

Selection for Resistance to the Rhizoctonia-Bacterial Root Rot Complex in Sugar Beet

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Abstract

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The Rhizoctonia-bacterial root rot complex continues to be a concerning problem in sugar beet production areas. To investigate resistance to this complex in 26 commercial sugar beet cultivars, field studies and greenhouse studies with mature roots from the field were conducted with *Rhizoctonia solani* anastomosis group 2-2 IIIB strains and *Leuconostoc mesenteroides*. Based on means for the 26 cultivars in the 2010 and 2011 field studies, fungal rot ranged from 0 to 8%, bacterial rot ranged from 0 to 37%, total internal rot ranged from 0 to 44%, and surface rot ranged from 0 to 52%. All four rot variables resulted in significant ($P < 0.0001$) cultivar differences. Based on regression

analysis, strong positive relationships (r^2 from 0.6628 to 0.9320; $P < 0.0001$) were present among the rot variables. When ranking cultivars, the most consistent rot variable was surface rot, because 12 of 13 variable-year combinations had significant ($P \leq 0.05$) correlations. When cultivar ranking in greenhouse assays was compared, there was frequently a positive correlation with storage data but no relationship with field results. Thus, the greenhouse assays will identify storage rot resistance but field screening will be required to find resistance to this rot complex in the field.

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is of considerable concern in sugar beet production areas in the United States and other areas of the world (9,15,38). Rhizoctonia root rot can lead to root yield losses of 50% or more but also seems to be on the increase and can be associated with losses in storage (9,21,27,38,41,43). In Idaho, Rhizoctonia root rot on mature roots tends to be associated with the *R. solani* anastomosis group (AG) 2-2 IIIB strains and is frequently accompanied by a bacterial root rot caused by *Leuconostoc mesenteroides* subsp. *dextranicum* (Bejerinck) Garvie which leads to a Rhizoctonia-bacterial root rot complex (36,38,39). The prevalence of this rot complex seems to be favored by warmer longer-season production areas, poor crop rotation, and surface irrigations (38,40).

The fungus *R. solani* survives from season to season as propagules in the soil or as mycelia in infested organic matter, while *L. mesenteroides* is widely distributed throughout the environment (11,13,19,39). The genus *Leuconostoc* is a gram-positive heterofermentative lactic acid bacterium commonly found in soils, sugar factories, fermenting vegetables, dairy products, manure, and wine (4,11,12,16,19,28,35,47). These bacteria are known to be important in the initial phase of fermentation but usually are superseded by other bacteria and yeast (2,6,16). A number of other bacteria and yeast associated with bacterial root rot in sugar beet roots do not cause rot on their own but will slow down rot caused by *L. mesen-*

teroides and inhibit *R. solani* (25,39). These bacteria and yeast in competition with *R. solani* may explain why the dry black rot associated with this fungus is typically restricted to the outside of the root and involves only 3 to 5% of the root mass (36,40). On the other hand, the wet-bacterial rot in this rot complex can be associated with up to >70% of the root mass being lost (40).

Our understanding of this rot complex is still developing; therefore, disease control efforts have been targeted at *R. solani*. Crop rotation (7,8,14,23,31,33) and in-furrow or post-emergent banded fungicide sprays (5,21,22,42,44) have been shown to be helpful in controlling Rhizoctonia root rot but relying more on host resistance would be desirable (17,24,29). Thus, to gain a better understanding of the Rhizoctonia-bacterial root rot complex and also how to evaluate sugar beet cultivars for resistance to this complex, a series of field and greenhouse studies were conducted to achieve the following objectives: (i) identify the best disease variable for determining cultivar selection for this rot complex, (ii) determine whether resistance to *R. solani* will also allow for control of bacterial root rot, and (iii) establish an assay under controlled conditions for investigating the rot complex and *Rhizoctonia-Leuconostoc* interactions.

Materials and Methods

Rhizoctonia inoculum. The eight *R. solani* AG-2-2 IIIB strains (F304, F508, F512, F517, F521 [this strain's internal transcribed spacer region is genetically identical to that for the R9 strain], F548, F551, and F552) used in the studies had been isolated in Idaho and characterized previously (38). The strains had been stored on sterile barley kernels at -80°C . To create inoculum, the strains were first grown on potato dextrose agar (PDA; Becton Dickinson & Co.) amended with streptomycin sulfate (MP Bio-medicals, Inc.) at 200 mg/liter for 10 days. Plugs from these plates were used to inoculate sterile barley (*Hordeum vulgare* L.) kernels. Before inoculation, the barley kernels had been soaked in tap water for 24 h, then autoclaved for 1 h at 121°C . The kernels were then autoclaved a second time for 1 h the next day. Plugs from the PDA cultures were placed with the barley kernels and incubated in the dark at 21°C for approximately 6 weeks. The kernels were then air dried and ground using a Thomas Wiley Laboratory Mill (model 4; GMI Inc.) with a 1-mm screen (modified with 5-mm holes drilled into it).

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Bacterial inoculum. The bacterial suspension of *L. mesenteroides* subsp. *dextranicum* strain B322 (39) isolated in Idaho was prepared by washing 48-h yeast-dextrose-calcium carbonate agar (YDC; 34) cultures grown at 30°C with sterile tap water. The suspension was adjusted to 10⁸ CFU/ml with sterile tap water using a BioPhotometer (Eppendorf AG) and confirmed with a 10-fold dilution series on YDC.

