Influence of Rhizoctonia-Bacterial Root Rot Complex on Storability of Sugarbeet

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ABSTRACT

A Rhizoctonia-bacterial root rot complex can lead to yield loss in the field but rots also have the potential to cause sucrose loss in storage. Thus, studies were conducted to investigate if combining sugarbeet roots suffering from this complex with healthy roots would compromise the ability of the healthy roots to retain sucrose. Over a three year period, root samples from three commercial cultivars were compared in storage as a healthy (eight healthy roots) or mixed (eight healthy roots + one rotted root) treatment inside an outdoor storage pile. The experiment was arranged as a split block (healthy in one half of block and mixed in the other) with the whole blocks arranged in a randomized complete block design with four replications. Treatments were sampled in December, January, and February and evaluated for discolored and frozen root area, weight loss, and sucrose reduction and recovery. When comparing the healthy to the mixed treatment over the nine year x sampling date combinations, the Wilcoxon signed-rank test indicated the median change for discoloration (7% increase), frozen area (14% increase), sucrose loss (5% loss), and recoverable sucrose (689 kg/ha less or 8% reduction) were significantly different from zero (P = 0.008, 0.031, 0.007, and 0.008, respectively). These data indicate that the Rhizoctonia-bacterial root rot complex can negatively affect neighboring healthy roots in storage leading to additional sucrose losses.

Additional Key Words: beet storage, Rhizoctonia root rot, bacterial root rot, *Leuconostoc*

Maintaining sucrose in sugarbeet storage piles under ambient conditions can be a challenge because of rot, respiration, and the buildup of impurities (Bugbee, 1982; Bugbee, 1993; Bugbee and Cole, 1976; Kenter and Hoffman, 2009; Klotz and Campbell, 2009; Klotz and Finger, 2004; Lafta and Fugate, 2009). In Idaho and Oregon production areas, about one-third of the crop is processed immediately after harvest while the remaining two-thirds of the crop is processed from storage piles that are maintained under ambient conditions from the end of October through early March (Peterson et al., 1984; Strausbaugh et al., 2010). Maintaining sucrose levels in stored sugarbeet roots can be a challenge because of new leaf growth, fungal and bacterial rot, freeze damage, and air flow restrictions (dirt, mud, weeds, and debris) which can lead to anaerobic conditions and hot spots detrimental to roots in storage (Bugbee, 1982; Bugbee, 1993; Bugbee and Cole, 1976; Klotz and Finger, 2004; Lafta and Fugate, 2009; Strausbaugh et al., 2008b).

The industry has largely used physical methods (indoor storage, stripping piles, covering piles, and ventilation) to alleviate storage problems (Bugbee, 1982; Peterson et al., 1980). However, losses costing growers and companies millions of dollars still occur (Anonymous, 2005; Bugbee, 1982). In Michigan during the 2004-05 storage season 300,000 tons of beet were lost costing growers approximately $26 million (Anonymous, 2005). Germplasm with resistance to storage rots and reduced sucrose loss have been released in the past (Akeson and Widner, 1981; Bugbee and Campbell, 1990; Campbell and Bugbee, 1988; Campbell and Bugbee, 1989; Wyse and Dexter, 1971), but current commercial cultivars still leave considerable room for improvement (Strausbaugh et al., 2009).

Abiotic and biotic conditions in the field prior to harvest can affect sugarbeet storability (sucrose loss, moisture loss, rot in tissue, etc.). Drought stress (Kenter et al., 2006; Kenter and Hoffman, 2008) and disease problems such as Aphanomyces root rot (Campbell and Klotz, 2006; Klotz and Campbell, 2009), Cercospora leaf spot (Smith and Ruppel, 1971), curly top (Strausbaugh et al., 2008a), Rhizoctonia root rot (Kenter et al., 2006), and rhizomania (Campbell et al., 2008; Strausbaugh et al., 2008b; Strausbaugh et al., 2009) can all increase sucrose loss in stored roots . Frozen tissue damage both in the field and storage piles can also lead to sucrose loss in storage (Bruijn, 2000; Buczys, 2007; Oldfield et al., 1971; and Wyse, 1978). Frozen tissue damage in storage has been shown to be worse if tissue is infested with *Beet necrotic yellow vein virus* (Strausbaugh et al., 2008b). Consequently, mixing rotted rots with healthy roots could potentially influence frozen tissue damage as well.

