Project Update – November 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 November 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 winter 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 has generated large amounts of RNAseq and Nanopore cDNA sequencing across seven life stages of SCN. We have used these data to improve the gene annotations of all existing SCN genomes. This annotation has resulted in a consistent 3-5% improvement in BUSCO scores across all genomes and lineages. Because each gene prediction was produced using the same pipeline, we were able run Orthofinder to cluster genes by identity to be able to assign gene names based on gene families. Thus, we now have improved annotations for all 9 SCN genomes that can be readily compared across genotypes. In addition, each gene has been annotated with functional information from NCBI NR and UniProt databases. Furthermore, we have produced a number of genome browser tracks that will be helpful to researchers, including bigwig expression tracks for RNAseq and Nanopore cDNA, two repeat tracks, known effector alignments, and all annotations used to produce the final output. Among these, we produced a robust repeats track that utilized all 9 SCN genomes to identify genome repeats, which should in theory improve transposon models, resulting in fewer spurious gene models.

In collaboration with the Mitchum Lab, the sequencing and analysis of pooled *SCN* populations has provided quick and promising results. In the previous report, we provided pool-seq results based on the alignment of the sequencing data to TN10 reference genome - published on SCNbase. However, alignment against one linear reference genome can lead to bias towards the alleles present in the reference haplotypes. Some procedures can be done to minimize reference bias: on one hand, pan-genomes are promising alternatives that cope with the reference bias issue, and that is one of reasons why our work on the SCN pan-genome is fundamental for the future of SCN genomics research. On the other hand, reference bias can be minimized by using multiple representative reference genomes. Therefore, we have been re-

estimating the population genetic parameters using multiple representative reference genomes from our new set of references (PA3 and MM26) related to the populations addressed in pool-seq analysis. We aim to identify any possible signatures of selection that were not previously detected due to reference bias. The Hudson group have also completed the raw single-juvenile SCN whole-genome sequencing data on the individuals supplied by the Mitchum group, completed the initial analysis and started the downstream analysis. Haplotype-based statistical approaches for selection scan (XP-EHH and Rsb) and related metrics were applied to the unphased SCN genomic data. Preliminary results showed high significant (FDR corrected: $p < 1E-5$) candidate regions under selection for virulence in SCN genome. These preliminary results parallel with pool-seq results, but with outstanding increase in resolution. Some of these candidate regions are probably harboring the genes with the most leading role in SCN adaptation to Peking type resistant soybeans (RHG1a/RHG4). Now, our analysis focuses on phasing the genotypes and estimating the population effective size (Ne). The first will increase the performance and resolution of haplotype-based statistical methods, and the latter will be the one of first attempts to estimate this important parameter in SCN populations. Our final analysis at this step would be introducing the probable genes involved in adaptation.

The Mitchum lab has continued to focus on using the available 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 plan for the few missing gland cell-specific library resources. Together, all gland transcriptomes will provide insights into transcriptional activity within the gland cells of the developing parasitic stages over multiple life stages. We have been making progress towards the development of this useful resource to allow comprehensive analyses. When combined with our developing genomic resources, these 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.

In a previous report, the Mitchum lab finalized a list of 71 candidate SCN virulence genes discovered from the Pool-Seq analysis and validated some of the exon SNPs present in select candidate genes by conducting Sanger sequencing of SNP-flanking PCR products amplified from genomic DNA of individual females from Pool-Seq SCN populations. For this reporting period, we have been testing the variation of these exon SNPs for their correlation to virulence, using individual females from unrelated SCN inbred populations (i.e., SCN populations not used in Pool-Seq) with same or similar virulence profiles (HG types). If those SNPs are important for virulence, we expect that the unrelated, adapted population will also contain the same homozygous (i.e., single peak at both SNPs) form, while the unrelated, un-adapted population may have a mixture of both homozygotes and heterozygotes. Concurrently, we are piloting RNA interference studies for select candidate genes, using *in vitro* double-stranded RNA-soaking of

pre-parasitic second-stage juveniles (ppJ2s) and host induced gene silencing of parasitic second-stage juveniles (pJ2s), in order to functionally test their role in virulence.