Isolations. Isolations for *R. solani* were conducted on PDA amended with streptomycin sulfate at 200 mg/liter. Isolations for *L. mesenteroides* were conducted on glucose, yeast extract, peptone agar (glucose, 10.0 g; yeast extract, 5.0 g; peptone, 5.0 g; sodium acetate 2.0 g; Tween 80, 0.25 g; MgSO₄ · 7H₂O, 0.2 g; MnSO₄ · 4H₂O, 0.01 g; FeSO₄ · 7 H₂O, 0.01 g; NaCl, 5.0 g; CaCO₃, 5.0 g; agar, 20 g; and 1,000 ml of reverse osmosis water; adjusted to pH 6.8) with bromocresol purple (0.04 g/liter) amended with tetracycline (0.2 mg/liter) and vancomycin (0.03 g/liter) to make it semiselective for *Leuconostoc* spp. (4,10).

Field cultivar trial. Because sugar beet cultivars had not been previously investigated for the Rhizoctonia-bacterial root rot complex, roots from 26 commercial cultivars (Table 1), 2 check lines (FC901/C817 = *R. solani*-susceptible check and FC705/1 = *R. solani*-resistant check; both checks provided by the United States Department of Agriculture–Agricultural Research Service sugar-beet program in Ft. Collins, CO), and a noninoculated commercial check ‘B-5’ (contact Betaseed Inc. with code for more information on this cultivar) were evaluated for Rhizoctonia-bacterial root rot in a field trial conducted in Kimberly, ID in 2010. Plots were

planted on 3 May in a randomized complete block design with six replications. The seed was treated with the insecticide clothianidin (Bayer CropScience) at 60 g a.i. per 100,000 seed and the fungicides metalaxyl (Bayer CropScience) at 15.6 g a.i. per 100 kg of seed and thiram (Bayer CropScience) at 250 g a.i. per 100 kg of seed to allow for good stand establishment and protection against early-season pest problems. The plots were planted to a density of 352,123 seeds/ha and thinned at the four-leaf growth stage to 117,374 plants/ha. Plots were single rows with 56-cm row spacing, 3 m in length, and irrigated with solid set handlines. Trials were managed using standard crop production practices. However, weed management did not include glyphosate, because the FC check lines were not glyphosate resistant. Weed control was accomplished with a preplant ethotron (42% ethofumesate) application of 2.33 liters/ha and hand weeding. Plants were inoculated on 28 June in the crown at the eight-leaf growth stage with dried ground barley inoculum of the *R. solani* AG-2-2 IIIB strain F517 at 0.6 g/plant. No soil was knocked into the crown area, because cultivation is no longer a common practice with growers having switched to glyphosate-resistant cultivars. No root or foliar diseases or pests were evident at the time of harvest other than the Rhizoctonia-bacterial root rot complex. The first 10 roots in each row were evaluated visually for surface rot (percentage of root surface with dry black rot) on 10 September from each plot. These roots were also bisected to determine visually the percentage of root mass associated with fungal rot (dry black rot) and bacterial rot (wet rot). Isolations from 15 roots were conducted to confirm the pres-

Table 1. Rhizoctonia and bacterial root rot measured on 10 September 2010 in 26 commercial sugar beet cultivars and 2 check cultivars or lines inoculated with *Rhizoctonia solani* anastomosis group 2-2 IIIB strain F517 in a field trial conducted in Kimberly, ID

Cultivar ^y	Internal root mass rotted (%) ^x			
	Fungal	Bacterial	Total	Surface ^x (%)
B-5	8.0 a	29.3 a	37 a	52 a
C-25	8.1 a	16.6 bc	25 b	49 ab
HM080006	5.7 a-c	10.4 b-g	16 b-d	44 a-c
HM080004	6.4 ab	19.4 b	26 ab	42 a-d
B-7	6.1 ab	8.4 c-h	14 b-f	36 b-e
C-28	5.9 a-c	10.7 b-f	17 bc	36 b-e
C-29	5.1 b-d	11.9 b-d	17 bc	35 b-e
HM080011	4.9 b-e	7.2 c-h	12 c-g	34 c-f
B-37	4.8 b-f	11.3 b-e	16 b-e	32 c-g
HH016	4.8 b-f	6.9 d-h	12 c-h	31 c-h
B-39	3.9 b-g	7.4 c-h	11 c-h	28 d-i
FC901/C817	4.8 b-f	6.2 d-h	11 c-h	27 e-j
C-19	4.0 b-g	5.4 d-h	9 c-h	27 e-k
SV007	3.3 c-h	7.9 c-h	11 c-h	26 e-k
C-27	2.9 d-h	3.5 d-h	6 c-h	22 e-k
B-38	2.5 e-i	2.1 e-h	5 e-h	20 f-k
HM070022	2.0 g-i	2.7 d-h	5 d-h	19 f-k
SV001	2.2 g-i	4.9 d-h	7 c-h	17 g-k
C-11	2.2 f-i	2.7 d-h	5 d-h	16 h-k
C-208	1.8 g-i	1.8 f-h	4 f-h	16 i-l
HH017	1.7 g-i	1.1 gh	3 gh	14 i-l
SV003	1.4 g-i	1.3 f-h	3 gh	14 i-l
C-204	1.6 g-i	0.9 h	3 gh	13 i-l
HH015	1.4 g-i	0.5 h	2 gh	13 i-l
C-12	1.6 g-i	4.2 d-h	6 c-h	13 i-l
B-34	2.0 g-i	1.9 e-h	4 f-h	12 j-l
HM070006	1.2 hi	0.8 h	2 gh	12 kl
FC705/1	0.1 i	0.0 h	0 h	11
Noninoculated check	0.0 i	0.0 h	0 h	01
Overall mean	3.5	12.4	10	24
<i>P</i> > <i>F</i> ^z	<0.0001	<0.0001	<0.0001	<0.0001

^x Fungal = percentage of rotted root mass associated with dry black rot caused by *R. solani*, Bacterial = percentage of rotted root mass associated with wet rot (relied on natural inoculum for *Leuconostoc mesenteroides*), Total = percentage of internal root mass associated with fungal and bacterial rot, and Surface = percentage of root surface with a dry black rot caused by *R. solani*. Means within a column followed by the same letter did not differ significantly based on least square means ($\alpha = 0.05$).

