Project Update – September 1st, 2023

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 September 1st, 2023:**

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. We are planning our next group meeting for the fall of 2023 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)

The Baum lab is continuing the gene expansion analyses mentioned in the previous report. We added 12 new expression tracks to JBrowse in SCNBase for the TN10 genome, all of which represent long read nanopore sequencing of cDNA. In an attempt to address some of the haplotype-related bloating issues in the current TN10 genome assembly, we have begun a reassembly and reannotation pipeline. Using both new nanopore sequencing (Male and Female TN10) and Pacbio from an earlier TN10 assembly, we created a new TN10 assembly that far surpasses the genome quality statistics of the previous assembly and fits well comparatively with the size of other assembled SCN genomes at 115Mb in 9 scaffolds. This genome has been annotated and is very close to the theoretical max for BUSCO scores for genome completeness across three lineages, though some tweaking of the annotation is necessary due to an influx of new data.

In a previous report, we mentioned that the Hudson group accomplished whole genome sequence from single juvenile (J2) nematodes. The Illumina sequence raw data from 382 individual worms from two selected SCN populations from the Mitchum group (i.e., MM1 and MM2) was generated at the Roy J. Carver Biotechnology Center, UIUC, and has passed quality control. After pre-processing analysis, the short reads were mapped to a newly generated SCN reference genome (PA3). Around 1.4 m raw markers were discovered across 384 individuals using the Sentieon pipeline. In down-tream analysis, after filtering and refinement, the overall fixation indices (Fst), nucleotide diversity and some haplotype based statistical methods (e.g., XP-EHH and RSB) were calculated to explore the signatures of selection between populations, during independently divergent selection from the PA3 ancestor for more than a decade. The populations now show significantly different phenotypes on Peking type resistant soybean. Our results confirm the significant genetic differentiation between the two populations at specific loci. We anticipate that this data could lead us to the gene(s) responsible for SCN adaptation on this type of resistance. Now we are finishing up the downstream analysis and starting to annotate the regions under selection. We are also making progress on the publication of the SCN pangenome thanks to a revised TN10 genome sequence that is more comparable to the seven assemblies we have generated on the new Hg types, however we need to redo all of the previous analysis on the new TN10 reference.

Project Update – September 1st, 2023

The Mitchum lab has been focused on using the SCN genomes to conduct genome analyses for the candidate virulence genes identified from the Pool-seq analysis described below. This involves manual annotation, mapping identified SNPs and predicting impacts on protein function, assessing candidates for signal peptides, subcellular localization, and esophageal gland expression.

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

The Baum lab continues to develop gland cell-specific library resources to provide insights into transcriptional activity within the gland cells of the developing parasitic stages over multiple life stages. We are developing a useful resource for this analysis. We hope that, when combined with our developing genomic resources, the data will provide a comprehensive analysis of the activity of the key genes (effectors) responsible for the nematode's development and evasion strategies in achieving its parasitic lifestyle.

The Mitchum lab now has a final list of candidate virulence genes discovered from the Pool-Seq analysis of the two pairs of SCN populations un-adapted or adapted to reproduce on resistant soybeans; included among these genes are known effectors as well as novel candidate effectors without functional annotation. Out of 316 significantly overly differentiated single nucleotide polymorphisms (SNPs), 273 SNPs were mapped to 71 unique gene_IDs (Hetglys). Interestingly, 58 out of 273 were exonic SNPs, some of which may alter protein translation possibly affecting SCN virulence traits. For this reporting period, we confirmed the SNPs for two candidate genes by conducting Sanger sequencing of PCR products flanking these SNPs, amplified from cDNA of bulk parasitic J2s as well as from genomic DNA of individual females. Each of the two candidate genes (both predicted to have a signal peptide without a transmembrane domain) harbors at least two exonic SNPs which seem to be dependent on the population's adaptation status. After carefully inspecting the sequencing chromatogram, we learned that the un-adapted population contained heterozygous SNPs having two alleles with different bases (i.e., double peaks were present at both SNP locations), whereas its adapted counterpart was homozygous (i.e., single peak at both SNPs). This validates our Pool-seq results and provides extra confidence to pursue these genes and test for their correlation to virulence in independent SCN populations (lab-reared and field populations) with same or similar virulence profiles (HG types), followed by molecular functional studies.