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is on the

increase in Europe and the United States (Bolton et al., 2010; Buddemeyer et al., 2004; Buhre et al., 2009; Führer Ithurrart et al., 2004; Ohkura et al., 2009; Strausbaugh and Gillen, 2009; Strausbaugh et al., 2011). In the Intermountain West (IMW) production area of Oregon and Idaho, Rhizoctonia root rot is accompanied by a bacterial root rot complex led by *Leuconostoc mesenteroides* subsp. *dextranicum* (Beijerinck) Garvie (Strausbaugh and Gillen, 2008; Strausbaugh and Gillen, 2009). In this IMW production area when the root rot complex is present, *R. solani* appears to only invade a small percentage (5 to 10% of the outer portion) of the root mass, but it appears to provide an entry point for bacterial invasion which frequently leads to considerable loss (up to 70% or more) of root mass (Strausbaugh and Gillen, 2009). This may be due to a restriction in *R. solani* growth as a number of bacteria from sugarbeet tissue have the potential to inhibit growth of *R. solani* (Lovic et al., 1993). A number of these same bacteria have also been shown to slow rot development associated with *Leuconostoc* (Strausbaugh and Gillen, 2008). In fact, the majority of the most common bacteria and yeast isolated from sugarbeet tissue slowed rot development by *Leuconostoc* in sugarbeet, except for the strictly aerobic acetic acid bacterium, *Gluconobacter* (Strausbaugh and Gillen, 2008).

With *R. solani* on the increase in the field, more of these rotted roots are ending up in storage piles. However, the potential impact of mixing roots with this particular complex with healthy roots is unknown. Thus, a three-year study was conducted to investigate the potential influence on root health and sucrose loss in healthy roots surrounding a rotted root.

MATERIALS AND METHODS

Treatments.

The experiment was arranged in a split block (healthy treatments in one half of block and the mixed treatments in the other) design with the whole blocks arranged in a randomized complete block design with four replications. The treatments were samples from three commercial sugarbeet cultivars (Table 1) stored with and without a rotted root inside an outdoor storage pile (described below) in Twin Falls, ID. The experimental unit was an eight-root sample (healthy = eight roots from normal appearing plants and no apparent root symptoms; mixed = eight healthy roots plus one rotted root). The experiment was conducted three times using roots grown in 2007, 2008, and 2009. In 2007, the three commercial sugarbeet cultivars used in the study were HH001, HH015, and HM070020. In 2008, HH015, HM070020, and C-12 were the cultivars utilized. In 2009, cultivars HH015, HM070017, and C-12 were used. The cultivar for the rotted root in the "mixed treatment" in 2007 and 2008 was unknown but in 2009 came from the cultivar HM080004.

Table 1. Percentage of discolored surface area on sugarbeet roots harvested in 2007, 2008, and 2009 and stored in an outdoor commercial pile at Twin Falls, ID with and without a rotted root.

	Surface discoloration (%)[‡]								
	December			January			February		
Cultivar[†]	Healthy[§]	Mixed	$P > F$	Healthy	Mixed	$P > F$	Healthy	Mixed	$P > F$
2007 roots									
HH001	5	4		12	25		15	31	
HH015	4	5		12	13		14	31	
HM070020	1	6		12	20		14	26	
Overall mean	3	5	0.229	12	19	0.198	15	30	0.042
2008 roots									
C-12	1	2		1	11		14	25	
HH015	0	0		5	16		16	34	
HM070020	4	0		2	15		14	29	
Overall mean	2	1	0.267	3	14	0.230	15	29	0.092
2009 roots									
C-12	0	0		2	4		7	59	
HH015	0	4		4	12		38	52	
HM070017	0	7		10	8		19	39	
Overall mean	0	4	0.081	6	8	0.452	21	50	0.085

† For more information on the coded cultivars contact the respective seed companies: C = ACH Seeds Inc., HH = Holly Hybrids, and HM = Hilleshog.

‡ Surface discoloration = percentage of root area covered by fungal growth or dark tissue discoloration. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated after 72 (Dec), 114 (Jan), and 151 (Feb) days in storage (DIS). The 2008 roots were evaluated 66, 97, and 128 DIS. The 2009 roots were evaluated 70, 99, and 129 DIS.

§ Healthy = roots healthy in appearance; Mixed = healthy roots stored with one rotted (Rhizoctonia-bacterial root rot complex) root; and $P > F$ was the probability associated with the F value. Data were analyzed in SAS (SAS Institute Inc., 2008) using the Proc GLIMMIX procedure. In December during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.198, 0.222, and 0.342, respectively) so the overall treatment means were compared. In January during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.671, 0.234, and 0.434, respectively) so the overall treatment means were compared. In February during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.657, 0.851, and 0.110, respectively) so the overall treatment means were compared.

Root samples.