^y All cultivar names are coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SV = SESVanderHave) but the respective companies can be contacted using the code to gain additional information on the cultivars. FC901/C817 (susceptible) and FC705/1 (resistant) were the *R. solani* check cultivars provided by the United States Department of Agriculture–Agricultural Research Service sugar beet program in Ft. Collins, CO. B-5 was the noninoculated check cultivar.

^z *P* > *F* was the probability associated with the *F* value. Data were analyzed in SAS using Proc GLIMMIX.

ence of *R. solani* and *L. mesenteroides* as described in the Isolation subsection.

The experiment was repeated in 2011 with the same methods and cultivars as in 2010. Seed were planted on 4 May and inoculated on the 23 June (eight-leaf growth stage). No root or foliar diseases or pests were evident at the time of harvest other than the Rhizoctonia-bacterial root rot complex. Disease evaluations were conducted on 12 September.

***R. solani* strain comparison.** In an effort to find a method for comparing *R. solani* and *L. mesenteroides* strains under controlled conditions, a greenhouse assay using mature sugar beet roots from the field was investigated. This root rot assay was conducted to compare eight *R. solani* AG-2-2 IIIB strains (F304, F508, F512, F517, F521, F548, F551, and F552) and the *L. mesenteroides* subsp. *dextranicum* strain B322 inoculated individually and in combination on roots of the commercial sugar beet B-5. The healthy-appearing mature roots used in the assay were grown in Kimberly, ID using standard production practices. The roots were hand dug and topped and inoculated on 3 September 2009. Each root was inoculated in three places (*R. solani* only, *L. mesenteroides* only, and *R. solani* + *L. mesenteroides*) in the shoulder (widest portion of root) of the root. A noninoculated check was also included. From each root, three plugs (8 mm in diameter and 24 mm in length) equally spaced around the root were removed from the shoulder of the root with a cork borer (Supplementary Figure S1). One hole was inoculated with 0.06 g of ground barley infested with an *R. solani* strain. A second hole was inoculated with 0.06 g of *R. solani* and a 0.2-ml suspension (10^8 CFU/ml) containing *L. mesenteroides*. A third hole was inoculated with just the 0.2-ml suspension of *L. mesenteroides*. The noninoculated check had plugs pulled and replaced but no inoculum was inserted. Once the plugs were replaced, they were then sealed with petroleum jelly (100% white petrolatum USP, distributed by Albertsons, Inc.). Each root served as an experimental unit and was arranged in a randomized complete block design with six replications. The roots were placed on the cement floor of a greenhouse (low and high temperature set points were 18 and 27°C, respectively) under a greenhouse bench and covered with a nylon tarp (which minimized desiccation but still allowed for some air exchange). A second nylon tarp was draped over the greenhouse bench to keep the tarp directly covering the roots out of direct sunlight. After 2 weeks, the roots were cross sectioned (sliced along the length of the plug; and the rotted area was measured perpendicular to the plug. At the time of rot evaluation, isolations from seven roots at all three inoculation points were conducted to confirm the presence of the inoculated organism or contaminants. The experiment was repeated the next year on 15 September 2010 using the same methods.

Greenhouse assay at harvest. The same 26 commercial sugar beet cultivars compared in the field assay were also compared in a greenhouse assay using methods similar to those described above in the *R. solani* strain assay subsection. The roots used in the assay were grown in American Falls, ID as part of a cultivar trial. The trial was managed using standard cultural practices and had been planted on 13 April 2009. The roots were harvested using a mechanical harvester on 28 September 2009 and inoculated the next day. The greenhouse assay maintained the roots in the same randomized complete block design with six replications that was used in the field. The experimental unit for the greenhouse assay was a single healthy-appearing mature root from each cultivar. From each root, three plugs were pulled and inoculated as described in the *R. solani* strain assay, while the *R. solani* AG-2-2 IIIB strain F508 and bacterial strain *L. mesenteroides* subsp. *dextranicum* strain B322 served as inoculum sources. After 3 weeks, the roots were cross sectioned (sliced along the length of the plug) and the diameter of the rotted area was measured perpendicular to plug. At the time of rot evaluation, isolations from 28 roots (all three inoculation points) were conducted to confirm the presence of the inoculated organism or contaminants. The assay was repeated the next year in 2010 with the same methods and cultivars

while using roots from a field planted on 16 April in American Falls, ID and harvested on 27 September and inoculated the next day.

Greenhouse assay after storage. In an effort to determine whether the greenhouse assay using roots at harvest was related more to conditions in the field or during storage, the greenhouse root rot assay in 2009 was conducted with a second set of roots from the same plots as the assay conducted at harvest. Storing the roots in a cold room at 4°C and 90% relative humidity for 60 days was the only change from the greenhouse assay at harvest time. The rest of the methods were the same as the greenhouse root assay at harvest, except the incubation period was reduced to 2 weeks, because the roots were in a weakened state from storage and some fungal contaminants were established on the root surface. The assay was repeated in 2010 using the same cultivars and methods as the previous year.

