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Interaction of *Rhizoctonia solani* and *Leuconostoc* spp. causing sugar beet root rot and tissue pH changes in Idaho

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Abstract: Late season Rhizoctonia root rot in sugar beet is a serious yield limiting disease problem caused by an interaction between *Rhizoctonia solani* and *Leuconostoc* spp. in Idaho. To better understand this interaction, the two most common *Leuconostoc* haplotypes were co-inoculated with 17 *R. solani* strains representing the range of genetic diversity established previously. The study was conducted twice by inoculating sugar beet roots in the field using a plug assay and measuring root rot and tissue pH. *L. mesenteroides* strain L12311 (17 mm of rot) had significantly ($P < 0.0001$) more rot than *L. pseudomesenteroides* strain L12487 (13 mm) when combined with the fungal strains. The *R. solani* anastomosis group (AG) 2–2 IIIB strains (16 mm of rot) led to significantly ($P < 0.0001$–0.0073) more rot than strains associated with other AGs: 2–2 IV (11 mm), 4 HG-I (5 mm) and 4 HG-II (3 mm). When *R. solani* AG-2-2 IIIB strains from three phylogenetic groups were compared, rot ranged from 15 to 17 mm and did not differ ($P = 0.1275$–0.5565). The pH for root tissue with at least 30 mm of rot was lower (4.0–4.2 ± 0.2–0.4) than tissue with < 2 mm of rot and water checks (6.2–6.4 ± 0.1–0.2). Both isolations and tissue pH suggest late season sugar beet root rot is primarily associated with *Leuconostoc* and secondary organisms. However, damage was minor without both *R. solani* AG-2-2 and *Leuconostoc* strains present when internal rot initiates.

Keywords: *Beta vulgaris*, *Leuconostoc*, Rhizoctonia root rot, *Rhizoctonia solani*, sugar beet

Résumé: La pourriture des racines causée par Rhizoctonia chez la betterave à sucre tard dans la saison est une maladie, résultant de l'interaction de *Rhizoctonia solani* et de *Leuconostoc* spp., qui limite sérieusement les rendements en Idaho. Afin de mieux comprendre cette interaction, les deux haplotypes les plus courants de *Leuconostoc* ont été co-inoculés avec 17 souches de *R. solani* représentant la gamme de diversité génétique préalablement établie. L'étude a été menée à deux reprises en inoculant, en champ, des racines de betteraves à sucre par la méthode du bouchon et en évaluant la pourriture des racines ainsi qu'en mesurant le pH des tissus. La souche L12311 de *L. mesenteroides* (17 mm de pourriture) affichait plus de pourriture ($P < 0.0001$) que la souche L12487 de *L. pseudomesenteroides* (13 mm), lorsque combinée aux souches fongiques. La catégorie d'anastomoses (AG) de *R. solani*, souches 2-2 IIIB (16 mm de pourriture) a engendré beaucoup plus de pourriture ($P < 0.0001$–0.0073) que les souches associées à d'autres AG: 2-2 IV (11 mm), 4 HG-I (5 mm) et 4 HG-II (3 mm). Lorsque les souches AG-2-2 IIIB de *R. solani* issues de trois groupes phylogénétiques ont été comparées, la pourriture a varié de 15 à 17 mm et était similaire ($P = 0.1275$–0.5565). Le pH du tissu racinaire affichant au moins 30 mm de pourriture était plus bas (de 4.0–4.2 ±0.2–0.4) que celui du tissu avec moins de 2 mm de pourriture et gestion de l'eau (de 6.2–6.4 ± 0.1–0.2). Les isolements et le pH des tissus suggèrent que la pourriture tardive des racines est principalement associée à *Leuconostoc* et à des organismes secondaires. Toutefois, lors de l'attaque initiale interne de la pourriture, sans les souches AG-2-2 de *R. solani* et de *Leuconostoc*, le dommage était minime.

Mots clés: *Beta vulgaris*, betterave à sucre, *Leuconostoc*, pourriture des racines causée par Rhizoctonia, *Rhizoctonia solani*

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Introduction

Rhizoctonia root rot (RRR) on sugar beet (*Beta vulgaris* L.) caused by *Rhizoctonia solani* Kühn can be a serious yield limiting problem worldwide (Kiewnick et al. 2001; Büttner et al. 2004; Führer Ithurrart et al. 2004; Ohkura et al. 2009; Strausbaugh et al. 2011a; Taheri and Tarighi 2012). In Idaho, the worst rot associated with *R. solani* occurs in the warmer, lower elevation, south-western production area where virtually entire sugar beet fields can be lost to RRR (Strausbaugh 2016). The rot in Idaho associated with *R. solani* late in the growing season has been shown to result from an interaction with *Leuconostoc* van Tiegham spp. and typically affects the side or bottom of the sugar beet root and not the crown (Strausbaugh and Gillen 2008, 2009). This contrasts with reports from northern regions of the USA (Montana, North Dakota and Minnesota) and the Great Lakes region where the disease typically begins in the crown, although infection can also develop at or below the soil line (Windels et al. 2009). Thus, the infection of sugar beet roots may differ between regions. The RRR not only negatively affects root and sucrose yield in the field but can also lead to additional losses in storage and factory processing (Cogan and Jordan 1994; Tallgren et al. 1999; Cescutti et al. 2005; Strausbaugh et al. 2011b).