The healthy-appearing commercial sugarbeet roots came from a disease-free (no visual symptoms) variety trial arranged in a randomized complete block design with eight replications and four-row plots conducted by the Amalgamated Sugar Co. LLC. The trial was conducted using standard crop production practices in American Falls, ID. The rotted roots were hand dug and topped and originated from a commercial production field near Weiser, ID suffering from the Rhizoctonia-bacterial root rot complex (Strausbaugh and Gillen, 2008; Strausbaugh and Gillen, 2009; Strausbaugh et al., 2011). Isolations from at least five neighboring roots with similar symptoms established that both *R. solani* and *Leuconostoc* were present in the commercial field each year. Isolations for *R. solani* were conducted on potato dextrose agar (Becton Dickson & Co., Sparks, MD) amended with 200 mg L^{-1} streptomycin sulfate using previously described techniques (Strausbaugh and Gillen, 2009). Isolations for *Leuconostoc* were conducted on a semi-selective medium, glucose-yeast extract-peptone (GYP) agar (Cai et al., 1999) amended with 0.2 mg L^{-1} tetracycline and 30 mg L^{-1} vancomycin, using previously described techniques (Benkerroum et al., 1993; Strausbaugh and Gillen, 2009). Rotted roots used in the storage work all had 60 to 80% of the root surface discolored by fungal and bacterial growth. The healthy roots for the storage work were mechanically topped and then dug with a shovel from the outside row of four-row plots. The center two rows were harvested with a two-row plot harvester at the same time the roots for the storage work were collected. The storage samples were placed in nylon mesh onion bags and piled inside a metal corrugated ventilation pipe (0.9 m diameter) on top of plywood. All samples in the tube were at least 6.1 m from the edge of the pile with the open end of the tube covered by straw bales. The pipe was located on top of a 30-cm layer of beet. The pipe was covered by roots piled to a height of 8 m. The pile was ventilated using the same perforated pipe placed 3.7 m on center. The storage pipe with the samples was placed in between ventilation pipes. The beet surrounding the pipe were commercial roots healthy in appearance (roots were normal shape and size and had no rot symptoms). The temperature inside the storage tube was recorded using a Hobo temperature sensor (Model H08-001-02; Onset Computer Corp., Bourne, MA) at 1 h intervals. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated on 11 Dec 07, 22 Jan 08, and 28 Feb 08 after 72, 114, and 151 days in storage (DIS), respectively. The 2008 roots were evaluated on 5 Dec 08, 5 Jan 09, and 5 Feb 09 after 66, 97, and 128 DIS, respectively. The 2009 roots were evaluated on 8 Dec 09, 6 Jan 10, 5 Feb 10 after 70, 99, and 129 DIS, respectively.

Ratings for discoloration and freeze damage.

After being retrieved from the storage pile on each sampling date, the roots were visually evaluated for surface discoloration as the percentage of root surface area associated with rot damage such as dry black rot, wet bacterial rot, and/or tissue covered with fungal growth. The percentage of root surface area associated with freeze damage (frost on surface and translucent tissue) was also visually established at the time of retrieval from storage.

Weight analysis.

Prior to placing the storage samples in the pile, each sample was weighed. The samples were reweighed when retrieved from the storage pile. The healthy roots in the mixed treatment were weighed separately from the rotted root. These weights were used to determine reduction in root weight for the healthy roots in all treatments.

Sugar analysis and yield.

Two eight-beet samples collected from each plot at harvest were submitted to the Amalgamated Tare Lab in Paul, ID. Percent sucrose was determined using an Autopol 880 polarimeter (Rudolph Research Analytical, Hackettstown, NJ) and a half-normal weight sample dilution and aluminum sulfate clarification method [ICUMSA Method GS6-3 1994] (Bartens, 2005). Conductivity was measured using a Foxboro conductivity meter Model 871EC (Foxboro, Foxboro, MA) and nitrate was measured using a multimeter Model 250 (Denver Instruments, Denver, CO) with Orion probes 900200 and 9300 BNWP (Krackler Scientific, Inc., Albany, NY). Percent sucrose for samples coming out of storage was determined by Amalgamated Research Inc. in Twin Falls, ID using gas chromatography, since polarimeter readings can be affected by impurities that accumulate during storage (Buczys, 2007; Shore et al., 1983). The gas chromatographic method was similar to ICUMSA Method GS4/7/8/5-2 [2002] with the following modifications: the internal standard used is D(-)- salicin [2-(hydroxymethyl)phenyl-β-D-glucopyranoside] and equal volumes (to ± 0.01 ml) of a solution of internal standard in dimethylformamide were dispensed into weighed samples and standards using a volumetric dispenser (Bartens, 2005). The gas chromatography analysis averaged 1.395% higher than the polarimeter reading on samples evaluated in previous work (Strausbaugh et al., 2008b). To establish percent reduction in sucrose at harvest versus storage, only samples from within the same plot were compared. Percent sucrose reduction was established using the following equation (Strausbaugh et al., 2010): % reduction in pounds of sucrose = (1-{[(% $\text{Sucrose}_{\text{storage sample}}$ – 1.395) x $\text{Weight}_{\text{storage sample}}$]/(% $\text{Sucrose}_{\text{harvest sample}}$ x $\text{Weight}_{\text{harvest sample}}$)}) x 100. Estimated recoverable sucrose (ERS) yield per ton of roots was calculated using [(extraction) x (0.01) x (gross sucrose/ha)]/(t/ha), where extraction = 250 + [[(1255.2) x (conductiv-

ity) – (15000) x (percent sucrose - 6185)]/[(percent sucrose) x (98.66 – [(7.845) x (conductivity)])]] and gross sucrose = [[(t/ha) x (percent sucrose)] x (0.01)] x (1000 kg/t).

Data analysis.