Data analysis. The SAS (version 9.2; SAS Institute Inc.) Univariate procedure was used to test for normality of the data. Disease variables were investigated using the SAS general linear mixed models procedure (Proc GLIMMIX). In the model statement, the fixed effect was treatment, the random effect was block, and the denominator degrees of freedom were calculated using the DDFM=KENWARDRODGER option. Mean comparisons were conducted using least squared means (LSMEANS) statement ($\alpha = 0.05$) while using the "lines" option to generate the output. Correlations based on regression analysis (Proc REG) and Spearman's coefficient of rank correlation and were also conducted with SAS. When means are followed by $\pm x$, x refers to the standard error.

Results

Field cultivar trial. Based on means from the 2010 and 2011 field studies, fungal rot ranged from 0 to 8%, bacterial rot ranged from 0 to 37%, total internal root rot ranged from 0 to 44%, and surface rot ranged from 0 to 52% (Tables 1 and 2). Based on regression analysis with the 2010 data, the positive relationships between surface rot and fungal ($r^2 = 0.8629$, $P < 0.0001$), bacterial ($r^2 = 0.7008$, $P < 0.0001$), and total internal root ($r^2 = 0.7890$, $P < 0.0001$) rots were all significant. Based on regression analysis with the 2011 data, the positive relationships between surface rot and fungal ($r^2 = 0.8798$, $P < 0.0001$), bacterial ($r^2 = 0.9164$, $P < 0.0001$), and total internal root ($r^2 = 0.9320$, $P < 0.0001$) rots were also significant. Based on regression analysis, there was also a positive relationship between fungal and bacterial rot in 2010 ($r^2 = 0.6628$, $P < 0.0001$) and 2011 ($r^2 = 0.8073$, $P < 0.0001$). All four disease variables resulted in significant ($P < 0.0001$) cultivar differences for both years (Tables 1 and 2). Based on surface rot, 'B-7', 'HM080006', and 'C-25' ranked lower than the susceptible check in both years. 'C-204', 'C-208', 'HH017', and 'HM070006' were among the most resistant cultivars and not significantly different from the resistant and noninoculated checks both years. The Spearman's rank coefficients for 21 of the 28 variable-year comparisons were significant (Table 3). The most consistent variable to use for cultivar ranking was surface rot, because 12 of 13 variable-year combinations were significant (Table 3). The other variables, in decreasing order of consistency, were fungal rot (11 of 13 were significant), total internal rot (9 of 13), and bacterial rot (8 of 13). Based on isolations from 30 roots (15 each year), *R. solani* could be confirmed in 73% of the roots while *L. mesenteroides* could be confirmed in 35% of the roots. Isolations were frequently hindered by bacterial and yeast contaminants.

***R. solani* strain comparison.** With the *Rhizoctonia* inoculation, all *R. solani* strains were pathogenic based on a comparison with the noninoculated check (Table 4). There were significant differences between strains at times but significant separation of strains was not consistent between years. With the *Rhizoctonia* + *Leuconostoc* inoculation, all strain combinations resulted in significant rot based on a comparison with the noninoculated check (Table 4). On average, the amount of rot doubled both years when *L. mesenteroides* was inoculated with *R. solani*. In 2009 and 2010 without

the noninoculated check, the *Rhizoctonia* inoculation had a mean rot of 12 ± 3 and 16 ± 2 mm, respectively, while the *Rhizoctonia* + *Leuconostoc* inoculation had a mean rot of 26 ± 2 and 32 ± 9 mm, respectively. When *L. mesenteroides* was inoculated alone, no rot occurred. Based on isolations from 14 roots (7 per year), *R. solani* could be confirmed 100% of the time in the *Rhizoctonia* inoculation, whereas it was isolated only 21% of the time when inoculated with *Leuconostoc*. On the other hand, *L. mesenteroides* could be confirmed 79% of the time in the *Rhizoctonia* + *Leuconostoc*

inoculation but was also isolated 50% of the time in the *Rhizoctonia* inoculation.

Greenhouse assay at harvest and after storage. The *Rhizoctonia* and *Rhizoctonia* + *Leuconostoc* inoculations resulted in rot in all roots for the greenhouse assays at harvest and differences between cultivars in both years (Table 5). After 60 days in storage, the same inoculations resulted in rot in all roots but differences between cultivars could only be established for the *Rhizoctonia* + *Leuconostoc* inoculation in 2009 (Table 6). Cultivar rankings for the

Table 2. Rhizoctonia and bacterial root rot measured during 12 September 2011 in 26 commercial sugar beet cultivars and 2 check cultivars or lines inoculated with *Rhizoctonia solani* anastomosis group 2-2 IIIB strain F517 in a field trial conducted in Kimberly, ID

