Project Final Report – November 26th, 2025

I. **Project Title:** An integrated approach to enhance durability of SCN resistance for long-term, strategic SCN management (Phase III)

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 updated SCNBase.org to include the most mature version of the TN10 genome, which includes our gene annotations, as well as the 8 additional SCN genomes. Additionally, the TN10 genome has been manually annotated, and high-quality gene models have been curated throughout the genome. To increase accessibility, all genomes, genes, transcripts, and proteins from every *H. glycines* genome were incorporated into SCNBase giving full access to all tools for each. This latest resource allowed a comprehensive analysis of SCN effectors based in expression at key life stages of the nematode. A new manuscript detailing these discoveries is being prepared for submission.

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 utilizing its combined genomic and transcriptomic data resources to aid in the ongoing efforts to identify and fine tune our effector candidates for the yeast two-hybrid approach in sub-objective 1.3. and has made progress conducting single-cell transcriptome sequencing of gland cells from different SCN life stages and populations. These sequence reads have aided the manual annotation of the latest TN10 genome and the identification of effector genes in the genome.

The Hudson group has successfully completed a comprehensive single-nematode whole-genome sequencing (WGS) study aimed at identifying the genetic basis of soybean cyst nematode (SCN) adaptation to major resistance genes, particularly *Rhg1-a* and *Rhg4* (part of Peking-type resistance). This work represents a substantial technical and biological advance for SCN genomics and has validated pooled sequencing (Pool-seq) approach to study diverse field populations. A robust protocol was developed to extract high-quality genomic DNA from individual SCN J2 juveniles, enabling construction of Illumina libraries from single nematodes. A total of 382 individuals from two long-term divergent SCN populations (MM1: virulent; MM2: avirulent) were sequenced. Reads were mapped to the latest PA3 reference genome, identifying approximately 650,000 high-quality variants across all individuals. Population genomic analyses including Fst, nucleotide diversity, XP-EHH, and Rsb, revealed highly significant genomic regions under selection (FDR < 1e-5). The fine resolution of single-nematode sequencing enabled the detection of haplotypes under selection with an average size of ~7.5 kb, generating a greatly refined list of candidate genes. Many of these genes have predicted roles in parasitism, although a substantial portion remains unannotated due to limited functional resources for plant-parasitic nematodes. The effective population size (Ne) of SCN was estimated using appropriate methods, with results converging around ~5 million, the first such estimate for a plant-parasitic nematode. This extraordinarily large Ne highlights extreme genetic hypervariability even in greenhouse-maintained SCN populations.

Complementary analyses, including approaches that bypass imputation, confirmed the major signals of selection and revealed additional haploblocks and candidate loci. We are now focused on preparing the manuscript for publication.

In parallel, the Hudson group has contributed to the generation of a complete SCN pangenome, harmonizing assemblies and annotations across multiple Hg types. A manuscript describing the pangenome and its comparative genomic features is currently under review. Updated TN10 genome resources were incorporated to ensure consistency among reference assemblies, supporting the future public release of these datasets on SCNBase. Building on insights from single-nematode sequencing and Kwon et al. (2024), a second major study was initiated using a Pool-seq approach to investigate SCN adaptation in genetically diverse field populations. A total of 72 SCN-infested soil samples were collected from two Iowa field sites (Ames and Kanawha). After cyst extraction, surface sterilization, and pooling, whole-genome shotgun sequencing was completed for 12 SCN populations, each with six biological replicates (72 pools). Preliminary quality control of raw reads has been completed, and downstream allele-frequency–based analyses have been carried out using PoPoolation and related pipelines. The results support the findings from the single-nematode dataset and clearly illustrate the impact of different resistance sources on selection pressure and nucleotide diversity. They also highlight the important role of agricultural practice, specifically rotation versus continuous soybean cultivation in shaping genomic patterns of selection in SCN. These Wormplasm populations have undergone long-term selection on diverse soybean resistance sources (PI 88788, Peking, PI 90763, wild *Glycine soja* accessions, etc.), providing a powerful system to evaluate the genomic basis of virulence evolution. This work enables comparative analyses across resistance backgrounds, identification of variants associated with complex virulence phenotypes, and a broader understanding of adaptive trajectories in SCN. The combined single-nematode WGS and Pool-seq studies provide unprecedented resolution into SCN population structure, effective population size, genomic diversity, and virulence adaptation. Together, these efforts significantly advance genomic resources for SCN and strengthen the foundation for understanding and managing virulence evolution in this major soybean pathogen.

