phylogenic analysis suggested that three RBPs from H. glysines were classed two branches, which indicative that the rbp genes of H. glycines may evolved from two different ancients or evolutionary pathways In situ hybridization showed that those transcripts of rbp genes accumulated exclusively within the dorsal esophageal glands cell of H. glycines. A semi-quantitative RT-PCR assay supported the finding that the expression of these genes was strong in pre-parasitic and early parasitic second stage juveniles and that it declined in further developmental stages of the nematode. Southern blot confirmed that rbp genes were nematode origin and existed as a single in genome of H. glycines. Subcellular localization by transient expression of RBP-GFP fusion protein on onion epiderm showed that Hg-RBP2, Hg-RBP4 and Hg-RBP6 were localized in nucleolus. RNAi mediated Hg-rbp-2 genes silencing were conducted, RT-PCR detection confirmed the decrease of Hg-rbp-2 transcription in nematode treated with dsRNA before 16h. Knocking down Hg-rbp-2 resulted in a 26.9% reduction of the number of nematodes that invaded the plants and a 31.5% suppression of the development of H. glycines females within roots compared to the GFP-dsRNA control.

Incidence and titer of viral infections within soybean cyst nematode culture collections and field populations.

Casey Ruark, *North Carolina State University*

Five viruses [ScNV, ScPV, ScRV, ScTV, and SbCNV-5] previously found to infect SCN populations in Illinois were also detected within soybean cyst nematode (SCN; Heterodera glycines) greenhouse and field populations from North Carolina (NC) and Missouri (MO). The prevalence and titers of viruses in SCN from 43 greenhouse cultures and 25 field populations were analyzed using qRT-PCR. Viral titers within SCN greenhouse cultures were similar throughout juvenile development, and the presence of viral anti-genomic RNAs within egg, second-stage juvenile (J2), and pooled J3 and J4 stages suggests active viral replication within the nematode. Viruses were found at similar or lower levels within field populations of SCN compared to greenhouse cultures of NC populations. Five greenhouse cultures [LY1, LY2, MM2, TN7, and TN22] harbored all five known viruses whereas in most populations a mixture of fewer viruses was detected. In contrast, three greenhouse cultures [MM21, MM23, MM24] of similar descent to one another did not possess any detectable viruses and primarily differed in location of the cultures (NC versus MO). Viruses ScNV, ScPV, and ScTV were also detected in Heterodera trifolii (clover cyst) and viruses ScPV and ScRV were detected in a greenhouse population of Heterodera schachtii (beet cyst), but none of the five SCN viruses were detected in other cyst, root-knot, or reniform nematode populations tested. Viruses were not detected within soybean host plant tissue. If nematode infection with viruses is truly more common than first considered, the potential influence on nematode biology, pathogenicity, ecology, and control warrants continued investigation.

SCN embryogenesis is not affected by hatching stimulants.

Nathan Schroeder, *University of Illinois Urbana-Champaign*

Hatching in the soybean cyst nematode (SCN) is incompletely responsive to hatching stimulants. A larger percentage of SCN eggs will hatch in soybean root diffusate compared to water; however, even in the presence of soybean, some SCN eggs will not hatch immediately. To better understand the process of hatching in SCN we established a detailed timeline of pre-hatch development. We recovered two-celled SCN embryos from three to four-week old females and recorded developmental progress in water as well as soybean root diffusate and the hatching stimulant ZnCl2. Eggs developed from a two-celled embryo to a fully developed J2 in approximately one week at 25°C. While the hatching stimulants did increase the percentage of SCN that hatched, there were no differences in the developmental timeline among the different solutions. Previous research demonstrated increased hatching of eggs recovered from the gelatinous egg mass compared to those found in cysts. Similar to our hatching stimulant data, we found no difference in the pre-hatch developmental timeline between these two sources of eggs. Our results suggest that pre-hatch development is independent of the presence of a soybean and oviposition location and that hatching stimulation acts primarily on the fully developed J2. To examine the development of the infective J2 stage, we dissected eggs following the molt from J1 to J2. We observed that the cuticle and nervous system of the pre-hatch J2 continue to develop within the egg after the onset of J1 ecdysis. We speculate that these structures are required for further development.