Cultivar ^y	Internal root mass rotted (%) ^x				Surface (%)
	Fungal	Bacterial	Total		
B-7	6.4 a	37.1 a	44 a	52 a	
HM080006	5.4 a-c	28.5 a-d	34 a-d	49 ab	
HH015	5.6 ab	36.6 a	42 ab	46 a-c	
C-19	5.0 a-c	33.8 a-c	39 a-c	44 a-d	
C-25	5.0 a-d	28.0 a-d	33 a-d	42 a-e	
SV001	5.3 a-c	28.0 a-d	33 a-d	41 a-f	
HM070022	4.1 b-g	34.6 a-c	39 a-c	40 a-f	
FC901/C817	4.2 a-f	28.0 a-d	32 a-d	38 a-g	
C-29	4.6 a-e	25.4 a-e	30 a-e	37 a-g	
B-37	4.9 a-e	26.6 a-e	32 a-e	36 a-g	
B-38	4.3 a-f	24.5 a-f	29 a-e	34 a-h	
C-12	4.6 a-e	15.6 d-h	20 c-h	29 b-i	
B-5	3.6 b-h	11.2 d-h	15 d-h	27 c-i	
HM080004	3.6 b-h	14.5 d-h	18 d-h	26 c-i	
SV003	3.3 c-h	18.8 b-g	22 b-f	25 d-i	
SV007	3.4 b-h	17.5 c-h	21 c-g	24 d-i	
C-28	3.4 b-h	15.3 d-h	19 c-h	21 e-j	
B-39	2.8 d-i	12.8 d-h	16 d-h	20 f-j	
C-27	2.2 f-j	11.6 d-h	14 d-h	18 g-j	
B-34	2.7 e-i	8.7 e-h	11 e-h	17 g-j	
HH016	3.6 b-h	11.2 d-h	15 d-h	17 g-j	
HM080011	1.8 h-k	4.8 gh	7 f-h	14 h-j	
C-208	1.8 g-k	3.6 gh	5 f-h	13 h-j	
C-204	1.7 h-k	6.1 gh	8 f-h	13 ij	
HH017	1.8 h-k	6.5 f-h	8 f-h	11 ij	
C-11	1.0 i-k	0.4 h	1 gh	3 j	
HM070006	0.2 jk	0.0 h	0 h	1 j	
FC705/1	0.0 k	0.0 h	0 h	1 j	
Noninoculated check	0.0 k	0.0 h	0 h	0 j	
Overall mean	3.3	16.9	20	26	
$P > F^z$	<0.0001	<0.0001	<0.0001	<0.0001	

^x Fungal = percentage of rotted root mass associated with dry black rot caused by *R. solani*, Bacterial = percentage of rotted root mass associated with wet rot (relied on natural inoculum for *Leuconostoc mesenteroides*), Total = percentage of root mass associated with fungal and bacterial rot, and Surface = percentage of root surface with a dry black rot caused by *R. solani*. Means within a column followed by the same letter did not differ significantly based on least square means ($\alpha = 0.05$).

^y All cultivar names are coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SV = SESVanderHave) but the respective companies can be contacted using the code to gain additional information on the cultivars. FC901/C817 (susceptible) and FC705/1 (resistant) were the *R. solani* check cultivars provided by the United States Department of Agriculture–Agricultural Research Service sugar beet program in Ft. Collins, CO. B-5 was the noninoculated check cultivar.

^z $P > F$ was the probability associated with the F value. Data were analyzed in SAS using Proc GLIMMIX.

Table 3. Spearman's rank correlation on 26 commercial sugar beet cultivars evaluated for the Rhizoctonia-bacterial root rot complex after being inoculated in the field during the 2010 and 2011 growing seasons with the *Rhizoctonia solani* anastomosis group 2-2 IIIB strain F517 in Kimberly, ID

Variable ^z	Internal							
	Fungal rot		Bacterial rot		Total rot		Surface rot	
	2010	2011	2010	2011	2010	2011	2010	2011
Fungal rot 2010	1.000
Fungal rot 2011	0.393**	1.000
Bacterial rot 2010	0.933**	0.420**	1.000
Bacterial rot 2011	0.263	0.918**	0.283	1.000
Total rot 2010	0.956**	0.423**	0.991**	0.269	1.000
Total rot 2011	0.274	0.930**	0.297	0.997**	0.285	1.000
Surface rot 2010	0.972**	0.422**	0.927**	0.317	0.948**	0.327*	1.000	...
Surface rot 2011	0.388**	0.966**	0.410**	0.956**	0.403**	0.963**	0.434**	1.000

^z Fungal rot = percentage of internal root mass associated with Rhizoctonia root rot, Bacterial rot = percentage of internal root mass associated with bacterial root rot, Total rot = fungal and bacterial rot combined, Surface rot = percentage of root surface area discolored by root rot, ** = r value significant with $P \leq 0.05$, and * = r value significant with $P \leq 0.10$. Although *R. solani* had been inoculated, the bacterial rot relied on natural infestation by *Leuconostoc mesenteroides*.

Table 4. Diameter of rotted area after 2 weeks in a greenhouse root assay from six *Rhizoctonia solani* anastomosis group (AG)-2-2 IIIB strains inoculated alone and in combination with *Leuconostoc mesenteroides* subsp. *dextranicum* strain B322 into mature sugar beet roots of commercial sugar beet 'B-5' hand harvested from disease-free field plots in 2009 and 2010 from Kimberly, ID

Strain ^y	Root rot (mm) ^x			
	<i>Rhizoctonia</i>		<i>Rhizoctonia</i> + <i>Leuconostoc</i>	
	2009	2010	2009	2010
F521	10 b	18 ab	24 ab	41 a
F551	ND	16 ab	ND	40 a
F552	ND	16 ab	ND	38 a
F548	13 ab	19 a	28 a	27 a
F508	10 b	13 b	29 a	24 a
F517	16 a	14 ab	22 b	21 a
Check	0 c	0 c	0 c	0 b
Mean	10	14	21	27
<i>P</i> > <i>F</i> ^z	<0.0001	<0.0001	<0.0001	0.0054

^x *Rhizoctonia* = root inoculated with *R. solani* strain. *Rhizoctonia* + *Leuconostoc* = root inoculated with *R. solani* strain plus *L. mesenteroides* subsp. *dextranicum* strain B322. Means within a column followed by the same letter did not differ significantly based on least square means ($\alpha = 0.05$); ND = no data.

^y *R. solani* AG-2-2 IIIB strains from Idaho; Check = noninoculated check and Mean = overall mean.

^z *P* > *F* was the probability associated with the *F* value. Data were analyzed in SAS using the Proc GLIMMIX procedure.