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 continued to develope tools for the scientific community to employ when conducting *in planta* SCN studies. In particular, 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. In addition, we have established the generation of composite soybean plants, which 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. Furthermore, we have been working to establish a reliable RNAi pipeline based on soaking in dsRNA. We are using a SCN gland-specific transcription factor as a proof of principle case study and plan to expand to a diverse panel of effectors.

The Mitchum group has initiated cloning and characterization studies for several virulence gene candidates identified above. These candidate virulence genes coding for putative effectors will be confirmed to be expressed in the nematode esophageal gland cells and moved forward for protein-protein interaction studies. Genome analyses will be carried out to determine copy number, gene structure, and organization in the new SCN genomes.

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)

Between 1 September and 31 October 2023 personnel in the Tylka laboratory removed mature soybean plants from the microplots at both experimental sites. The plants were run through a plot combine to obtain the seed, which will be used to plant the microplots in 2024. Also, two separate 10-core soil samples were collected from each microplot, one to use to determine SCN egg population densities and the other to test the virulence of the SCN population in each microplot on several SCN-resistant soybean genotypes. The soil samples to determine egg

population densities will be processed at Iowa State University and the samples to assess virulence will be sent to the University of Missouri for HG type testing.

Our research group had two meetings during this reporting period to finalize figures and tables associated with the analysis of this 4-year rotation project, and we hope to submit a manuscript for publication of the results in early 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)

Tylka gave 5 radio interviews with ag media personnel between September 1 and October 31, 2023. The interviews were with the National Association of Farm Broadcasters, the American Ag Network, RFD Radio Network, AgriTalk, and the Brownfield Radio Network. In each interview the loss of effectiveness of PI 88788 SCN resistance was discussed.

Mitchum hosted Gil Gullickson, contributing editor for The Furrow magazine (a John Deere publication), for a behind the scenes look at ongoing research supported by this project and SCN Coalition efforts. Gil is writing a story to translate some of the results from this project that will highlight the importance of gaining new insights into the genes controlling virulence in the nematode and how this knowledge can inform soybean breeders as new sources of SCN-resistant soybeans are developed and deployed in strategic rotations.

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

The Soybean Breeding and Genetics group at the University of Illinois is coordinating the screening of the SCN Regional Test entries, which is being done at the University of Missouri. A total of 78 lines are being tested for HG 7 screening, 44 lines are being tested for HG 2.5.7, and 18 lines for HG 1.2.5.7. Additionally, samples were collected and shipped for protein and oil content and molecular markers analyses. Our group is compiling all the phenotypic data for the SCN Regional trials entries. All maturity and lodging notes were completed at each location, and the datasheets with instructions for data submission were sent to cooperators. In late October we started harvesting the SCN plots in Urbana, and we plan to finish during the first week of November.

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 F_1 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 2023, we grew over 10,000 F_3 plants at our nursery in Hawaii, and all plants were sampled for marker assisted selection. Over 750 plants were selected carrying *rhg1-a*, *rhg2*, and *Rhg4*. Plant rows from these selections will be

grown during the winter of 2023/2024, and preliminary yield trails will be conducted during the summer of 2024. Additionally, we are actively identifying and introgressing new and novel QTL/genes into our breeding programs' elite cultivars for cultivar development, including the new SCN resistance gene *GmSNAP02*.

The Soybean Breeding and Genetics group at the University of Illinois is currently harvesting the preliminary yield trials containing the experimental lines that carry the *rhg1-a/Rhg4* and the Rhg1*/G. soja* SCN resistance gene combinations. These PYT were grown in two different locations in the state of Illinois. Additionally, single plants from F4 populations containing these same gene combinations were previously selected with molecular markers, were harvested to be grown in plant rows next year.