Project Update – October 31st, 2022

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

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

III. **Brief Description of Accomplishments as of October 31st, 2022:**

A description of relevant progress for principle and co-principal 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. We had a group meeting in March of 2022 to discuss current research progress and goals and we are on track to continue our cutting-edge research in soybean cyst nematode biology, management, and breeding for novel resistance.

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

Sub-objective 1.1: Combine, compare, and catalogue the genomes that compromise the SCN pan-genome. (Hudson, Baum, Mitchum)

Previously the Baum group reported on gene family expansion and contraction across 13 plant-parasitic species of the Tylenchomorpha. We now have annotated these genes including secretory status, effector homology, nuclear localization, gene variability across 15 populations, and expression across various stages of the *H. glycines* lifecycle. We found 551 gene expansions in sedentary nematodes differentiate them from migratory nematodes, 124 gene expansions in cyst nematodes differentiate them from root-knot nematodes, and 1,100 gene expansions differentiates *H. glycines* from *Globodera* species. One interesting finding from this analysis lies with the inability of nematodes to produce their own cholesterol. To obtain this vital resource, plant-parasitic nematodes must acquire cholesterol from their hosts. We found that a gene family of expanded and secreted genes in cyst nematodes may be involved with this process. SCNBase has undergone many updates since our last report. We have modified the naming schemes of the predicted proteomes, transcriptomes, and genomes so that they are consistent across genomics tools and more intuitive for users to access. To further disseminate *H. glycines* genomics resources, we collaborated with Wormbase to host the most current *H. glycines* genome assembly and annotation. To better understand the biology of male and female *H. glycines* nematodes, we previously reported a genome assembly and annotation project for each sex. We now assessed differences in expression between the sexes. In this analysis there were numerous on/off differences in expression with one sex having high expression and the other sex completely lacking expression: 512 genes in females and 744 genes in males were silenced. A comparison of expressed genes revealed 6,543 genes were upregulated in females and 6,920 were upregulated in males. Many expression differences were tied to sex-related gene functions, though few have been reported in the literature for nematodes in the Tylenchomorpha.

Previously, the Mitchum lab employed a dual effector prediction strategy that coupled the traditional secreted protein prediction strategy with a newly developed nematode effector prediction tool, N-Preffector, to identify novel effector candidates in a *de novo* transcriptome assembly of the pre-parasitic and parasitic life stages of *H. glycines* with potential roles in virulence. From this analysis, 1,383 SignalP positive, N-Preffector positive candidates were identified, of which 210 were upregulated in parasitic juvenile life stages (Gardner et al., 2018). This transcriptome analysis, which preceded the release of the pseudomolecule genome assembly generated through this project (Masonbrink et al., 2021), represented sequences from the whole-nematode. Since then, in collaboration with the Baum lab, we

also generated a gland cell-specific RNA-seq resource for *H. glycines* representing an avirulent and virulent population (Maier et al., 2021). Thus, to further narrow the 210 candidates to those that may be expressed in the nematode esophageal gland cells and likely function in virulence, we carried out an *in silico in situ* analysis by cross comparing this list with the gland RNA-seq dataset. Effectors upregulated in the transcriptomic data but missing from the gland data were eliminated. This *in silico* comparison narrowed down the list of candidate effectors which were Signal P, N-Preffector positive with some evidence of expression in nematode gland cells to 123 candidates. The predicted candidate effector protein sequences were further analyzed for nuclear localization signals (NLSs). One or more NLSs were predicted in 32 putative effectors. Sequences hitting to known effector sequences of plant-parasitic nematodes or housekeeping genes were rejected, reducing the list to eight novel candidate effectors with high to moderate expression in the gland RNA-seq dataset. We mined the SCN pseudomolecule genome assembly to determine the gene structures and genomic organization of these sequences. These were selected for further analysis in sub-objective 1.3 below.

Sub-objective 1.2: Resequencing of the genomes and transcriptomes of virulent SCN populations and conduct comparative analyses. (Hudson, Mitchum, Baum)

Building upon our prior success with generating novel SCN gland cell-specific libraries, we are developing additional SCN life-stage specific libraries which will provide important transcriptomic data on development stages not previously available. Currently underway in the Baum lab are SCN gland cell-specific libraries for the pre-infective J2 life stage, for both the avirulent (PA3) and virulent (MM10) populations previously used. This will provide valuable data on the early stages of nematode development and effector activation, which can then be compared to later parasitic stages. Additionally, we are now able to separate, identify and collect subventral glands, specifically. We can then generate subventral gland cell-specific RNA-seq libraries, which will identify which transcripts are specific to the subventral gland. We can then use that as a "subtraction" for the genes that are transcribed in our libraries generated from both types of gland cells, at the same life stage, and can infer from that which genes are transcribed in the dorsal glands. Having this gland specific transcriptome will be immensely powerful in elucidating effector timing and function, given what we already know about the SCN parasitic lifestyle. Effectors involved in host invasion and initiation of the syncytium are typically thought of as being produced in the subventral glands and active early in the SCN life cycle. Effectors that are involved in syncytial maintenance and host defense suppression are thought of as being produced in the dorsal gland, which becomes active later in the SCN life cycle. By finally having a well-defined transcriptome for each cell type, we can once-and-for-all confirm these observations and potentially find additional novel subventral and dorsal-specific effectors. Additionally, by also having these cell-specific transcriptomes that vary by virulence, we can identify temporally variant effectors that also may exist in different configurations based on virulence.

