Project Update – April 15th, 2025

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 April 15th, 2025:**

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 Mitchum lab continued comparative genomics analyses using the above SCN pan-genome resources generated through this project to catalogue candidate virulence genes identified in pool-seq mapping studies.

The Hudson lab has slightly delayed SCN pangenome paper, but we hope that it will be submitted in the next month or so. It looks very promising, so we want to do a good job. We have built on the poolseq approach we developed with Melissa to look at the wormplasm in the microplots. Greg was able to ship us soil samples and we have sequenced 72 of them with deep Illumina sequencing. Analysis is in progress. Following the successful completion of a single-nematode population genomic analysis, the Hudson group has initiated a second study employing a pooled sequencing (Pool-seq) approach to investigate soybean cyst nematode (SCN) adaptation. This study builds upon findings from the initial work, which identified candidate genes linked to SCN virulence against rhg1-a- and Rhg4-mediated resistance and highlighted a high effective population size, suggesting extensive genetic diversity within SCN populations. For this new effort, 72 SCN-infested soil samples were collected from two independent field sites in Iowa: Ames and Kanawha. Cyst extractions and purification were performed following established nematological protocols. Cysts were then surface-sterilized, pooled by sample, and prepared for whole-genome shotgun sequencing. Sequencing has been completed for 12 SCN populations, each with six biological replicates, totaling 72 pooled samples. Preliminary analyses of raw reads have been performed, and downstream population genomic analyses are currently underway using the PoPoolation pipeline. The SCN populations in the wormplasm experiment represent a broad spectrum of virulence profiles, having been exposed over several years to various soybean resistance sources—including PI 88788, Peking, PI 90763, wild soybean accession, etc. This dataset offers a unique opportunity to dissect the genomic basis of SCN adaptation and selection under long-term resistance pressure and to uncover mechanisms driving changes in virulence.

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The Baum lab has updated SCNBase.org to include the most mature version of the TN10 genome, which includes our latest manual annotations, as well as the 8 additional Hudson genomes.

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

The Mitchum lab has confirmed a correlation between exon SNPs in select candidate virulence genes and 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).

The Hudson lab has investigated the genetic variation within two regions of interest (ROIs) in the soybean cyst nematode (SCN) genome, focusing on loci previously associated with increased virulence (Kwon et al., 2024). Specifically, we targeted genomic intervals on chromosomes 3 and 6, where strong signatures of selection were detected. For each ROI, we extracted a 5 kb window centered around the most significant SNPs and analyzed their local sequence diversity using a graph-based approach. The graphs were constructed from seven high-quality de novo assemblies, with TN10 serving as the reference. Subgraphs representing the ROI on chromosome 6 revealed multiple structural variants (SVs), including three large (654 bp, 818 bp, and 722 bp) and three smaller insertions or deletions (83 bp, 133 bp, and 110 bp), which we refer to as L-1 through L-3 and M-1 through M-3, respectively. Within the chromosome 6 ROI, the structural variants segregated into two major haplotypes. The first haplotype, observed in TN22, TN10, and MM26, consists of the variants M-1, L-1, and L-2. The second haplotype, shared by OP50, TN8, TN20, TN7, and PA3, includes M-2, M-3, and L-3. These contrasting configurations may underline functional differences in virulence among SCN populations. However, since each genome assembly was generated from pooled individuals, it remains uncertain whether these patterns represent true, individual-level haplotypes. Thus, further validation is required to confirm the biological significance of the structural divergence observed in the graph. To address this, our next step is to align PacBio HiFi long reads from the MM26 and PA3 populations to the pangenome graph. Although the sequencing libraries were prepared from pooled nematodes, each HiFi read corresponds to a single molecule derived from a single individual, preserving individual haplotypic segments. By aligning these reads to the graph and extracting those that traverse the ROI, we aim to identify whether these two haplotypes are supported by real reads. This will involve quantifying read depth and clustering alignments based on their traversal of variant paths through the graph. If validated, this approach not only confirms population-level haplotypes but also establishes a strategy for recovering real haplotypes in systems where sequencing single individuals remains technically challenging. Confirming the presence of distinct haplotypes in these virulence-associated regions would provide strong support for the functional role of structural variation in SCN pathogenicity.

The Baum lab is utilizing its combined genomic and transcriptomic data resources to aid in the ongoing efforts to identify and fine tune our effector candidates for our yeast two-hybrid approach in sub-objective 1.3. This approach reflects the vast amounts of data we have developed and made available for SCN omics-based research.