SCN resistance management: A farm perspective.
George Bird, *Michigan State University*

Soybean Cyst Nematode (SCN) is a key pest of soybeans in the U.S.A. It has been managed with resistant varieties since shortly after its initial discovery in North Carolina. Unfortunately, however, cultivars derived from a single source of resistance have been used excessively, resulting in development of highly aggressive populations and suboptimal bean yields. In 2014-15, Michigan State University, Diagnostic Services processed 59 soybean soil samples for SCN Type analysis. Sixty-one percent of the populations were classified as Type 2; whereas, 32%, 2% and 5% were classified as Types 1.2, 1, and 0, respectively. A 2015 national survey of more than 1,000 soybean growers across seventeen states indicated that more than two-thirds of them plant SCN resistant varieties, but the majority are unaware of the source of resistance for the varieties they use in relation to the SCN Types present on their farms. This indicates a distinct need for a national SCN Resistance Management Coalition designed to resolve the problem through education, facilitation and persuasion technologies. The presentation: 1) includes an econometrics assessment of a hypothetical 3,000 acre soybean/corn/ wheat farm, 2) options for resolving the problem and 3) assessment of the constraints associated with adoption of SCN resistance management strategies and tactics at the on-farm, regional and national levels.

Grower perceptions of SCN: the 1990s versus 2015.
Kaitlyn Bissonnette, *Iowa State University*

A survey of United States soybean growers was conducted in 1997 and 1998 and then again in 2015 to assess grower perceptions regarding the soybean cyst nematode (SCN) and its management. In each survey, respondents were asked a series of questions pertaining to awareness of the SCN, soil sampling and scouting methods, and use of general SCN management practices. In the 1997 survey, prior to the first concerted SCN awareness campaign (SCN Coalition), 53% of all respondents deemed SCN to be a somewhat important or important pest, but only 35% reported scouting or soil sampling for SCN. Of those who reported having SCN, 55% indicated they were doing nothing to manage it. In 1998, one year into the SCN Coalition, the perceived importance of SCN increased by 13% and the incidence of scouting or soil sampling increased by 6%. Management practices used by growers with SCN in their fields changed drastically from 55% reporting doing nothing in 1997 to 56% using SCN-resistant varieties and 41% rotating to non-host crops in 1998. In 2015, only 34% of surveyed growers reported scouting or sampling for SCN. The use of the most commonly reported management practices to control SCN in 2015 was notably increased from the 1998 survey, with 71% using non-host crops and 59% growing SCN-resistant varieties in 2015. The information collected in these surveys is extremely useful to guide development of effective SCN educational materials to reduce yield impacts attributed to SCN damage."

Nematicide seed treatments alter Heterodera glycines development within roots

Jared Jensen, *Iowa State University*

Inhibition of nematode development is an important component of the activity of a nematicide. The current method of root clearing and nematode staining with acid fuschin often results in variable nematode counts among users. Furthermore, with this method assessing the developmental stages of nematodes can only be done by making subjective visual comparisons of size and shape of the worms. Preliminary tests using a newer method, root maceration and sieving, of 15-day-old soybeans infected with soybean cyst nematode (SCN), Heterodera glycines, yielded a nematode recovery of about 150% compared to counting stained nematodes in the same root samples. A stack of sieves with pore sizes 250, 150, 75, 45, and 25 micrometers each, captured adult females, J4 females, J4 males and J3s, swollen J2s, and parasitic J2s, respectively, allowing for accurate isolation and quantification of SCN developmental stages. Experiments were conducted in which untreated susceptible soybean seeds or seeds treated with Avicta or Clariva were grown in SCN-infested soil for 20 days after which roots were macerated and nematode development was assessed and they were counted. Results showed slower nematode development, in the form of decreased proportions of J4s and young adult females, in roots grown from both types of treated seeds compared to the untreated control. Slowing in planta SCN development may allow for healthier root development, therefore reducing the effects of SCN damage. The ability to isolate and objectively quantify specific SCN developmental stages proves to be a useful tool in the assessment of new nematode management products.