The SAS Univariate procedure (SAS Institute Inc., 2008) was used to test for normality of the data. The data were also subjected to analysis of variance (ANOVA) using the SAS generalized linear mixed models procedure (Proc GLIMMIX). In the model statement the fixed effects were treatment, cultivar, and the treatment by cultivar interaction. The random effects were block and the block by treatment and block by cultivar interactions. In the model statement, the denominator degrees of freedom were calculated using the DDFM=KENWARDRODGER option. Mean comparisons were conducted using least square means ($\alpha = 0.05$) while using the "Lines" output option. To investigate trends in the data, Wilcoxon signed-rank tests and regression analyses were conducted (SAS Institute Inc., 2008).

RESULTS

Temperature.

During the 2007/2008 storage season, root temperature remained above 0°C for 52 days (Fig. 1, Plate A). Temperatures then turned cold and remained consistently cold with 88 of the next 94 days remaining below 0°C. During the 2008/2009 storage season, temperatures were above 0°C for the first 66 days (Fig. 1, Plate B). Temperatures then remained below 0°C for the rest of the storage period. During the 2009/2010 storage season, temperatures were above 0°C for the first 47 days and then were below 0°C for the rest of the storage period except for 1 day (Fig. 1, Plate C).

Surface discoloration.

On all nine year x sampling date combinations the healthy- and mixed-root treatments could be compared across the three cultivars since there were no significant interactions (P ranged from 0.110 to 0.851; Table 1). On the February sampling date, the mixed-root treatment had more surface discoloration than the healthy-root treatment with 2007 roots ($P = 0.042$) but with 2008 roots ($P = 0.092$) and 2009 roots ($P = 0.085$) differences were only evident at the 10% level (Table 1). Earlier sampling dates did not consistently identify differences between treatments but there was a trend for the mixed mean to be higher. To test this trend, all nine sampling dates were compared using the Wilcoxon signed-rank test. The median change (7% discoloration) between the healthy and mixed treatments was significantly different from zero ($P = 0.008$). Cultivars did not differ on any of the sampling dates at the 5% level.

Figure 1. Average daily temperature (°C) next to sugarbeet storage samples inside the storage tube from 1 October 2007 to 28 February 2008 (A), 1 October 2008 to 5 February 2009 (B), and 29 September 2009 to 5 February 2010 (C) in an outdoor pile in Twin Falls, ID. Arrows designate when storage samples were retrieved.

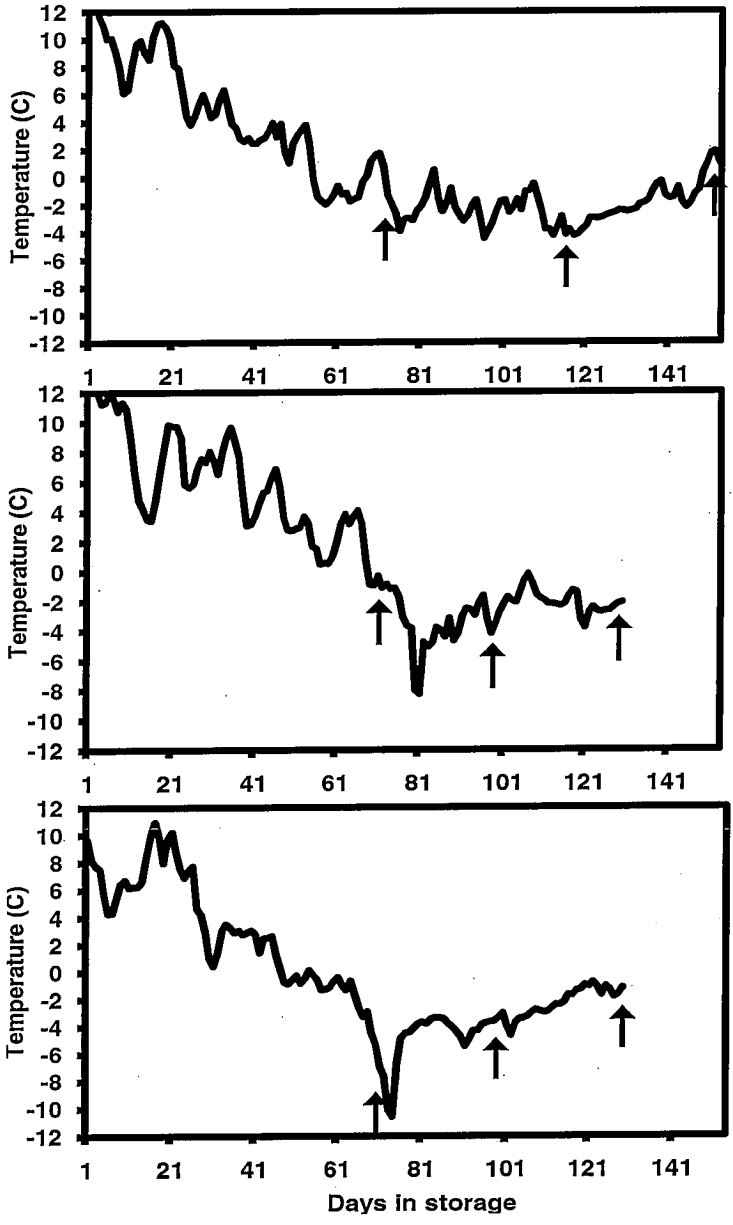

Table 2. Percent of frozen surface area on sugarbeet roots harvested in 2007, 2008, and 2009 and stored in an outdoor commercial pile at Twin Falls, ID with and without a rotted root.