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

In the Mitchum lab, multiple full-length virulence gene candidates were cloned from the cDNAs of parasitic juveniles and used to generate host-induced gene silencing (HIGS) constructs or double-stranded RNA for silencing gene targets in juveniles through soaking methods. In a first set of bioassays, one HIGS construct targeting a candidate virulence gene showed promising results in reducing the fecundity of females (23-46% reduction) developing on the transgenic roots transformed with the HIGS construct for our target gene. A second set of composite soybean plants in both a resistant and susceptible soybean background were generated to test reproducibility of the results; again, we observed a reduction in fecundity of females but to a lesser extent (10-13%); additional HIGS and soaking experiments are underway to further validate reductions and evaluate silencing in nematodes developing on the transgenic roots.

In the Baum lab, we are preparing to analyze protein-protein interactions of 220 soybean cyst nematode (SCN) effector proteins in soybean using a high-throughput Cre recombinase-based yeast two-hybrid (CrY2H) system. To date, both positive and negative controls have been successfully cloned into the requisite vector (pEntry) and subsequently transferred into the destination vector via Gateway cloning. Whole plasmid sequencing has confirmed that these genes are cloned in-frame. We have transformed these constructs into CrY2H-compatible yeast strains and optimized the culture conditions, including dropout and other selective media, which are essential for detecting genuine protein-protein interactions. One of the main challenges we are addressing is the occurrence of false-positive results caused by auto-activator—proteins that independently activate reporter genes without a true interaction partner. To mitigate this issue, we are currently developing a negative selection strategy to minimize the impact of auto-activators. Following this optimization, we will proceed to screen the cloned SCN effectors against a soybean cDNA library. This will help identify effector–host protein interactions, offering insights into SCN parasitism and potential targets for enhancing soybean resistance

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

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 2025. Scaboo, Tylka, and Mitchum assisted with the review and editing of the manuscript draft in preparation for submission in the next reporting period.

In October 2024, mature soybean plants were collected by the Tylka lab from the microplots at both Iowa microplot experiments. The plants were run through a plot combine to obtain the seed, which was weighed and then saved for use in 2025. Also, two separate 10-core soil samples were collected from each microplot in both experiments at the time of harvest. One set of soil samples were used to determine SCN egg population densities and the other set to test the virulence of the SCN populations in the microplots on the HG type indicator lines. The soil samples collected to determine egg population densities were processed and counted at Iowa State University by Tylka lab personnel in March 2025. The soil samples collected to assess

virulence of the SCN populations in the microplots were sent to the University of Missouri SCN Diagnostics facility for HG type testing in November 2024, and those results are expected to be received by the end of June 2025.

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 numerous interviews with print and radio ag media personnel and several in-person and virtual online presentations to seed company personnel and independent crop consultants about the situation with SCN and resistant varieties in the Midwest. The loss of effectiveness of PI 88788 SCN resistance was discussed in every presentation as was the research being conducted with NCSRP funding in this project to develop strategic rotations to the main effectiveness of SCN resistance in the future. Mitchum and Scaboo continued discussions to develop a public-private partnership with Corteva on a project to evaluate different soybean product concepts that can be used in rotation to increase the profitability of soybean for producers and protect the future of resistant sources. This ultimately will lead to an integrated approach to enhance durability of SCN resistance for long-term, strategic SCN management resulting in a significant return on investment through increased profitability of soybean for producers, which are two of the primary goals of this NCSRP project.

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

After harvest, all collaborators sent their data on yield and agronomic data. These data along with data on protein, oil, greenhouse data on SCN resistance, egg counts and HG types on soil was compiled and analyzed at UIUC. The final electronic version of the 2024 SCN regional trials report was sent to collaborators in December of 2024, and the printed version was distributed at the Soybean Breeders' Workshop in February 2025.

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)

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. This year, we sent one high yielding line with Peking resistance, and two lines with a combination of *rhg 1-b* from 88788 and two *G. soja* genes (*cqSCN-007* and *cqSCN-006*), to commercial increases in 2025. We have more lines with these gene combinations in our pipeline, which will go for preliminary and advanced testing in 2025. In addition, with support from the Illinois Soybean Association, we are adding one more genes to each of these two gene combinations in order to enhance pathogen resistance in our soybean lines. We are now working on combining the *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. We sent F2 seed to Puerto Rico for increases, and we will have F3 and F4 populations in the field this season.