New tools for evaluating the efficacy and mode of action of seed applied nematicides.

Breann Bender, *Bayer*

The landscape of seed-applied nematicides has evolved dramatically over the past ten years, but there remains a great need for Soybean Cyst Nematode (SCN) protection. A single solution may not be the best answer. Developing seed treatments that complement both current and future genetics for SCN is critical. The impact of SCN on soybeans is greatest early in the season, so protection for the young seedling is an absolute need. ILeVO® provides a direct impact on nematodes in the seed zone, even showing season-long reduction in nematode numbers in the field. As roots continue to grow, Poncho®/VOTiVO® provides extended protection through a biological component. Greenhouse and field studies have shown significant reduction in nematode pressure and reproduction indexes when combining ILeVO and Poncho/VOTiVO. The diversity of methods available to combat SCN is itself a driver of innovation as we must work to not only better understand one particular MoA but also how it might work in combination with others. Standard nematode assays simply focusing on the number of eggs collected from an infested plant do not always tell the whole story. Quantitative root stains, directionally targeted or even differentially timed inoculations are among the tools we are developing to provide a better picture of how a product or group of products will ultimately perform in the field. Further development of these tools and techniques will improve our ability to provide the grower with targeted solutions to a problem that is much more diverse and complicated than initially envisioned.

Enhancing performance of PI88788 with clariva complete beans.
Dale Ireland, *Syngenta Crop Protection*

Genetic resistance to Soybean Cyst Nematode (Heterodera glycines) has been the primary method US soybean growers have used combating soybean cyst nematode (SCN); the number one soybean pathogen. Soybean Cyst Nematode host resistance was significantly improved with Plant Introduction 88788 (PI88788). Today it is estimated that over 95% of US SCN resistant commercial soybean varieties depend on SCN resistance genes from PI88788. Through the heavy use and success of PI88788 over the last two decades, it has begun to lose its effectiveness; these SCN populations are termed "HG Type 2" populations. Soybean growers today require additional tools to provide the same level of protection once provided by SCN-resistant genetics alone. During the development of Syngenta's most recent SCN solution, Clariva Complete Beans, nearly 80% of SCN infested fields from across the Midwest where data were collected were infested by HG Type 2 SCN populations. But multi-year replicated data reveal within the HG Type 2 population pressure, Clariva Complete Beans continued to provide strong performance by offering 4.5% yield increase when compared against the fungicide/insecticide base check treatment. Today US soybean growers need additional tools beyond PI88788 based genetics to manage SCN. Seed-applied nematicides, such as Clariva Complete Beans, show promise retrieving some of this lost protection.

Introduction of Aveo EZ, a new and novel seed treatment for management of soybean cyst nematode.
Scott Halley, *Valent USA*

Valent USA plans to introduce to the market place in 2017 a novel branded product for management of Soybean Cyst Nematode (SCN). The product will be sold under the trade name Aveo EZ and protects against root damage caused by nematodes including SCN. The first use will be as a seed protection coating to be applied to soybean seed before planting. Aveo EZ contains the biological organism Bacillus amyloliquefaciens strain PTA-4838. Greenhouse generated data show SCN number reduction on plant samples. Multi-site field studies demonstrated soybean yield increased when management strategy included a seed protection component with the active ingredient in Aveo EZ.

Evaluation of cover crops for hosts and population reduction of soybean cyst nematode.
Krishna Acharya, *North Dakota State University*