2009 and 2010 *Rhizoctonia* inoculations at harvest were positively related in 6 of the 8 year-treatment combinations associated with storage data, whereas there were no relationships with all 16 year-treatment combinations associated with field data (Table 7). When considering the *Rhizoctonia* + *Leuconostoc* inoculations at harvest, the cultivar rankings showed no correlation with data from the field but did have a significant positive correlation twice with storage data.

In 2009 and 2010, when *L. mesenteroides* was inoculated alone, the number of roots with rot ranged from 4 to 15% in roots from the 26 commercial sugar beet cultivars, depending on year and inoculation time (*data not shown*). Because of the low incidence of rot associated with the *Leuconostoc* inoculation, analysis of variance and mean separation were not attempted with these data.

Based on isolations from 112 roots when *R. solani* was inoculated individually, *R. solani* could be confirmed 66% of the time, while 28% of the time *L. mesenteroides* was also present. Isolations from these same roots for the *Rhizoctonia* + *Leuconostoc* inoculation indicated that *L. mesenteroides* could be confirmed in 61% of the samples, whereas *R. solani* was only identified in 12% of the samples. Isolations from the *Leuconostoc* inoculation indicated that *L. mesenteroides* could be found in 100% of the samples, whereas *R. solani* was not isolated. Isolations where *R. solani* was involved were frequently hindered by bacterial and yeast contaminants. No rot or contaminants were evident in the noninoculated checks.

Across the greenhouse studies at harvest and after storage, contaminating fungal rot other than the dry black rot associated with

Table 5. Diameter of rotted area after 3 weeks in a greenhouse root assay with roots inoculated at harvest time with *Rhizoctonia solani* strain F508 inoculated alone or in combination with *Leuconostoc mesenteroides* subsp. *dextranicum* strain B322 into mature sugar beet roots of 26 commercial sugar beet cultivars harvested from disease-free field plots in 2009 and 2010 from a cultivar trial in American Falls, ID

Cultivar ^y	Root rot (mm) ^x			
	<i>Rhizoctonia</i>		<i>Rhizoctonia</i> + <i>Leuconostoc</i>	
	2009	2010	2009	2010
HH015	27 a-e	35 ab	24 b-f	43 a
C-11	27 a-e	36 a	15 f	42 ab
B-5	45 a	14 gh	24 b-f	41 ab
B-34	29 a-e	26 b-f	16 ef	36 a-c
HH016	23 c-e	26 b-f	18 c-f	34 a-d
HM080011	31 a-e	30 a-d	19 b-f	32 a-e
HM070006	23 c-e	20 d-h	17 d-f	32 a-e
B-37	29 a-e	30 a-c	34 ab	32 a-e
HM070022	12 ef	13 gh	16 d-f	30 a-e
C-25	41 a-c	17 f-h	23 b-f	30 b-e
C-27	42 a-c	21 c-h	39 a	29 b-f
C-19	27 a-e	29 a-e	20 b-f	25 c-g
C-204	34 a-d	32 ab	26 a-f	25 c-g
B-38	46 a	26 b-f	32 a-d	25 c-g
HM080006	24 b-e	19 e-h	16 d-f	25 c-g
B-7	18 de	14 gh	19 b-f	24 c-g
HH017	42 a-c	14 gh	23 b-f	23 d-g
C-28	28 a-e	22 c-g	24 a-f	23 d-g
C-29	44 ab	29 a-e	30 a-e	23 d-g
SV003	37 a-d	22 c-g	26 a-f	22 d-g
SV007	30 a-e	22 c-g	33 a-c	22 d-g
C-208	33 a-d	26 a-e	34 ab	20 e-g
C-12	47 a	26 a-f	22 b-f	18 fg
HM080004	27 a-e	32 ab	14 fg	17 fg
B-39	24 b-e	25 b-f	25 a-f	17 fg
SV001	22 c-e	10 hi	18 c-f	13 gh
Noninoculated check	0 f	0 i	0 g	0 h
Overall mean	30	23	22	26
<i>P</i> > <i>F</i> ^z	0.0452	<0.0001	0.0441	<0.0001

^x *Rhizoctonia* = root inoculated with *R. solani* strain F508. *Rhizoctonia* + *Leuconostoc* = root inoculated with *R. solani* strain F508 plus *L. mesenteroides* subsp. *dextranicum* strain B322. Means within a column followed by the same letter did not differ significantly based on least square means ($\alpha = 0.05$).

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

^z *P* > *F* was the probability associated with the *F* value. Data were analyzed in SAS using Proc GLIMMIX.

Table 6. Diameter of rotted root area after 2 weeks in a greenhouse root assay with roots inoculated after 60 days in storage with *Rhizoctonia solani* strain F508 inoculated alone or in combination with *Leuconostoc mesenteroides* subsp. *dextranicum* strain B322 into mature sugar beet roots of 26 commercial sugar beet cultivars harvested from disease-free plots in the 2009 and 2010 cultivar trials in American Falls, ID

Cultivar ^y	Root rot (mm) ^x			
	<i>Rhizoctonia</i>		<i>Rhizoctonia</i> + <i>Leuconostoc</i>	
	2009	2010	2009	2010
HM080011	16	16	30 b-e	22
C-28	21	18	21 f-j	20
C-11	17	19	17 ij	20
C-204	18	18	28 b-g	19
HH015	15	13	31 b-d	18
HH016	14	14	15 ij	18
HH017	14	15	21 f-j	18
SV001	15	15	20 f-j	17
C-208	17	14	30 b-e	17
C-29	14	14	32 bc	17
B-39	12	16	29 b-f	17
B-37	18	19	21 e-j	16
B-38	16	16	36 ab	16
B-7	13	12	18 h-j	15
SV007	18	13	24 c-i	15
HM080004	16	16	22 d-j	15
C-12	20	17	21 e-j	14
SV003	17	15	23 c-i	14
B-34	15	16	17 ij	14
C-27	16	15	44 a	14
HM070006	14	12	14 j	14
HM070022	14	11	20 f-j	14
HM080006	15	12	23 c-i	13
C-25	21	13	27 b-h	13
B-5	17	15	19 g-j	12
C-19	17	13	28 b-g	12
Check	0	0	0 k	0
Mean	16	14	23	15
<i>P</i> > <i>F</i> ^z	0.0766	0.2155	<0.0001	0.1631