To perform single SCN-J2 genome sequencing, The Hudson group tested different DNA extraction methods and kits to find a method that gave sufficient DNA quality and quantity to get whole genome data on a single nematode. Genomic DNA samples from multiple kits were then sent to the Roy J. Carver Biotechnology Center on campus to assess first DNA quality, then quality of the library construction process, and finally trial sequencing to select the proper kit. We are now collecting 200 DNA samples from "MM1" SCN population and expect to receive a second population after finishing the first one. We will likely be able to send the whole samples (400/two populations) for sequencing around mid-November. The annotations and assemblies for the seven SCN Hg types have been finalized and are ready to submit once collaborators have completed analysis.

The Mitchum lab completed the preliminary bioinformatics analysis of the Pool-Seq data derived from sequencing two pairs of SCN populations unadapted or adapted to reproduce on resistant soybeans. We have been running multiple software packages to identify genomic regions and candidate genes

potentially involved in overcoming resistance. By calculating population differentiation estimated from single nucleotide polymorphism (SNP) data, we identified five genomic regions spanning four chromosomes which contained distinct peaks formed by clusters of SNPs, indicating strong signatures of selection. Some candidate regions were detected in both pairs of SCN populations, while others were unique genomic regions under selection in each contrast. Interestingly and as expected, some of these include genes known to be involved in plant defense suppression. Therefore, we hypothesize that these genomic regions and their genes may have undergone selection pressure to overcome soybean resistance to SCN. We are currently using other tools to confirm extra evidence of selection in these candidate regions.

Sub-objective 1.3: Validate and characterize genes associated with SCN virulence and evaluate their utility as novel resistance targets. (Mitchum, Baum)

The Baum group has reported that characterization of the function of 28B03 effector family has been advanced to a natural stopping point to publish the first extensive report on this effector's function during parasitism. A complete manuscript is being finalized. Furthermore, we are exploring how the knowledge gained for 28B03 can be used to develop novel management tools against SCN. In addition, we are adapting new techniques for use in our laboratory to functionally study SCN virulence determinants identified in our genomic assessments described above. For this purpose, we have streamlined the use of soybean hairy roots as parts of composite plants as a powerful tool to study SCN genes. Also, we are in the process of constructing a set of cloning and expression vectors to be used in the composite hairy-root plants. These vectors will allow ease of cloning and gene transfers between the different vectors which will aid the high throughput study of their functions in soybean roots. Also, we are establishing different approaches to determine and study the interacting soybean proteins for SCN effectors. Finally, we are establishing a reliable methodology to routinely silence SCN genes by soaking them in double-stranded RNA. All these advances will aid the further functional characterization of the genes identified in our genomic studies described above and will unravel the mechanisms that determine SCN virulence. Such knowledge is critical when designing novel SCN control tools.

The manuscript submitted by the Mitchum lab during the past quarter - Verma A, Lin M, Smith D, Lee C, Walker JC, Hewezi T, Davis EL, Hussey RS, Baum TJ, Mitchum MG. A novel cyst nematode effector (2D01) targets the Arabidopsis HAESA receptor-like kinase was revised and accepted for publication in the journal *Molecular Plant Pathology* this quarter. It was also selected by the Editor in Chief for a highlight on the British Society of Plant Pathology website emphasizing the scientific/societal significance and impact of this study and images from the paper will be featured on the cover of the December 2022 issue. Further characterization of the 8 novel effector candidates identified under objective 1.1 is underway. We have profiled the expression of these genes in SCN throughout the life cycle and initiated studies to confirm where they effectors potentially localize within host cells after secretion by the nematode.

