I. **Project Title:** Translating Basic Research Knowledge into Soybean Resistance to the Soybean Cyst Nematode.

II. Principle Investigator: ***Thomas J. Baum;*** Iowa State University***;*** 1344 ATRB***;*** 2213 Pammel Dr.***;*** Ames, IA 50011

Phone: 515-294-5420 ***Email:*** tbaum@iastate.edu
Affiliation: Department of Plant Pathology, Entomology and Microbiology

III. Brief Description of Accomplishments as of May, 2025:

This project seeks to express in plants a multipartite cell toxin kill gene under the control of separate nematode-responsive promoters with the goal to render these plans resistant to nematode infection. In order to activate the expression of the kill gene under the control of two or three promoters specifically in the syncytium, one of the required strategies is to split gene sequences in two inactive components and then use available technologies to reconstitute the gene function in cells where both halves are present. The project, thus, is progressing along the two objectives of (1) Testing soybean promoters for nematode responsiveness and (2) Reconstituting the cell toxin in planta.

Objective 1) Test promoters and verify their reported specificities

From the published data from other labs, we are testing various syncytium specific promoters via promoter-GUS expression in transgenic tobacco and soybean hairy roots and subsequent soybean cyst nematode infection to select our potential candidates. In the last report, we reported that we have finished testing two published promoters in tobacco and soybean hairy roots. Testing of more such promoters is underway. We need these promoters later on to drive the expression of kill gene halves.

Objective 2) Reconstitution of inactive halves of the kill gene

We have finalized the delineation of gene fragments (protein halves) of one candidate kill gene. Currently we have ten gene fragments cloned into a vector, driven by a dexamethasone-inducible promoter. These gene fragment constructs have been confirmed through whole plasmid sequencing.

These constructs have been transformed into *Agrobacterium tumefaciens* for testing in tobacco plants. Next, we will assess whether these gene fragments are forming proteins using western blotting and then will determine if these fragments do no longer elicit any hypersensitive response (HR) defense. Following this, we will test the same constructs also in soybean. We are also creating additional gene cassettes for testing. So far, we have optimized the Agroinfiltration

protocol in tobacco with the full-length kill gene to induce HR (Figure 1) and are ready to test kill gene fragments for their ability to elicit HR.

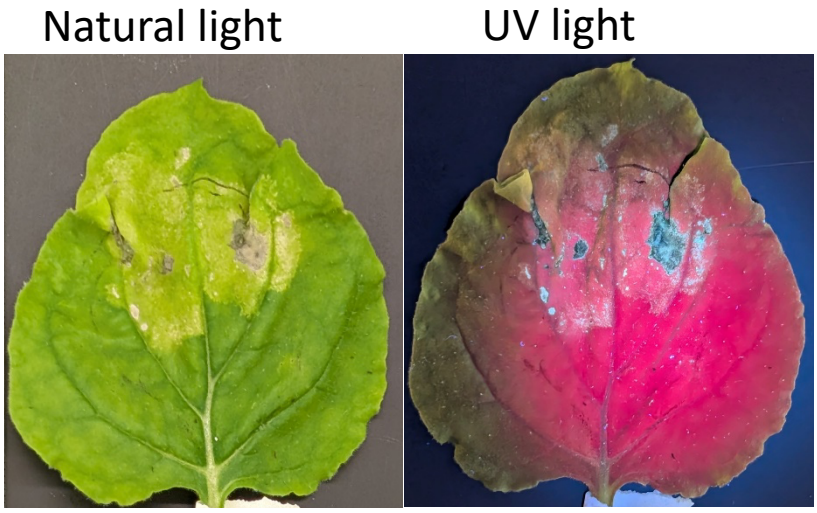

Figure 1. Full-length kill gene showing a hypersensitive response (HR) defense in tobacco leaf tissue. HR is shown as lesions in the leaf. The full construct was transiently expressed in *Nicotiana benthamiana* using dexamethasone-inducible promoters. We are now ready to test kill gene fragments in this HR assay.