Project Update – July 31st, 2021

I. **Project Title:** An integrated approach to enhance durability of SCN resistance for long-term, strategic SCN management (Phase II)

II. **Principle and Co-Principle Investigators:** Dr. Andrew Scaboo, Dr. Melissa Mitchum, Dr. Brian Diers, Dr. Thomas Baum, Dr. Gregory Tylka, Dr. Matthew Hudson, and Dr. Andrew Severin

III. **Brief Description of Accomplishments as of July 31st, 2021:**

A description of relevant progress for principle and co-principle investigators is below for each objective and sub objective in our proposal. Our team has made tremendous progress in accomplishing our research goals, conducting field experiments, publishing refereed journal articles, and communicating our results to scientists and soybean producers even during the challenges faced due to the current COVID-19 pandemic. We had our last group research meeting in April of 2021, and our next group meeting will be scheduled for the fall/winter of 2021.

Objective 1: Identify SCN virulence genes to better understand how the nematode adapts to reproduce on resistant varieties.

1.1: Sequence, curate and annotate SCN reference genomes for each common HG type (*Severin, Hudson, Baum, Mitchum*)

The Baum and Severin groups have already reported previous SCN genome-related publications (SCN reference genome, Masonbrink *et al*., 2019; and SCNBase, Masonbrink et al 2019) that resulted from collaborative work. For this reporting period, we would like to announce another significant milestone that we achieved during this funding period; the publication of our most detailed*, chromosome-level* assembly of the TN10 *H. glycines* genome in a high impact journal, Molecular Ecology Resources (Masonbrink RE, Maier TR, Hudson M, Severin A, Baum T. A chromosomal assembly of the soybean cyst nematode genome. Mol Ecol Resour. 2021 May 25. doi: 10.1111/1755-0998.13432. Epub ahead of print. PMID: 34036752.). As a consequence, all associated resources were publicly released on SCNBase, including: BLAST and JBROWSE capability for the genome assembly, predicted proteome, genome, transcriptome, and alignments for ribosomal genes, genome structural variation, SNPs and INDELs, alignment of X12 gene models, effector alignments, ncRNAs, multiple repeat predictions, and every putative input gene annotation. This will serve as a major informative resource to the entire SCN community worldwide.

Simultaneously, we are working on another project; we previously surveyed SNP and INDEL variation from 15 *H. glycines* populations. We created SNP/INDEL-modified versions of the TN10 genome to represent pseudo-genomes of these 15 populations. Using these, we identified genomic variation that affects gene structure and its potential for secretion. We have incorporated this information into a larger work that assesses the extent of gene family duplication and contraction that has occurred in SCN and related species at three phylogenetic nodes. Overall, the comparisons include sedentary vs migratory nematodes, root knot vs cyst nematodes, and Globodera species vs SCN. In our preliminary work, we identified 551 gene family expansions in sedentary nematodes vs migratory outgroups, 124 gene expansions in cyst nematodes vs root knot nematodes, and 479 gene family expansions in SCN vs Globodera species. These data are currently being prepared for submission and will result in the release of 323 columns of data describing every gene in SCN, across 15 SCN populations, and gene families among 13 related parasitic nematodes. We are also working on collecting and analyzing data to distinguish gene expression patterns and genomic structure between male and female *H. glycines*

nematodes, which may provide new targets for resistance development. We previously assessed gene expression differences in a non-feeding (male) vs. feeding (female) population, revealing 6,039 upregulated genes in males and 5,881 upregulated genes in females. As a complement to this approach, we sequenced male and female genomes using Nanopore long reads. We now have draft genome assemblies for male and female SCN nematodes, for which we can use male- and female-specific RNA-seq samples to create distinct sex-specific gene annotations. This dataset will provide a precise clarity on the genomic and transcriptomic differences between male and female nematodes, further enhancing our understanding of SCN sex determination and SCN genes involved in manipulating the host. We will continue to explore these data further for the upcoming reporting period.

The Hudson group, in collaboration with the Severin/Baum group, now has all seven of the target genotype assemblies completed and frozen. The Severin/Baum group assisted with manual curation using tools from their TN10 assembly workflow. Additionally, where possible, the genomes were improved by polishing with Illumina short reads using algorithms currently employed by large-scale genome projects. Alternate haplotype contigs were purged to generate seven assemblies each containing 9 pseudomolecules that correspond to the 9 SCN chromosomes. All seven assemblies have genome completeness scores that exceed those of the published TN10 assembly. Annotation is now underway to generate the predicted proteomes for comparison across the 8 SCN lines.