	Frozen surface area (%)[‡]								
	December			January			February		
Cultivar[†]	Healthy[§]	Mixed	$P > F$	Healthy	Mixed	$P > F$	Healthy	Mixed	$P > F$
2007 roots									
HH001	0	0		54	90		10	25	
HH015	0	0		25	61		9	28	
HM070020	0	0		35	59		10	20	
Overall mean	0	0	NA	38	70	0.105	10	24	0.034
2008 roots									
C-12	0	0		10	60		20	6	
HH015	0	0		46	55		40	76	
HM070020	0	0		17	15		6	12	
Overall mean	0	0	NA	24	43	0.239	22	32	0.412
2009 roots									
C-12	44	100		100	100		60	100	
HH015	50	65		100	100		74	88	
HM070017	55	80		100	100		84	100	
Overall mean	50	82	0.095	100	100	NA	73	96	0.098

† For more information on the coded cultivars contact the respective seed companies: C = ACH Seeds Inc., HH = Holly Hybrids, and HM = Hilleshog.

‡ Frozen root surface area = percentage of root surface area associated with frozen tissue. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated after 72, 114, and 151 days in storage (DIS). The 2008 roots were evaluated 66, 97, and 128 DIS. The 2009 roots were evaluated 70, 99, and 129 DIS.

§ Healthy = roots healthy in appearance; Mixed = healthy roots stored with one rotted (Rhizoctonia-bacterial root rot complex) root; and $P > F$ was the probability associated with the F value. Data were analyzed in SAS (SAS Institute Inc., 2008) using the Proc GLIMMIX procedure. In December during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = NA, NA, and 0.630, respectively) so the overall treatment means were compared. In January during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.879, 0.171, and NA, respectively) so the overall treatment means were compared. In February during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.431, 0.221, and 0.359, respectively) so the overall treatment means were compared. NA = no analysis since all data were the same.

Table 3. Percent root weight reduction on sugarbeet roots harvested in 2007, 2008, and 2009 and stored in an outdoor commercial pile at Twin Falls, ID with and without a rotted root.

	Root weight reduction (%)[‡]								
	December			January			February		
Cultivar[†]	Healthy[§]	Mixed	$P > F$	Healthy	Mixed	$P > F$	Healthy	Mixed	$P > F$
2007 roots									
HH001	12	11		13	14		14	18	
HH015	9	9		14	15		15	17	
HM070020	9	10		17	15		13	17	
Overall mean	10	10	0.790	14	14	0.908	14	17	0.023
2008 roots									
C-12	12	15		16	20		18	21	
HH015	15	14		16	15		20	20	
HM070020	15	13		18	17		19	20	
Overall mean	14	14	0.931	17	17	0.575	19	20	0.794
2009 roots									
C-12	13	13		14	13		13	14	
HH015	12	11		15	14		12	16	
HM070017	16	16		17	16		17	18	
Overall mean	14	14	0.910	15	14	0.591	14	16	0.304

† For more information on the coded cultivars contact the respective seed companies: C = ACH Seeds Inc., HH = Holly Hybrids, and HM = Hilleshog.

‡ Percent reduction in root weight in relation to that determined at harvest. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated after 72, 114, and 151 days in storage (DIS). The 2008 roots were evaluated 66, 97, and 128 DIS. The 2009 roots were evaluated 70, 99, and 129 DIS.

§ Healthy = roots healthy in appearance; Mixed = healthy roots stored with one rotted (Rhizoctonia-bacterial root rot complex) root; and $P > F$ was the probability associated with the F value. Data were analyzed in SAS (SAS Institute Inc., 2008) using the Proc GLIMMIX procedure. In December during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.340, 0.470, and 0.816, respectively) so the overall treatment means were compared. In January during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.367, 0.075, and 0.977, respectively) so the overall treatment means were compared. In February during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction (P = 0.877, 0.438, and 0.587, respectively) so the overall treatment means were compared.

Frozen root area.

On all nine year x sampling date combinations the healthy- and mixed-root treatments could be compared across the three cultivars since there were no interactions (*P* ranged from 0.171 to 0.879; Table 2). With the 2007 roots on the February sampling date there was more (*P* = 0.034) frozen root tissue with the mixed sample. Other sampling dates did not consistently identify differences between treatments at the 5% level, but there was a trend for the mixed mean to be higher. To test this trend, all nine sampling dates were compared using the Wilcoxon signed-rank test. The median change (14% surface area) between the healthy and mixed treatments was significantly different from zero (*P* = 0.031). The only differences between cultivars were with the 2008 roots in the January and February samplings (*P* = 0.049 and 0.032, respectively). With the 2008 roots and January sampling, HH015 (50% frozen) had more frozen root surface area than C-12 (35%) and HM070020 (16%). With the 2008 roots and February sampling, HH015 (58% frozen) had more frozen root surface area than C-12 (13%) and HM070020 (9%).

