Sugarbeet Cultivar Evaluation for Bacterial Root Rot

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ABSTRACT

Bacterial root rot of sugarbeet (*Beta vulgaris*) caused by *Leuconostoc mesenteroides* subsp. *dextranicum* is a disease problem recently described in the Intermountain West region of the United States. To ameliorate the impact of bacterial root rot on sucrose loss in the field, storage piles, and factories, studies were conducted to establish an assay for identifying host resistance. In 2006 and 2007, 21 commercial cultivars were evaluated in a petri dish laboratory assay. Root slices were inoculated with *L. mesenteroides* and the diameter of the rotted area was measured after 72 and 96 h. The cultivars were arranged in a randomized complete block design with 4 replications. With 2006 roots after 96 h, the average rot diameter ranged from 16 to 34 mm (LSD = 5) depending on cultivar. With 2007 roots after 96 h, the average rot diameter ranged from 10 to 48 mm (LSD = 7) depending on cultivar. The cultivar ranking between years was correlated at 72 (r = 0.47, *P* = 0.03) and 96 (r = 0.43, *P* = 0.05) h. The assay allowed for reliable cultivar separation regardless of whether one, two, three, or four roots were used per replication. The laboratory assay has the potential to allow host resistance to bacterial root rot caused by *L. mesenteroides* to be improved in sugarbeet.

Additional key words: Beta vulgaris, lactic acid bacteria, acetic acid bacteria, host resistance

Traditionally, bacterial root rot in sugarbeet (*Beta vulgaris* L.) roots has been attributed to *Pectobacterium betavasculorum* [Thomson et al.] Gardan et al., syn. *Erwinia carotovora* [Jones] Bergy et al. subsp. *betavasculorum* Thomson et al. (Gardan et al., 2003; Ruppel et al., 1975; Thomson et al., 1977; Whitney, 1986). However, an additional bacteria-associated root rot in sugarbeet that affects roots both in the field and storage has recently been described and found to be more prevalent than *Erwinia* in the Intermountain West (IMW) region of the United States (Strausbaugh and Gillen, 2008; Strausbaugh and Gillen, 2009). The primary bacterial root rot organism identified was *Leuconostoc mesenteroides* subsp. *dextranicum* (Beijerinck) Garvie, while *Lactobacillus plantarum* Hammes & Hertel and *Gluconobacter asaii* Mason & Claus were frequently isolated and were found to be pathogenic, but caused less rot. Although these lactic acid and acetic acid bacteria may cause rot in the sugarbeet roots on their own, they have frequently been associated with fungal root rots such as Rhizoctonia root rot in the field (Strausbaugh and Gillen, 2009) and *Aspergillus fumigatus* under high temperature (35°C or greater) storage conditions (Halloin and Roberts, 1995). A survey of sugarbeet roots with rot at harvest in Idaho found 6% of the root tissue was rotted when only fungi were isolated, but 68-71% was rotted when bacteria were isolated alone or along with fungi (Strausbaugh and Gillen, 2009). Thus bacterial root rot can lead to considerable root mass loss in the Amalgamated Sugar Companies production area in western Idaho and eastern Oregon (Strausbaugh and Gillen, 2008; Strausbaugh and Gillen, 2009).

Bacterial root rot in the IMW has been observed in the field and recently harvested roots (Strausbaugh and Gillen, 2008; Strausbaugh and Gillen, 2009). However, this bacterial complex is likely to progress in storage when ambient temperatures remain above freezing. The dextran produced by *L. mesenteroides* subsp. *dextranicum*, along with the impurities that build up in roots, will make these roots a challenge to process in a sugar factory (Cescutti et al., 2005; Cogan and Jordan, 1994; Tallgren et al., 1999). With significant losses occurring in the field, storage, and factory (costs for enzymes, clogged filters, and reduced extraction), management guidelines need to be established for bacterial root rot. However, developing management guidelines is complicated by the lack of information published on this bacterial root rot problem.