The Mitchum group has continued to help develop and provide nematode materials for genome sequencing efforts.

1.2: Generate sufficient genetic material of virulent SCN populations selected on different types of resistance (*Mitchum, Baum*)

In Phase I of this project (under Obj 2.3 of the previous project) the Mitchum group identified a HG type 1.2.5.7 field population and continuously selected this population in the greenhouse on either a susceptible soybean line (SCN inbred population MM-BD1), a soybean line containing the *rhg1* resistance gene from PI 88788 (SCN inbred population MM-BD2), a soybean line containing the *rhg1* and *Rhg4* resistance genes from PI 437654 (Hartwig) (SCN inbred population MM-BD3), and a soybean line containing resistance genes on chromosomes 15 and 18 from wild soybean *G. soja* (inbred population MM-BD4) (Meinhardt et al, 2021 *Plant Disease*). These inbred populations have been maintained on a monthly turn over schedule since they were started. Thus, the original SCN field population and this series of SCN populations selected for virulence on each set of resistance genes has been continuously reared during this project period.

As a complementary approach to our transcriptomic analysis (outlined under 1.3), we are conducting a Pool-Seq strategy which pools individual nematodes from the same population to increase the amount of DNA required for sequencing. Pool-Seq will provide genome-wide polymorphic data and variant allele frequencies across populations to help pinpoint the candidate regions important for virulence. Four populations, including the aforementioned HG type 1.2.5.7 field population (MM26) and the inbred population MM-BD3, along with the avirulent (MM1) and virulent (MM2) inbred populations used for our transcriptomic analysis, will undergo Pool-Seq to help us map to candidate virulence regions important for overcoming the Peking-type (*Rhg4*-mediated) resistance. We have determined that sequencing virgin female nematodes which have successfully adapted on their respective hosts, as opposed to other life stages, would further bias their virulence, and provide us with a better resolution of virulence traits among different populations. Therefore, we have been harvesting virgin females from these four populations and have been optimizing DNA extraction protocols in preparation for Pool-Seq.

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1.3: Resequence the genomes and transcriptomes of virulent SCN populations described in 2.2 and conduct comparative analyses (*Severin*, *Hudson, Mitchum, Baum*)

The Mitchum group is focused on identifying SCN virulence genes used by the nematode to overcome the Peking-type (*Rhg4*-mediated) resistance. Our comparative transcriptomic analysis comprises (1) differential expression and (2) variant call analysis by utilizing RNA-seq data generated from the early parasitic stages of virulent and avirulent SCN populations adapted on soybean recombinant inbred lines (RILs) that only differ at the *Rhg4* locus (i.e., a resistant RIL with a resistant *Rhg4* allele and a susceptible RIL containing a susceptible *Rhg4*). The differential expression analysis has allowed us to narrow down to 59 genes upregulated in the virulent SCN population. These genes were cross-compared with the nematode gland-specific RNA data sets to select 14 genes of special interest, categorized into three subgroups: (1) putative effectors involved in defense suppression, (2) putative enzymes related to reactive oxygen species, and (3) putative vitamin B-associated genes. These genes will be validated with qRT-PCR to confirm upregulation in the virulent population. From our variant call analysis to discover single nucleotide polymorphisms (SNPs) and insertions and deletions (INDELs) that may contribute to virulence, we previously found that 5,506 SNPs and 287 INDELs were unique to the avirulent nematode and 5,854 SNPs and 280 INDELs were exclusive to the virulent nematode, while those in common from both were 9,093 SNPs and 203 INDELs. Now that we have 14 genes of special interest, we are further analyzing these SNPs and INDELs to see if they are present in these genes.

For this reporting period, the Baum and Severin groups would like to announce publication of the MPMI Resource Announcement, entitled "Esophageal Gland RNA-seq Resource of a Virulent and Avirulent Population of the Soybean Cyst Nematode, *Heterodera glycines,"* (Mol Plant Microbe Interact, 2021 Apr 26. doi: 10.1094/MPMI-03-21-0051-A.). We are exploring the RNA-seq dataset derived from a comparison of SCN gland cell transcriptomics of a virulent (MM10) and avirulent (PA3) population. This data analysis is published in the above Resource Announcement paper. Briefly, with this submission, we have announced the availability of a unique gland-specific RNA-seq dataset for the SCN community, which provides an expression snapshot of gland cell activity during early infection of a virulent and avirulent SCN population. This represents a highly valuable resource for researchers examining effector biology and nematode virulence. Within the Resource Announcement, we have outlined a few initial intriguing gene expression differences between the two populations. Across all replications of these gland cell RNA-seq libraries, there are 96 mRNAs (which correlate to 115 genes) upregulated in the PA3 libraries versus the MM10 libraries. Conversely, there are 41 mRNAs (which correlate to 38 genes) that are upregulated in MM10 vs PA3. The data generated continue to be analyzed and is being used by the Baum lab in several avenues to progress omics research within the lab.

