PROJECT PROPOSAL
SUGARBEET RESEARCH AND EDUCATION BOARD OF MINNESOTA AND NORTH DAKOTA
**FY 2018 – 2019**

**Project Title:**
DOES *RHIZOCTONIA SOLANI* INOCULUM DENSITY INFLUENCE EFFECTIVENES OF RESISTANCE AT THE SEEDLING STAGE

**Project Number:** New Project

**Project Leader:**
Dr. Kimberly M. Webb, Research Plant Pathologist
USDA-ARS, PA Soil Management and Sugar Beet Research Unit,
Crops Research Laboratory
1701 Centre Ave.
Fort Collins, CO 80526
Phone: (970) 492-7141
Email: kimberly.webb@ars.usda.gov

**Other Personnel Involved:**
Dr. Kirk Broders, Assistant Professor
Dept. Bioagricultural Sciences and Pest Management, Colorado State University
Fort Collins, CO 80526
Phone: (970) 491-0850
Email: Kirk.Broders@colostate.edu

Dr. James Woodhall, Assistant Professor
Dept. Entomology, Plant Pathology, and Nematology, Univ. of Idaho
Parma Research and Extension Center
Parma, ID 83660
Phone: (208) 722-6701
Email: jwoodhall@uidaho.edu

**Project Location:** USDA-ARS, Crops Research Laboratory, Fort Collins, CO

**Justification for Research:** (*For new projects only)*

The amount of *Rhizoctonia solani* in the soil and how much is needed to cause disease in sugar beet is relatively unknown. This is mostly because of the usually low inoculum densities natually found in soil and the difficulty of quantifying *R. solani* from the soil. However, characterizing the inoculum density of *R. solani* in the soil necessary to cause Rhizoctonia diseases, or to break down resistant varieties, will help researchers and growers make better pest management decisions when producing sugar beet.

**Summary of Literature Review:** (*For new projects only)*

In sugar beet, *Rhizoctonia solani* Kühn not only causes Rhizoctonia crown and root rot of mature roots (Panella and Lewellen 2005) but can also cause damping-off in germinating seedlings (Herr 1996). *R. solani* is endemic in growing areas across the United States and is an increasing problem world-wide. While plant breeding for Rhizoctonia disease resistance provides the most effective control to date, resistant germplasm provides protection primarily to mature beets only (Ruppel and Hecker 1994) and most of this germplasm is not resistant at the seedling stage (Panella and Lewellen 2005; Panella and Ruppel 1996). Only recently has a germplasm resistant to Rhizoctonia seedling damping-off been reported (Nagendran et al. 2009).

*R. solani* is a ubiquitous soilborne fungal pathogen and considered to be a species complex that contains related but genetically distinct sub-specific groups based on hyphal anastomosis reactions and pathogenicity to particular plant species. On sugar beet, *R. solani* AG 2-2 (both interspecific groups IIIB and IV) are most commonly associated with causing Rhizoctonia crown and root rot whereas *R. solani* AG-4 primarily causes Rhizoctonia seedling damping off (Hanson and McGrath 2011; Herr 1996, O'Sullivan and Kavanagh 1991). However, *R. solani* AG 2-2 has been reported to be increasingly important in causing Rhizoctonia seedling damping off as well as AG 4 (Hanson and McGrath 2011). The relative amount of *R. solani* in the soil and how much is needed to cause disease in sugar beet is relatively unknown (Carol Windels; Frank Martin; personal communication). This is partially due to typically low inoculum densities of *R. solani* natually found in soil, and that tools are generally unable to detect such low levels of the pathogen (Weinhold 1977; Paulitz and Schroder 2005). Artificial inoculation of sugar beet is a common practice to elicit Rhizoctonia crown and root-rot for screening of breeding materials and germplasm for disease resistance (Pierson and Gaskill, 1961; Ruppel et al. 1979). However, most of these studies have not characterized what natural infection rates are necessary for creating Rhizoctonia epidemics in the field. Boosalis and Scahren (1959) have reported that they were able to recover 18X as much plant debri, that was infected with *R. solani,* from soil where Rhizoctonia disease(s) occured as compared to soils that had low incidence of disease. And Naiki and Ui (1975) reported that highest numbers of *R. solani* sclerotia can be found in soils closer to diseased beets than at increasing distances away from infected beets; and that healthy beets had the lowest numbers of sclerotia associated with them. However, neither of these studies tested what particular infection levels of *R. solani,* were required for Rhizoctonia crown and root rot development. Likewise, it has been shown that different types of inoculum preparations (i.e. sclerotia, artificial inoculum using colonized cereal grains, living mycelial fragments etc.) could influence the amount of *Rhizoctonia* diseases that can occur in soils (Chet and Baker 1980). One important factor in disease development may be the inluence of the infective propagule size of the *R. solani* inoculum and corresponding number of infective particles on the rate and/or severity of disease development. For example, Wijetunga and Baker (1979) previously showed that less disease occurs when small propagules of mycelial fragments (<250mm) were used as an inoculum source compared to large fragments (>250mm).