Lactic acid bacteria are widespread in soil and manure and on plant surfaces (Chen et al., 2005; Cogan and Jordan, 1994; Holt et al., 1994; Mundt and Hammer, 1968; Sever-Busson et al., 1999). Given the wounds and bruising on sugarbeet roots from defoliating, scalping, harvesting,

and piling, it is surprising that not more bacterial rot occurs in storage piles. These storage observations may be partly explained by a recent study which establishes that bacterial root rot only occurred in field trials when *Rhizoctonia solani* Kuhn was present in roots (Strausbaugh and Gillen, 2009). Bacterial root rot in the 2007 field trial showed that bacterial rot could be substantial in the field confirming rot observed in field surveys (Strausbaugh and Gillen, 2009). In the 2008 field trial less bacterial root rot was observed, but the growing season was cooler than in 2007. Thus, temperature may play a role in rot development. In culture, the optimum growth temperature for *L. mesenteroides* is 20-30°C (Holt et al., 1994). Research has also shown that having just manure in a field does not lead to bacterial root rot (Strausbaugh and Gillen, 2009). The field trials with *R. solani* also showed that bacterial root rot could be a problem under normal moisture conditions provided by sprinkler irrigation (Strausbaugh and Gillen, 2009). Although normal irrigation can allow for bacterial rot development, excessive irrigation (furrow irrigating for longer than 24 h) can lead to anaerobic conditions in fields and substantial bacterial rot (Strausbaugh and Gillen, 2008). Anaerobic conditions in storage piles can also lead to substantial breakdown of roots (Cole and Bugbee, 1976). Other than trying to limit fungal root rots and anaerobic conditions, little can be suggested to minimize this problem at this time. Therefore developing cultivars with good host resistance would be valuable to help control bacterial root rot in sugarbeet. A preliminary report indicated that host resistance in sugarbeet might be available in some sugarbeet cultivars (Strausbaugh et al., 2008). Thus, a two-year study was conducted to examine the feasibility of screening sugarbeet cultivars for resistance to bacterial root rot.

MATERIALS AND METHODS

Field conditions

Sugarbeet roots of commercial cultivars were screened for susceptibility to *L. mesensteroides* subsp. *dextranicum* isolate B322 (Strausbaugh and Gillen, 2008; GenBank accession EU196393). The 2006 roots were grown in Nampa, ID in plots planted on 27-28 March 2006 in a randomized design. The plots were planted to a density of 352,123 seeds/ha, and thinned to 117,374 plants/ha. Plots were four rows wide (56-cm row spacing) and 10.5 m long and irrigated with wheel lines. Trials were managed using standard crop production practices described previously (Strausbaugh et al., 2006). No root or foliar diseases were evident at the time of harvest. The 2006 roots were harvested by hand on 12 Oct from an outside row of a single plot. The roots were stored at 4°C and 90% relative humidity, until they were screened in mid-January 2007.

The experiment was repeated in 2007 with roots from plots in American Falls, ID that were planted on 30 April 2007. The roots were harvested on 1 Oct 2007 and screened in mid-January 2008.

Cultivar rot assay

The rot assay was established as a randomized complete block design with four replications. There were 22 treatments (21 cultivars plus a non-inoculated check from cultivar B31). Each replication in the rot assay was taken from a single root. The roots were rinsed in 0.6% sodium hypochlorite solution for 1 min and then rinsed once in sterile reverse osmosis water. The roots were then allowed to air dry in the laminar hood. All exterior portions of the root were then removed. Eight to 10 mm thick and 45 to 70 mm in diameter cross sections from the center of each root (just below the widest portion of the root) were then removed. The slices were placed inside a 15 x 100 mm Petri dish on sterilized (121°C for 30 min) filter paper moistened with sterile well water. A 2 mm diameter by 3 mm deep hole was poked in the center of the slice with a sterile tooth pick. A standard hole size was maintained by comparison with a marked reference toothpick. A sterile tooth pick dipped in a 48 h old culture of *L. mesenteroides* subsp. *dextranicum* isolate B322 (Strausbaugh and Gillen, 2008; GenBank accession EU196393) grown on Difco Lactobacilli MRS agar (MRS; Becton, Dickson, & Co., Sparks, MD) at 30°C was poked into the hole and removed. A drop of sterile well water was then placed in the hole. The Petri dishes containing the inoculated slice from each replication were placed into a ziplock plastic bag (FoodHandler Inc., Melville, NY), and bags were closed and incubated at 30°C. If the filter paper began to dry out, it was rewetted with sterile well water. The diameter of the rotted area was measured after 72 and 96 h. The bacteria in the rotted area in each slice were streaked onto MRS and incubated at 30°C to confirm the identity of the pathogen and show no other pathogens were present.