Root weight reduction.

On all nine year x sampling date combinations the healthy- and mixed-root treatments could be compared across the three cultivars since there were no interactions (*P* ranged from 0.340 to 0.977; Table 3). With the 2007 roots on the February sampling date there was more (*P* = 0.023) weight loss with the mixed sample. Other sampling dates did not consistently identify differences between treatments at the 5% level and there were no trends (*P* = 0.375) evident across the nine sampling dates based on the Wilcoxon signed-rank test. With the 2009 roots on the December sampling date, HM070017 (16% loss) lost more (*P* = 0.008) weight than C-12 (13%) and HH015 (12%). On the other sampling dates, there were no differences between cultivars identified.

Sucrose reduction.

With the 2007 roots on the December sampling date there was a treatment by cultivar interaction (*P* = 0.027), so the treatments were compared within each cultivar. On the other eight year x sampling date combinations the healthy- and mixed-root treatments could be compared across the three cultivars since there were no interactions (*P* ranged from 0.171 to 0.942; Table 4). With the 2007 roots on the December sampling date the mixed treatment had 7% and 13% more sucrose reduction with HH015 (*P* = 0.005) and HM070020 (*P* = 0.031), respectively. With the 2007 roots on the January and 2008 roots on the December sampling dates, there was 7% and 5% more sucrose reduction with the mixed treatment, respectively. The other sampling dates did not consistently identify differences between treatments but there was a trend for the mixed treatment to have

more sucrose reduction. To test this trend, all nine sampling dates were compared using the Wilcoxon signed-rank test. The median change (5% loss of sucrose) between the healthy and mixed treatments was significantly different from zero ($P = 0.007$). Differences among cultivars were only evident with the 2008 roots on the December sampling (P = 0.012) with C-12 (15% reduction) having less reduction than HH015 (20%) and HM070020 (23%).

Estimated recoverable sucrose (ERS).

With the 2007 roots on the December sampling date there was a treatment by cultivar interaction ($P = 0.029$), so the treatments were compared within each cultivar. On the other eight year x sampling date combinations the healthy- and mixed-root treatments could be compared across the three cultivars since there were no interactions (P ranged from 0.120 to 0.959; Table 5). With the 2007 roots on the December sampling date the mixed treatment had 1,450 and 1,441kg/ha less estimated recoverable sucrose with HH015 ($P = 0.044$) and HM070020 ($P = 0.032$), respectively. With the 2007 roots on the January and 2008 roots on the December sampling dates, there was less ERS with the mixed treatment at the 10% level. The other sampling dates did not consistently identify differences between treatments but there was a trend for the mixed treatment to have less ERS. To test this trend, all nine sampling dates were compared using the Wilcoxon signed-rank test. The median change (689 kg/ha reduction) between the healthy and mixed treatments was significantly different from zero ($P = 0.008$). On three of the sampling dates cultivar differences were evident. On the January sampling with 2007 roots, HH001 (6,807 kg/ha) yielded less ERS than HH015 and HM070020 (7,805 and 7,578 kg/ha, respectively). On the February sampling with 2007 roots, HH001 (5,572 kg/ha) yielded less ERS than HH015 and HM070020 (7,777 and 6,924 kg/ha, respectively). On the February sampling with 2009 roots, HH015 (7,949 kg/ha) yielded less ERS than C12 (9016 kg/ha) but not HM070017 (8,340 kg/ha).

Regression analyses.

Based on regression analysis with 2007 roots in January and February, discolored surface area had a positive relationship ($r^2 = 0.252$, $P = 0.012$ and $r^2 = 0.951$, $P < 0.0001$, respectively) with frozen root surface area. With 2007 and 2008 roots, surface discoloration in the February sampling had a weak positive relationship ($r^2 = 0.193$, $P = 0.032$ and $r^2 = 0.234$, $P = 0.016$, respectively) with sucrose reduction. With 2008 roots, surface discoloration in the February sampling had a weak negative relationship ($r^2 = 0.268$, $P = 0.010$) with ERS. With 2008 roots in January and 2009 roots in February, frozen surface area had a weak positive relationship ($r^2 = 0.208$, $P = 0.025$ and $r^2 = 0.171$, $P = 0.045$, respectively) with weight loss. With 2007 roots in January

Table 4. Percent sucrose reduction in sugarbeet roots harvested in 2007, 2008, and 2009 and stored in an outdoor commercial pile at Twin Falls, ID with and without a rotted root.