Rhizoctonia along with other fungi and bacteria can alter the pH of their environment to some extent (Stiles 1994; Raspor and Goranovič 2008; Davidzon et al. 2010; Papadimitriou et al. 2016; Tardi-Ovadia et al. 2017). *Alternaria, Candida, Colletotrichum* and *Rhizoctonia* are fungi that can alkalize their environment, while *Botrytis* spp., *Fusarium oxysporum, Penicillium* spp. and *Sclerotinia sclerotiorum* acidify it (Davidzon et al. 2010; Tardi-Ovadia et al. 2017; Vylkova 2017; Xue et al. 2018). Acetic and lactic acid bacteria such as *Acetobactor, Gluconobacter, Lactobacillus* and *Leuconostoc* can also acidify their environment (Stiles 1994; Papadimitriou et al. 2016). Although some fungal species may grow over a wide pH range, pH-controlled gene expression can occur in fungal systems and their ability to degrade certain types of substrate can be strongly reduced at low pH (Kok et al. 1992; Akimitsu et al. 2004; Peñalva and Arst 2004; Miyara et al. 2008; Niture et al. 2008; Peñalva et al. 2008; Kubicek et al. 2014). Enzymes can also be deactivated by conformational changes in suboptimal pH even though fungal growth may be occurring (Niture et al. 2008). Thus, low pH can lead to low or even undetectable activities of hemicellulase, pectinase and pectin lyase, while cellulase activity may not be influenced by pH 4.0 (Lisker et al. 1975; Bugbee 1990; Kok et al. 1992). Since cell wall-degrading enzymes produced by fungi facilitate plant infection and improve virulence (Akimitsu et al. 2004; Kubicek et al. 2014; Xue et al. 2018), pH changes in sugar beet root tissue associated with the *R. solani*–*Leuconostoc* interaction were investigated to gain a better understanding of what may be occurring during late season rot.

The AG of *R. solani* primarily associated with RRR on mature roots has been 2–2 IIIB in Idaho and other production areas (Führer Ithurrart et al. 2004; Pfähler and Petersen 2004; Windels and Brantner 2005; Buhre et al. 2009; Bolton et al. 2010; Kluth et al. 2010; Strausbaugh et al. 2011a; Taheri and Tarighi 2012). However, strains from other *R. solani* AG have also been reported: 2–2 IV, 4 HG-I and 4 HG-II (Windels and Nabben 1989; Rush et al. 1994; Strausbaugh et al. 2011a). In Idaho, the four counties (Cassia and Minidoka counties in south-central Idaho; Bingham and Power counties in south-eastern Idaho) with the highest sugar beet production are also the same counties with the highest potato production (United States Department of Agriculture–National Ag Statistics Service). The RRR in these major production areas tends to be rather minor and superficial on the sugar beet roots and associated with *R. solani* AG-4 strains (Strausbaugh et al. 2011a). The areas with the worst RRR in Idaho are located in the south-western sugar beet production area where *R. solani* AG-2-2 IIIB strains combine with strains from *Leuconostoc mesenteroides* van Tiegham late in the growing season to create a synergistic interaction (Strausbaugh and Gillen 2009; Strausbaugh et al. 2011a; Strausbaugh 2016). The *L. mesenteroides* strains have been the primary strains associated with the rot complex since they tend to be isolated 85–88% of the time (Strausbaugh 2016). However, *Leuconostoc pseudomesenteroides* Farrow strains have also been isolated 6–15% of the time from the rot complex (Strausbaugh 2016). Representative haplotypes from these two *Leuconostoc* spp. and other less frequently isolated species were evaluated on sugar beet for pathogenicity and virulence individually and in combination with *R. solani* AG-2-2 IIIB strain F517 (Strausbaugh 2016). However, strain F517 only represents a small portion of genetic diversity of the *R. solani* strains associated with RRR in Idaho (Strausbaugh et al. 2011a). Thus, a more comprehensive comparison with *R. solani* strains representing a greater portion of the genetic diversity in Idaho was conducted along with the evaluation of root tissue pH.

Materials and methods

2017 pathogenicity study

To investigate the interaction between strains of *R. solani* and *Leuconostoc* spp., a field study was established on the United States Department of Agriculture–Agricultural Research Service North Farm (latitude 42.552883°

longitude −114.358293°, elevation 1190 m) near Kimberly, ID, in a field that has Portneuf silt loam soil and had been used to grow barley the previous year. In the spring, the field had been ploughed and then fertilized (with 100.8 kg ha^{-1} N and 123.3 kg ha^{-1} P_2O_5) and roller harrowed on 11 April. Seed of the *R. solani* susceptible (Strausbaugh 2016) commercial sugar beet cultivar B-7 (Betaseed Inc., Kimberly, ID) was planted with 56 cm between rows on 3 May 2017 to a density of 352 272 seed ha^{-1} and thinned to 117 424 plants ha^{-1} on 27 May. The field was managed using standard cultural practices described in the 2017 sugar beet grower's guide book (Amalgamated Sugar Co., Boise, ID). The experiment was arranged as a randomized complete block design with five replications. A total of 54 treatments were evaluated: a non-inoculated water check, two *Leuconostoc* strains inoculated individually, and 17 *R. solani* strains inoculated individually and in combination with each of the *Leuconostoc* strains (Table 1). The two *Leuconostoc* strains (*L. mesenteroides* strain L12311 haplotype 11 and *L. pseudomesenteroides* strain L12487 haplotype 23) were chosen since they represent the predominant haplotypes associated with RRR on sugar beet in Idaho (Strausbaugh 2016). The 17 *R. solani* strains were chosen since they represent the genetic diversity observed in a previous study (Strausbaugh et al. 2011a) for the following AG found in Idaho: 2–2 IIIB, 2–2 IV, 4 HG-I and 4 HG-II (Table 1). An individual root served as the experimental unit.