^x *Rhizoctonia* = root inoculated with *R. solani* strain F508. *Rhizoctonia* + *Leuconostoc* = root inoculated with *R. solani* strain F508 plus *Leuconostoc mesenteroides* subsp. *dextranicum* strain B322. Means within a column followed by the same letter did not differ significantly based on least square means ($\alpha = 0.05$).

^y All cultivar names are coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleleshog, and SV = SESVanderHave) but the respective companies can be contacted using the code to gain additional information on the cultivars. Check = noninoculated check and Mean = overall mean.

^z *P* > *F* was the probability associated with the *F* value. Data were analyzed in SAS using Proc GLIMMIX.

R. solani was present in 21% of the *Rhizoctonia* inoculations, 7% of the *Rhizoctonia* + *Leuconostoc* inoculations, and none of the *Leuconostoc* inoculations. Based on 60 isolations from the contaminating fungal rots, *Rhizopus* spp. and *Aspergillus* spp. were present 52 and 48% of the time, respectively.

Discussion

Previous work evaluating *Rhizoctonia* root rot in sugar beet roots has been focused on fungal rot (3,9,30,32) but, recently, a bacterial root rot process was discovered and described (39). Cultivar evaluation for bacterial root rot for stored roots has been described (37) but it is not applicable to large screening nurseries and likely does not relate to the field. The current study evaluates the *Rhizoctonia*-bacterial root rot complex in both the field and storage environments. Although there was a strong relationship among the four (fungal, bacterial, total, and surface) variables evaluated in the field, surface rot was the best for cultivar separation based on rankings, had the largest range of values, and also performed as well as any of the other variables for mean separation. Rating the root surface also is faster and easier, because the root does not need to be bisected as it does for the other three variables.

In the 2010 and 2011 field studies, the four *Rhizoctonia*-bacterial disease variables (fungal, bacterial, total internal, and surface

rot) all resulted in significant ($P < 0.0001$) cultivar differences. Based on regression analysis, strong positive relationships ($P < 0.0001$) were present among the disease variables. When ranking cultivars, the most consistent variable was surface rot, because 12 of 13 variable-year combinations had significant correlations. The greenhouse assay at harvest with mature roots from the field allowed for good cultivar separation with *R. solani* inoculations both with and without *Leuconostoc*. After the roots had been stored for 60 days, cultivar separation with the greenhouse assay was marginal or not possible. When cultivar ranking in the greenhouse assays were compared, there was frequently a positive correlation with storage data but, when compared with field results, there was no relationship. Thus, the greenhouse assay seems to relate to storage and will not substitute for conducting field screening. Fungal strain comparisons with the greenhouse assay at harvest indicated that all *R. solani* strains were pathogenic but establishing consistent differences between strains across years was problematic. When conducting comparisons with both *R. solani* and *L. mesenteroides* strains in the future, work will likely have to be done with mature roots in the field because, if roots are removed from the field, there is a storage response and not a field response.

Previous studies have shown that the bacterial root rot in sugar beet appeared to frequently be associated with *Rhizoctonia* root rot in the field (36,40). However, the fungal rot was only associated with 3 to 5% of the root mass and the majority of the rot, 6 to > 70% of the root mass, was related to bacterial rot (40). The field studies in 2010 and 2011 support these earlier findings, because fungal rot means ranged from 0 to 8% and bacterial rot means ranged from 0 to 37%. The regression analysis also indicated there was a strong positive relationship ($P < 0.0001$) between the surface, fungal, and bacterial rots.

Although there is a link with *Rhizoctonia* root rot, bacterial root rot appeared to occasionally be initiated without the presence of fungal rot at times in previous field collections and studies (39,40). In the six studies conducted under greenhouse conditions with *L. mesenteroides* inoculated individually, there was only a low frequency (0 to 15% of roots) of bacterial root rot established and isolations always confirmed the presence of *L. mesenteroides*. These data fit with what has been seen in previous field observations and studies (39,40). However, when *L. mesenteroides* was combined with *R. solani*, there was rot in every root and each root had twice as much internal rot as when *R. solani* was inoculated alone. Thus, these data provide support that *R. solani* has a synergistic interaction with *L. mesenteroides*, which helps establish the bacterial root rot phase. However, the data also indicate that there may be some inhibition by the *L. mesenteroides* associated with bacterial root rot. When *R. solani* was inoculated with *L. mesenteroides*, *R. solani* could only be isolated from 12% of the samples in greenhouse assays at harvest and after storage. However, when *R. solani* was inoculated individually, it was isolated from 66% of the samples; however, *L. mesenteroides* was also isolated from 28% of these samples. Because the roots were not sterilized prior to inoculation, contaminating dust from the air and root surface could have gotten in the inoculation site when the plug was removed and replaced. However, the noninoculated checks and the *L. mesenteroides*-only inoculations did not have problems with contaminants. These data help clarify the synergistic role *R. solani* appears to play in allowing other organisms to invade and become established in the root tissue.

