Project Update – August 1st, 2024

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. Eliana Monteverde, Dr. Thomas Baum, Dr. Gregory Tylka, Dr. Matthew Hudson

III. **Brief Description of Accomplishments as of August 1st, 2024:**

A description of relevant progress for principal 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.

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)

In our latest report, the Baum lab finished computational annotations for the nine SCN genomes and had begun to manually annotate genes for TN10 using webapollo in order to get a better "true" representation of SCN genes and proteins. We finished this manual annotation over five rounds with great effort to produce accurate transcripts, each with start codons, stop codons, and exons representing the majority of RNA-seq or long-read nanopore RNA alignments. We created 18,170 transcripts from 17,182 genes with complete BUSCO scores for eukaryota at 92.5%, 73.9% complete for metazoa, and 71.2% complete for nematoda. These counts and scores improved from the automated gene prediction, with genes down from 17,602, transcripts down from 22,074, eukaryota complete up from 91%, metazoan up from 71.9% complete, and nematoda up from 67.5% complete. The overall statistics of the TN10 gene annotation have changed quite dramatically, with 76% of transcripts having a functional annotation, up from 70.6% in the previous TN10 computation gene prediction. The next steps are to adapt this TN10 manual annotation to the other 8 genomes and integrate this data into SCNBase.org. Currently, the computational gene predictions for these 8 genomes are being integrated into SCNBase for release, the annotations for which will be subsequently updated to reflect the manually annotated genes from TN10.

The Hudson group's recent analysis of the SCN pangenome has identified key protein domains associated with the genes under positive selection. These domains play crucial roles in various molecular processes, including DNA replication, signaling pathways, sensory perception, and immune system functions such as pathogen recognition. Additionally, we have predicted and characterized over 1,200 secreted proteins in SCN, collectively known as the 'secretome.' This secretome characterization provides a comprehensive dataset to facilitate future research in identifying potential effectors responsible for SCN virulence. Following the completion of these analyses, we aim to finalize and submit the manuscript for revision by the end of August.

The Mitchum lab also submitted a revised manuscript to publish the Pool-seq study, which has now been accepted for publication in the peer-reviewed journal *Molecular Ecology*.

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

The Baum lab previously has developed gland cell-specific library resources that were successfully used in our analysis of potential novel effector targets and genome annotation. We seek the completion of our library time points to include a pre-parasitic time point (freshly hatched J2) to investigate effector targets being expressed prior to invasion. We are also exploring the feasibility of developing a J4 parasitic female time point to look at effector targets being expressed during late syncytial maintenance and plant immune suppression stages. For this purpose, we are pursuing a novel methodology to purify gland cells.

The Huson group previously completed the downstream analysis of a whole-genome single nematode sequence dataset, leading to the identification of candidate genes associated with SCN adaptation to *rhg1-a/Rhg4*-type resistance in soybean. We have now finalized our analysis of population effective size, estimating it to be approximately 5 million. To our knowledge, this is the first estimation of population effective size in plant parasitic nematodes. This extraordinarily large number indicates significant genetic hypervariability in SCN, even in inbred lines. We employed various approaches to validate our downstream analysis, specifically bypassing imputation analysis. These methods largely confirmed our previous results and also identified new candidate haploblocks and genes under selection. Our updated list of candidate genes now includes a higher proportion of genes with known or probable functions in plant parasitism. However, a considerable number of genes within the haploblocks under selection yet remain unannotated. Our attempts to obtain reliable functional annotations using protein BLAST and 3-D structure similarity tools have been unsuccessful thus far due to the poor database for PPNs. We are now focusing on a subset of these genes with potentially crucial roles to confirm their impact on adaptation to resistant soybean. Additionally, we are continuing to investigate structural variants in the SCN genome within this dataset, which represents novel research in the field of plant parasitic nematode studies.

The Mitchum lab continued testing the exon SNPs in select candidate virulence genes for their possible correlation to SCN virulence phenotypes (HG Types) using individual virgin females isolated from multiple, un-related SCN inbred populations (i.e., populations not used in the Pool-Seq study). Remarkably, one candidate gene strongly correlated to virulence on Peking and/or PI 90763 in several unrelated SCN inbred populations, in addition to the original Pool-Seq populations from which we validated the exon SNPs. For this reporting period, we continued finalizing the testing of several more SCN population-specific correlation experiments to solidify our claim that this candidate gene may be involved in virulence.

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

As previously reported, the Baum lab has established the *in vitro* gene silencing process in soybean cyst nematode (SCN) to rapidly screen the nematode's virulence/parasitism in the

early stage of infection. The established process was applied to other SCN genes that are highly expressed in the gland cells at an early stage of infection. We successfully silenced these genes, and subsequently, a penetration assay was used to study the effect of gene silencing on nematode infection in soybean plants. The results showed that silenced nematodes penetrate soybean roots less than non-silenced nematodes, suggesting a correlation between silencing of the expression of the tested gene and successful parasitism. Apart from this, we also tested different dsRNA concentration to effectively silence a gene we found that even a 2 mg/ml concentration of dsRNA was sufficient to achieve gene silencing.