A cork borer plug (8 mm diameter × 24 mm deep) on the shoulder of the root (where the root meets the soil) was pulled to allow for inoculation on 1 August. For the water check, 0.1 mL of sterile well water was placed in the hole. For the fungal checks, a 2 × 2 mm piece of mycelial mass was placed in the hole with 0.1 mL of sterile well water. The mycelia were produced by growing the fungus for 10 days in potato dextrose broth (product no. 1.00510.0500, EMD Chemicals Inc., Gibbstown, NJ) using a shaker on the bench top at 22°C. The mycelia had been rinsed with sterile well water prior to use. For the bacterial checks, 0.1 mL of a 10^8 cfu mL^{-1} *Leuconostoc* strain suspension was inoculated. The bacterial inoculum was prepared using yeast-dextrose-calcium carbonate agar (YDC) as described previously (Strausbaugh et al. 2013a). For the combination treatments, both the fungal and bacterial strains were placed in the hole in the same amount as the individual inoculations. Following inoculation, the plug was replaced and sealed with petroleum jelly (Unilever, Greenwich, CT). The roots were dug and bisected through the inoculation site on 12 September to measure the amount of rot with a ruler perpendicular to the plug. To complete Koch's postulates, 45 isolations (5, 10, 10 and 20 isolations from the non-inoculated water checks, fungus only, bacteria only and combination treatments, respectively) were conducted on fungal and bacterial media. A 10 × 10 mm cube was cut from

Table 1. Background for fungal and bacterial strains utilized in the pathogenicity studies[a].

Strain	Genus	Species	AG	Group	Year	Origin
F16	*Rhizoctonia*	*solani*	2-2 IIIB	PG 2	2004	Minidoka, ID
F20	*Rhizoctonia*	*solani*	4 HG-I	4 HG-I	2004	Gooding, ID
F24	*Rhizoctonia*	*solani*	2-2 IV	2-2 IV	2004	Mora, ID
F27	*Rhizoctonia*	*solani*	4 HG-II	4 HG-II	2004	Bowmont, ID
F30	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2004	Homedale, ID
F32	*Rhizoctonia*	*solani*	4 HG-I	4 HG-I	2004	Nyssa, OR
F311	*Rhizoctonia*	*solani*	4 HG-II	4 HG-II	2005	Gooding, ID
F321	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2005	Mt. Home, ID
F501	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2006	Nampa, ID
F503	*Rhizoctonia*	*solani*	2-2 IIIB	PG 3	2006	Nampa, ID
F508	*Rhizoctonia*	*solani*	2-2 IIIB	PG 2	2006	Heyburn, ID
F514	*Rhizoctonia*	*solani*	2-2 IIIB	PG 2	2006	Mt. Home, ID
F517	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2006	Grandview, ID
F521	*Rhizoctonia*	*solani*	2-2 IIIB	PG 2	2006	Grandview, ID
F548	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2006	Jerome, ID
F551	*Rhizoctonia*	*solani*	2-2 IIIB	PG 1	2006	Jerome, ID
F552	*Rhizoctonia*	*solani*	2-2 IIIB	PG 3	2006	Jerome, ID
L12311	*Leuconostoc*	*mesenteroides*	N/A	Haplotype 11	2012	Mt. Home, ID
L12487	*Leuconostoc*	*pseudomesenteroides*	N/A	Haplotype 23	2012	Burley, ID

[a]The *Rhizoctonia* and *Leuconostoc* strains from sugar beet were characterized in previous studies (Strausbaugh et al. 2011a; Strausbaugh 2016). AG = *Rhizoctonia solani* anastomosis group. N/A = not applicable. Group = phylogenetic group (PG) or AG established in previous studies (Strausbaugh et al. 2011a; Strausbaugh 2016). Year = year of collection. Origin = nearest town to collection site.

the leading edge of the rotted tissue and surface sterilized using 0.5% NaOCl (vol/vol) for 1 min, followed by rinsing the cube in sterilized well water for 1 min, and removing the surface tissue. For fungal isolations, a 2 × 2 mm piece of the surface sterilized tissue was placed onto potato dextrose agar (PDA; Becton Dickson & Co., Sparks, MD) amended with streptomycin (200 mg L^{-1}) on the bench top at 22°C and evaluated for the next 7 days. For bacterial isolations, a 2 × 2 mm piece of the surface sterilized tissue was macerated in a drop of sterile well water and then streaked onto Difco Lactobacilli MRS agar (Becton Dickson & Co) and incubated at 30°C for at least 2 days.

2018 pathogenicity study

The 2017 field study was repeated in 2018 in a different area (latitude 42.552863° longitude −114.356953°, elevation 1187 m) of the same field utilized the previous year. This area of the field had been in barley in 2017. The materials and methods for this study were the same as those described for the 2017 pathogenicity study. The field was planted on 24 April. The roots were inoculated on 22 August and evaluated on 18 October.