The Hudson group has made the finished genome assemblies available to collaborators in the group. Annotation is necessary to understand the results of transcriptome and resequencing work, and is the next step in the genomics process.

1.4: Validate and characterize genes associated with SCN virulence and evaluate their utility as novel resistance targets (*Mitchum, Baum*)

The Mitchum group continued the characterization of novel stylet-secreted effectors of the soybean cyst nematode *Heterodera glycines* parasitome, 16B09 and 2D01. These effectors belong to a highly

expanded superfamily of novel effectors, share the same gene structure, harbor conserved protein domains, and exhibit the same spatial and temporal expression in the dorsal gland cell during parasitism. This group of effectors was also identified as upregulated in the RNAseq data set as potentially involved in virulence (outlined under 1.3). Thus, we conducted a full transcriptome and genome analysis of this group of SCN genes. A search of the latest nine-scaffold, 158 Mb pseudomolecule assembly of the *H. glycines* TN10 genome identified 20 full-length (4 exon-3 intron gene structure) or partial copies of this effector gene superfamily (Masonbrink et al., 2021). Twelve members of this family were present on scaffold 5/Chromosome 1 and eight on scaffold 6/Chromosome 6. A sequence similarity tree constructed using the neighbor-joining method of the Clustal Omega program (Sievers et al., 2011) and including the original SCN parasitome sequences revealed three distinct subgroups. These subgroup sequences clustered together in the genome and may share functional similarities with some members involved in manipulating host developmental programs and others involved in suppressing host defenses. The variation observed across SCN populations is consistent with the highly expanded and diversified nature of this gene family. A manuscript describing the results of the functional characterization and genome analysis of this effector family is in preparation. This effector family will be a focus of comparative genomics analysis across populations of SCN differing in virulence on resistant soybean.

The Baum group has been characterizing the robust defense suppression and comprehensive re-engineering of the feeding site, which are two of the hallmarks of the successful cyst nematode infection. The cyst nematode achieves this by producing a large number of effector molecules and delivering them into soybean host cells via its mouth spear. These effectors specifically target host factors and modulate their functions. Generating an in-depth understanding of how individual effectors help the cyst nematode establish and maintain infection is a difficult but necessary task that will reveal vulnerable “nodes” in host signal transduction pathways that can be altered via either breeding or molecular approaches. As a part of this project, we are actively involved in conducting in-depth molecular characterization of the 28B03 effector family. Our analysis has shown that members of this effector family are robust defense suppressors. Our work has also revealed that a member of this family achieves its defense suppressive ability by interfering with a previously uncharacterized kinase cascade in plants. We continue to characterize the interactome associated with the kinases from this cascade as it will reveal signal transduction pathways that this particular effector modulates. To identify such an interactome, we are establishing a “proximity labeling assay system” in our laboratory, which is the latest and the most advanced technique to identify protein interactors *in planta*. In short, we have developed multiple fusion constructs in which we have fused an unmodified kinase, a dead version of this kinase as well as a truncated version of this kinase to a highly active derivative of the biotin ligase enzyme. As a control, we have fused the GUS marker gene to this biotin ligase enzyme in the same orientation. All these fusion constructs are expressed stably in Arabidopsis using the native promoter of the kinase in question. We have confirmed the expression of the GUS marker gene in the transgenic Arabidopsis lines showing that our fusion constructs are functional. We have also confirmed transcription of the other fusion constructs with the kinase in focus by RT-PCR. Finally, using the Western blot analysis we have confirmed the expression of all the fusion constructs. Currently, we are identifying homozygous lines expressing these constructs. Simultaneously, we are in the process of assessing the activity of the biotin ligase enzyme in our transgenic plants. We will begin protein interaction work shortly after such confirmation. We believe that establishing and characterizing such a system will prove pivotal for all our *in planta* protein interaction studies involving other effectors, particularly in soybean.

Simultaneously, we are employing state-of-the-art confocal microscopy technique to pinpoint the sub-cellular localization of the proteins involved in this kinase cascade. For this purpose, we are developing various constructs fused with compatible fluorescent proteins. Since some of these proteins seem to be vesicle localized, conducting co-localization studies using confocal microscopy will assist us to investigate their functions in more detail.