	Sucrose reduction (%)[‡]								
	December			January			February		
Cultivar[†]	Healthy[§]	Mixed	$P > F$	Healthy	Mixed	$P > F$	Healthy	Mixed	$P > F$
2007 roots									
HH001	11	13	0.134	22	25		35	40	
HH015	7	14	0.005	11	21		22	28	
HM070020	10	23	0.031	16	22		33	37	
Overall mean	9	17		16	23	0.031	30	35	0.152
2008 roots									
C-12	10	19		33	45		42	50	
HH015	20	20		27	43		41	45	
HM070020	20	25		32	33		49	58	
Overall mean	17	22	0.024	31	40	0.172	44	51	0.223
2009 roots									
C-12	25	24		29	28		31	34	
HH015	22	22		30	31		29	35	
HM070017	26	26		28	33		34	39	
Overall mean	24	24	0.864	29	31	0.404	31	36	0.173

† For more information on the coded cultivars contact the respective seed companies: C = ACH Seeds Inc., HH = Holly Hybrids, and HM = Hilleshog.

‡ Percent reduction in sucrose in relation to that determined at harvest. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated after 72, 114, and 151 days in storage (DIS). The 2008 roots were evaluated 66, 97, and 128 DIS. The 2009 roots were evaluated 70, 99, and 129 DIS.

§ Healthy = roots healthy in appearance; Mixed = healthy roots stored with one rotted (Rhizoctonia-bacterial root rot complex) root; and $P > F$ was the probability associated with the F value. Data were analyzed in SAS (SAS Institute Inc., 2008) using the Proc GLIMMIX procedure. In December 2007, there was a treatment by cultivar interaction ($P = 0.027$) so the treatment means were compared within each cultivar. In December during the 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.227$ and 0.942, respectively) so the overall treatment means were compared. In January during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.171$, 0.172, and 0.211, respectively) so the overall treatment means were compared. In February during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.927$, 0.848, and 0.662, respectively) so the overall treatment means were compared.

Table 5. Estimated recoverable sucrose in sugarbeet roots harvested in 2007, 2008, and 2009 and stored in an outdoor commercial pile at Twin Falls, ID with and without a rotted root.

	Estimated recoverable sucrose (kg/ha)‡								
	December			January			February		
Cultivar†	Healthy§	Mixed	$P > F$	Healthy	Mixed	$P > F$	Healthy	Mixed	$P > F$
2007 roots									
HH001	7,914	7,728	0.136	6,982	6,633		5,779	5,364	
HH015	9,730	8,913	0.044	9,300	8,196		8,094	7,460	
HM070020	9,601	8,160	0.032	8,993	7,994		7,088	6,759	
Overall mean	9,082	8,267		8,424	7,607	0.057	6,987	6,528	0.142
2008 roots									
C-12	10,589	9,440		7,859	6,384		6,865	5,804	
HH015	10,050	9,977		9,160	7,189		7,384	6,855	
HM070020	9,698	9,167		8,268	8,205		6,304	5,086	
Overall mean	10,112	9,528	0.088	8,430	7,260	0.225	6,851	5,915	0.205
2009 roots									
C-12	10,046	10,158		9,496	9,626		9,202	8,831	
HH015	9,106	9,152		8,211	7,965		8,317	7,583	
HM070017	9,708	9,673		9,394	8,802		8,662	8,019	
Overall mean	9,619	9,662	0.876	9,033	8,818	0.422	8,727	8,144	0.184

† For more information on the coded cultivars contact the respective seed companies: C = ACH Seeds Inc., HH = Holly Hybrids, and HM = Hilleshog.

‡ Percent reduction in root weight in relation to that determined at harvest. Sugarbeet were harvested and put into storage on 1 October 2007, 1 October 2008, and 29 September 2009. The 2007 roots were evaluated after 72, 114, and 151 days in storage (DIS). The 2008 roots were evaluated 66, 97, and 128 DIS. The 2009 roots were evaluated 70, 99, and 129 DIS.

§ Healthy = roots healthy in appearance; Mixed = healthy roots stored with one rotted (Rhizoctonia-bacterial root rot complex) root; and $P > F$ was the probability associated with the F value. Data were analyzed in SAS (SAS Institute Inc., 2008) using the Proc GLIMMIX procedure. In December 2007, there was a treatment by cultivar interaction ($P = 0.029$), so the treatment means were compared within each cultivar. In December during the 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.392$ and 0.959, respectively) so the overall treatment means were compared. In January during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.179$, 0.212, and 0.120, respectively) so the overall treatment means were compared. In February during the 2007, 2008, and 2009 studies, there was no treatment by cultivar interaction ($P = 0.906$, 0.856, and 0.677, respectively) so the overall treatment means were compared.

and February and 2009 roots in February, frozen surface area had a weak positive relationship ($r^2 = 0.380$, $P = 0.001$; $r^2 = 0.201$, $P = 0.028$; and $r^2 = 0.154$, $P = 0.050$, respectively) with sucrose reduction. With 2008 roots in December and January and 2009 roots in December and February, weight loss had a significant positive relationship ($r^2 = 0.206$, $P = 0.026$; $r^2 = 0.164$, $P = 0.050$; $r^2 = 0.630$, $P < 0.001$; and $r^2 = 0.561$, $P < 0.001$, respectively) with sucrose reduction. With 2008 roots in January and 2009 roots in February, weight loss had a weak positive relationship ($r^2 = 0.256$, $P = 0.012$ and $r^2 = 0.188$, $P = 0.034$, respectively) with ERS.

