PCR-BASED DETECTION OF *RHIZOCTONIA SOLANI* IN SOIL

Ashok K. Chanda and Jason R. Brantner

Assistant Professor and Research Fellow, respectively
University of Minnesota, Northwest Research and Outreach Center
Crookston, MN 56716

Rhizoctonia damping-off and crown and root rot (RCRR) caused by *Rhizoctonia solani* AG 2-2 have been the most common root diseases on sugarbeet in Minnesota and North Dakota for several years (1-2, 4). Disease can occur throughout the growing season and reduces plant stand, root yield, and quality. Warm and wet soil conditions favor infection. Control options include rotating with non-host crops (cereals), planting partially resistant varieties, planting early when soil temperatures are cool, improving soil drainage, and applying fungicides as seed treatments, in-furrow (IF), or postemergence. An integrated management strategy should take advantage of multiple control options to reduce Rhizoctonia crown and root rot.

OBJECTIVES

1) To assess the inoculum potential of soil infested with varying levels sclerotia of *R. solani*
2) To compare the efficiency of two different kits for isolating DNA from soil for real-time PCR

MATERIALS AND METHODS

Growth chamber trial. Inoculum of *R. solani* AG 2-2 was prepared by growing the fungus on sterilized, moistened, barley grain for ~2 weeks, drying, removing sclerotia from the surface of the barley grain, and then grinding the sclerotia in a coffee grinder. Ground sclerotia was added to a field soil (wheatville very fine sandy loam) at 0, 1, 10, 100, 1000, and 10000 mg sclerotia/kg dry weight of soil. Each infested field soil was added to six 10 x 10 x 10 cm pots (~350 cc soil/pot). Seed was sown (25 seed/pot, 6 replicate pots/infested soil treatment) and then another 250 cc of the appropriate infested soil was added over the seed to each pot. Pots were arranged in a randomized block design and incubated in a growth chamber at 77°F with a 14-hour photoperiod for 4 weeks. Pots were watered once or twice daily to keep soil moisture high to favor infection by *R. solani*. Seedlings were counted every day during emergence and three times per week thereafter. Dying seedlings were removed and assayed in the laboratory to verify presence of *R. solani*. Four weeks after planting, remaining seedlings were removed from soil, washed, and rated on a 0-3 scale (0 = no disease, 3 = hypocotyl completely necrotic/plant dead). The number of seedlings that died during the 4-week assay along with the ratings after 4 weeks were used to calculate a root rot index (RRI, 0 = no disease, 100 = all plants died during the 4-week assay). Another weighted Rhizoctonia soil index was calculated based on the week after planting in which seedlings died (the earlier seedlings died, the more they raised the Rhizoctonia soil index) (Harveson et al., 2014).

Soil DNA isolation. PowerMax® and PowerLyzer® powerSoil® DNA isolation kits were purchased from MO BIO Laboratories Inc. (Carlsbad, CA). Manufacturer's protocols were followed for PowerMax® kit, except that only 5 g of soil was used as starting material. Final DNA was eluted in 5 mL of Solution C6 and stored at -20 °C until downstream application. For PowerLyzer® powerSoil® kit, 250 mg of soil was used as starting material and final DNA was eluted in 100 µL of Solution C6 and stored at -20 °C until further use.

Real-time PCR. Primers and probe specific for internal transcribed spacer (ITS) region of *R. solani* anastomosis group (AG) 2-2 used in this study were developed by Budge et al. (2009). All real-time PCR assays were set up as duplicate 20 µL reactions using LightCycler® 480 Probes Master (Roche Life Science) following manufacturer's protocols. 20x Custom TaqMan® Gene Expression Assay (contains 18 µM each primer and 5 µM 6-FAM™ dye-labeled TaqMan® MGB probe) were obtained from Life Technologies (Carlsbad, CA) and 1 µL of DNA template were used in the assay. Thermal cycling parameters include 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Fungal DNA was extracted from *R. solani* AG 2-2 IIIB and IV

cultures and a 10-fold dilution series was prepared with highest concentration as 10 ng/µL and lowest concentration as 0.1 pg/ µL. Real-time PCR assays were performed in Roche LightCycler® 480 System.

RESULTS AND DISCUSSION

Growth chamber trial. By the end of four weeks, only 32 % stand was left in lowest concentration of sclerotia (1 mg/kg dry weight soil) compared to complete loss of stands in all other treatments by two weeks after planting (Fig. 1). As the sclerotial dosage increased (≥ 100 mg) more pre-emergence damping-off was observed. The lowest concentration of sclerotia also resulted in moderate disease level, while all higher concentrations resulted in severe disease with complete loss of stands. The weighted Rhizoctonia soil index was also low for 1 mg level compared to all higher concentrations of sclerotia (Tab. 1).