Therefore we propose assays that will add *R. solani* at known inoculum densities to greenhouse soil samples (using an artificial barley inoculum) and to correlate this with the infective rate of *R. solani* required to elicit Rhizoctonia seedling damping off and Rhizoctonia crown and root rot in sugar beet.

**Objectives:**

**Objective 1:** Characterize infection rates of *R. solani* that is necessary to elicit Rhizoctonia seedling damping off and (potentially) breakdown resistance in the soil.

**Materials and Methods:** *(Briefly describe)*

**Propagule colonization with *Rhizoctonia solani***

For inoculum preparation, hydrated hulless barley grain is prepared by soaking barley with distilled water over night in mushroom bags, then autoclaved for 1h at 121°C. The autoclaved barley grains are allowed to cool for 24h and then inoculated with a prepared liquid culture of *R. solani.* To prepare liquid inoculum, agar plugs (7 mm diameter) from each *R. solani* isolate are placed into 200mL potato dextrose broth (PDB) and shaken at 25°C for ~5-7 days. Liquid *R. solani* inoculum is then poured over the prepared hulless barley and incubated for 14-21 days at 28°C. Infested barley is then removed from mushroom bags and dried for 5 to 7 days at room temperature, and finally ground using a Wiley Mill, making sure to completely sterilize the grinder with 70% ethanol between each treatment being prepared. A negative (un-inoculated) control will be prepared by autoclaving the hydrated hulless barley, inoculating with PDB and then drying and grinding as described above. Once we have established enough artificial inoculum for each treatment this preparation will be used to make ratios of infected *R. solani* to sterilized soil by adding infected barley particles at rates to maintain a range of infection at different inoculum densities.

**Soil inoculation and sugar beet pathogenicity assays to determine infective rate of *R. solani*.**

Pasteurized potting soil (Farfard #2-SV, American Clay Works) will be prepared as described by Hanson and Hill (2004). Artificial *R. solani* inoculum will be prepared as described above and the number of infective particles (infection rate) of the inoculum will be tested using a serial dilution plating assay as described by Webb et al. (2015) and using Ko and Hora's media (Ko and Hora, 1971). After quantification, inoculum will be added to one of 4 soil samples in amounts that will correlate to 2, 10, 20, and 200 infected particles per gram (i.p./g.) of soil respectively. Un-inoculated barley will be used as a negative control and added at the same rates. 455g of inoculated soil will be placed into each of four flats per inoculum density. Flats will be immediately watered by adding as much water as possible and allowing it to completely drain through. Flats will then be watered gently in order to make sure that the entire flat is completely moistened prior to seeding sugar beet. Using a pre-made template, we will create 49 "holes" into each flat ~1-2cm into soil and then place 1 seed per "hole" for each variety. Monogerm sugar beet varieties will be used to ensure that a single seedling is produced per seed planted. Germplasm tested will include 1 susceptible germplasm (1997A051) and 3 resistant germplasm (FC708CMS, FC715CMS, FC721CMS). Inoculated flats will be placed into a greenhouse in a split-split plot experimental design and scored for the number of live plants germinated at 7, 10, 14, 21 days after inoculation. To determine disease severity, the % germinated plants from the number of seeds planted will be calculated at each evaluation date.