Host resistance and crop rotations are common practices for managing soybean cyst nematode (SCN), Heterodera glycines, in North Dakota, but the role of cover crops in SCN management is not fully understood. To evaluate this, twenty-one cover crop species and cultivars plus two susceptible soybean cultivars were planted in two naturally infested soils (initial population densities: 5,000 and 10,000 eggs/100 cm3) from Richland and Cass counties, ND, in a growth chamber at 27 oC for 35 days. Host range was evaluated based upon numbers of SCN white females on roots and the effect on population reduction was determined by comparing initial and final SCN egg population densities. Out of the tested crops, SCN white females were not found on roots of annual ryegrass, camelina, carinata, ethiopian cabbage, faba bean, foxtail millet, radish, rape dwarf essex, red clover, sweet clover, triticale, and winter rye. Cowpea, crimson clover and turnips showed very few white females ranging from 1 to 13, but Austrian winter pea, field pea, forage pea and hairy vetch showed some levels of reproduction (white females: 13 to 173). SCN reproduced less in all tested cultivars compared to the susceptible checks (827 to 1,251). All the tested cover crops reduced the SCN population except field pea (Aragon) and forage pea which increased the population from 10,000 to 18,240 eggs/100 cm3 soil for Aragon and to 25,560 eggs/100 cm3 for forage pea. Cover crops which were non-host or poor host will be evaluated in the microplot study for reducing SCN populations.

Reproduction of Three Heterodera glycines types on commercial cultivars with PI88788 as the source of resistance genes under greenhouse conditions.
Emmanuel Byamukama, *South Dakota State University*

The soybean cyst nematode (SCN; Heterodera glycines) is the main soybean production constraint in South Dakota and in the U.S. The main SCN management practice is use of resistant cultivars. However, with the majority of available cultivars having PI88788 as the only source of SCN resistance genes, host resistance may not be as effective across different nematode phenotypes. A greenhouse study to determine the response of 34 commercial SCN resistant cultivars was set up by inoculating these cultivars with the three most prevalent Heterodera glycines (HG) types 0, 7, 2.5.7 in South Dakota. Williams 82 was included as the susceptible check. Plants were maintained in a water bath kept at 27-28 oC for 35 days. A female index for each cultivar and HG type was obtained by dividing the number of cysts on a commercial cultivar by the cysts on the susceptible check. All cultivars had a resistant (FI<10%) or moderately resistant (FI=10-30%) response for HG type 0 inoculation. The majority of the cultivars had a moderately resistant response under the HG types 7 and 2.5.7 inoculations. The FI for the moderately resistant cultivars inoculated with HG type 0 was less than 20% whereas for HG types 7 and 2.5.7, FI ranged between 11 and 30%. These results indicate that the use of resistant cultivars to manage SCN is still effective, however, this practice should be integrated with other tactics such as rotation with non-SCN hosts and rotation within resistant cultivars for sustainable SCN management.

PLENARY IV

The challenges of SCN: Considering the past and the present in charting a collaborative course for the future.
Ed Anderson, *Iowa Soybean Association and North Central Soybean Research Program*

The 2016 SCN Conference: Adapting to an Evolving Pathogen has provided a venue for bringing together interested and engaged academic and company researchers, students, farmers, crop advisors and soybean checkoff leaders to consider the past, the present and the future of SCN management. At all times, but especially at this time of lower commodity prices and industry consolidation, farm landowners and operators must consider every opportunity to optimize their productivity, profitability and sustainability. Posing an annual loss risk potential of greater than $1 billion in North America, SCN is a significant and constantly evolving threat to soybean production. Charting a successful future for controlling SCN is predicated on understanding the past and present states of management across the soybean industry. The presentations, posters and conversations during this conference have provided great background in setting the stage for meaningful strategic and tactical planning for the future. Future management of SCN will require holistic and integrated approaches through strong public and private partnerships. With committed participation, the reformation of an SCN Coalition can facilitate the coordination of surveys, communication and outreach, and basic and applied research that will facilitate the development and delivery of pathogen biology, host/pathogen interactions, traits, tools and technologies, products and management solutions for SCN. The future of SCN management is dependent on farmers and crop advisors assessing fields and modifying management practices, in combination with public/private partnerships that will coordinate holistic solutions across native and transgenic traits, chemical and biological control, equipment and digital agriculture, and cultural practices.

POSTER ABSTRACTS

HOST

Poster #1

Soybean cyst nematode in dry bean (Phaseolus vulgaris L.): Understanding and managing underground enemies in the era of genomics.

