Project Update – March 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 March 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 December 4th of 2020, and our next group meeting is scheduled for April 2021 for research planning and group discussions.

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*)

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

The Hudson group has the multi-genome assembly project close to completion, with manually curated assemblies completed or under way for all seven of the target genotypes as well as the original TN10. For MM26, PA3 and OP50, we have finalized, frozen assemblies ready for annotation. For TN8, curated annotation is complete and a final step to remove duplicate haplotigs is under way. For TN7, TN20 and TN22, final steps of manual curation of the assemblies are in progress. The quality of each assembly is better than the previously published TN10 assembly and close to the quality of the current, manually curated TN10 assembly. Overall, we are very close to eight full high-quality assemblies of SCN lines corresponding to all of the major HG types.

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 a new chromosome-level assembly of the TN10 *H. glycines* genome, with assembly and annotation statistics that are much improved over the previous version. This genome has all the existing contigs incorporated into a chromosome. We believe that this latest genome contains the highest confidence SCN gene models publicly available, since the gene models in this version were created from a consensus of nine separate annotations. The manuscript describing these latest results has been submitted and is currently under review. (Preprint DOI: 10.22541/au.161538368.83631935/v1). As part of this release, a large number of additional resources have become available on SCNBase to complement this genome assembly, including a predicted proteome, genome, and transcriptome BLAST capability, and a number of downloadable alignments in JBROWSE, including ribosomal genes, genome structural variation, SNPs and INDELs, alignment of X12 gene models, effector alignments, ncRNAs, multiple repeat predictions, and every prospective gene annotation. We surveyed SNP and INDEL variations from 15 distinct *H. glycines* populations and have created SNP/INDEL-modified versions of the TN10 genome to represent

pseudo-genomes from these 15 populations. With these new pseudo-genomes, we used the TN10 genes to identify genomic variation that affects gene structure and coding potential. These pseudo-proteomes were subjected to bioinformatic screens for secretion and can potentially lead to a greater understanding of how variation in signal peptide presence can vary across *H. glycines* populations, and perhaps how they could confer virulence. This work is currently being written up for publication submission. Simultaneously, we are also working on collecting and analyzing data to distinguish gene expression patterns between male and female *H. glycines* populations, which may provide new targets for resistance development. Additionally, differences in the male vs. female populations can be looked at as differences in a non-feeding (male) vs. feeding (female) population. Our most current analysis of this data has revealed multiple genes that are differentially expressed in males vs females. Specifically, we have identified 6,039 genes upregulated in males and 5,881 genes upregulated in females. 43 of the genes upregulated in males previously were identified as effectors, while 29 of the genes upregulated in female stages were identified as effectors. We will continue to explore this data further for the upcoming reporting period.

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) Mitchum 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) for more than 12 generations. 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. A manuscript describing these populations was submitted and accepted to the scientific journal *Plant Disease*.

Meinhardt C, Howland A, Ellersick M, Scaboo A, Diers B, Mitchum MG. Resistance gene pyramiding and rotation to combat widespread soybean cyst nematode virulence. *Plant Disease* 2021; https://doi.org/10.1094/PDIS-12-20-2556-RE

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*). With the aid of the newly annotated pseudomolecule SCN genome assembly, we have finalized the differential expression analysis which has allowed us to generate a list of potentially important differentially expressed genes (DEGs) containing genes that may be specific to *Rhg4*-mediated resistance as well as genes that may be important for virulent nematodes growing on resistant soybeans regardless of the type of resistance. This final list of DEGs was further prioritized for virulence effectors through cross-comparison with the nematode gland-specific RNA data sets (i.e., *in silico* subtraction for gland-specific genes) from the Baum group. Consequently, these DEGs will be

