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

Kimberly M. Webb
USDA-ARS, Sugar Beet Research Unit, Fort Collins, CO

In sugar beet, *Rhizoctonia solani* Kühn not only causes Rhizoctonia crown and root rot of mature roots 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 & Hecker, 1994) and most of this germplasm is not resistant at the seedling stage (Panella & Ruppel, 1996; Panella, Ruppel & Hecker, 1995). Only recently has a germplasm resistant to Rhizoctonia seedling damping-off been reported (Nagendran, Hammerschmidt & McGrath, 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 & McGrath, 2011; Herr, 1996; O'Sullivan & 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. 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 (Paulitz & Schroder, 2005; Weinhold, 1977). 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 & 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. Likewise, 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 & Baker, 1980).

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 are necessary to elicit Rhizoctonia seedling damping off and (potentially) breakdown resistance in the soil (**Completed**)

Materials and Methods

Propagule colonization with *Rhizoctonia solani*

For inoculum preparation, hydrated hulless barley grain was prepared by soaking barley with distilled water over night in mushroom bags, then autoclaved for 1h at 121°C. The autoclaved barley grains were 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 were placed into 200mL potato dextrose broth (PDB) and shaken at 25°C for ~5-7 days. Liquid *R. solani* inoculum was then poured over the prepared hulless barley and incubated for 14-21 days at 28°C. Infested barley was then removed from mushroom bags and dried for 5 to 7 days at room temperature, then ground using a Wiley Mill that was sterilized between isolate treatments with 70% ethanol between each treatment. A negative (un-inoculated) control was prepared by autoclaving the hydrated hulless barley, inoculating with PDB and then drying and grinding as described above.

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

Two experiments were performed. For each experiment, pasteurized potting soil (Farfard #2-SV, American Clay Works) was pre-measured and dried fully in a soil oven set at XX°C for ~5-7days. Artificial *R. solani* inoculum was prepared as described above and the number of infective particles (infection rate) of the inoculum was tested using a serial dilution plating assay as described by Webb et al. (2015) using Ko and Hora's media (Ko and Hora, 1971). After quantification of the infective rate of *R. solani* on the barley inoculum this rate was used to infect the dried sterilized soil at A) 2, 10, 20, and 200 infected particles per gram of soil (i.p./g.) and B) 0, 1, 2, and 10 i.p./g. of soil for each separate experiment respectively. Un-inoculated barley was used as a negative control for the first experiment and added at the same rates.

For each experiment, 455g of inoculated soil for each inoculum density were placed into each of four flats. Flats immediately watered by adding as much water as possible and allowing it to completely drain through then watered gently to make sure that the entire flat was completely moistened prior to seeding sugar beet. Using a pre-made template, 49 "holes" that were ~1-2cm deep were made in each flat in which 1 seed per was placed for each variety. Monogerm sugar beet varieties were used to ensure that a single seedling was produced per seed planted. For experiment A, 2 susceptible germplasm (1997A051 and 1978A045) were planted and for experiment B, 1 susceptible (1997A051) and 3 resistant germplasm (FC708CMS, FC715CMS, FC721CMS) were planted. Inoculated flats were 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 (dai; experiment A) or 7 and 14 dai (experiment B). To determine disease severity, the % germinated plants from the number of seeds planted were calculated at each evaluation date and analyzed for significant differences using SAS statistical software.

Results and Discussion.

All inoculum studies have been completed and data analysis for significant differences in treatments are currently in progress. Preliminary findings suggest that there are difference in virulence of the two *R. solani* AG 2-2 IIIB isolates with R-9 being more virulent than R-1 but

both are less virulent than the AG 4 isolate (F307). R-9 was able to cause a significant reduction in sugar beet seedlings at 2 i.p./g. of soil on both susceptible varieties whereas R-1 needed between 2-10 i.p./g. of soil. 1978A045 was more susceptible (to both isolates) than 1997A051 as it had a greater amount of seedling death as compared to the uninoculated controls. In the second experiment R-9 was able to further reduce sugar beet stands even at 1 i.p/g. soil. Some lines showed more resistance to *R. solani* infection than others with FC708 appearing to be the more resistant. However, all lines showed a significant reduction in alive seedlings when inoculated with 10 i.p./g. soil indicating that resistance is breaking down at higher inoculum loads. Statistical analysis is currently in progress.

Reference List

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

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

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

Nagendran,S., Hammerschmidt,R. & McGrath,J.M. (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.

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

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

Panella,L., Ruppel,E.G. & Hecker,R.J. (1995). Registration of four multigerm sugar beet germplasm resistant to Rhizoctonia root rot: FC716, FC717, FC718, and FC719. *Crop Science* **35**:291-292.

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

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

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

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

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