Project Update – October 31st, 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 October 31st, 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)

The Baum lab have adapted our TN10 manual annotation to the other 8 SCN genomes the Hudson group is working on and have integrated this data into SCNBase.org. The computational gene predictions for these 8 additional genomes have been integrated into SCNBase for release, the annotations for which have been updated to reflect the manually annotated genes from TN10.

The Mithcum lab published the following paper - Kwon KK, Viana JPG, Walden KO, Usovsky M, Scaboo AM, Hudson ME, Mitchum MG. Genome scans for selection signatures identify candidate virulence genes for adaptation of the soybean cyst nematode to host resistance. 2024. Molecular Ecology, 33:17: e17490.

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

The Baum lab is continuing to develop their gland-cell-specific library resources. This includes the completion of library time points to include novel effector targets expressed at these unexplored time points. Additionally, we are utilizing our pooled genomic and transcriptomic data to identify candidate effector genes of the soybean cyst nematode for high-throughput protein-protein interaction studies. We have identified 220 effector candidates based on criteria such as the presence of signal peptides, high and specific expression in gland cells during developmental stages, and cellular localization. These effectors are expected to modulate host defenses, alter hormone signaling, and restructure plant cellular architecture, which are critical for nematode parasitism.

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)

The Baum lab is currently preparing to analyze the protein-protein interactions of 220 SCN effector proteins within the soybean plant using a high-throughput yeast two-hybrid Cre recombinase-based system. Optimization of experimental conditions is underway, and both positive and negative controls have been successfully cloned in the pEntry vector and subsequently into the destination vector using the Gateway cloning. Further sequencing will be done to confirm that these genes are cloned in-frame, which is a critical step for ensuring proper interactions. We will then plan to screen the identified effectors against a soybean cDNA library to map effector-host protein interactions, furthering our understanding of SCN parasitism and identifying potential targets.

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. We observed a significant reduction in the fecundity of females developing on the transgenic roots transformed with the HIGS construct for our target gene. The eggs recovered from transgenic and non-transgenic control roots were re-inoculated back to wild-type soybeans to evaluate the progeny for any defects in parasitism and the test results will become available in the next reporting period. A second set of composite soybean plants in both a resistant and susceptible soybean background have been generated to test reproducibility of the results and evaluate silencing in nematodes developing on the transgenic roots.

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

Between 1 August and 31 October 2024 personnel in the Tylka laboratory removed mature soybean plants from the microplots at both experiments. The plants will be run through a plot combine to obtain the seed. Also, two separate 10-core soil samples were collected from each microplot in both experiments, one to determine SCN egg population densities and the other to test the virulence of the SCN population on several SCN-resistant soybean genotypes. The soil samples to determine egg population densities will be processed at Iowa State University in upcoming months and the samples to assess virulence have been sent to the University of Missouri for HG type testing. 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.

Project Update – October 31st, 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 4 interviews with print ag media personnel at the Farm Progress Show in Boone, IA on August 27 and gave one presentation at a Syngenta VIP education event at the show on the same day. He also gave 4 hour-long presentations at a Pioneer meeting on September 12. On October 1, Tylka gave an interview to George Bower of KICD radio in northwest Iowa about what farmers should be doing relating to SCN from October through December 2024. In every presentation and interview mentioned above the loss of effectiveness of PI 88788 SCN resistance was discussed.

Mitchum partnered with The SCN Coalition to highlight how Checkoff investments through this NCSRP project have paved the way for important research breakthroughs in identifying the resistance-thwarting genes in SCN and what it means for growers. The article was published in here August 20, 2024: https://www.thescncoalition.com/news/2024/08/20/breakthrough-scn-research-pest-resilience-genes/

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

During July-August period we took data for flower color, pubescence and height on all accessions that were planted in Urbana. In September we finished our maturity notes and all trials in Urbana are being harvested during the last week of October. All collaborators will send their data on yield and agronomic traits in the next months, for final analysis and compiling.

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. During the August -September period we collected data on flowering, lodging and maturity on 25 lines on advanced trials and 152 lines in preliminary trials. All these lines contained either Peking type resistance, or a combination of *rhg 1-b* from 88788 and two *G. soja* genes that confer resistance to several SCN HG types. We finished harvesting at the end of October, and we will be processing yield data in the following weeks to make experimental line selections to send to USDA uniform trials. We also genotyped our F_3 and F_4 populations for these two types of resistance, and we selected 208 plants out of a total of 2527 plants with Peking type resistance, and 321 plants out of a total of 1731 plants with the 2 *G. soja* genes + *rhg 1-b* gene combination. 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 three gene Peking stack. We are also adding the CHR10 gene to the *rhg1-b* + 2 gene *G. soja* combination. Crosses were made in July, F1 seed was harvested and will be sent to winter

nurseries for generation advance. In 2025 we will be genotyping and selecting plants with the desired gene stacks.

The Scaboo group, during the summer of 2024 grew over 5,000 F_3 plants for marker assisted selection of important genes *rhg1-a, rhg1-b, rhg2, Rhg4, and GmSNAP02*. Over 200 plants were selected carrying *rhg1-a*, *rhg2*, and *Rhg4* plus additional genes. Plant rows from these selections will be grown during the winter of 2024/2024, 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.