subject to a high-throughput screening which will commence shortly. 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 the virulent SCN harbors 72,232 SNPs and 4,564 INDELs while the avirulent SCN contains 73,026 SNPs and 4,513 INDELs, relative to the reference genome with statistical significance. Since the last reporting period we have been able to filter out the majority of the SNPs and INDELs: 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. We believe that SNPs and INDELs unique to the virulent nematode that are present at a relatively high or low frequency may be of special interest. Moreover, those that are common in both nematodes, but are significantly higher or lower in the virulent SCN relative to the avirulent SCN may be interesting as well. For this reporting period, we are now collaborating with the Severin group to predict the effect of these SNPs and INDELs on amino acid changes (e.g., synonymous vs. nonsynonymous mutations). Clearly, SNPs and INDELs that result in nonsynonymous mutations are more likely to contribute to virulence. As a complementary approach to our transcriptomic analysis, we are conducting Pool-seq which pools individual nematodes from the same population to increase the amount of DNA and accurately estimate the population allele frequencies. This sequencing strategy has successfully been utilized by potato cyst nematode (PCN) researchers to map to a region containing several candidate virulence genes from experimentally adapted PCN populations. Similarly, Pool-seq applied to our unique SCN populations will allow us to pinpoint candidate regions important for SCN virulence. In preparation for this strategy, we have been harvesting nematodes from these populations and optimizing protocols for DNA extraction and sequencing.

The Hudson's group preliminary analysis of the genomes has already produced interesting results, with effector genes aligning to the same strand of the same chromosome across multiple lines (synteny). This result is important for understanding and tracking the evolution of virulence in SCN. Included among these effectors are Hgg23, G19B10, GLAND15, GLAND16 and GLAND17, glutathione peroxidase, G11A06, and GLAND5, these effectors aligned to the same chromosomes across all the different strain assemblies. A few effectors such as Hgg-25, G33E05 25A01, GLAND7, and G4G05, and GLAND18 had more variation in results including alignments to multiple chromosomes. These results need to be confirmed using the final, higher quality versions of the assemblies.

For this reporting period, the Baum and Severin groups continued to explore the RNA-seq dataset derived from a comparison of SCN gland cell transcriptomics of a virulent (MM10) and avirulent (PA3) population. We have developed this data analysis into a Resource Announcement paper, which we have submitted to the "Molecular Plant-Microbe Interactions"(MPMI) journal where it is currently under review. 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. We are currently pursuing these differences and examining the data for the identification of novel effector candidates.

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

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As candidate SCN virulence genes are identified by the Mitchum group, gene function studies are being conducted using biochemical and/or genetic analysis to not only better understand the mechanisms of virulence, but to also evaluate these gene targets as vulnerable points of disruption in the SCN life cycle as a means to enhance resistance in soybean. The Mitchum group continued our characterization of novel stylet-secreted effectors of the soybean cyst nematode *Heterodera glycines* parasitome, 16B09 and 2D01. 16B09 and 2D01 belong to the same superfamily of effectors, highly expanded in the genome, share the same gene structure, harbor conserved protein domains, and exhibit the same spatial and temporal expression in the dorsal gland cell during parasitism. Host-induced gene silencing (RNAi) of 16B09 demonstrated a requirement of this effector protein for successful parasitism. Protein interaction studies identified a specific interaction of 2D01 with a plant plasma membrane-associated protein kinase. We further demonstrate that this protein kinase is expressed in feeding sites and plants unable to produce this kinase showed increased resistance to nematodes. The identified protein kinase is involved in the signaling pathway which is extremely important for both abscission and lateral root emergence and this process activates a number of cell wall modifying enzymes that are important for these processes. Both lateral root emergence and abscission require cell wall expansion and dissolution. These processes would be advantageous for the nematode as we know one of the key characteristics for syncytium formation is cell wall dissolution as it allows fusion of surrounding cells to form feeding site. We have now confirmed the subcellular localization of Hs2D01dSP in the cytoplasm of the plant cell and the protein kinase to the plasma membrane and demonstrated that that these two proteins interact in plants. A comparative genomics analysis of this effector family across populations of SCN differing in virulence on resistant soybean is currently under investigation.

