DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR DETECTION AND QUANTIFICATION OF *RHIZOCTONIA SOLANI* IN SOIL

Project Description (Continuing): This research seeks to develop a method for detection and quantification of DNA of *Rhizoctonia solani* in soil and evaluate its potential value for predicting inoculum (disease) potential in growers' fields.

Project Leader: Ashok K. Chanda, University of Minnesota, NWROC, Crookston

Other Personnel Involved: Jason Brantner and other personnel at NWROC and sugarbeet cooperative agriculturists for help in identifying fields for sampling

Project Location: University of Minnesota, NWROC and growers' fields for sampling

Objectives: To develop a real-time PCR assay for detection and quantification of *R. solani* AG 2-2 directly from soil samples for use in predicting inoculum potential

Materials and Methods: Preliminary experiments will focus on establishing a procedure to successfully extract DNA from soil and plant tissue samples that can be used in real-time PCR. Procedures have been published (see literature search above) but PCR inhibitors can be a problem in different soils and plant tissues. In follow up experiments, natural field soil will be infested with various inoculum levels of *R. solani*. Samples will be taken from each infested soil and used for DNA extraction and real-time PCR. Results from real-time PCR will be correlated with soil assays using sugarbeet seedlings to assess inoculum potential, similar to the Aphanomyces soil index. Twenty-five seed will be sown per pot, pots will be incubated under conditions favorable for *Rhizoctonia* (high soil moisture, 75-77 °F), and dying seedlings will be counted and assayed in the laboratory for verification of *R. solani* infection.

Soil samples will be collected from growers' fields (8 fields in ACSC; 4 in MDFC; 4 in SMBSC) with a history of RCRR (fields will be identified with the help of agriculturists from each cooperative) that will be planted with sugarbeets in 2018. Real-time PCR-based quantification levels of *Rhizoctonia solani* will be correlated with actual disease levels observed in the fields at the end of the growing season. Soil assays using sugar beet seedlings will also be conducted as described above and the soil index value will be correlated with real-time PCR-based quantification levels to assess inoculum potential.

Time Line of Anticipated Accomplishments:

2018
July: Collect soil samples from growers' fields (by this time Rhizoctonia should be visible on plants)
August: Perform soil DNA extractions
September-October: Real-time PCR and assess Rhizoctonia crown and root rot incidence and severity
November-December: Analyze data and write Sugarbeet Research & Extension Reports
November 2018-January 2019: Conduct growth chamber assays for Rhizoctonia soil indexing

Progress Toward Objectives of On-going Projects:

2017: We located 16 fields with active Rhizoctonia root rot based on the information from agriculturists from ACSC (8 fields), MDFC (4 fields), and SMBSC (4 fields). From each field, 5 soil cores were taken at a depth of 6 inches representing approximately 1 acre area. Each soil core was divided in to 0-2 inch, 2-4 inch and 4-6 inch sub-samples. In each field at least 1 or 2 soil cores were taken from where beets were apparently healthy. In total, we collected 240 soil samples from all 16 fields (16 x 15). Currently we are isolating DNA from these soil samples. At each sampling point (16 fields x 5 sites per field = 80) where we

collected soil cores, we also collected approximately 1 gallon of soil to determine Rhizoctonia root rot index (RRI) values using a growth chamber assay. In 2017, we waited to collect soil samples until we saw some Rhizoctonia disease in the fields and we are hopeful that this year's data will be more useful for this project to develop a predictive soil assay.

- We rated 10 sugarbeet roots for root rot severity at the time of soil sampling. The mean root rot ratings ranged from 0 to 7.0 and root rot incidence values ranged from 0 to 100 %.
- Currently, we are working on DNA isolations and real-time PCR assays to measure the DNA levels of *R. solani* present in these soil samples. DNA levels will be correlated with RRI, root rot rating, and root rot incidence values. If we find a significant positive correlation of DNA levels of Rhizoctonia with root rot and incidence values, we can use this real-time PCR test as a reliable predictor for Rhizoctonia inoculum present in the soil.

2016: We located 16 fields with a history of Rhizoctonia root rot based on the best knowledge of the agriculturists from ACSC (8 fields), MDFC (4 fields), and SMBSC (4 fields). From each field, 5 soil cores were taken at a depth of 6 inches representing approximately 1 acre area. Each soil core was divided in to 0-2 inch, 2-4 inch and 4-6 inch sub-samples. In total, we collected 240 soil samples from all 16 fields (16 x 15). Total soil DNA was isolated from all 240 samples. At each sampling point (16 fields x 5 sites per field = 80) where we collected soil cores, we also collected approximately 1 gallon of soil to determine Rhizoctonia root rot index (RRI) values using a growth chamber assay.

- We rated 10 sugarbeet roots for root rot severity toward the end of season. The mean root rot ratings ranged from 1.12 to 3.72 and root rot incidence values ranged from 18 to 68 %.
- DNA isolation and Rhizoctonia quantification from all 240 samples using real-time PCR has been completed.
- We found significant correlation at the field level between RRI and root rot ratings ($r = 0.59$ and $r^2 = 0.34$), and RRI and root rot incidence values ($r = 0.56$ and $r^2 = 0.32$).
- The lowest Ct value of 27.02 (highest Rhizoctonia DNA) was found in one field in MDFC area (MD4).
- There was significant correlation ($r= 0.24$; $r^2= 0.06$) between RRI and DNA of *R. solani*, and root rot rating and DNA of *R. solani* ($r = 0.31$; $r^2= 0.11$).

Budget Requested: $35,868

LABOR: SALARIES (19,800.00) AND FRINGE (5,997.00)
EQUIPMENT (OVER $250.00): NONE
SUPPLIES: 8,470.00 (DNA ISOLATION KITS, PCR REAGENTS AND SUPPLIES ETC.)
TRAVEL: 601.00
LEASES: NONE
OTHER: 1,000.00

TOTAL: $35,868.00