

HILL, AMY L.<sup>1</sup>, KIMBERLY M. WEBB<sup>1\*</sup>, JULIE LAUFMANN<sup>2</sup>, LINDA E. HANSON<sup>3</sup> and LEE PANELLA<sup>1</sup>, <sup>1</sup>USDA-ARS, Sugarbeet Research Unit, 1701 Centre Avenue, Fort Collins, CO 80526, <sup>2</sup>U.S. Forest Service, 1409 Westfield Drive, Fort Collins, CO 80526, <sup>3</sup>USDA-ARS, Sugarbeet and Bean Research, East Lansing MI 48824. **Long term preservation of a collection of *Rhizoctonia solani*, using cryogenic storage.**

## ABSTRACT

The fungus *Rhizoctonia solani* Kühn is an important plant pathogen on a number of crops and maintaining an extensive collection of reference isolates is important in understanding relationships of this pathogen with multiple hosts. While a number of long-term storage methods have been developed, most of these require frequent transfer, require the entire sample to be removed from storage, and can cause changes to morphological or pathogenic characteristics after several years. Several methods were tested to determine the best way to prepare and store *R. solani* isolates using a cryogenic storage. Three *R. solani* isolates were stored in liquid nitrogen using a combination of one of three pre-treatments and one of two post treatments. Pre-treatments were 1) fast cool, where samples were directly plunged into liquid nitrogen 2) slow cool, where samples are slowly brought to -160°C and then placed in liquid nitrogen and 3) refrigerator/freezer, samples are first placed at 4°C, and then stored at -20°C. Post-treatments included 1) fast warm, where samples were warmed in a water bath at 70°C or 2) slow warm, where samples were warmed at room temperature. After 30 days the number of seeds that had *R. solani* germinating on the (% viability) was measured and indicated that the method of sample preparation had no impact on effectiveness of liquid nitrogen storage. Therefore, the entire Sugarbeet Research Units *R. solani* culture collection containing one hundred nine isolates of *R. solani* were placed into individual cryotubes, plunged directly into the liquid nitrogen, and stored at -160°C in liquid nitrogen. At 60 days, 5, and 10 years, all isolates were removed from storage, placed directly on PDA plates and examined at 2 and 5 days after plating. Percentage of barley grains from which *R. solani* germinated measured. At 10 years, a subset of isolates was also inoculated to a susceptible (FC901) and a resistant (FC703) sugar beet cultivar. Roots were rated for symptoms on a scale of 0 (no visible symptoms) to 7 (plant dead and root completely rotted through). After 5 years, only one isolate (Sc-1) had a significant decrease in viability as compared to the percent growth 60 days after initial storage. At 60 days it had 93% growth and at 5 years it had only 69% growth ( $p=0.0213$ ). The rest of the isolates had comparable percent growth. There were 35 out of 109 isolates that had 100% viability when originally put into storage and this viability remained unchanged throughout the entire 10 years of the study (~32% of the isolates tested). Another 35 out of 109 (36%) isolates had no significant changes in viability during the study although this amount was less than 100 percent growth. However, 35 out of 109 isolates did have significant decreases in percent growth at 10 years. 10 isolates had less than 10% growth when tested at 10 years with one isolate (1556) having no growth at all in both replicates tested ( $p\text{-value}=0.0410$ ). Isolate Sc-1 which was the only isolate to lose viability at 5 years also had a decreased viability at 10 years of only 9% growth ( $p\text{-value}=0.0035$ ) All isolates tested maintained approximately the same level of virulence to both the susceptible and resistant varieties of sugar beet used for this study. However, two of the isolates (RZC65 and RZC68), were not significantly more virulent than the negative control used (sterile barley) therefore they could be considered as avirulent isolates ( $p<0.05$ ). This indicates that for permanent storage, cryogenic methods are well suited for the preservation of *R. solani* culture collections.