Project Update – April 1st, 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 April 1st, 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. We are planning our next group meeting for the spring of 2024 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)

In our latest report, the Baum lab produced improved gene annotations for the nine SCN genomes, leveraging the expanded TN10 transcriptome readily available to us. However, given the computational nature of these predictions, errors were abundant among the nine's annotations. To obtain a more comprehensive genetic overview of SCN and its genic content, we embarked on a program of manual annotation using WebApollo with the TN10 genome. Currently, we are nearing completion, with approximately 75% (85.7Mb/115Mb) of the genome manually annotated, resulting in the curation of 12,888 genes. Through manual annotation, we identified the limitations of current gene prediction software in the context of SCN gene prediction. SCN defies conventional splicing norms, with 20-30% of splice sites being non-canonical, while massive, highly similar gene families introduce challenges in RNA-seq mapping. Predictors also struggle with operons, and frequently search too far for start and stop codons in this compact genome. While about 50% of the TN10 genome's computational annotation was not necessary to modify, many erroneous transcript models were eliminated. Upon completion of the TN10 manual gene annotation, we will use these nearly perfect gene annotations to enhance the annotations of the remaining eight genomes.

In our last report, we highlighted our downstream analysis of a whole-genome single nematode sequence dataset. Following the identification of candidate genetic regions under selection for adaptation on Peking type resistant soybeans (RHG1a/RHG4), the Hudson group completed the downstream analysis. The high resolution and statistical power from single SCN J2 sequencing allowed us to pinpoint small haplotypes under selection (average ~ 8 KB), resulting in a narrower and more precise candidate gene list. We estimated the population effective size using various methods, and we are in the process of confirming it with other software – this

population size is likely at least in the hundreds of thousands, even in this greenhouse-maintained inbred population, showing the degree of diversity that is retained in SCN populations. Due to limited functional annotation in the TN10 and PA3 SCN genomes, we are now developing better functional annotation of the candidate genes using protein blast and 3-D structure similarity tools. Additionally, we are analyzing the effect of SNPs on protein structure and function. With a list of candidate genes under selection, our next step is to determine their function and role in SCN-Soybean interaction, along with assessing the impact of corresponding SNPs on their function. Analyzing and understanding structural genome variants in the population is now in progress, something that is now possible in SCN research for the first time. Our joint publication led by the Mitchum group and including the Scaboo group on poolseq analysis of the selected populations (the previous generation of population analysis) is now under revision as detailed elsewhere in this report. While the poolseq work is ground breaking and the first of its kind, the single nematode genome data will resolve the loci described in that research in far greater detail, and potentially find genes and / or markers underlying virulence in the population. The SCN pangenome has been completed, we are currently close to finalizing coherent assemblies and annotations between the different genomes to be able to launch a public resource on SCNBase. A manuscript describing the pangenome is in preparation, with extensive comparative analysis between the Hg type reference assemblies.

As previously mentioned by the Hudson group, alignment against one reference genome (for example, the TN10 genome used for Pool-Seq mapping) can lead to reference bias. In order to minimize reference bias, independently confirm our Pool-Seq results, and possibly detect more relevant and refined candidate genes, the Mitchum lab repeated the entire Pool-Seq analysis using a new set of reference genomes (PA3 and MM26). Because the two unrelated avirulent and virulent SCN population pairs used in the Pool-Seq study were derived from the progenitors PA3 and MM26 (i.e., ancestors), those two pairs are closely related to these new genomes and are, therefore, more appropriate for apples-to-apples comparison, instead of mapping to an unrelated TN10 genome. Repeating the analysis with these two new references led to the discovery of mostly identical candidate genes that were located on the same chromosomal regions, as identified from the TN10-mapped Pool-Seq study. Interestingly, we were able to find additional candidate genes which were not discoverable from mapping to the TN10 genome. The Mitchum lab has also submitted a manuscript to publish the Pool-seq study and has received positive, constructive feedback from the reviewers; we will submit a revised manuscript very soon.

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

The Baum lab has developed gland cell-specific library resources for two developmentally important time points (parasitic J2 and J3), and representing two diverse populations of soybean cyst nematode, PA3 (avirulent, Hg type 0) and MM10 (virulent, Hg type 1-7). We are successfully using these resources in our analysis of potential novel effector targets, and strategies for attacking such targets. To allow for comprehensive analysis of the key targets responsible for SCN parasitic and evasion strategies, we seek the completion of our library time points to include a pre-parasitic time point (freshly hatched J2) to investigate effector targets

being expressed prior to invasion. We are also exploring the feasibility of developing a J4 parasitic female time point to look at effector targets being expressed during late syncytial maintenance and plant immune suppression stages. This later time point may be harder to achieve, given the fragility of the gland cell at this late time point, and may possibly require hand-dissection to isolate this cell type. Successful generation of libraries from this later cell type remains uncertain.

