Progress Report April 2026

- Project Title: **Cyst Nematode Single-Cell Omics**
- Lead PI: Thomas Baum

This project set out to decipher single cell gene expression specifics in the soybean feeding cells (the syncytium) of the soybean cyst nematode (SCN; Objective 1) and in the SCN gland cells that produce the disease-inducing nematode secretions(so-called effectors) (Objective 2). The Baum lab continued to make great progress towards both objectives in Year 2 of our project.

Objective 1) Perform transcriptomic analyses in soybean cells after SCN infection

As reported earlier, for this objective, we joined forces with scientists Mark Libault at the University of Missouri, Khalid Meksem at Southern Illinois University and Tarek Hewezi at the University of Tennessee and collaboratively made solid progress analyzing gene expression specifics in single cells from different SCN-infected soybean cultivars. The continued generation of sequence data and their analyses continues to proceed on track and is allowing first intriguing insights. We have analyzed the data collected in Year 1 and have established preliminary identification of cell type clusters of infected soybean root tissues from these data. We are just now beginning to explore these clusters to identify cell types that may consist of the SCN feeding cells, i.e., the early developing syncytial cells we are targeting to discover. An additional successful and surprising outcome from this exploration of the single cell sequence data generated from Objective 1 is that we are able to detect large quantities of nematode cells from this same experiment. Therefore, we can assemble nematode cell types into their own clusters. This was unexpected, given the scope of this experiment, but could be impactful enough to change our approach to Objective 2. In short, we were able to identify sufficient gland cells in these assays and are studying the gland cell gene expression now.

Objective 2) Perform transcriptomic and genomic analyses in the SCN gland cells that produce so-called effectors.

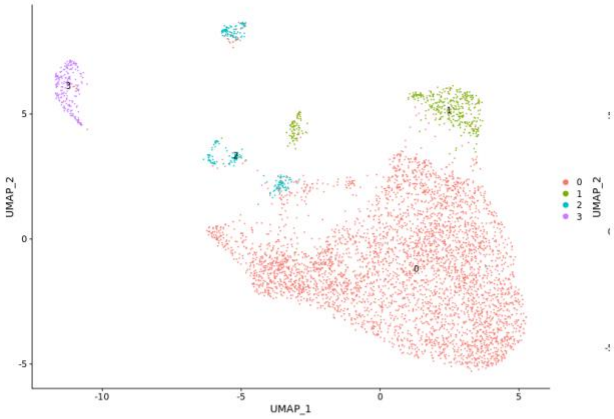
In pilot experiments, utilizing SCN infective juveniles, we have generated next-generation sequencing libraries for the two gland cell nuclei types, the dorsal and the subventral gland cells. We successfully sequenced those libraries to verify identity using gene tags that are unique to those two gland cell types. We have demonstrated that we can, in fact, sort our transcripts by gland cell type and identify deep sequence pools for each gland cell type (Figure 1). Next steps will be to refine these cell/nuclei type assemblies and explore how complete these assemblies are with respect to all known SCN effectors for a given life

stage. With this success in hand, we are now preparing to apply this novel technique to additional life stages of SCN, involving a more extensive experimental setup. While labor intensive, this will clearly define the expression of the nematode's effector repertoire and the related regulatory genes across the SCN life cycle.

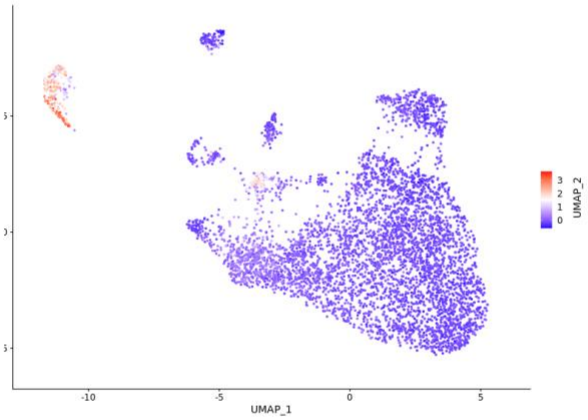
All in all, the proposed work is proceeding very nicely and is on track to be successful. We continue to be excited about our discoveries and are looking forward to sharing additional insights.

Figure 1. Cluster analysis of sorted SCN gland nuclei

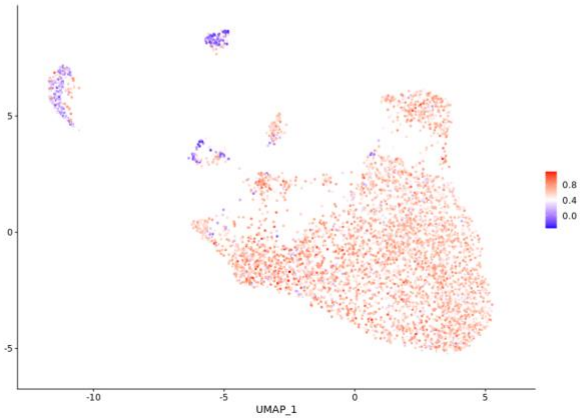
A. SCN Nuclei Clusters



B. Subventral Gland Marker Gene Expression



C. Dorsal Gland Marker Gene Expression



Four clusters of nuclei type were formed (Panel A) via UMAP analysis when assembling the SCN single nuclei sequencing data, of which cluster 3 associated with known subventral gland gene markers (Panel B) and cluster 0 associated with known dorsal gland gene markers (Panel C). In Panels B and C, red is denoted as higher expression.