Objective 2. Determine what combinations of resistance genes would be beneficial in variety rotations to enhance the durability of SCN resistance in soybean.

2.1 Evaluate how rotations of various resistance gene combinations impact SCN field population densities and virulence profiles (*Diers*, *Scaboo*, *Tylka*, *Mitchum*)

Activities by the Tylka group for subobjective 2.1 at Iowa State University from November 1, 2020 through July 31, 2021 were as follows. At the conclusion of the 2020 growing season, two separate multiple-core soil samples were taken from each microplot in the two experiments that were conducted in Iowa. The first set of soil samples were used to determine end-of-season SCN population densities in each plot, and the second set of samples were sent to the University of Missouri for HG type testing to determine if and how the soybean genotypes grown in the microplots in 2020 might have shifted the virulence phenotypes of the SCN populations originally added to the microplots in spring 2019.

Preliminary data analysis was completed for each of the experiments, and some trends in changes in SCN population densities were observed. In both experiments, the highest SCN population densities in fall 2020 occurred in microplots in which the susceptible soybean variety was grown. The microplots that were continuous with the same resistance from 2019 had higher SCN population densities in fall 2020 than the microplots that had rotated resistant varieties from 2019. The lowest population densities were found in plots where PI90763 resistance was grown in 2020. The SCN population densities in plots with continuous PI90763 also were lower than those that had previously had rhg1-b, rhg1-b + soja, and rhg1-b + soja + ch10 in 2019 and were then rotated to PI90763 in 2020.

The results of the HG Type test results on the SCN populations in the soil samples collected from the microplots at harvest in 2020 show promising results. The SCN population in the soil in the experiment at the Kanawha, IA location had an HG type of 1.2 with a range of 35-80% female index on PI88788 and 7-40% female index on Peking. The SCN population in the soil in the experiment at the Ames, IA location had an HG type of 1.2.3 with a range of 27-80% female index on PI88788, 12-73% female index on Peking and 1-47% female index on PI 90763. The plots with rhg1-a + rhg4 and PI90763 had the highest female index on Peking and PI90763, while every plot had a female index greater than 10% on PI88788 in experiments at both locations and a female index greater than 10% on Peking at the Ames location. Increased virulence on PI437654 was not observed. After 2020 we observed increased SCN reproduction and some slight changes in female index (virulence) between microplots with rotated and with continuous cropping sequences.

In May 2021, each microplot was planted according to the deployment sequence being used in all microplot experiments in the project. This sequence included a third year of planting the same variety in half of the microplots (continuous sequence) and a rotation sequence of varieties to what was planted in 2019 in the remaining half of the microplots. At 30 days after planting, each row in each microplot was thinned to 30 plants and those with fewer than 30 plants were recorded.

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During this period, the Diers group finished the 2019 rotation experiment and grew the 2020 experiment. We also recently received the HG type data from the fall 2019 plots as the results had been delayed until now because of Covid. Although the results have not been fully analyzed, a few observations can be reported. One is that the nematode population in all plots could overcome PI 88788 resistance and the female index (FI) on this source ranged from 10% to 96%. The fact that the nematodes could overcome PI 88788 resistance was expected because this was the HG type of the nematodes used to inoculate the plots. There were six plots that had nematodes that could overcome Peking resistance and the two plots with the highest FI on Peking (37-23%) were planted to a line with Peking type resistance. We are still waiting for the HG type data from the fall 2020 soil samples and these results should be available by the second week of August.

In Missouri, the Scaboo group harvested Fall 2020 soil samples for SCN egg counts, HG type tests were processed, and data was collected. The egg count data has shown that the susceptible plots have the highest egg densities followed by *rhg1-b* plots. The PI 90763, *rhg1-a* + *Rhg4*, and *rhg1-b* + *G.soja* + ch.10 treatments had the lowest population densities and the highest reduction in SCN egg densities compared to initial levels. In continuous treatments, the highest reduction in egg counts was observed in PI 90763 and *rhg1-a* + *Rhg4*. Similarly, the highest reduction in percentage change in the egg counts is in those treatments rotated with PI 90763. Interestingly, *rhg1-a* + *Rhg4* treatments rotated with *rhg1-b* or its multiple gene stacks showed the largest reduction in egg counts that have been observed in the treatments with *rhg1-b* stacked with other resistance loci. The SCN HG type data has shown that the highest shifts in virulence were in continuous PI 90763 and continuous *rhg1-a+Rhg4* plots. In continuous *rhg1-a+Rhg4* treatment, increased virulence on PI 90763 and Peking has been observed. Similarly, in continuous PI 90763 treatment, an increase in virulence on Peking and PI 88788 has been observed. The seeds obtained for the third-year field season experiment have been checked, packaged and were planted in the microplots during June of 2021.