Root tissue pH

Samples for pH were collected at the time the roots were bisected to measure root rot in both 2017 and 2018, but obtaining liquid to pH from all roots proved to be too laborious. Thus, pH samples were collected individually from the non-inoculated water checks and the first 4 roots from each replicate (20 roots total) that had <2 mm of rot and compared with readings from the first 4 roots from each replicate (20 roots total) that had at least 30 mm of rot. Both years, all the roots used for pH that were associated with at least 30 mm of rot came from combination treatments. A 15 mm × 15 mm cube of tissue was cut from the sugar beet root next to the point of inoculation. A 100 µL drop of juice from the root tissue was extracted using a garlic press and evaluated with a LAQUA Twin pH meter (Model 2103AL, Spectrum Technologies, Inc., Plainfield, IL) to establish the ambient pH of the root tissue sample.

Data analysis

The data were analysed for normality using the Univariate procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Homogeneity of variance was determined using Levene's test. Analysis of variance was performed using the SAS generalized linear models procedure (Proc GLM). Mean comparisons were conducted using Fisher's protected least significant difference ($\alpha = 0.05$). Mean comparisons across treatments were conducted using single degree-of-freedom orthogonal contrast statements. When means are followed by ± *x, x* refers to the standard error.

Results

Pathogenicity studies

The sugar beet root rot data for the 2017 and 2018 pathogenicity studies were not significantly different ($P = 0.2781$). Thus, the 2017 and 2018 studies were analysed together since the interactions were not significant (year × block, $P = 0.0650$; year × treatment, $P = 0.1602$; block × treatment, $P = 0.9971$) and Levene's test indicated the variances did not differ ($P = 0.5736$). Significant differences ($P < 0.0001$) among the treatments were evident in Table 2. There was no rot in the non-inoculated water checks (Table 2, Fig. 1). The rot associated with the individual inoculations for both bacterial strains and 13 of the 17 fungal strains was minor enough that it did not differ significantly from the non-inoculated water check (Table 2, Fig. 1). Strains F501, F503, F508 and F514 were the only fungal strains inoculated individually that led to rot that was significantly different than the non-inoculated water check. With combination treatments, 10 of the 34 combinations (six with *L. mesenteroides* strain L12311 and four with *L. pseudomesenteroides* strain L12487) were not significantly different from the non-inoculated water check (Table 2). Of the top 20 ranking treatments with the most rot, only one treatment (F514 individually) was not a combination treatment. Of the 19 combination treatments in the top 20, 12 combinations were with *L. mesenteroides* strain L12311 (Fig. 1) and seven were with *L. pseudomesenteroides* strain L12487.

When comparing the bacterial strains across the combination treatments in Table 3, *L. mesenteroides* strain L12311 averaged 17 mm of rot, which was significantly ($P < 0.0001$) more than the 13 mm of rot associated with *L. pseudomesenteroides* strain L12487. When comparing the *R. solani* AG across the combination treatments in Table 3, 2–2 IIIB strains (16 mm of rot) led to significantly (P ranged from <0.0001 to 0.0073) more rot than strains from 2–2 IV (11 mm), 4 HG-I (5 mm) and 4 HG-II (3 mm). AG-2-2 IV strains were also more virulent (P ranged from 0.0001 to 0.0019) than strains from the two AG-4 subgroups. The rot associated with strains from the AG-4 subgroups did not differ from the non-inoculated water check. When comparing strains from the three phylogenic groups within AG-2-2 IIIB, rot ranged

Table 2. Pathogenicity tests were conducted via Idaho field studies in 2017 and 2018 to investigate 54 treatments comparing *Leuconostoc* and *Rhizoctonia solani* strains inoculated into the commercial sugar beet cultivar B-7.

Treatment	*Leuconostoc*[a]	*Rhizoctonia*	Root rot (mm)
41	L12487	F501	38 a
27	L12311	F514	38 a
24	L12311	F501	37 ab
29	L12311	F521	31 a-c
42	L12487	F503	29 b-d
47	L12487	F548	29 b-d
23	L12311	F321	29 b-d
30	L12311	F548	26 c-e
49	L12487	F552	23 c-f
21	L12311	F30	22 d-f
32	L12311	F552	22 d-g
46	L12487	F521	19 e-g
26	L12311	F508	19 e-h
43	L12487	F508	18 e-i
33	L12311	F24	16 f-j
25	L12311	F503	16 f-k
10	None	F514	14 g-k
31	L12311	F551	11 h-m
22	L12311	F16	11 h-m
39	L12487	F16	11 i-n
50	L12487	F24	10 i-o
40	L12487	F321	10 i-p
7	None	F501	10 i-p
38	L12487	F30	10 i-p
44	L12487	F514	9 j-q
28	L12311	F517	9 j-q
9	None	F508	8 k-r
8	None	F503	8 k-r
34	L12311	F20	8 l-s
4	None	F30	7 l-s
11	None	F517	7 l-s
5	None	F16	7 l-s
45	L12487	F517	7 l-s
16	None	F24	6 l-s
51	L12487	F20	6 l-s
54	L12487	F311	6 l-s
37	L12311	F311	6 l-s
6	None	F321	6 m-s
13	None	F548	6 m-s
18	None	F32	5 m-s
52	L12487	F32	5 m-s
15	None	F552	4 m-s
12	None	F521	4 m-s
48	L12487	F551	4 m-s
17	None	F20	3 m-s
14	None	F551	3 m-s
35	L12311	F32	3 m-s
20	None	F311	3 n-s
53	L12487	F27	2 o-s
2	L12311	None	2 p-s
36	L12311	F27	2 p-s
19	None	F27	1 q-s
3	L12487	None	1 rs
1	None	None	0 s
P > *F*[b]			<0.0001
LSD (α = 0.05)			8

(*Continued*)

Table 2. (Continued.)