Objective 2: Complete the evaluation of how rotations of various resistance gene combinations impact SCN field population densities and virulence profiles. (Diers, Scaboo, Tylka, Mitchum)

To start the 4th year of the project, the Tylka group planted each microplot with the 4th year planting scheme (same as year 2) in May 2022 and collected multi-core soil samples from each microplot in the experiments conducted in central Iowa and north central Iowa. After 30 days, plants in each microplot were thinned to 30 plants per row, monitored throughout the rest of the growing season, and hand weeded to control weed populations. Soybeans at both locations were harvested in late October and two soil samples were collected from each microplot. One set of soil samples from each experiment will

be processed at Iowa State University to determine the end-of-season SCN egg population density in each microplot, and the other samples were sent to the University of Missouri for HG type testing to determine how the virulence profiles (or HG types) of the SCN population in each microplot may have been affected (shifted) by the soybean genotypes grown in the microplots in 2022 and in previous years. HG Type test results for samples taken at harvest in 2021 were received in mid-June 2022. Preliminary results revealed increased virulence in many of the SCN populations at both locations from 2020, although those grown in rotation with rhg1-a + rhg4 (Peking-type) showed a reduced female index (FI). SCN populations in the two experiments were HG Type 1.2 or 1.2.3 and the FIs ranged from 5-51% on Peking in Kanawha, 3-68% on Peking in Ames, 12-66% on PI 88788 in Kanawha and 10-65% on PI 88788 in Ames. The average FI on PI 88788 was reduced from 2020 in every treatment in Kanawha, while at Ames the female indices of all treatments were reduced except for those grown in rotation with the Rhg1-b pyramided alleles and PI 90763 combination.

The fourth year of tests with the different gene combinations were planted by the Diers group in the rotation field this spring and the plots were maintained through the growing season. Soil samples will be taken soon to study the impact of the rotations on the nematode population levels and HG types.

The fourth year of the field experiment was planted by the Scaboo group this spring to the continuous and rotated schemes as conducted previously in 2020. Soybeans were recently harvested and soil samples will be collected in the near future for SCN egg count and HG type testing. We will begin processing these samples directly after they are collected. SCN egg count data will be available shortly after processing to quantify egg density from each microplot. HG type testing will take several months to increase and characterize the SCN population from each microplot.

Objective 3: Translate the results of objectives 1-3 to the SCN Coalition to increase the profitability of soybean for producers and inform growers on effective rotation schemes designed to protect our resistant sources. (Tylka, Mitchum)

Melissa Mitchum continued to chair the planning committee of the 2022 National Soybean Nematode Conference, and Tylka is a member of the committee as well. The conference will be held in mid-December 2022. The planning committee has identified and invited speakers on important soybean nematode-related topics, which include the loss of effectiveness of SCN resistance in the US. One ISU graduate student working on this NCSRP-funded project will present results from all three states participating in the field microplot studies of Objective 2 of this project at the conference. One UGA graduate student working this NCSRP-funded project will present results from mapping SCN virulence genes of Objective 1 of this project at the conference.

Between April 1 and September 30, 2022 Tylka gave 15 interviews with ag media personnel. In each interview, the loss of effectiveness of PI88788 SCN resistance was mentioned or discussed in detail, and the NCSRP-funded research also was mentioned when time allowed.

Objective 4: Organize tests of experimental lines developed by public breeders in the north central US states and Ontario. (Diers)

The Diers group sent seed to cooperators for the 2022 SCN Regional Test. This test includes 225 entries that range from MG 0-IV. The regional test cooperators grew the tests over the summer and are in the process of harvesting them. We forwarded data sheets to the cooperators and they will use them to send us their test data and we will analyze the data across environment and summarize this information in a report that will be sent out in December.

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Objective 5: Diversify the genetic base of SCN resistance in soybean by developing and evaluating germplasm and varieties with new combinations of resistance genes in high-yielding backgrounds. (Diers, Scaboo)

The Scaboo group has now completed successful crossing attempts (3 backcrosses) using PI 90763 as a donor parent, and LD11-2170 and SA13-1385 as recurrent parents, for three major genes associated with resistance to virulent nematode populations (rhg1-a, rhg2, and Rhg4). For each crossing attempt, we have identified desirable F1 plants using marker assisted selection, and we have sped up the process by utilizing our winter nurseries in Hawaii and Puerto Rico for the last two years. During the summer of 2022, we grew over 100 plant rows derived from selected plants, and our first yield trials of this material will be in the summer of 2023. Additionally, we tested over 85 new and advanced SCN resistant lines in state, regional, and national yield trials during 2022. Harvest is currently over halfway competed, and analysis and advancements will commence during the next quarter.

The Diers group has continued to work to diversify SCN resistance away from PI 88788 based resistance in Midwestern adapted cultivars. To do this, we have continued to select for the major SCN resistance genes rhg1-a and Rhg4. During the past summer, we tested over 5000 plants to select these genes and selected over 950. These selected plants will be advanced to plant rows during next growing season. In addition, in advanced yield tests we evaluated 35 experimental lines that carried both rhg1-a and Rhg4 and 22 lines with two SCN resistance genes from G. soja.