Progress Report – April 30, 2024

Objective 1: Establish baseline knowledge of microbial communities associated with SCN cysts in NY state

In conjunction with the Fall 2023 SCN survey, we assisted in collecting samples and processed soil samples from 33 fields across 10 counties in NY (Table 1). The results detected SCN cysts in only 7 fields in three counties (Tioga, Montgomery, and Herkimer), with only three fields, two in Tioga County and one in Montgomery County, showing substantial egg counts above 1000. Because only a handful of cysts were isolated, these were used to reproduce the nematode on plants in order to perform HG typing for the SCN survey to identify what strains of the nematode are present in NY state. These results suggest SCN is still in the early stages of spread in NY but has spread and reached more northern counties (Montgomery, Herkimer) in the middle of the state. This does provide an opportunity to establish a baseline of native microbial communities present in soils in grower fields in NY state before high levels of SCN develop. From each field, a subset of approximately 250g soil was sent for soil nutrient/health analysis (pH, nutrients, organic matter) at Cornell and a subsample of 10g was frozen in tubes or in glycerol solution (for culturing) for analysis of soil microbiome.

The last (April 30, 2024) progress report described the results from sequencing of DNA isolated from the soil samples to analyze fungal and bacterial communities. We identified a set of core bacterial and fungal taxa found across a majority of soil samples in NY state and also found a trend showing differences in the microbial communities between soil in SCN infested versus uninfested fields. While these differences could also be due to soil parameters, the SCN infested fields were from seven samples from several different counties in NY state clustered together rather than with other uninfested samples from the same county, suggesting that the presence of SCN may be causing this change in microbial communities. This past quarter, we have focused on isolating fungi and bacteria from soil samples from these seven fields. We have isolated and frozen stocks of ~200 fungi and ~200 bacterial cultures. Work on isolating DNA and sequencing ITS barcode region for taxonomic identification is underway and we have identified ~50 to genus and species and hope to complete identification by early fall. This will enable us to identify cultures of bacteria or fungi that are either known as nematode parasitic taxa or were found only in the SCN soils. These will be further tested in Objective 2.

Objective 2: Screen fungi for antagonism of the soybean cyst nematode

1.2 Screen fungi for production of nematicidal and hatch inhibitory metabolites

We have begun screening filtrates from fungi isolated from soil using J2 toxicity assays. We have screened raw filtrates from approximately 50 fungal isolates to date. Five isolates have shown significantly greater mortality of J2 exposed to the raw fungal filtrates of a fermentation broth compared to the media (1/4 Czapek-Dox broth) control (Figure 1). In the previous reporting period, one of these isolates whose raw filtrate showed high toxicity to J2 in bioassays was grown up in rice media for one month. Raw extraction using a 1) water (RW), 2) a relatively polar solvent butanol (RB), and two nonpolar solvents, ethyl acetate (RE) and hexane (RH), as

well as a distilled water control, were then tested in toxicity bioassays with J2 worms. Results showed that the ethyl acetate fraction (RE) had significantly higher mortality than the water extract. We are in the process of continuing to test fractions of this isolate as well as growing up these other five isolates that showed activity in rice media for bioactivity guided fractionation.

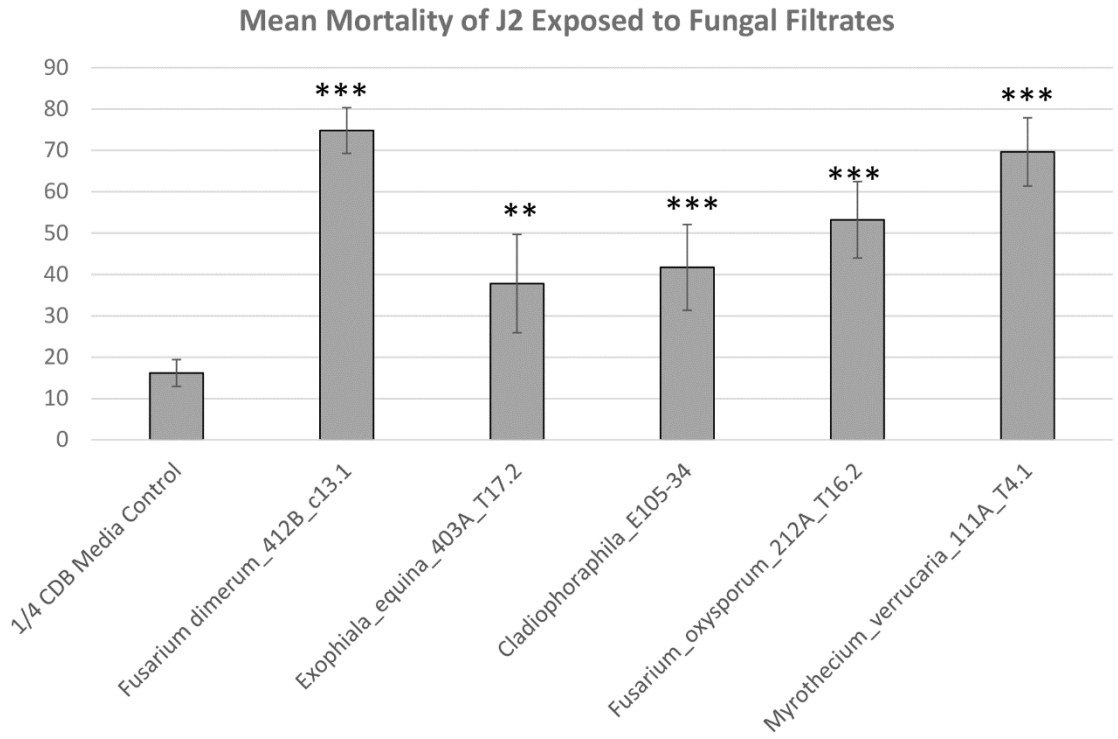

Figure 1. Mean and standard deviation of mortality at 72 hours of exposure of SCN J2 to raw filtrates (media broth) of cultures grown in ¼ strength Czapek-Dox broth for two weeks.

Table 1. Soil sampling for SCN survey (Fall 2023) showing 33 fields sampled in 10 counties across NY.

Sample number	County	# Cysts	Viable Eggs Counts
42H	Chautauqua		
42L	Chautauqua		
43	Chautauqua		
44	Chautauqua		
7	Chautauqua		
15	Cortland		
16	Cortland		
17	Cortland		
18	Cortland		
19	Cortland		
10	Cortland		
11	Cortland		
45	Erie		
46	Erie		
38	Herkimer		
D	Herkimer	2	44
33	Madison		
28	Montgomery	19	2080
30	Montgomery		
40	Montgomery		
41	Montgomery		
B	Montgomery	5	210
A	Montgomery		
35	Otsego		
37	Otsego		
C	Saratoga		
47	Tioga	20	1400
48	Tioga	4	91
49	Tioga	5	51
50	Tioga	87	2100
12	Tompkins		
14	Tompkins		
20	Tompkins		