[a]*Leuconostoc* = *Leuconostoc* spp. (L12311 = *L. mesenteroides*, L12487 = *L. pseudomesenteroides*) and *Rhizoctonia* = *Rhizoctonia solani* strains from the following anastomosis groups: AG-2-2 IIIB (F16, F30, F321, F501, F503, F508, F514, F517, F521, F548, F551, F552), AG-2-2 IV (F24), AG4 HG-I (F20, F32) and AG4 HG-II (F27, F311). None = water.

[b]P > *F* was the probability associated with the *F* value. The means followed by the same letter did not differ significantly based on Fisher's protected least significant difference (LSD; α = 0.05). The sugar beet root rot data for the 2017 and 2018 pathogenicity studies were not significantly different (P = 0.2781). The interactions also were not significant (year × block, P = 0.0650; year × treatment, P = 0.1602; block × treatment, P = 0.9971). Since Levene's test indicated the variances did not differ (P = 0.5736), the two studies were analysed together.

from 15 to 17 mm and the three groups were not significantly different (P ranged from 0.0001 to 0.0019). In both years, isolations from the non-inoculated water checks were negative for the presence of *R. solani* and *Leuconostoc*. Isolations from the individual fungal inoculations were positive 60–70% of the time for *R. solani* depending on year and negative for *Leuconostoc*. Isolations from the individual bacterial inoculations were 80% positive for *Leuconostoc* in both years and negative for *R. solani*. Isolations not positive for the target organism were impacted by bacterial and yeast contaminants. Isolations from combination inoculations were only positive for *Leuconostoc* and/or bacterial and yeast contaminants and negative for *R. solani*.

Root tissue pH

In 2017, the pH for the non-inoculated water checks and sugar beet roots with < 2 mm of rot was 6.3 ± 0.2 and 6.2 ± 0.2, respectively. These readings were higher than the 4.2 ± 0.4 pH for roots with at least 30 mm of rot. In 2018, the pH for the non-inoculated water checks and sugar beet roots with < 2 mm of rot was 6.3 ± 0.1 and 6.4 ± 0.1, respectively. These readings were higher than the 4.0 ± 0.2 pH for roots with at least 30 mm of rot.

Discussion

A previous study (Strausbaugh 2016) had investigated the interaction of various *Leuconostoc* haplotypes versus *R.*

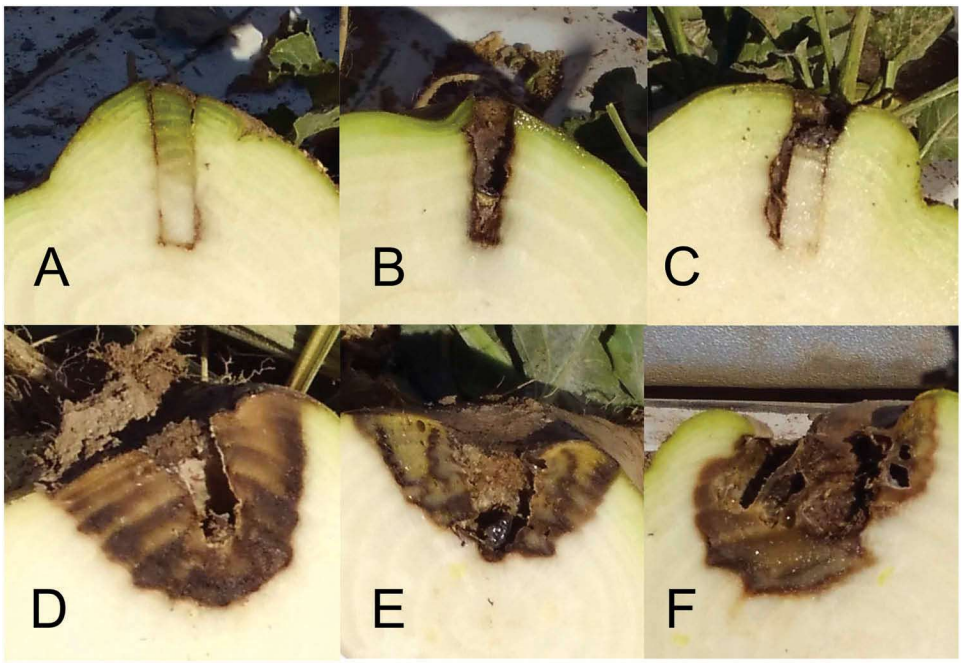

Fig. 1 (Colour online) Bisected roots from the 2018 field study with plug inoculations in the commercial sugar beet cultivar B-7 for the non-inoculated water check (Panel A), *Rhizoctonia solani* anastomosis group (AG) 2–2 IIIB strain F552 inoculated individually (Panel B), *Leuconostoc mesenteroides* strain L12311 inoculated individually (Panel C), and *L. mesenteroides* strain L12311 inoculated in combination with each of the following *R. solani* AG-2-2 IIIB strains: F552 (Panel D), F514 (Panel E) and F501 (Panel F).