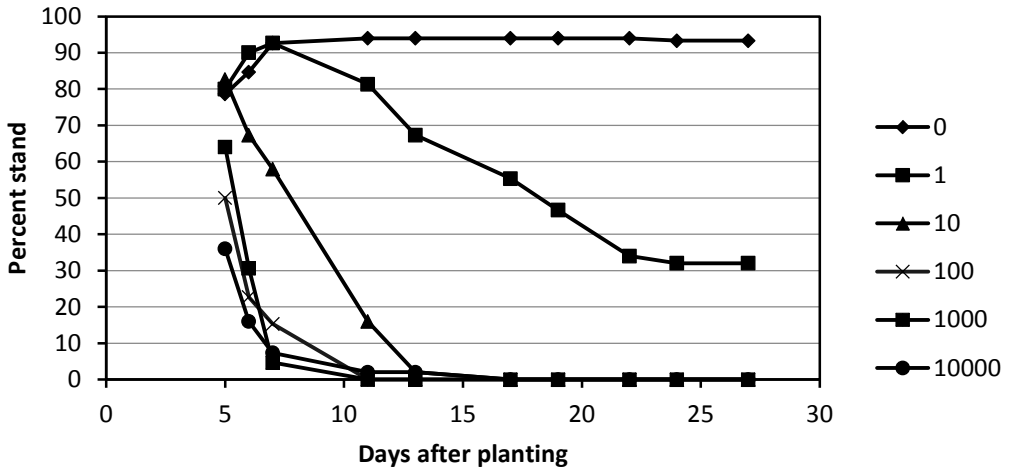

Fig 1. Effect of soil infested with varying levels of sclerotia of *R. solani* (mg/kg dry weight of soil) on stand establishment.

Table 1. Inoculum potential of soil infested with varying levels of sclerotia of *R. solani*.

Sclerotia (mg/kg soil)	RRI[Y]	WR-Index[Z]
0	1	1
1	67	37
10	100	84
100	100	95
1000	100	98
10000	100	94

[Y] RRI: Root Rot Index (0 = no disease, 100 = all plants died during the 4-week assay)
[Z] WR-Index, Weighted Rhizoctonia index developed by Harveson et al. (2014)

DNA isolation. We have detected *R. solani* AG 2-2 DNA in soil DNA extractions from soils infested with ground sclerotia of *R. solani* at concentrations of 1, 10, 100, 1000, and 10000 mg /kg dry weight soil. Both DNA isolation methods resulted in detection of DNA of *R. solani* AG 2-2 by real-time PCR with different efficiencies. A Ct value ≥ 35 is considered to be negative for detection. We did not detect any DNA of *R. solani* from non-infested soil. When sclerotia were 1 mg/kg of soil only PowerMax® kit was able to detect but not PowerLyzer® kit. Except for 100 mg of sclerotia, DNA isolations from PowerMax® kit had lower Ct values compared to PowerLyzer® kit (Tab. 2). This could be attributed to use of 5 g of starting material for PowerMax® kit as compared to 250 mg for PowerLyzer® kit.

Table 2. Comparison of two different DNA isolation kits for soil infested with sclerotia of *R. solani*.

	Ct value[z]	
Sclerotia (mg/kg soil)	**PowerMax®**	**PowerLyzer®**
0	35.00	35.00
1	34.00	35.00
10	31.80	33.18
100	29.37	28.55
1000	23.89	27.59
10000	20.40	24.16

[z] Threshold cycle value ≥ 35 means negative or no detection

Real-time PCR. The primers and probe used in this study were very specific for ITS region of *R. solani* AG 2-2. The lowest detection limit was 0.001 ng (1 pg) of DNA of *R. solani*. There was a difference of about 1 Ct value between AG IIIB and IV ISGs for the same DNA concentration (Tab. 3). We did not find any PCR inhibitors from soil DNA isolation procedures. This was evaluated by spiking the DNA isolated from non-infested soil with 10 ng of AG 2-2 IIIB or AG 2-2 IV DNA and the resultant Ct values were very close to those obtained for 10 ng of DNA of AG 2-2 IIIB or AG 2-2 IV (data not shown).

Table 3. Real-time PCR threshold cycle (Ct) values for DNA of *R. solani*

	Ct value[z]	
DNA concentration	**AG 2-2 IIIB**	**AG 2-2 IV**
10 ng	17.94	18.92
1 ng	21.24	22.18
0.1 ng	24.65	25.70
0.01 ng	28.13	29.01
0.001 ng	31.37	32.10
0.1 pg	35.00	35.00

[z] Threshold cycle value ≥ 35 means negative or no detection

We would like to use this real-time PCR assay to assess the inoculum potential of growers' fields. Since the distribution of *R. solani* in soil is very non-uniform and patchy, we will be sampling several sites within each field to detect inoculum levels of *R. solani*. Knowing if the soil is infested with *R. solani* will greatly benefit growers to make informed choices about proper management practices such as selecting a tolerant variety and proper fungicide application.

ACKNOWLEDGEMENTS

We thank the Sugarbeet Research and Education Board of Minnesota and North Dakota for funding in support of this research, the University of Minnesota, Northwest Research and Outreach Center for providing facilities and equipment; and Katie Sheetz for technical assistance.

LITERATURE CITED

1. Brantner, J.R. and C.E. Windels. 2011. Plant pathology laboratory: summary of 2009-2010 field samples. 2010 Sugarbeet Res. Ext. Rept. 41:260-261.

2. Brantner, J.R. and C.E. Windels. 2009. Plant pathology laboratory: summary of 2007-2008 field samples. 2008 Sugarbeet Res. Ext. Rept. 39:250-251.

3. Budge, G.E., Shaw, M.W., Colyer, A., Pietravalle, S., and N. Boonham. 2009. Molecular tools to investigate *Rhizoctonia solani* distribution in soil. Plant Pathol. 58:1071-1080.

4. Crane, E., Brantner, J.R., and C.E. Windels. 2013. Plant pathology laboratory: summary of 2011-2012 field samples. 2012 Sugarbeet Res. Ext. Rept. 43:169-170.

5. Harveson, R.M., Nielsen, K.A., and K.M. Eskridge. 2014. Utilizing a Preplant Soil Test for Predicting and Estimating Root Rot Severity in Sugar Beet in the Central High Plains of the United States. Plant Dis. 98:1248-1252.