Root number rot assay

To determine if increasing the number of roots per replication reduces variation and therefore increased the precision of the assay allowing for better cultivar separation, two additional studies were conducted. Roots from two cultivars, C3 and B31, were collected from the 2007 cultivar trial in American Falls, ID. The study was set up as a randomized complete block design with five replications. Two cultivars were compared when one, two, three, and four root slices were evaluated per cultivar within each replication. Each root slice came from a different root. When multiple root slices for a cultivar were utililzed for

a treatment within a replication, data from these slices were averaged prior to analysis to establish a data point (Fig. 1). A root slice for each cultivar that did not receive bacteria served as a non-inoculated check within each replication. The rot assay was conducted as described in the previous section. Bacteria from the rotted area in each slice were streaked onto MRS and incubated at 30°C to confirm the identity of the pathogen and show no other pathogens were present. The experiment was repeated with a second set of roots from the 2007 cultivar trial.

Yield variables

To determine yield variables for the field plots, the center two rows were mechanically topped and then harvested with a 2-row plot harvester (adapted to two rows from a commercial three-row Hesston 565 harvester; AGCO Corp., Duluth, GA) on 17 and 1 Oct in 2006 and 2007, respectively . Two 8-beet samples per plot were collected for sugar analysis during harvest and submitted to the Amalgamated Tare Lab in Paul, ID. Percent sugar was determined using an Autopol 880 polarimeter (Rudolph Research Analytical, Hackettstown, NJ) and a half-normal weight sample dilution and aluminum sulfate clarification method

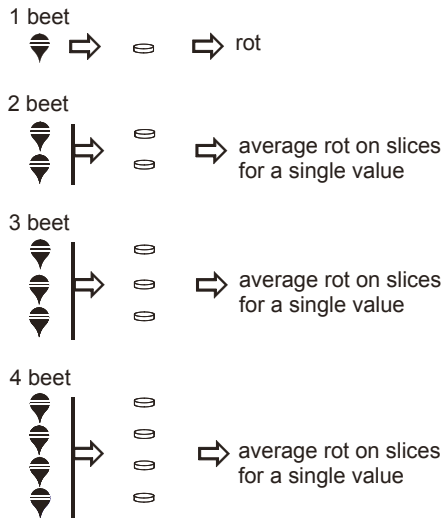

Fig. 1. In the Root Number Rot Assay two cultivars were compared when one, two, three, and four root slices (each from a separate root) were evaluated per cultivar within a replication. When multiple root slices were utilized for a treatment, data for these slices were averaged prior to analysis to establish a single value.

[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). Recoverable sugar yield was estimated based on root yield, percent sugar, and conductivity.

Data Analysis

Data were analyzed in SAS (SAS Institute Inc., 2008) using the general linear models procedure (Proc GLM) and Fisher's protected least significant difference ($\alpha = 0.05$) was used for mean comparisons. Correlations based on Spearman's coefficient of rank correlation and linear regression analyses (Proc Reg) were conducted with SAS.

RESULTS

Cultivar rot assay

At both the 72 and 96 h readings, the 2006 and 2007 experiments were significantly different ($P = 0.03$ and 0.03, respectively). Thus, the data were analyzed and presented by year (Table 1). Significant differences between cultivars were evident at 72 and 96 h in both studies. The rot diameter was two to four times greater among the more susceptible cultivars. At 72 ($r = 0.47$, $P = 0.03$) and 96 ($r = 0.43$, $P = 0.05$) h the cultivar rankings for rot were weakly correlated between years based on Spearman's Correlation Coefficient. In both the 2006 and 2007 assay, the non-inoculated checks had no rot. In both studies only *L. mesenteroides* colonies were present on MRS test plates and no contamination was evident.

Root number rot assay

The two experiments did not differ for the one, two, three, and four beet treatments at either the 72 or 96 h rating (Table 2). Thus, the data for the two experiments were analyzed together (Table 3). The two cultivars were always significantly different (P ranged from 0.01 to 0.05; Table 3) no matter if one, two, three, or four roots were used per replication with both ratings. At the 72 h rating, the coefficient of variation was not reduced when the number of roots was increased to four (Table 3). At the 96 h rating, the coefficient of variation was lower when three or four roots were used per replication (Table 3). In both studies only *L. mesenteroides* colonies were present on MRS plates and no contamination was evident. In both assays, the non-inoculated checks had no rot.

Table 1. Rot measured from *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322 in a laboratory assay on 21 commercial sugarbeet cultivars harvested from Nampa, ID in 2006 and American Falls, ID in 2007 from root rot-free plots.