Table 3. Single degree-of-freedom orthogonal contrasts to establish the response of different treatment subgroups from pathogenicity tests conducted in 2017 and 2018 Idaho field studies conducted with the commercial sugar beet cultivar B-7.

Variable[a]	Contrast (rot mean in mm)	Difference (%)	*F*	P > *F*
Leuconostoc spp.	*L. mesenteroides* (17) vs. No bacteria (6)	65	154	<0.0001
	L. pseudomesenteroides (13) vs. No bacteria (6)	54	66	<0.0001
	L. mesenteroides (17) vs. *L. pseudomesenteroides* (13)	24	18	<0.0001
R. solani AG	2-2 IIIB (16) vs. 2–2 IV (11)	31	7	0.0073
	2-2 IIIB (16) vs. 4 HG-I (5)	69	75	<0.0001
	2-2 IIIB (16) vs. 4 HG-II (3)	81	99	<0.0001
	2-2 IV (11) vs. 4 HG-I (5)	55	10	0.0019
	2-2 IV (11) vs. 4 HG-II (3)	73	15	0.0001
	4 HG-I (5) vs. 4 HG-II (3)	NS	1	0.3344
AG 2–2 IIIB subgroups	PG 1 (15) vs. PG 2 (16)	NS	<1	0.5565
	PG 1 (15) vs. PG 3 (17)	NS	2	0.1275
	PG 2 (16) vs. PG 3 (17)	NS	1	0.3175

[a]*R. solani* AG = *Rhizoctonia solani* anastomosis group. NS = not significant. The *R. solani* AG and phylogenetic subgroups (PG) were established in a previous study (Table 1; Strausbaugh 2016; Strausbaugh et al. 2011a). Contrast = orthogonal contrasts used to compare subgroups of the 54 treatments (Table 2) involving *Leuconostoc* and *Rhizoctonia solani* strains and a water check.

solani AG-2-2 IIIB strain F517, which left the interaction versus a genetically diverse range of *R. solani* strains (Strausbaugh et al. 2011a) unexplored. To fill this knowledge gap, a genetically diverse set of *R. solani* strains was compared in field studies individually and in combination with the two primary *Leuconostoc* haplotypes via rot and tissue pH in sugar beet roots. The synergistic interaction between either *Leuconostoc* strain and the *R. solani* AG-2-2 IIIB strains allowed for the most rot to occur and lowered the 6.2–6.4 pH of root tissue with < 2 mm of rot down to 4.0–4.2 in tissue with at least 30 mm of rot. Individually, both the bacterial and fungal strains primarily led to minor rot (8 mm or less) that was not significantly different from the non-inoculated water check (0 mm). On the other hand, 19 of the 20 top ranking treatments that led to the most rot were *R. solani*–*Leuconostoc* combination treatments. Combinations with *L. mesenteroides* led to more rot (17 mm) than those with *L. pseudomesenteroides* (13 mm). Likewise, combinations with AG-2-2 IIIB strains led to more rot (16 mm) than rot with strains in other AG: 2–2 IV (11 mm), 4 HG-I (5 mm) and 4 HG-II (3 mm). Both the isolation data and tissue pH suggest *Leuconostoc* spp. and subsequent bacterial and yeast contaminants were present in the rotted root tissue. However, without *R. solani* AG-2-2 strains being present

when internal rot initiates, *Leuconostoc* strains did very little damage.

This is the first investigation to show that *R. solani* AG-4 strains could not lead to a synergistic interaction when paired with *Leuconostoc* spp., while some combinations of *R. solani* AG-2-2 IIIB and IV strains with *Leuconostoc* spp. could lead to a synergistic interaction. In Idaho sugar beet fields where AG-4 predominates, rot tends to be restricted to the root surface and RRR tends not to be severe in these production areas (Strausbaugh et al. 2011a). In Idaho fields where AG-2-2 IIIB strains predominate and RRR can be severe, there tends to be considerable internal rot late in the season (Strausbaugh and Gillen 2009; Strausbaugh et al. 2011a; Strausbaugh 2016). The ability of some AG-2-2 strains to interact synergistically with *Leuconostoc* spp. may help explain the field observations in Idaho.

A previous study (Strausbaugh 2016) which had investigated the interaction of various *Leuconostoc* haplotypes versus one *R. solani* AG-2-2 IIIB strain F517 utilized fungal inoculum produced on sterile barley kernels in conjunction with the same plug-inoculation technique used in the present study. By introducing dried-ground inoculum on barley, an additional potential food source was introduced for the pathogens involved in the interaction. Thus, in the present study the fungal inoculum was introduced as mycelia cut from a mycelial mass produced in shake culture and rinsed prior to use. Despite the fungal inoculum differences, both inoculation approaches led to similar synergistic interaction results and conclusions. The internal rot symptoms evident with this inoculation approach were similar to those observed in sugar beet fields with RRR on mature roots. The only roots in the study with large cavities and cracks similar to that associated with RRR in the field late in the season were roots inoculated with both *R. solani* and *Leuconostoc* spp.