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 was working to analyze protein-protein interactions of soybean cyst nematode (SCN) effector proteins in soybean using a high-throughput Cre recombinase-based yeast two-hybrid (CrY2H) system. For this purpose, 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 requisite 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 (AAs) -proteins that independently activate reporter genes without a true interaction partner. To mitigate this issue, we began developing a negative selection strategy to minimize the impact of auto-

activators. Specifically, we synthesized a pGAL2-URA3 fragment and are integrating it into the yeast genome. The URA3 gene encodes orotidine-5'-phosphate decarboxylase, which converts 5-fluoroorotic acid (FOA), a uracil analog, into the toxic compound 5-fluorouracil. Because URA3 expression is driven by the pGAL2 promoter activated by AAs, the addition of FOA selectively kills yeast cells containing AAs. At the end of the funding period, we so far have synthesized the required gene cassette and are seeking new funding avenues to continue this work. In addition, the Baum lab performed functional characterizations of two separate SCN effector proteins that both serve functions in inactivating plant defenses. These efforts have identified two parasitic strategies that the nematode uses to infect plants. Of particular relevance is that RNAi inactivation of one of these effectors renders nematodes almost completely unable to infect plants. The mechanistic insights into these effector functions validate them as suitable targets for anti-nematode intervention. For that effector, we performed a Y2H assay and identified seven interacting proteins in soybean. Now, those interactors are being characterized further to study their role during SCN interactions.

Building on the results reported by Kwon et al. (2024), the Mitchum group expanded the functional characterization of high-priority candidate SCN virulence genes through a combination of molecular, genomic, and expression-level analyses. Full-length coding sequences of multiple candidate genes were successfully cloned to enable downstream functional assays. We further assessed allelic variation and zygosity patterns of SCN individuals across a broader panel of independently-derived SCN inbred populations adapted on resistant soybean genotypes. These analyses revealed distinct patterns of homozygosity and heterozygosity at key loci, which appear to correlate with virulence phenotypes and may reflect population-specific evolutionary pressures. In parallel, we conducted detailed temporal and spatial expression profiling using both quantitative expression assays and in situ hybridization spanning SCN parasitic life stages, thereby determining key parameters for gene silencing experiments to validate the functional relevance of these candidate genes in virulence through assessments of their impact on nematode development on soybean roots. Together, these efforts have extended our knowledge on the genetic basis of SCN adaptation to major soybean resistance genes opening the door to molecular-based diagnostics that may enable future prescriptive SCN management approaches. Mekidani Jacob Salu, a PhD student in the Department of Plant Pathology at the University of Georgia's Center for Applied Genetic Technologies, working under the mentorship of Dr. Melissa Mitchum is a newly named 2025 Foundation for Food & Agriculture Research (FFAR) Fellow - sponsored by Corteva - Salu is advancing his PhD work on the functional characterization of candidate SCN virulence genes and the genetic basis of fitness costs associated with adaptation to resistant soybean cultivars, building on the results generated through this NCSRP project. His research aims to support the development of durable resistance strategies and inform real-time nematode diagnostics, contributing to more sustainable SCN management. You can read his story here: https://storymaps.arcgis.com/stories/b7771df22d6c4628a84d2933c856918b