Shalu Jain, *North Dakota State University*

Soybean cyst nematode (SCN; Heterodera glycines Ichinohe) is a devastating pathogen of soybean which can live and reproduce on the roots of common bean. SCN can cause significant reductions in plant growth and seed yield in both crops. Identification of genetic factors associated with SCN resistance could help develop bean varieties with higher levels of SCN resistance. Gene expression profiling was conducted on untreated and SCN inoculated roots of pinto bean genotypes, PI533561 and GTS-900, resistant and susceptible to SCN reproduction, respectively using RNA-seq. In the inoculated roots of PI533561, a total of 353 genes were differentially expressed while 990 genes were differentially expressed in SCN-inoculated roots of GTC-900. Some of the differentially expressed genes were nucleotide-binding site leucine-rich repeat resistance proteins, WRKY transcription factors, pathogenesis-related proteins and heat shock proteins with well-known role in resistance mechanisms in different plants. Relative expression profiles of some important gene families were evaluated using qPCR and were in complete agreement with the RNA-seq data. This is the first report describing differential transcriptional regulation of genes putatively involved in the SCN-common bean host-parasite interaction. In our breeding efforts towards screening for resistance, 116 advanced breeding lines of different market classes of common bean indicated diverse responses to SCN with 21% resistant and 52% moderately resistant lines while 25% lines were susceptible based on female index. The resistant genotypes can be utilized as sources of resistance for development of SCN resistant varieties adapted to the northern Great Plains.

Poster #2

The Iowa State University SCN-resistant soybean variety trial program.

Gregory Tylka, *Iowa State University*

Soybean varieties that are resistant to the soybean cyst nematode (SCN), Heterodera glycines, allow less than 10 percent nematode reproduction compared to susceptible varieties. However, there is no minimum level of SCN reproduction commercial soybean varieties must allow to be labelled as "SCN resistant" in the United States. Resistance to SCN is conferred by several soybean genes, and the number of copies of a set of resistance genes also affects the level of resistance. Not all SCN-resistant varieties provide equal nematode control or yield. Iowa State University evaluates the nematode control and yield of SCN-resistant soybean varieties in experiments in SCN-infested fields throughout Iowa. Sixty or more resistant varieties and two or three susceptible varieties are grown in 4-row-wide by 17-foot-long plots replicated four times per variety in each experiment. Three experiments are conducted across northern Iowa each year, three across central Iowa, and three across southern Iowa. SCN egg population densities are determined at planting and at harvest in each plot, as is yield. Collecting soil samples in each plot to measure changes in SCN population densities is required to accurately assess the effectiveness of resistance of the varieties because results of 30-day greenhouse experiments do not correlate well with field results (R2 = 0.32). A report summarizing the results of the experiments is

prepared and distributed in the Iowa and Illinois Farmer Today newspapers each January. Results also are posted online at www.isuscntrials.info. This work is funded, in part, by the soybean checkoff through the Iowa Soybean Association.

MANAGEMENT

Poster #3

Biocontrol activity of Pseudomonas strains: From genomic sequencing, biocontrol activity screening to mode of action study.

Christopher Taylor, *The Ohio State University*

Plant-parasitic nematodes are of great economic importance due to their negative effect on agricultural production worldwide. Many root-associated bacteria, including members of the Pseudomonas genus, have been found to have biocontrol activity against plant-parasitic nematodes. Previous work conducted in the Taylor laboratory identified 45 different strains of Pseudomonas isolated from plant, soil and water samples exhibiting biocontrol activity against fungi, oomycetes, bacteria and/or the model nematode, Caenorhabditis elegans. Genomic sequencing and annotation for these strains have been completed. We performed a comparative genomic analyses of all 45 Pseudomonas strains along with a few previously sequenced Pseudomonas reference strains. A phylogenetic tree depicting the relationships of all the sequenced strains was developed. Core genomes and genes specific to each sub-clade have been identified. We have also examined the annotated genomes for biocontrol and plant-growth regulator genes to explore diverse mechanisms related to biocontrol and plant growth promoting (PGP) activity. Phenotypic assays are being done to support gene function or PGP designations. These assays include examining C. elegans lethal activity, nematode hatching, ACC deaminase activity and auxin activity (using a DR5::GUS auxin Arabidopsis indicator line to detect auxin production by Pseudomonas). Also, a greenhouse biocontrol experimental platform was successfully established for studying the plant-parasitic nematode biocontrol properties of the collected Pseudomonas strains. From 15 independent sets of greenhouse assay, we successfully identified seven Pseudomonas strains that significantly suppress activity of the soybean cyst nematode (Heterodera glycines). These strains will be used for future mode of action studies and field trail.