**Time Line of Anticipated Accomplishments:** All experiments are expected to be completed in the first year of study.

**Progress Toward Objectives On-going Projects:** (*Please list*)

| **Budget:** | USDA | SBREB |
|---|---|---|
| Labor (13%; Part-time 180-day employee) | $0 | $1,500.00 |
| Equipment (over $250.00) | $0 | $0 |

| | | |
|---|---|---|
| Supplies | $5,000.00 | $0 |
| Travel | $1,500.00 | $1,500.00 |
| TOTAL | $6,500.00 | $3,000.00 |

Literature Cited


1. Boosalis, M. G. and A. L. Scharen. 1959. Methods for microscope detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. *Phytopathology* 49:192-198.

2. Chet, I. and R. Baker. 1980. Induction of suppressiveness to *Rhizoctonia solani* in soil. *Phytopathology* 70:994-998.

3. Hanson, L. E. and A. L. Hill. 2004. *Fusarium* species causing Fusarium yellows of sugarbeet. *Journal of Sugarbeet Research* 41, no. 4:163-178.

4. Hanson, L. E. and J. M. McGrath. 2011. Rhizoctonia seedling disease on sugar beet. *International Sugar Journal* 113:28-33.

5. Herr, L. J. 1996. Sugar Beet Diseases Incited by *Rhizoctonia* spp. In *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control*, eds. Sneh, B., S. Jabaji-Hare, S. Neate, and G. Dijst, 341-349. (Netherlands: Kluwer Academic Publishers).

6. Ko, W. H. and F. K. Hora. 1971. A selective medium for the quantitative determination of *Rhizoctoia solani* in soil. *Phytopathology* 61:707-710.

7. Nagendran, S., R. Hammerschmidt, and J. M. McGrath. 2009. Identification of sugar beet germlasm EL51 as a source of resistance to post-emergence Rhizoctonia damping-off. *European Journal of Plant Pathology* 123:461-471.

8. Naiki, T. and T. Ui. 1975. Ultrastructure of sclerotia of *Rhizoctonia solani* Kuhn invaded and decayed by soil microorganisms. *Soil Biol Biochem* 7:301-304.

9. O'Sullivan, E. and J. A. Kavanagh. 1991. Characteristics and pathogenicity of isolates of *Rhizoctonia* spp. associated with damping-off of sugar beet. *Plant Pathology* 40:128-135.

10. Panella, L. and R. T. Lewellen. 2005. Fusarium Yellows. In *Genetics and Breeding of Sugar Beet*, eds. Biancardi, E., L. G. Campbell, G. N. Skaracis, and M. De Biaggi, 93-95. (Enfield, NH: Science Publishers, Inc.).

11. Panella, L. and E. G. Ruppel. 1996. Availability of germplasm for resistance against *Rhizoctonia* spp. In *Rhizoctonia Species: Taxomony, Molecular Biology, Ecology, Pathology and Disease Control*, eds. Sneh, B., S. Jabaji-Hare, S. Neate, and G. Dijst, 515-527. (Netherlands: Kluwer Academic Publishers).

12. Paulitz, T. C. and K. L. Schroder. 2005. A new method for the quantification of *Rhizoctonia solani* and *R. oryzae* from soil. *Plant Disease* 89:767-772.

13. Pierson, V. G. and J. O. Gaskill. 1961. Artificial exposure of sugar beets to *Rhizoctonia solani*. *Journal of the A. S. S. B. T.* XI:574-590.

14. Ruppel, E. G. and R. J. Hecker. 1994. Rhizoctonia root rot on sugarbeet cultivars having varied degrees of resistance. *Journal of Sugarbeet Research* 31:135-142.

15. Ruppel, E. G., C. L. Schneider, R. J. Hecker, and G. J. Hogaboam. 1979. Creating epiphytotics of *Rhizoctonia* root rot and evaluating for resistance to *Rhizoctonia solani* in sugarbeet field plots. *Plant Disease Reporter* 63:518-522.

16. Weinhold, A. R. 1977. Population of *Rhizoctonia solani* in agricultural soils determined by a screening procedure. *Phytopathology* 67:566-569.

17. Webb, K. M. and F. J. Calderon. 2015. Mid-infared (MIR) and near-infared (NIR) detection of *Rhizoctonia solani* AG 2-2 IIIB on barley-based artificial inoculum. *Applied Spectroscopy* 69:1129-1136

18. Wijetunga, C. and R. Baker. 1979. Modelling of phenomena associated with soil suppressive to *Rhizoctonia solani*. *Phytopathology* 69:1287-1293.