Objective 3. Translate the results of objectives 1-3 to the SCN Coalition to increase the profitability of soybean for producers.

3.1: Inform growers on effective rotation schemes designed to protect our resistant sources (*Tylka, Mitchum*)

The Mitchum lab helped to produce The SCN Coalition's "Let's Talk Todes" Research Collection video series now released online https://www.thescncoalition.com/lets-talk-todes/research-collection. In this 7 video series, soybean growers and scientists (nematologists, breeders, plant geneticists, extension pathologists) who are battling SCN explain the checkoff-funded research they are conducting that's focused on bringing new tools to soybean growers in the fight against parasitic nematodes. Video topics include: 'What is a virulent nematode?', 'Why does the source of resistance matter?', 'What SCN resistant tools are being created?', 'Genome editing can be an effective tool to combat SCN', 'The Tode farm is a rich genetic resource for researchers', 'Unlocking the power of Tode Spit', 'Soybean Breeders focused on sustainability'. During this reporting period, MorganMyers deployed promotions of the "Let's Talk Todes" Research Collection through a 7-week digital campaign (5/23/21-7/10/21). The campaign content was seen 2,656,014 times across web and social media. The digital ads and social posts generated 969,057 video views and 2,100 website page views. Mitchum assisted with content for three press releases and conducted five Ag media interviews including with Delta Farm Press, Adams on Ag

Live, Brownfield Ag News, Iowa Business Radio Network, and Michigan Ag Today. Mitchum assisted with content for a 3-page article published by Gil Gulickson in Successful Farming, "It Starts With SPIT" highlighting the research directed at developing new sources of resistance to SCN. She also participated in a Webinar "Let's Talk Todes: Actively Managing SCN" alongside SCN Coalition leaders Sam Markell and Greg Tylka and hosted by Farm Journal's Agritalk with Chip Flory targeted at crop consultants, agronomists, extension agents, and growers.

The Tylka group conducted 24 radio and newspaper/magazine interviews from November 2020 through July 2021. The loss of effectiveness of PI88788 SCN resistance was discussed and this current NCSRP-funded research project was mentioned and described whenever time/space permitted. In the fall of 2020 The SCN Coalition began posting short videos about SCN biology, management, and research on the project website www.TheSCNCoalition.com. The first seven videos of the SCN Coalition's "Let's Talk Todes" video collection were made available online at www.thescncoalition.com/lets-talk-todes in October 2020 and received >930,000 views from October through November 2020. The NCSRP-funded SCN research project is described by Tylka in one of these videos (see https://youtu.be/4PpvvavwwHc). Also, SCN Coalition communications experts, a videographer, and Gil Gullickson, reporter from Successful Farming magazine, traveled to Athens, Georgia in November 2020 to videotape University of Georgia professors Melissa Mitchum, Richard Hussey, Zenglu Li, Bob Kemerait, and Wayne Parrott as well as a Georgia soybean farmer to create numerous additional videos for the "Let's Talk Todes" video collection. The videos focusing on research, including this research project, can be viewed at www.thescncoalition.com/lets-talk-todes/research-collection. See https://youtu.be/0YqEM0CcvtY for the video in which Melissa Mitchum explains this current, NCSRP-funded research project.

Objective 4. Coordinate the testing of publicly developed SCN resistant experimental lines.

4.1: Organize tests of experimental lines developed by public breeders in the north central US states and Ontario *(Diers)*

During the period of the grant, the results from the 2019 SCN Regional Test were sent to the University of Illinois by collaborators, summarized, and the first version of the report was provided to cooperators on the 19th of December 2020. This version included the agronomic, composition and resistance test results. This version was followed by the distribution of the final version on the 6th of January 2019 which included the results of testing the egg number and HG type of nematode populations in field environments.

The 2020 SCN Regional Test was planned, seed was sent to cooperators, data were returned and the results were summarized in a report. The 2020 test included a total of 184 entries that ranged from MG 0 to IV. The initial version of the report was sent to cooperators on December 10th and the final version was delivered on January 12th. These timely deliveries of results are important so cooperators can make decisions on selections in time for winter crosses and nurseries.

Plans have been made for the 2021 SCN Regional Test. This test includes 242 entries that range from MG 0 to IV. The tests were organized and the seed was shipped to the University of Illinois by cooperators and then redistributed to cooperators. The tests have been planted and are currently growing in the field.