DISCUSSION

The Rhizoctonia-bacterial root rot complex can lead to severe yield loss in the field (Strausbaugh and Gillen, 2009) but putting roots affected by this root rot complex in with healthy roots in an outdoor storage pile has the potential to lead to additional sucrose loss as shown in this study. When comparing the healthy to the mixed treatment over the nine samplings, the Wilcoxon signed-rank test indicated the median change for discoloration (7% increase), frozen area (14% increase), sucrose loss (5% loss), and ERS (689 kg/ha less or 8% reduction) were significantly different from zero ($P = 0.008$, 0.031, 0.007, and 0.008, respectively). The only variable not influenced by having a rotted root next to healthy roots was root weight loss ($P = 0.375$). Therefore, the Rhizoctonia-bacterial root rot complex is not only a management concern in the field but also in storage for sugarbeet roots held under ambient conditions.

A number of variables (discolored and frozen root surface area, sucrose loss, and ERS) were negatively affected in the healthy roots stored next to a rotted root. Most comparisons between the healthy and mixed treatments on the nine-sampling dates could not be consistently proven to be different based on ANOVA, but with the mixed treatment there was usually a negative trend with these four variables. These trends were all found to be significant since the Wilcoxon signed-rank test indicated the median change was always different from zero. Finding that ANOVA could not consistently prove differences were significant should not be viewed as unusual since the differences in most comparisons between the healthy and mixed treatment were less than 10%.

Since these roots could not be readily observed and volatiles were not collected while the roots were in storage, it is not clear if the effects were direct and/or indirect. In the mixed treatment, fungal and/or bacterial growth could have grown or oozed from the rotted root onto healthy roots in direct contact. We did not observe the spread of *R. solani* type growth in storage nor did we find any reported in the literature. However, a mixture of saprophytic fungi along with some potential pathogens (such as *Botrytis* sp., *Pennicil-*

lium sp., *Fusarium* sp., etc.) was clearly active on the rotted root along with bacterial ooze. This mixture of fungal growth and bacterial ooze did contact surrounding roots in storage. In addition, volatile materials such as ethylene released by the compromised root or microbial organisms may have also impacted the surrounding roots (Fugate et al., 2010; Fukuda et al., 1993). A median increase of 7% discoloration may not sound like much of a change but when just 20% of the root surface is covered by fungal growth, respiration can be increased 100% (Mumford and Wyse, 1976). In our observations, root discoloration incorporated discolored tissue along with tissue covered by fungal and/or bacterial growth but did not include frozen or translucent tissue. Since these root discoloration responses tended to overlap, no attempt to separate them was made. However, an increase in discoloration indicates a potential for a large increase in respiration. Also, a 14% median increase in frozen root surface area was associated with the mixed treatment. Figure 1 shows that the roots were exposed to temperatures below -2°C which can lead to irreversible damage (loss of cellular contents) and increased respiration rates (Wyse, 1978). As compromised tissue (rotted and/or frozen) accumulates during prolonged storage, sucrose is lost to respiration and also becomes more difficult to extract because of the buildup of impurities such as dextran, glucose, fructose, and raffinose (Klotz and Campbell, 2009). Thus, a median sucrose loss (5% loss) and ERS reduction (689 kg/ha less or 8% reduction) in the mixed versus the healthy treatment fits with responses seen in the literature.

Rhizoctonia root rot is on the increase in Europe and the United States (Bolton et al., 2010; Buddemeyer et al., 2004; Buhre et al., 2009; Führer Ithurrart et al., 2004; Ohkura et al., 2009; Strausbaugh and Gillen, 2009; Strausbaugh et al., 2011) potentially leading to more rotted rots ending up in storage piles. However, managing the sucrose loss associated with rotted roots entering storage piles will likely have to focus on excluding rotted roots from piles and managing Rhizoctonia root rot in the field. Since *R. solani* AG-2-2 IIIB can attack a number of our rotation crops (Engelkes and Windels, 1996; Nelson et al., 1996; Ohkura et al., 2009; Strausbaugh et al., 2011), crop rotation alone will likely need to be supplemented with other control measures. The application of fungicides such as azoxystrobin at planting can delay early infection and enhance establishment of vigorous stands, but does not completely prevent infections (Kirk et al. 2008, Windels and Brantner 2005). The use of resistant cultivars would be a preferred means of control but "specialty cultivars" with tolerance to *R. solani* tend to suffer from poor yield potential and have had other performance issues as well (Strausbaugh et al., 2011). Thus, storage losses associated with rotted roots provides additional evidence for the need to improve management options for Rhizoctonia root rot.

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DISCLAIMER

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