In the Mitchum lab, full-length virulence gene candidates were cloned from the cDNAs of parasitic juveniles and subsequently sequenced to confirm the presence of significant SNPs detected through pool-seq, along with any additional SNPs. Utilizing these clones, primers were designed for cloning these candidate genes into host-induced gene silencing (HIGS) vectors. One HIGS construct targeting a candidate virulence gene was completed and composite soybean plants were generated for nematode infection assays. Additionally, as a complementary/alternative approach, DNA templates for dsRNA synthesis were prepared for RNAi by soaking to test functionality of these candidates in virulence/parasitism of SCN. Multiple attempts have been made to confirm silencing of gene targets in soaked specimens and further optimization studies are underway.

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

In October 2023, plants from each microplot were carefully removed and bundled then threshed to determine yield quantity. The harvested seeds were saved to use in the experiments (after checking for purity) in microplot experiments in 2024. Also in October, two different soil samples were collected from each microplot in each of the two microplot field experiments conducted in Iowa. One set of samples were processed at Iowa State University to determine the end-of-season SCN egg population density (egg number) in each microplot. The second set of samples were sent to the University of Missouri SCN Diagnostics facility for HG type testing to assess how the soybean genotypes grown in the microplots in 2023 affected or shifted the virulence of the SCN populations and how the virulence phenotypes differed from the virulence phenotype of the SCN populations used to infest the microplots initially.

Overall, SCN population densities continue to increase in the microplots, and year-to-year differences occur among plots with rotated soybean genotypes. Also, results varied somewhat between locations again. For most microplots, rotated treatments had lower SCN egg numbers than treatments planted with the same genotypes continuously. Gene pyramid rhg1-b+G. soja+Chr.10 rotated with PI 90763 (*rhg1-a, Rhg4, rhg2*) had the lowest SCN egg numbers in both experiments. However, this rotation caused the virulence of the SCN population to increase, as evidenced by elevated SCN female index (FI) values. In the Ames experiment, the initial SCN population used to infest the microplots had a FI of 7 on PI 90763, and the continuous PI 90763 treatment and the rotation of pyramid 2 with PI 90763 caused the FI to increase over three field seasons. The FI on PI 88788 remained well above 10 across all

microplots, even in SCN populations not exposed to the rhg1-b gene, which PI 88788 possesses. Additional shifts in virulence were observed but were less substantial in comparison to those described above. HG Type test results of the SCN populations in samples collected from the 2023 microplots have not been received yet. The results of this important rotation study for the first four years have been analyzed and Dr. Pawan Basnet and Dr. Monica Pennewitt, with support from our group, are planning to publish this research in *Plant Disease* during 2024.

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)

Mitchum had several interviews with ag media personnel related to research outputs under this project including The SCN Coalition and communicated new developments in soybean resistance to SCN to a group of extension agents working directly with soybean producers. These workshops and interviews not only discussed the loss of effectiveness of PI 88788 SCN resistance but highlighted the ongoing research and new discoveries in soybean resistance and nematode virulence to address this situation.

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

The Monteverde group compiled the entry lists for soybean lines that will be entered in the SCN regional tests and received the seed from collaborators. Seeds were then repackaged and sent to collaborators for planting at test locations. In 2024, the final test entry list included approximately 160 experimental lines and checks ranging from MG0-IV, that will be evaluated in 30 locations across 10 states and one Canadian province. Soil samples at each testing location were collected by collaborators and submitted to the SCN Diagnostics Lab at the University of Missouri for HG typing and SCN egg counting. Additionally, data for flower color, pubescence and height is being collected on each site.

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. (Monteverde, Scaboo)

At the soybean breeding program in UIUC, we are testing promising high yielding lines containing combinations of three SCN resistant genes in multi-environment trials. We have a total of 13 lines that are being tested across multiple environments in soybean Uniform Trials. Some of these lines carry the *rhg1-a*, *rhg2*, and *Rhg4* combination, and six lines containing *rhg1-b* from 88788 with other two *G. soja* genes (*cqSCN-006* and *cqSCN-007*). We have more of these lines in our pipeline and we are currently genotyping our plant populations for these gene combinations. In addition, in 2024 we decided to add one more gene to each of these two combinations in order to enhance pathogen resistance in our soybean lines. We are now working on combining GmSNAP02 gene, previously identified by the Scaboo group in Missouri, to the *rhg1-a*, *rhg2*, and *Rhg4* stack. We are also adding the CHR10 gene to the *rhg1-b/cqSCN-006/cqSCN-007* combination. Crosses were made in July, and in 2025 we will be genotyping and selecting plants with the desired gene stacks.

Project Update – August 1st, 2024

After analyzing the data from harvest, in 2024 we will be sending promising high yielding lines containing combinations of three SCN resistant genes to Uniform and SCN Regional trials. The lines we selected are 13 in total, seven carrying the *rhg1-a*, *rhg2*, and *Rhg4* combination, and six lines containing *rhg1-b* from 88788 with other two *G. soja* genes (*cqSCN-006 and cqSCN-007*). In addition, we have more lines in our pipeline with these two different gene combinations. In 2024 we will be testing a total of 25 lines with both combinations in advanced trials, and 152 In preliminary trials. We will also be genotyping our plant rows and populations for these gene combinations.