Cultivar[†]	Diameter of bacterial rot (mm)[‡]			
	72 h		96 h	
	2006	2007	2006	2007
HH004	21 b-e	35 a	26 b-d	48 a
HH005	18 d-g	21 b	24 b-e	25 b
B4	14 hi	20 b	18 f-h	22 bc
B32	19 c-g	15 cd	23 c-e	20 b-d
B30	16 g-i	19 bc	20 e-h	20 b-d
C17	27 a	14 c-e	34 a	18 c-e
HM070001	22 b-d	12 d-f	28 bc	17 c-f
HM070014	23 ab	11 d-f	29 ab	15 d-f
HH003	20 b-f	12 d-f	24 b-e	16 c-f
B28	17 e-i	13 d-f	21 d-g	15 d-f
HH002	22 bc	10 d-f	28 bc	14 d-f
C21	15 g-i	9 f	21 e-g	12 ef
HM070004	16 g-i	9 f	20 e-h	12 ef
B31	16 g-i	10 d-f	20 e-h	12 ef
SX001	14 i	11 d-f	16 h	12 ef
B26	13 i	10 d-f	16 h	12 ef
SX005	16 f-i	10 d-f	23 c-e	11 ef
C2	18 e-h	10 d-f	22 d-f	11 ef
HH001	16 g-i	10 d-f	20 e-h	10 f
HM070015	16 f-i	10 d-f	20 e-h	10 f
HM070007	14 i	9 f	17 gh	10 f
Non-inoculated check	0 j	0 g	0 i	0 g
$P > F$[§]	< 0.01	< 0.01	< 0.01	< 0.01
LSD ($P < 0.05$)	4	5	5	7

[†] All cultivar names are coded (B = Betaseed, C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex) but the respective companies can be contacted using the code to gain additional information on the cultivars.

[‡] Root pieces that were not inoculated did not develop rot. Inoculated pieces that developed rot all contained *Leuconostoc mesenteroides* subsp. *dextranicum* when reisolation was done and did not contain contaminants.

[§] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value. NS = not significantly different. Means followed by the same letter did not differ significantly based on Fisher's protected least significant difference value with $P < 0.05$.

Table 2. Comparison of experiments for rot measured from *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322 on commercial sugarbeet cultivars harvested in 2007 from root rot-free plots in American Falls, ID based on tests with one, two, three, and four beet per replication, respectively.

	72 h[†]				96 h			
Variable	1 beet	2 beet	3 beet	4 beet	1 beet	2 beet	3 beet	4 beet
Experiments ($P > F$) [‡]	0.06	0.43	0.39	0.33	0.12	0.78	0.60	0.49
Variance ($P > \chi^2$)	0.32	0.61	0.57	0.16	0.91	0.59	0.57	0.23

[†] Root pieces that were non-inoculated checks did not develop rot (data not shown). Inoculated pieces that developed rot all contained *Leuconostoc mesenteroides* subsp. *dextranicum* when reisolation was done and did not contain contaminants.

[‡] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value. $P > \chi^2$ is the probability associated with Bartlett's Test for homogeneity of variance.

Table 3. Diameter of rot (mm) caused by *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322 on commercial sugarbeet cultivars harvested in 2007 from root rot-free plots in American Falls, ID based on tests with one, two, three, and four beet per replication, respectively.

	72 h†				96 h			
Cultivar †	1 beet	2 beet	3 beet	4 beet	1 beet	2 beet	3 beet	4 beet
B31	14	13	14	13	16	16	17	16
C17	10	10	10	10	11	11	11	12
P > F§	0.04	0.02	0.02	0.03	0.05	0.04	0.01	0.02
CV	21	19	20	19	26	30	18	18

† All cultivar names are coded (B = Betaseed and C = ACH Seed Inc.) but the respective companies can be contacted using the code to gain additional information on the cultivars.

‡ Root pieces that were non-inoculated checks did not develop rot (data not shown). Inoculated pieces that developed rot all contained *Leuconostoc mesenteroides* subsp. *dextranicum* when reisolated and did not contain contaminants.

§ *P > F* was the probability associated with the F value. CV = coefficient of variation.

Rot assay versus yield variables

When comparing the yield variables for the individual plots with the rot data for roots from the same plots, regression analysis did not reveal any significant relationships with the Nampa roots (Table 4). There was a significant positive relationship between the area of rotted tissue and conductivity at American Falls (Table 5), although the r^2 value was only 0.26.