Poster #4

Effect of commerical seed treatments on SDS and SCN in Tennessee soybean fields.

Heather Kelly, *University of Tennessee*

In 2014-2015 the efficacy of different fungicide/nematocide seed treatments were evaluated at 2 different field locations in Tennessee. One location was inoculated with Fusarium solani f. sp. glycines (causal agent of sudden death syndrome) and irrigated to promote infection and the other location was naturally infested with Heterodera glycines (soybean cyst nematode). Treatments included (1) Cruiser Maxx Beans + Vibrance 500 FS, (2) Clariva + Cruiser Maxx Beans + Vibrance 500 FS, (3) Clariva + Mertect + Cruiser Maxx Beans, (4) Trilex, (5) Trilex + ILeVo (6) Trilex + Poncho + Votivo, (7) Trilex + Poncho + Votivo + ILeVo and a non-treated check. Trials were planted in early May and cyst counts, foliar disease ratings, and yield were recorded each year. Differences and similarities across treatments and the non-treated check will be discussed.

Poster #5

First detection of soybean cyst nematode on dry bean (Phaseolus vulgaris L.) in a commercial field in Minnesota.

Guiping Yan, *North Dakota State University*

In July 2016, irregular patches of stunted and yellowed plants were observed and reported by a grower in a commercial dark red kidney bean field in Sherburne County, Minnesota. Examination of root tissue revealed many white to yellow females of cyst nematodes. Five soil samples were collected, and nematodes were extracted from soil. Population densities of cyst nematodes ranged from 1,630 to 3,840 eggs and juveniles/100 cm3 soil. The soil samples were mixed thoroughly, and the composite soil with 2,112 eggs (31 cysts)/100 cm3 soil was planted to the three most widely grown kidney bean cultivars, Montcalm, Red Hawk and Pink Panther, each in 5 replicates. After 32 days of growth maintained at 27 °C with 16-h light/day, females were extracted from roots and counted. The numbers of white-yellow females on Montcalm, Red Hawk and Pink Panther were 429 ± 180, 452 ± 147 and 620 ± 131, respectively, demonstrating a population increase on these dry bean cultivars. Nematodes from soil and roots were examined morphologically and molecularly, and identified as soybean cyst nematode (SCN), Heterodera glycines. SCN is the most destructive pathogen of soybean in the U.S. Dry bean was known as a host of SCN under artificially infested soil conditions in North Dakota. This represents the first occurrence of SCN in a commercial dry bean production field in Minnesota. SCN distribution is increasing in the major U.S. dry bean growing area of Minnesota/North Dakota, and will likely become a major yield-limiting threat, especially in highly susceptible kidney bean.

Poster #6

Increasing Soybean Cyst Nematode awareness in North Dakota through a grower-based sampling program.

Brandt Berghuis, *North Dakota State University*

Planted soybean (Glycine max) acreage in North Dakota (ND) increased approximately ten-fold since the early 1990's to approximately 6M acres in 2014. In 2003, soybean cyst nematode (SCN: Heterodera glycines) was first identified in the state, and by 2012, it had been confirmed in 12 ND counties. Despite the geographic spread and the yield threat presented by SCN, a limited awareness and a negative social stigma inhibited SCN soil-sampling and utilization of management tools among growers in much of the state. In order to increase soil-sampling among growers and better understand the distribution of SCN in ND, a free and anonymous grower-based sampling program was established in 2013. Pre-paid SCN sample bags were distributed to growers through all ND Cooperative Extension County offices, the North Dakota Soybean Council offices, and at field days and other events. Egg count data (eggs/100cc) were mailed directly to the submitting grower and a low-resolution map distribution map of egg data was created and publically distributed. The number of submitted samples were 193, 579, and 943 in 2013, 2014, and 2015, respectively, and are expected to exceed 1,000 in 2016. Approximately, 30% of submitted samples had at least 50 eggs /100cc soil. Of those, egg levels in approximately 50% of the samples exceeded 200 eggs/100cc and 10 % exceeded 10,000 egg/cc. Between 2013 and 2015, sampling was done in 39 of the 53 ND Counties, and we estimate 19 counties are positive for SCN.