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. Currently, 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. Proteins in the near vicinity of the active biotin ligase will be biotinylated and then will be purified using streptavidin beads. Biotinylated proteins will then be identified using mass-spectrometry, which will identify the complete ‘interactome’ of this particular

kinase. We believe that establishing and characterizing such a system will prove pivotal for all our *in planta* protein interaction studies involving other effectors.

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*)

The experimental lines with resistance gene combinations developed in Objective 1 during Phase I of this project and tested in four different rotation schemes with experimental lines containing various resistance gene combinations in a greenhouse study was published in the journal *Plant Disease*.

Meinhardt C, Howland A, Ellersick M, Scaboo A, Diers B, Mitchum MG. Resistance gene pyramiding and rotation to combat widespread soybean cyst nematode virulence. *Plant Disease* 2021; https://doi.org/10.1094/PDIS-12-20-2556-RE

This published study, which served as the basis for the field trials in Phase II of this project, identified potential alternative resistance gene combinations that when used in rotation could reduce the selection pressure on the SCN population thereby slowing nematode adaptation to resistant varieties.

At the conclusion of the 2020 growing season, two separate multi-core soil samples were collected from each microplot in the experiments conducted in central Iowa and in north central Iowa by the Tylka group. One set of soil samples from each experiment were processed at Iowa State University to determine the end-of-season population density of SCN in each microplot. The second set of soil samples from each experiment 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 (HG types) of the SCN populations originally added to the microplots in the spring of 2019. Preliminary data analysis was completed for each of the two field experiments in Iowa, and some trends in SCN population density were observed. In both experiments, the highest SCN population densities occurred in microplots in which the susceptible soybean variety was grown. The microplots that had continuous cropping of the same resistance in 2019 and 2020 had higher SCN population densities than the microplots that had different resistant varieties grown in them in 2019 and 2020. The lowest population densities were found in microplots where soybeans with SCN resistance from PI90763 were grown. Also, the microplots in which soybeans with PI90763 resistance were grown also were lower than those that previously had soybeans with rhg1-b, rhg1-b + soja, and rhg1-b + soja + ch10 SCN resistance grown in 2019 and then were rotated to soybeans with PI90763 SCN resistance in 2020. The results of the HG Type tests on the SCN populations in the soil samples collected from the microplots at harvest in 2019 revealed that almost all of the SCN populations in the soil at both experimental locations Iowa had an HG Type of 1.2 with a range of female indices of 20-69% on PI88788 and 3-36% on Peking. The plots in which soybeans with rhg1-a + rhg4 and PI90763 SCN resistance were grown had the highest female indices on Peking and PI90763, but the SCN population in every plot had a female index greater than 10% on PI88788. No elevated reproduction on PI437654 was detected. HG Type test results of the SCN populations in soil samples collected at harvest in 2020 are not yet available. Preparation and planning for the 3rd growing season is currently underway.

In IL, the Diers group is preparing and distributing seed for all collaborators for the 2021 SCN resistance source rotation study. This will be the third year of the rotation study and we will be rotating the plots

back to what was grown in them during 2019. We recently received the HG type results 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 10.1% 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.

In Missouri, fall 2020 soil samples were taken for SCN egg counts and HG type tests and were processed. The egg count data has shown that the susceptible plots have the highest egg densities followed by the *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. SCN HG type data has shown that 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 an entry list has been prepared.

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 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'. Several press releases and media interviews are underway.

Tylka conducted 18 radio and newspaper/magazine interviews from October 2020 through March 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 to November 2020. Our current 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)*

The results from the 2020 SCN Regional Test were received from cooperators and summarized in a report by the Diers group. 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 will include 242 entries that range from MG 0 to IV. The tests have been organized, the seed has been received from cooperators and will be shipped to cooperators soon. Arrangements also have been made to test the lines for SCN resistance in a greenhouse at the University of Illinois.