The isolation and contaminant data indicate that *R. solani* frequently allowed not only bacteria to get established in the root but fungal contaminants as well. When *R. solani* was inoculated individually in the greenhouse assays at harvest and after storage, fungal contaminants (*Rhizopus* spp. and *Aspergillus* spp.) became established in 21% of these inoculations, whereas this was observed in only 7% of the *Rhizoctonia* + *Leuconostoc* inoculations. In the noninoculated checks and when *L. mesenteroides* was inoculated individually, there were no fungal contaminants observed. In Michigan fields, *R. solani* was recently documented to interact

Table 7. Spearman's rank correlation for 26 commercial sugar beet cultivars using variables evaluated in the field, greenhouse, and storage in Idaho

Variable (year, treatment) ²	Greenhouse assays ³							
	Inoculated at harvest				Inoculated after 60 days in storage			
	<i>Rhizoctonia solani</i>		<i>R. solani</i> + <i>Leuconostoc</i>		<i>R. solani</i>		<i>R. solani</i> + <i>Leuconostoc</i>	
	2009	2010	2009	2010	2009	2010	2009	2010
2009 R at harvest in GH	1.000
2010 R at harvest in GH	0.201	1.000
2009 R + L at harvest in GH	0.607**	0.134	1.000
2010 R + L at harvest in GH	-0.071	0.182	-0.183	1.000
2009 R after storage in GH	0.516**	0.274	0.346*	-0.035	1.000
2010 R after storage in GH	0.452**	0.510**	0.271	0.022	0.437**	1.000
2009 R + L after storage in GH	0.454**	0.345*	0.582**	-0.240	0.198	0.058	1.000	...
2010 R + L after storage in GH	-0.043	0.459**	0.143	-0.042	0.003	0.473**	0.103	1.000
2010 R in field, percent fungal	-0.021	-0.150	-0.069	-0.014	0.104	-0.059	0.028	-0.162
2010 R in field, percent bacterial	0.034	-0.132	-0.016	-0.162	0.201	0.003	-0.010	-0.212
2010 R in field, percent total	0.030	-0.128	-0.036	-0.128	0.188	0.001	-0.001	-0.172
2010 R in field, percent surface	-0.008	-0.203	-0.001	-0.075	0.100	-0.136	0.088	-0.186
2011 R in field, percent fungal	-0.169	-0.195	-0.064	-0.110	-0.014	-0.382*	0.094	-0.295
2011 R in field, percent bacterial	-0.225	-0.243	0.017	-0.149	-0.017	-0.455**	0.180	-0.321
2011 R in field, percent total	-0.232	-0.236	0.003	-0.144	-0.011	-0.451**	0.156	-0.317
2011 R in field, percent surface	-0.149	-0.236	-0.036	-0.119	0.011	-0.427**	0.183	-0.399

³ *Leuconostoc* = *Leuconostoc mesenteroides*, ** = *r* value significant with $P \leq 0.05$, and * = *r* value significant with $P \leq 0.10$.

² R = inoculated with *R. solani*, R + L = inoculated with both *R. solani* and *Leuconostoc mesenteroides*, at harvest in GH = inoculated at harvest in the greenhouse assay, at storage in GH = inoculated after 60 days in storage in the greenhouse assay, in field = inoculated at eight-leaf growth stage in field, percent fungal = percentage of internal root mass with dry black fungal rot caused by *R. solani*, percent bacterial = percentage of internal root mass with wet bacterial rot, percent total = percentage of internal root mass with fungal or bacterial rot, and percent surface = percentage of rotted root surface.

with *Rhizopus stolonifer*, leading to additional rot (18). In Idaho, *Rhizoctonia solani* appears to facilitate the entry of both bacterial and fungal contaminants, which leads to increased rot in root tissue (36,39,40).

Bacterial rots typically contain other bacteria or yeast that get established in the rotted tissue along with *L. mesenteroides* (39). However, previous studies show bacteria in the soil, root surface, and contaminated rotted tissue cause little or no rot on their own but will reduce rot caused by *R. solani* or *L. mesenteroides* (25,26,39,46). Following the initiation of the fermentation or rotting process started by *L. mesenteroides*, this bacterium is typically superseded by other bacteria and yeast (1,2,16,20). These bacteria and yeast that supersede *L. mesenteroides* likely explain some of the difficulty in isolating both *R. solani* and *L. mesenteroides*. These bacteria also likely explain why *R. solani* is limited to just the outer portion of the root in Idaho and reduced the frequency of fungal contaminants in greenhouse assays. To limit the influence of contaminating bacteria and fungi, the greenhouse assay conducted at harvest time potentially would benefit from a shorter incubation period such as 2 weeks rather than 3 weeks.

When *Rhizoctonia* root rot was limited by resistance, the bacterial root rot was also limited in comparisons between the disease variables based on both regression and Spearman's coefficient of rank analysis. This observation is important because good resistance to *R. solani* alone should allow for control of the *Rhizoctonia*-bacterial root rot complex. Traditionally, *Rhizoctonia* root rot has been evaluated by placing roots into disease classes based on a 0-to-7 scale (32) or a 0-to-9 scale (9). In the present study, percentage data were utilized so that both fungal and bacterial rot could be assessed simultaneously and in a continuous manner. However, if identifying *Rhizoctonia* root rot resistance is the goal, then all approaches mentioned should work. When selecting for resistance in the field, surface observations on the root should be sufficient, because both surface and internal observations were highly related. Hopefully good resistance can be incorporated into a higher percentage of the commercial cultivars, because many cultivars were more susceptible than the resistant check. To supplement host resistance, good crop rotations (7,8,14,23,31,33,45) and fungicide applications (5,21,22,42,44) will likely be needed. Also, resistance to rot in the field appears to be different from controlling rot in storage. Thus, research to control rot in the field and storage will have to be considered independently.

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