In the field study, the sugar beet roots were inoculated in the shoulder of the root rather than in the crown or petioles. In Idaho sugar beets, RRR associated with *R. solani* and *Leuconostoc* typically affects the side or bottom of the sugar beet root and not the crown and petioles (Strausbaugh and Gillen 2008, 2009). When the RRR nursery has been conducted in Idaho, dried barley inoculum (Ruppel et al. 1979) along with soil via cultivation were placed in the crowns of the sugar beet plants and infection still typically occurred on the side of the roots. This contrasts with a report from the Red River Valley of North Dakota and Minnesota, where *R. solani* infection was described as usually beginning at the bases of petioles and moving into the sugar beet crown rather than attacking the roots initially (Bugbee 1990). Thus, the infection of sugar beet roots may differ between regions or may have changed over time.

In non-inoculated water checks with no rot, isolations confirmed that *R. solani* and *Leuconostoc* were not present. When *R. solani* and *Leuconostoc* strains were inoculated individually very little rot was present and the introduced organism was the only pathogen reisolated. In cases when the inoculated strain was not recovered, isolations were primarily compromised by the presence of bacterial and yeast contaminants. The pH recorded for tissue with < 2 mm of rot for sugar beet roots in the field was 6.2–6.4, which was consistent with the 6.3 pH in the non-inoculated water checks and the pH of 6.0–6.8 reported for healthy tissue in sugar beet roots in other studies (Fife and Frampton 1935; Cole and Bugbee 1976; Bugbee 1990).

In *R. solani–Leuconostoc* combination treatments a synergistic interaction led to more rot than individual inoculations. Isolations from this rotted tissue identified the presence of *Leuconostoc* and/or bacterial and yeast contaminants and no *R. solani*, which confirms what was observed in previous studies (Strausbaugh 2016). The ambient pH in root tissue with at least 30 mm of rot (all roots were from combination treatments) was lowered down to 4.0–4.2. In a sugar beet greenhouse study with *R. solani* AG-2-2, the pH in infected petiole tissue increased to 8.4 and crown tissue increased to 7.1, while root tissue dropped from 6.5 down to 5.8 (Bugbee 1990). While the trend for root tissue to drop in pH is similar to the present field study, the pH reduction in the present study with rotted root tissue seems to be greater. The potting mix and conditions in the greenhouse study (Bugbee 1990) would likely have provided a considerably different environment from that found in an Idaho field study, which might explain the differences. In a storage experiment with the sugar beet roots held at 26°C, the pH in sugar beet root tissue dropped to ~5.0 under aerobic conditions and 4.0 with anaerobic conditions over a 21-day period as bacterial growth increased (Cole and Bugbee 1976). These pH changes with sugar beet roots in storage mirrored what occurred in the present study with *R. solani–Leuconostoc* sugar beet root rot on mature roots in the field.

The ambient pH of host tissue can be important since pH-controlled gene expression can occur in fungal systems (Kok et al. 1992; Peñalva and Arst 2004; Akimitsu et al. 2004; Miyara et al. 2008; Niture et al. 2008; Peñalva et al. 2008; Kubicek et al. 2014; Vylkova 2017). Suboptimal pH can also deactivate enzymes by conformational changes even though fungal growth may

be occurring (Niture et al. 2008). In sugar beet, the cell wall-degrading enzymes of *R. solani* AG-2-2 have been shown to directly correlate with virulence (El-Abyad et al. 1997). In bean tissue, polygalacturonase (PG) was noted to occur at high levels during initial infection with *R. solani* followed by cellulase (Cx) and then pectin lyase (PL) (Lisker et al. 1975). After 18 days as pH increased from 6.0 to 8.0, the PG activity disappeared (Lisker et al. 1975). Lisker et al. (1975) determined that enzyme production and activity were optimal at a pH of 4.0 and 5.0 for PG, 4.0 and 5.5 for Cx and 8.0 and 7.5 for PL, respectively. With *R. solani* AG-4, the pH optima for PG-I, PG-II, pectin esterase and PL were 4.8, 5.4, 7.7 and 8.4, respectively (Marcus et al. 1986). With a *R. solani* AG-2-2 culture, Bugbee (1990) found the optimum pH in culture was 8.0 for PL. Bugbee (1990) also determined that PL was much more active and in larger quantities than PG in both culture and infected sugar beet tissue. Recently, a novel fungal enzyme was discovered in *R. solani* and classified as a *p*-coumaroyl esterase (Nieter et al. 2017). This novel enzyme had maximum activity for hydrolysis of methyl ferulate at pH 6.0, but it was no longer stable when ambient pH was lowered to 4.0 (Nieter et al. 2017). When *R. solani* AG1-IA, the causative organism of peanut sheath blight, was investigated an increase in pH was recorded in decayed peanut tissues (Xue et al. 2018). Other reports have also noted that an increase in host ambient pH was critical for fungal pathogenicity (Prusky et al. 2001; Eshel et al. 2002; Tardi-Ovadia et al. 2017). In three different growth media, the growth rate of *R. solani* AG1-IA was significantly correlated with an increase in pH (Xue et al. 2018). In a study with *R. solani* AG-2-2 IIIB, fungal growth was optimal at pH 5.5 and was observed to raise the pH of buffered malt extract broth (MEB) from 4.5 to 5.2 (Wantanabe et al. 2011). Thus, the lowering of ambient pH in rotted sugar beet root tissue in the field studies goes against what would be expected if *R. solani* was the dominant organism and is consistent with not isolating *R. solani* from the combination treatments.