Poster #7

SCN diagnostics, plant and nematode screening services.

Clinton Meinhardt, Division of Plant Sciences and Bond Life Sciences Center, *University of Missouri*

Each year, a microscopic parasite known as the soybean cyst nematode (SCN), costs more than $1 billion in yield losses to soybean producers. SCN has spread to nearly all soybean producing states and continues to expand its reach wherever soybeans are grown. Once in a field, the nematode's robust survival tactic means it is there to stay. This requires farmers to proactively manage the nematode population level through soil sampling, population typing, planting SCN-resistant soybeans, and crop rotation to reduce its impact on yield. SCN Diagnostics is a partnership with the University of Missouri and the College of Agriculture, Food, and Natural Resources. It was created to provide timely, high quality plant and nematode screening services to different parties related to the seed and biotech industries, researchers, crop advisors, and farmers. Whether you are developing SCN-resistant varieties by breeding or biotechnology, we offer validated screening services using a well-established pipeline to help our clients develop and deliver superior SCN-resistant soybeans to the marketplace. SCN Diagnostics maintains the United States largest culture collection of SCN populations, providing our clients with tailored screening options to help meet their desired goals. We also offer egg count testing and population typing for SCN, as well as soil testing for other plant-parasitic nematodes. For more information, please visit our website at scndiagnostics.com.

Poster #8

Survey of internet resources on the soybean cyst nematode.

Kaitlyn Bissonnette, *Iowa State University*

Access to relevant and impactful informational resources is vital to implement effective management practices to reduce the effects of the soybean cyst nematode (SCN) on soybean production. A survey was conducted in 2016 to assess the content of internet resources currently available regarding SCN and its management from four categories of information providers: government organizations, soil laboratories, seed and chemical companies, and universities. Sixty-one electronic publications were assessed, seven government-based, thirteen soil laboratories, fourteen seed and chemical companies, and twenty-seven universities. Each publication was examined for mention of twenty-one important topics and subtopics within signs and symptoms, SCN biology, SCN management, and sampling techniques. A majority, but not all, of the publications surveyed mentioned stunting (84%), yellowing (84%), and yield loss (82%) as symptoms. Lack of symptoms was only mentioned in 57% of publications. Less than half of the publications (30%) mentioned that SCN-resistant cultivars reduce reproduction of SCN, but 75% recommend using SCN-resistant cultivars for SCN management. Crop rotation (89%) was the most commonly recommended management practice, with many sources listing available non-host crops (80%). Implementing weed management strategies (54%) and the use of nematicide seed treatments (62%) were also common management strategies mentioned. Universities consistently covered more technical topics, such as HG typing (70%), how to soil sample (74%), and the life cycle of SCN (67%). This information is vital to developing current and accurate SCN publications to enhance grower awareness and improve the effectiveness of SCN management.

Poster #9

Distribution, virulence phenotypes and genetic structure of Heterodera glycines in China.