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

Baum –

During this most recent phase of the project, the Baum group has focused on developing tools for the scientific community to employ when conducting *in planta* SCN studies. Most notably, we have constructed a set of GATEWAY-compatible vectors to facilitate cloning of a gene of interest *in frame* with different epitopes to perform functional analyses on soybean roots (epitopes of choice: eGFP / 3xHA or miniTurboID-V5 at either N or C terminus). The newly developed vector series can express a given gene of interest through the highly constitutively expressed GmUbi promoter. Those vectors allow the rapid selection of transgenic roots via the mCherry fluorescent protein or via the expression of the novel non-invasive reporter gene RUBY (which produces a red pigmentation and therefore does not require any particular microscope) located on the same T-DNA as the gene-of-interest. Generation of composite soybean plants accelerates functional analyses of a given gene-of-interest since it does not require any sterile precautions compared to *in vitro* hairy root culturing and is closer to real-life conditions since those transgenic roots are generated directly from a wild-type plant. To confirm functionality of these vectors, we successfully expressed different subcellular markers in soybean roots

(nuclear, actin, microtubule, plasma membrane, endoplasmic reticulum and plasmodesmata). Along with these vectors, we are establishing different approaches to determine and study the interacting soybean proteins for SCN effectors, such as immunoprecipitation or proximity-labelling followed by mass-spectrometry. Also, we have developed a second series of these same vectors expressing a gene-of-interest through a dexamethasone-inducible promoter allowing us to fine-tune expression of the gene-of-interest.

The Mitchum group has continued characterization of 8 novel effector candidates identified under objective 1.1. Several of these candidate effector genes were confirmed to be expressed in the nematode esophageal gland cells and their subcellular localization in plant cells was determined in transient expression assays. Genome analyses were carried out to determine copy number, gene structure, and organization in the genome. A manuscript describing this work has been prepared for publication.

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)
To start the 5th year of the project, the Tylka group planted each microplot in May 2023 with the 5th year treatment scheme (which was the same as that used in years 1 and 3) and collected multi-core soil samples from each microplot in the experiments in central and north central Iowa. The number of plants in each microplot were thinned to 30 per row 30 days after planting, and the microplots were monitored throughout May, June, July, and August then hand weeded as needed to control weed populations. The HG Type test results for soil samples collected at harvest in 2022 were received in mid-June 2023. Preliminary analysis revealed increased virulence from 2021 in SCN populations in many of the treatments at both locations. The SCN populations in the two microplot experiments had varying HG types from a type 2 to 1.2.3 and the female indices (FI) ranged from 8-53% on Peking in Kanawha, 5-83% on Peking in Ames, 10-85% on PI 88788 in Kanawha and 13-83% on PI 88788 in Ames. There also was an increase in the FI on PI 90763 in the SCN populations in most plots in the Ames experiment, with a range of 0.5-62%, and in Kanawha with a range of 0.1-59%. The average FI on most Plant Introduction lines were greater compared to what they were in 2020 in both the Ames and Kanawha experiments. Further statistical analyses currently are being conducted.

Additionally, the Tylka, Scaboo, Monteverde, and Mitchum groups have had regular group meetings for reviewing analyzed data from this project in preparation of submitting a manuscript for publication.

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)
Between April 1 and August 31, 2023, Tylka gave 6 presentations to 259 people and 5 interviews with ag media personnel. In each presentation and interview, the loss of effectiveness of PI 88788 SCN resistance was mentioned or discussed in detail, and the NCSRP-funded research also was mentioned when time allowed.

Mitchum conducted an interview with Successful Farming to highlight the work of this project for an article focused on SCN adaptation and new management strategies, specifically fighting the nematode by rotating resistant varieties. Kheeman Kwon, a PhD student with Mitchum presented a poster at the 2023 Society of Nematologists meeting highlighting the experiments in objective 1 of this project that will inform effective rotation schemes.

Project Update – September 1st, 2023

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 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 2023, the final test entry list included approximately 205 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 was 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)

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 are currently growing in two locations in Missouri during the summer of 2023. We have also identified several lines in our breeding program with the desirable three gene stack, confirmed phenotype of resistance, and selected them for advanced testing across multiple states in 2023. Additionally, we are actively identifying and introgressing new and novel QTL/genes into our breeding programs' elite cultivars for cultivar development.

The Monteverde group is committed to developing experimental lines and cultivars with resistance to a wide range of SCN population types. We are currently selecting for the major SCN resistance genes *rhg1-a* and *Rhg4*. This summer we tested a total of 5712 plants for these genes using molecular markers, and a total of 721 plants were selected. These plants will be advanced to plant rows in the next growing season and will be further evaluated for other traits of interest. We will also evaluate 24 experimental lines carrying the *rhg1-a/Rhg4* combination, and 25 lines with different combinations of *Rhg1* with two SCN resistance genes from *G. soja*.