For low pH rotted sugar beet root tissue, the presence of *Leuconostoc* along with lactic (*Lactobacillus*) and acetic acid (*Acetobacter* and *Gluconobacter*) bacteria and yeast (*Candida* and *Pichia*) contaminants (Strausbaugh and Gillen 2008) matches the isolation data from the combination treatments. Growth optima for *L. mesenteroides* subsp. *dextranicum* strains can vary from a pH of 4.2 to 6.5 (Demirci and Hemme 1995). *Leuconostoc* spp. would be expected to predominate during the initial phases of the rotting-fermentation process, but they tend to die off rapidly when pH reaches 4.0 and lower (MacDonald et al. 1990; Gardner et al. 2001). Other lactic acid bacteria associated with rotting sugar beet tissue like *Lactobacillus* will tolerate pH down to 3.0 (MacDonald et al. 1990; Strausbaugh and Gillen 2008). At a pH of 4.0, the acetic acid bacteria (*Acetobacter* and *Gluconobacter*) and yeast (*Candida* and *Pichia*) contaminants known to be associated with sugar beet root rot would also grow well (Raspor and Goranovič 2008; Strausbaugh and Gillen 2008; Liu et al. 2012; Mamlouk and Gullo 2013; Qvirist et al. 2016; Belda et al. 2017; Wang et al. 2018). If yeast cell number is high or at least equal to the cells of lactobacilli, growth of lactobacilli is inhibited (Thomas et al. 2001). Thus, as fermentation and rotting proceed, *Leuconostoc* will typically be superseded by other bacteria and yeast (Gardner et al. 2001; Andesogan et al. 2003; Breidt 2004; Amoa-Awua et al. 2007; Jung et al. 2012), which likely explains why bacterial and yeast contaminants and not *Leuconostoc* were isolated from rotted sugar beet root tissue at times. These observations are consistent with what has been observed previously when conducting isolations from rotting sugar beet root tissue affected by the *R. solani–Leuconostoc* interaction (Strausbaugh et al. 2013a; Strausbaugh 2016).

Isolating *L. mesenteroides* and *L. pseudomesenteroides* from sugar beet root tissue should be conducted with appropriate caution since *Leuconostoc* spp. can potentially be human pathogens (Kumudhan and Mars 2004; Taneja et al. 2005; Albanese et al. 2006; Bou et al. 2008; Tholpady et al. 2010; Shin et al. 2011; Taşkapilioğlu et al. 2011; Deng et al. 2012; Wong et al. 2012; Yang et al. 2015; Ino et al. 2016; Barletta et al. 2017; Karbuz et al. 2017; Haslam and Geme 2018). *Leuconostoc* spp. have a large presence in foods and the environment and are generally regarded as safe (Hemme 2012). However, *Leuconostoc* spp. previously considered to be of low pathogenic potential for humans have emerged as sporadic pathogens (Arias and Murray 2015). Human infections are found primarily in immunocompromised individuals and infants with underlying disorders such as prematurity and gastrointestinal abnormalities (Haslam and Geme 2018). Thus, those working with *Leuconostoc* spp. are encouraged to exercise caution.

For the first time the synergistic rot response of a genetically diverse group of *R. solani* AG-2-2 IIIB strains was proven to be consistent with both *L. mensenteroides* and *L. pseudomesenteroides* strains. When this same *R. solani* AG-2-2 IIIB strain diversity was screened against the best sources of sugar beet host resistance, FC709-2 was resistant to all strains, while weaker sources of resistance exhibited

weak fungal strain-resistance source interactions at times (Strausbaugh et al. 2013b). Therefore, host resistance to *R. solani* in mature sugar beet roots should be sufficient to control this root rot problem even though *Leuconostoc* spp. along with other bacteria and yeast have the potential to lead to considerable rot damage (Strausbaugh and Gillen 2008). Unfortunately, most commercial sugar beet cultivars only contain low to intermediate levels of resistance to *R. solani* (Ruppel et al. 1979; Strausbaugh et al. 2013b). Maintaining a higher level of resistance in commercial sugar beet cultivars is problematic since resistance is quantitatively inherited, associated with yield drag, and resistance to other disease problems such as Aphanomyces root rot, Cercospora leaf spot, curly top and rhizomania is also needed for cultivar approval (Hecker and Ruppel 1975; Panella 2005; Lein et al. 2008; Strausbaugh et al. 2013b). Thus, control of RRR through other management options such as the use of fungicide applications (Kiewnick et al. 2001; Stump et al. 2004; Windels and Brantner 2005; Kirk et al. 2008; Bolton et al. 2010; Arabiat and Khan 2016; Cointe et al. 2016; Liu and Khan 2016; Bartholomäus et al. 2017; Khan et al. 2017; Stump 2018) and crop rotation (Ruppel 1985; Rush and Winter 1990; Engelkes and Windels 1996; Buddemeyer et al. 2004; Buhre et al. 2009; Kluth and Varrelmann 2010) will continue to be necessary to limit losses.

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