Progress on grant over entire reporting period. Progress during last period (Q4) specified, but we experienced some challenges due to covid19 - below.

**Objective 1: Identify biologically derived nematicides and anti-fungal compounds**
**1.2 Bioactivity guided fractionation to isolate active compounds**
Based on initial chemical screening of the top ten isolates exhibiting egg-hatch bioactivity, we selected one isolate (E417\_70, isolate D, *Ilyonectria*), which had among the highest egg-inhibition activity and also performed well *in planta* in cone-tainer assays for bioactivity guided fractionation to obtain a purified compound. In order to have sufficient material for nuclear magnetic resonance imaging after fractionation, production of filtrate for this fungus was scaled up to produce approximately 5L. The filtrate was run through a C18 HPLC column and partitioned into five different fractions, which were re-tested against nematodes. The first two fractions (F1 = 100% water and F2=75% water; 25% methanol) were identified to have activity (Figure 1). Each of these fractions (F1 and F2) was then run through the HPLC column and fractionated into 14 and 17 fractions respectively, which were retested for egg-hatch activity. The 13th fraction (F1) and the 15th fraction (F2), respectively, were found to retain activity (Figure 1).

***Quarter4:*** These were further fractionated in 50 fractions that are currently being tested for bioactivity. If analysis of HPLC peaks shows single peaks in the active fractions, we will move forward with structure determination using nuclear magnetic resonance. We also scaled up production of another isolate of *Ilyonectria* (A216\_25) show raw extract was previously analyzed by HPLC and compared to both the other isolate of *Ilyonectria*(Isolate D; E417.17) and the chemical profiles of a previously studied species (*Ilyonectria radicicola*). This clearly identified two peaks of the known nematicidal compounds radicicol in I. radicicola and a peaks of similar size in both other isolates of Ilyonectria, but the peak in strain A216\_25 has a slightly different profile and may represent a related but novel compound. Thus, we also plan to perform bioactivity guided fractionation on this strain to identify the active compound and to continue this work over summer 2020.

**Objective 2: Test combinations of effective biological and chemical agents for dual activity against SCN and SDS**

**2.1 Test combinations of high-performing biological agents:**
**Activity against SCN:** From cone-tainer assays, we identified three isolates (D, E, and T) that performed well and robustly at both low (3,000 eggs/100cc) and high (10,000 eggs/100cc soil) egg-densitites. We next performed greenhouse pot experiments, growing the soybean for 65 days to assess effects on egg-density over multiple nematode generations as well as to evaluate effects on plant growth and pod formation (yield) compared to a no-fungus control and the fungal biocontrol agent (MeloCon® WG). MeloCon® WG is a product formulated with spores of the fungus *Purpureocillium lilacinum*applied to soil as either chemigation or as a drench or by drip irrigation and is approved for use against nematodes in various vegetable crops and vineyards. Results from our first greenhouse experiment showed that two isolates (E and T), as well as the mixture, performed as well as applied at 40x the application rate of our isolates (Figure 2A). In this first experiment nematode egg-densities were below 25,000 egg/root biomass (grams). All of the fungal treatments performed significantly better than the no-fungus control, reducing nematode densities by nearly one half (Figure 2A).

**Activity against SDS:**We screened 8 isolated in a cone-tainer assays against SDS and identified two isolates that substantially increased plant survival and biomass (Figure 3).

***Quarter 4:*** We harvested a repeat trial of this experiment that resulted in higher nematode densities (>50,000 egg/root biomass (g). This trial was conducted during winter months (Dec – March 2019) with greater levels of artificial lighting and plants may have been under some stress, potentially contributing to higher observed SCN levels. In this trial, we also included several other commercial products as controls, including the bacterial biological control product Poncho VoTiVO®, and the fungal fermentation product DiTera®. All our isolates and the mixture of all three isolates performed better than the bacterial biological control product Poncho VoTiVO and comparable to the fungal fermentation product DiTera (Figure 2B). Only isolate E at low inoculation level (1 x 105), however, performed as well as MeloCon® WG under these higher levels of SCN. One consideration is that the MeloCon® WG product was applied at the recommended dosage, which is 400 times (4.1 x107) the concentration that isolate E was applied (1 x 105). These results could suggest stronger efficacy and translate into production and cost savings for both product producers and farmers. Future experiments will test them at an equivalent dosage.

**Objective 3: Optimize formulation and delivery methods:**

**3.1 Test seed coating treatments and amended spore formulations:**

As part of objective 2, we used two methods of application in greenhouse assays. Spores were applied both as a spore drench one week after seeding and as a lyophilized (freeze-dried) dry spore formulation combined with a carrier (powered dry milk). The formulation is similar to that used for the MeloCon® WG product and can be either wetted and applied as a drench or mixed directly into soil at planting. We applied it as a soil drench approximately 1 week after seedling emergence. We also tested the lyophilized spore formulation for germination rate and spores within the formulation were found to have equivalent germination rates. The formulated isolate T performed nearly as well as the simple spore drench in the greenhouse assay (Figure 2B) and likely has longer stability given that the spores are lyophilized. We are currently testing viability of the spores after different time periods to determine effective shelf life, but results are promising and indicate that fungal spores can be lyophilized to preserve shelf-life and retain efficacy.

**Achievements:**

During this final project period, we completed analysis of all plant bioassays with fungi against the SCN and prepared a publication on these results.

**Challenges:**

***Note on progress during Covid-19:*** During the last reporting period, we were able to harvest and analyze our last greenhouse experiment. Unfortunately, covid-19 stopped most experimental work on the project starting in mid-March. We were able to finish some chemical fractionations and to grow up some additional fungi for chemical analysis, but work in the laboratory and greenhouse has been very limited. We have primarily been working on analysis and writing of a manuscript on the plant testing of isolates in both the cone-tainer and greenhouse assays which was recently submitted to Phytopathology. Graduate student Deepak Haarith also completed his thesis on fungal biocontrol of SCN, which includes some of this work.We also met with the Office of Technology transfer at UM for an initial assessment of patentability and to assess future experiments that might be needed for patenting. We discussed repeating the greenhouse assay as equal concentrations of our isolates and MeloCon WG to determine if it does actually have better efficacy. We are not beginning to return to work in the laboratory. A small amount of additional supplemental funding (~5 to 10K) would help us to repeat this experiment and to continue chemical analysis to isolate active compound in the *Ilyonectria* isolates.