**Objectives:**

MROP Project Report 05/28/2019

**Objective 1: Test candidate egg-parasites in greenhouse trials for ability to reduce nematode densities and improve plant growth and yield**

1. **Test efficacy and survival of candidate SCN egg parasite biocontrol strains in greenhouse assays**

A greenhouse experiment using the six best performing isolates (three toxin-producing and three parasitic) fungal strains from cysts that were used in previous greenhouse study was conducted using two different levels of spore-inoculation: 1) Low = 3000 SCN eggs and 10^5 spores/cone and 2) High = 10000 SCN eggs and 10^6 spores /cone.  These plants were also growth for 60 days to allow two generations of the SCN.  Several controls were used, including a no fungus control, a commercial biological control (Poncho/VoTIVo) and a commercial chemical control (ILeVo). The female index (FI) or number of cysts formed and egg-density (#eggs/100cc soil) were recorded. Plant health parameters such as plant height, pod production, and live/dead status were also measured.

**1.2 Optimize formulation and delivery methods**
During the past reporting period, we reported results on two methods, ~1% hyphal inoculum mixed directly into soil, and a spore suspension of 1 x 106 spores was applied directly to soil surrounding the roots approximately 1 week after emergence.  The repeat experiment used spore delivery, as this method will be more cost-effective and easily implemented in soybean production systems in Minnesota, and confirmed that this is an effective method of application.

**Objective 2: Test root endophytes antagonistic to *F. virguliforme* SDS pathogen alone and in combination with the SCN in greenhouse assays**

A greenhouse assay of 10 endophytic fungi that were antagonistic to the SDS pathogen were tested in a greenhouse assay.  Soil was amended with spores of SDS and endophytic fungi were inoculated soaking seeds overnight in a solution of ~1x106 spores/mL to promote early colonization of roots by the fungus.  Five replicates each for both noninoculated and inoculated treatment were used.

**Objective 3:  Identify biologically derived nematicides and anti-fungal compounds**
Ten strains with high toxicity or inhibition of egg-hatch were selected and scaled up for batch fermentations for chemical analysis and filtrate was extracted using both a polar extraction (methanol) and a nonpolar extraction (ethyl-acetate) solvent.  The resulting fractions were re-tested for activity.  We have begun further chemical characterization of the active fractions in collaboration with Dr. Christine Salomon.

**Outreach Activities for this Period:**
Future Farmers of America April, 2019 – Gave presentation on SCN and potential biocontrol fungi to Future Farmers of America students visiting UM campus.

**Achievements:**

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During this reporting period, we repeated full-pot greenhouse bioassays for 6 of the best performing isolates in the 60-day trial at two different nematode and fungal inoculum levels (Low and High).  We completed an initial experiment to test endophyte isolates for antagonism to the SDS pathogen and identified optimal methods for infection with the SDS pathogen.  We continued chemical analysis of the top 10 isolates that showed toxicity to the SCN and fractionated and tested different fractions for bioactivity for further analysis.

**Objective 1: Test candidate egg-parasites in greenhouse trials for ability to reduce nematode densities and improve plant growth and yield**

1. **Test efficacy and survival of candidate SCN egg parasite biocontrol strains in greenhouse assays**

Results from repeat greenhouse experiment showed that in nematode conducive soils under Low inoculation levels, nearly all the isolates significantly reduced the number of cysts formed by almost a half compared to no-fungus control (Figure 1;blue stars), while under High inoculation level, only three isolates were significantly better than no-fungus control (Figure 1; T, N, F; purple stars). These results confirmed the previous trial results showing that these three isolates are among the best performing and demonstrate that results are reproducible. Two of these isolates (N and T) also showed growth promoting properties and one (T) promoted earlier flowering and pod development.

**1.2 Optimize formulation and delivery methods**

The greenhouse experiments for egg-parasites used spore delivery, as this method will be more cost-effective and easily implemented in soybean production systems in Minnesota, and confirmed that this is an effective method of application.

**Objective 2: Test root endophytes antagonistic to *F. virguliforme* SDS pathogen alone and in combination with the SCN in greenhouse assays**

During the first greenhous trial of SDS infection, we unfortunately did not have sufficient germination rates to statistically assess results of different endophyte strains, but had symptoms of SDS develop on some plants and have developed an effective SDS inoculation method. We have set up another trail with the endophyte *Pochonia chlamydosporia* to test delivery methods of both the endophyte via spore soaking, amending to soil as either hyphal inoculum or spores, and spore dip of the emerging radical and application of liquid spores to the area around the roots one week after emergence. For this experiment, we will quantify the amount of *P. chlamydosporia* able to colonize the roots using qPCR to assess which method best promotes endophyte colonization before running a trial on all isolates.

**Objective 3:  Identify biologically derived nematicides and anti-fungal compounds**
Ten strains with high toxicity or inhibition of egg-hatch were selected and scaled up for batch fermentations for chemical analysis and filtrate was extracted using both a polar extraction (methanol) and a nonpolar extraction (ethyl-acetate) solvent.  The resulting fractions were re-tested for activity.  We have begun further chemical characterization of the active fractions in collaboration with Dr. Christine Salomon.

**Challenges:**

We had some issues with our first greenhouse trial of the SDS pathogen, which we had not previously worked with.  We have hopefully worked out these difficulties and will repeat the experiment this summer.