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

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In the last 6 months, the Tylka lab received the results of HG type testing of the SCN populations collected from soil samples taken from 71 of the 72 microplots (35 microplots in two different experiments conducted in Iowa) in the fall of 2024. We are still awaiting results for the final HG type test. The Tylka lab also planted, maintained, and soil sampled the 36 microplots in both experiments in the 2025 growing season in hopes that funding would be secured for HG typing of the SCN populations again after the 2025 season (i.e. allowing data collection after one more year of treatment effects). Tylka solicited off-cycle funding ($8,520) from the United Soybean Board to pay for the HG type testing on the SCN populations in these samples, but the request was unsuccessful. The soil samples will be kept in cold storage for 12 more months in case funding to have the HG type testing completed somehow becomes available. After that time has passed, the samples will be offered to Dr. Matthew Hudson's lab for "wormplasm" testing and the samples will be discarded upon completion of those analyses. In October of 2025, our manuscript that describes this work and its results was accepted with minor revisions in the journal Plant Disease. We are in the process of addressing the requested revisions for re submission in December of 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 continued to conduct numerous print and radio interviews about SCN, its management, and the loss of effectiveness of the common PI 88788 SCN resistance. Whenever time (in radio interviews) or space (in interviews for print pieces) permitted, this NCSRP-funded project was mentioned.

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

In 2024, the Breeding team at UIUC coordinated and executed all the phases of the North Central Regional Trials. We worked with all the university cooperators across the region to distribute seed, and establish trials in both field and greenhouse environments. These multi-location trials captured a range of SCN populations and environmental conditions, enabling robust evaluation of soybean lines for resistance. Throughout the season, cooperators collected data on agronomic traits and yield, soil samples for all trials were sent for egg count and HG typing, and experimental lines were analyzed for SCN resistance. Following harvest and greenhouse assay completion, we centralized and curated datasets from all locations, conducted preliminary statistical analyses. Results were compiled in a 2024 SCN Regional Test Report, which was distributed across all cooperators. These activities advanced the long-term goal of strengthening regional SCN resistance evaluation and supporting the deployment of more durable resistance in North Central U.S. soybean breeding programs.

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. At the

beginning of 2025, we released two soybean varieties with different sources of SCN resistance, which went to seed increases this season (Table 1). These lines were evaluated in multi-environment trials as part of the SCN Regional Trials in 2024.

Table 1: Soybean varieties that went to commercial increases in 2025.

Line	**Commercial name**	**Traits**	**RM**
LD20-4542	Illini 1942N	Rhg1-b + qSCN-006 + qSCN-007	1.9
LD21-7458	Illini 3703Np	Rhg1-a + Rhg4 + Rhg2 (Peking)	3.7

We are currently processing the 2025 harvest data where a total of three Peking type varieties (*Rhg1-a + Rhg4 + Rhg2*) and two varieties carrying the *Rhg1-b + cqSCN-006 + cqSCN-007* gene combination were evaluated in advanced yield trials. 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 + cqSCN-006 + cqSCN-007* three-gene stack. Currently, we have 17 BC3F1 lines growing in the greenhouse carrying the *Rhg1-b + cqSCN-006 + cqSCN-007 + CHR10* four-gene stack, and 24 BC2F1 lines carrying the *Rhg1a + Rhg4 + rhg2 + GmSNAP02* four-gene stack.

Publications from our group

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Joffrey Mejias, Djampa K.L. Kozlowski, Jackson Goshon, Thomas R. Maier and Thomas J. Baum 2025. A user-friendly software to accurately count and measure cysts from the parasitic nematode *Heterodera glycines*. *Sci Rep* 15, 4468. https://doi.org/10.1038/s41598-025-88289-6

Alexandra Margets, Jessica Foster, Anil Kumar, Tom R Maier, Rick Masonbrink, Joffrey Mejias, Thomas J Baum, Roger W Innes 2024. The Soybean Cyst Nematode Effector Cysteine Protease 1 (CPR1) Targets a Mitochondrial Soybean Branched-Chain Amino Acid Aminotransferase (GmBCAT1). *MPMI* 37(11), 751-764 https://doi.org/10.1094/MPMI-06-24-0068-R

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