The Mitchum lab has been 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 are 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)

As proposed by Baum Lab previously, we developed an RNA interference (RNAi) pipeline based on dsRNA soaking to characterize nematode effector genes. We have established this pipeline, detailing the optimal probe size and conditions necessary for effective gene silencing via dsRNA soaking. Recently, we successfully silenced an SCN gland-specific transcription factor as a proof of concept. Furthermore, we have expanded our efforts to successfully silence other gland-specific effector gene candidates in SCN. Our work demonstrates the robustness and versatility of our RNAi approach. To assess the RNAi effect on nematode parasitism, we have developed a penetration assay using soybean seedlings. This assay showed that after gene silencing, treated nematodes struggled to penetrate the plant roots compared to non-soaked nematodes. We observed less nematode penetration in dsRNA soaking compared to the control nematodes (no soaking), suggesting a correlation between silencing of the expression of the tested gene and successful parasitism. This assay has proven highly effective in observing the gene silencing effects with precision.

In 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 both yeast two-hybrid and host-induced gene silencing vectors. Presently, the construction of these vectors containing the candidate genes is in progress. Additionally, DNA templates for dsRNA synthesis have been prepared for RNAi by soaking to test functionality of these candidates in virulence/parasitism of SCN.

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)

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In October 2023, plants from each microplot were carefully removed and bundled then threshed to determine yield quantity. The harvested seeds were saved to use in the experiments (after checking for purity) in microplot experiments in 2024. Also in October, two different soil samples were collected from each microplot in each of the two microplot field experiments conducted in Iowa. One set of samples were processed at Iowa State University to determine the end-of-season SCN egg population density (egg number) in each microplot. The second set of samples were sent to the University of Missouri SCN Diagnostics facility for HG type testing to assess how the soybean genotypes grown in the microplots in 2023 affected or shifted the virulence of the SCN populations and how the virulence phenotypes differed from the virulence phenotype of the SCN populations used to infest the microplots initially.

Overall, SCN population densities continue to increase in the microplots, and year-to-year differences occur among plots with rotated soybean genotypes. Also, results varied somewhat between locations again. For most microplots, rotated treatments had lower SCN egg numbers than treatments planted with the same genotypes continuously. Gene pyramid rhg1-b+G. soja+Chr.10 rotated with PI 90763 (rhg1-a, Rhg4, rhg2) had the lowest SCN egg numbers in both experiments. However, this rotation caused the virulence of the SCN population to increase, as evidenced by elevated SCN female index (FI) values. In the Ames experiment, the initial SCN population used to infest the microplots had a FI of 7 on PI 90763, and the continuous PI 90763 treatment and the rotation of pyramid 2 with PI 90763 caused the FI to increase over three field seasons. The FI on PI 88788 remained well above 10 across all microplots, even in SCN populations not exposed to the rhg1-b gene, which PI 88788 possesses. Additional shifts in virulence were observed but were less substantial in comparison to those described above. HG Type test results of the SCN populations in samples collected from the 2023 microplots have not been received yet.

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 Diseases during 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)

Between October 2023 and March 2024, Greg Tylka conducted 7 interviews with radio and newspaper/magazine journalists and podcast hosts and gave 17 presentations to farmers and agribusiness groups (some in person, some online) about SCN. The widespread loss of effectiveness of PI 88788 SCN resistance and its consequences was discussed in every interview and presentation, and Objective 2 of our NCSRP-funded research project was mentioned and described whenever time/space in the presentation or interview permitted.

Mitchum had several interviews with ag media personnel for news releases and radio related to research outputs under this project including the Soybean Research and Information Network, The SCN Coalition, and Alpha Ag. These releases and interviews not only discussed the loss of

effectiveness of PI 88788 SCN resistance, but highlighted the ongoing research and new discoveries in genetic resistance to address this situation.

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

Between December and January, we completed the analysis for the 2023 tests. Final data tables were sent to cooperators and the report book was formatted and printed. In February, cooperators submitted the lists of soybean lines that they would like entered in the 2024 SCN Regional tests. Cooperators meet at Soybean Breeders Workshop to discuss the SCN Regional Tests and to finalize the 2024 entry lists. The final entry lists were sent to cooperators in March, and seed was repackaged at the University of Illinois according to test and amount needed for each cooperator. During late March we will be shipping the seed to the cooperators.

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*.

After analyzing the data from harvest, in 2024 we will be sending promising high yielding lines containing combinations of three SCN resistant genes to Uniform and SCN Regional trials. The lines we selected are 13 in total, seven carrying the *rhg1-a*, *rhg2*, and *Rhg4* combination, and six lines containing *Rhg1-b* from 88788 with other two *G. soja* genes (qSCN-006 and qSCN-007). In addition, we have more lines in our pipeline with these two different gene combinations. In 2024 we will be testing a total of 25 lines with both combinations in advanced trials, and 152 In preliminary trials. We will also be genotyping our plant rows and populations for these gene combinations. We are currently preparing for the 2024 planting.