DISCUSSION

The bacterial root rot assay with tooth pick inoculation appeared to be an effective way to detect differences between sugarbeet cultivars for bacterial root rot. With roots produced in different environments and

Table 4. Regression analysis for bacterial root rot caused by Leuconostoc mesenteroides subsp. dextranicum isolate B322 at 96 hours and yield variables for the 2006 sugarbeet roots from Nampa, ID.

Independent variable†	Dependent variable	Slope	Intercept	r^2	Probability
Sucrose at harvest	Root rot	-1.2	41.4	0.04	0.38
Nitrates at harvest	Root rot	0.0	22.4	0.00	0.98
Conductivity at harvest	Root rot	2.2	20.5	0.00	0.82
Tons/ha	Root rot	0.2	8.5	0.05	0.34
ERS/ha	Root rot	0.0	13.5	0.01	0.65

†ERS = estimated recoverable sucrose. Root rot = bacterial root rot caused by *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322.

Table 5. Regression analysis for bacterial root rot caused by *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322 at 96 hours and yield variables for the 2007 sugarbeet roots from American Falls, ID.

Independent variable†	Dependent variable	Slope	Intercept	r^2	Probability
Sucrose at harvest	Root rot	-3.4	67.5	0.10	0.16
Nitrates at harvest	Root rot	0.0	7.4	0.10	0.16
Conductivity at harvest	Root rot	39.2	-22.8	0.26	0.02
Tons/ha	Root rot	0.3	6.8	0.01	0.70
ERS/ha	Root rot	0.0	33.9	0.04	0.37

† ERS = estimated recoverable sucrose. Root rot = bacterial root rot caused by *Leuconostoc mesenteroides* subsp. *dextranicum* isolate B322.

years, the ranking of the cultivars for resistance to bacterial root rot was similar based on Spearman's rank correlation coefficient. The assay should provide a starting point for the selection of resistance to this type of bacterial root rot. Given the length of time roots were stored prior to testing, these data and method may be more appropriate for identifying resistance in storage than the field.

Bacterial root rot in the field has been associated with an increase in rotted sugarbeet root mass in the IMW (Strausbaugh and Gillen, 2009). Bacterial root rot can also lead to losses in storage and additional loss when the roots get to the factory. A factory in Nampa, ID in 2008 went from processing 9,900 metric tons of sugarbeet a day down to 3,600 metric tons when roots with bacterial rot were processed. With the potential for substantial losses occurring in the field, piles, and factory, management options for bacterial root rot need to be established. There appear to be considerable differences in reaction to bacterial root rot in sugarbeet cultivars in the rot assay and a previous study (Strausbaugh et al., 2008). Some individual cultivar responses (ex. C17 and HH004 in Table 1) varied between years. However, cultivar responses may reflect differences in environment (the 2006 and 2007 roots came from different production areas and years) or heterogeneity between cultivar seed lots. There was little variation between replications for cultivars within a year, allowing for significant cultivar separation both years. The rot assay was able to provide a similar ranking of cultivars with roots harvested from different environments. Thus, host resistance in commercial cultivars and this laboratory rot assay should provide a useful starting point for examining resistance to bacterial root rot in sugarbeet cultivars.

The yield variables (percent sucrose, nitrates, conductivity, tonnage, and kg of sucrose harvested) did not have a relationship with bacterial rot development, except for conductivity in the American Falls trial. Thus, the differences between cultivars apparently reflect different levels of reaction and not just a response to differences in sucrose concentration in the roots. The differences in rot between cultivars could be detected with just one root per replication, but as three and four roots per replication were utilized the coefficient of variation was reduced at 96 h. Some current commercial cultivars may be more heterogeneous than those utilized in this study. Depending on the material being screened, screening more roots per replication may be desirable. Since resistance to *Pectobacterium*, syn. *Erwinia*, has been identified and incorporated into sugarbeet cultivars in the past (Lewellen et al., 1978; Whitney and Lewellen, 1978; Whitney and Mackey, 1989), perhaps finding resistance to *Leuconostoc* based bacterial root rot should

not be considered unusual. Ideally, this rot assay will lead to improved host resistance to bacteria root rot in sugarbeet and less sucrose loss in the field, storage piles, and factory. However, the data presented may be most applicable to bacterial root rot resistance in storage. Additional studies should be conducted to determine if resistance to bacterial root rot in the field and storage are similar, if some strains are more virulent than others, and whether resistance to *Pectobacterium* confers resistance to *Leuconostoc*.

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