Dong Wang, *Nematology Institute of Northern China*

The soybean cyst nematode (SCN), Heterodera glycines, has been a major pathogen of soybean. It is hard to control and can cause serious yield reductions and great economic losses worldwide. A survey for SCN was undertaken in 150 demonstration counties in China during 2012-2014. The results demonstrate some new distributions of SCN, which indicate that SCN has been reported in 23 provinces including Anhui, Beijing, Gansu, Guizhou, Guangxi, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Jiangxi, Jilin, Liaoning, Neimenggu, Ningxia, Shaanxi, Shandong, Shanxi, Shanghai, Sichuan, Xinjiang, Yunnan, and Zhejiang. H.glycines virulence phenotypes can be described in two ways. One is the race determination test that uses four soybean lines to categorize H.glycines into 16 "races". The HG type designation is similar, but includes seven soybean lines rather than four. In China, previous data revealed the occurrence of nine H.glycines races including races 1 through 7, 9 and 14. In 2013, we identified 8 HG Types: HG Types 0, 1.2.3.5.7, 1.2.5.7, 1.3.7, 2.5.7, 2.7, 5.7 and 7. Recently, a genetic polymorphism test on 318 individuals of 16 SCN populations with mtDNA COI gene was used to analyse population genetic structure of SCN in China. The results reveal genetic differentiation among SCN populations, but gene flow and geographical isolation are not considered as major reasons for genetic differentiation. Genetic variability was mainly observed within populations, not between populations, which is due to passive transport of cysts by human activities, water or wind.

Poster #10

Genetic, functional and taxonomic diversity of fungi associated with the soybean cyst nematode in midwest soybean-corn production systems.

Weiming Hu, *University of Minnesota*

Plant-parasitic nematodes are among the most important and difficult to manage plant pathogens. The soybean cyst nematode (SCN) is a major pathogen of soybean that decreases soybean yield both through direct infection of roots and also by vectoring or promoting other microbial diseases of soybean. There is great need for development of effective and environmentally sustainable management strategies for the SCN, but development of effective biocontrol is hampered by a lack of knowledge of nematode control microorganisms. The goal of this research is to characterize key genetic, functional and taxonomic groups of fungi under different crop rotation systems and to investigate their roles in controlling SCN populations. The research utilizes a long-term crop rotation experiment of corn and soybean that includes annual rotation, 5 year rotation, and continuous monoculture. For each treatment plot, bulk soil, SCN cysts, soybean and corn roots, rhizosphere soil, and rhizoplane soil directly adjacent to the root surface was collected and the fungal communities were investigated by metabarcoding of fungal ITS1 region. In addition, fungi were cultured from SCN cysts and J2, roots, and soil. The composition of fungal communities in soybean roots, corn roots, rhizosphere soil, and rhizoplane soil were differentiated from each other in NMDS analyses. Fungal communities in the rhizosphere also shifted with increasing years of monoculture, showing increased relative abundance of Hypocreales and Pezizales in Ascomycetes and Agaricales in Basidiomycetes. The composition of taxa found in SCN cysts differed when using metabarcoding versus culture-dependent methods, suggesting some uncultivable fungi may inhabit cysts.

Poster #11

Molecular detection of soybean cyst nematode in North Dakota.

Guiping Yan, *North Dakota State University*

The soybean cyst nematode (SCN), Heterodera glycines, is a major pathogen limiting soybean production in North Dakota. Other cyst nematodes such as sugar beet cyst nematode (H. schachtii), clover cyst nematode (H. trifolii), cereal cyst nematode (H. avenae) and Cactodera cyst nematode (Cactodera spp.) are hard to be distinguished from SCN by scientists without extensive training in nematode taxonomy using the traditional microscopic method. A DNA-based research was carried out to differentiate SCN from other closely related cyst-forming nematodes and to detect SCN in field soils with low population densities. Five published SCN-specific primers were screened. The primer pair SCNF1/SCNR1 showed high specificity to SCN and did not amplify DNA from 36 isolates of other nematode species including 12 cyst species and 10 species commonly found in ND fields. This primer pair was then used to develop a molecular assay for detecting SCN in DNA extracts of soils at a wide range of population densities (0-20,323 eggs and juveniles/100 cm3 soil) collected from 35 fields in five counties. Grinding the field soil coupled with addition of PCR inhibitor removal reagents to soil DNA extracts followed by nested PCR enabled SCN detection at as low as ≤ 12 SCN eggs/100 cm3 soil, including three samples in which SCN was not detectable by the traditional method. The PCR assay not only provides a method to separate SCN from other closely related cyst nematodes but also detects SCN directly from soils with low densities, obviating conventional nematode extraction from soil and microscopic identification.

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