Sugar beet root rot at harvest in the US Intermountain West

Carl A. Strausbaugh and Anne M. Gillen

Abstract: Root rot in sugar beet (*Beta vulgaris*) causes significant losses worldwide. To assess the distribution of root rot fungi and their relationship to bacterial root rot, commercial sugar beet roots with rot symptoms were collected at harvest time in the Intermountain West. Isolations for both fungi and bacteria were conducted using standard microbiological techniques, and the root area rotted was assessed. A subset of fungal isolates was tested for pathogenicity to sugar beet in greenhouse assays and field trials with and without manure. In the field survey of rotting beets, the fungi most frequently associated with root rot included *Fusarium* spp. (*Fusarium oxysporum* and *Fusarium acuminatum* with 24% and 15% of isolates, respectively), *Geotrichum* spp. (16% of isolates), *Rhizoctonia solani* (15% of isolates), and *Mucor* spp. (14% of isolates). However, only *R. solani* isolate F321 (AG-2-2IIIB) consistently caused rot in greenhouse pathogenicity tests. In the field survey, a mean of 6% of the root tissue had rotted for individual roots when fungi were isolated individually, whereas mean root rot was 71% and 68% when bacteria were isolated individually or in combination with other organisms, respectively. In field trials, roots inoculated with F321 averaged 3%–5% fungal rot, whereas the percentage of root tissue with bacterial rot was 6%–78%, which supports survey observations. Manure did not lead to root rots in the field. Traditionally, fungal root rots have been the main focus of breeding programs; however, because of the root area rotted by lactic acid bacteria, especially *Leuconostoc*, these bacteria should not be ignored in breeding efforts.

Key words: Leuconostoc, *Lactobacillus*, *Beta vulgaris*, *Rhizoctonia*, fermentation, lactic acid bacteria.

Résumé : Le pourridié de la betterave à sucre (*Beta vulgaris*) entraîne d'importantes pertes partout dans le monde. Afin d'évaluer la distribution des champignons qui causent le pourridié et leur relation au pourridié bactérien, on a collecté, à l'époque de la récolte, des racines de betteraves cultivées commercialement dans la région de l'Intermountain West américain, betteraves qui portaient les symptômes du pourridié. Les champignons et les bactéries ont été isolés selon les techniques microbiologiques standards et les portions de racines pourries ont été étudiées. Un sous-ensemble d'isolats fongiques a été évalué quant à sa pathénogénicité à l'égard de la betterave à sucre au cours de tests biologiques effectués en serre et d'essais au champ, avec et sans fumier. Lors des études sur le terrain, les champignons les plus souvent associés au pourridié de la betterave incluaient *Fusarium* spp. (*Fusarium oxysporum* and *Fusarium acuminatum* avec 24 % et 15 % des isolats, respectivement), *Geotrichum* spp. (16 % des isolats), *Rhizoctonia solani* (15 % des isolats) et *Mucor* spp. (14 % des isolats). Toutefois, seulement l'isolat F321 (AG-2-2IIIB) de *R. solani* causait invariablement le pourridié lors des tests de pathogénicité effectués en serre. L'étude menée au champ a révélé que, en moyenne, 6 % du tissu de chaque racine était pourri lorsque les champignons étaient isolés séparément. Par contre, la moyenne du pourridié variait de 71 % à 68 %, respectivement, lorsque les bactéries étaient isolées séparément ou avec d'autres organismes. Lors d'essais au champ, les racines inoculées avec F321 ont affiché un taux moyen de pourridié fongique de 3 % à 5 % tandis que le taux de tissu racinaire affichant le pourridié bactérien variait de 6 % à 78 %, ce qui supporte les observations de l'étude. Au champ, l'application de fumier n'a pas engendré le pourridié. Traditionnellement, les pourridiés fongiques ont fait l'objet de presque toute l'attention lors de programmes de sélection, mais, étant donné la portion de racines infectée par les bactéries lactiques, particulièrement par *Leuconostoc*, ces dernières devraient susciter l'intérêt quant aux efforts consentis à la sélection.

Mots-clés : Leuconostoc, *Lactobacillus*, *Beta vulgaris*, *Rhizoctonia*, fermentation, bactéries lactiques.

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Introduction

Root rot of sugar beet (*Beta vulgaris* L.) is of considerable importance worldwide. The primary causal agents of fungal root rot in sugar beet include *Rhizoctonia solani* Kühn, *Aphanomyces cochlioides* Dreschs., and *Fusarium* spp. (Harveson 2006). Other fungi that have also been associated with root rot in sugar beet include *Phoma betae* Frank, *Pythium* spp., *Rhizopus stolonifer* (Ehr. ex Fr.) Lind, *Sclerotium rolfsii* Sacc., *Verticillium albo-atrum* Reinke & Berth., *Phytophthora drechsleri* Tucker, *Phymatotrichum omnivorum* (Shear) Dug., and *Rhizoctonia crocorum* (Pers.) DC. ex Fr. (Schneider and Whitney 1986). Bacterial root rot in sugar beet has traditionally been attributed to *Pectobacterium betavasculorum* (Thomson et al.) Gardan et al. (syn. *Erwinia carotovora* (Jones) Bergey et al. subsp. *betavasculorum* Thomson et al.), but recently, *Leuconostoc mesenteroides* subsp. *dextranicum* (Beijerinck) Garvie has also been found to cause root rot in sugar beet (Ruppel et al. 1975; Strausbaugh and Gillen 2008; Tallgren et al. 1999; Thomson et al. 1977; Whitney 1986; Whitney and Mackey 1989).

Yield losses from Rhizoctonia root rot alone can amount to ≥ 50% in sugar beet (Büttner et al. 2004). Field observations in the Intermountain West (IMW) suggest root rot in sugar beet is of considerable importance, particularly in furrow irrigated fields and fields with short rotations. With the growth of the dairy industry in this region and changes in cropping practices, more corn (*Zea mays* L.) is being grown in rotation with sugar beet. *Rhizoctonia solani* anastomosis group (AG) 2-2IIIB, considered the leading cause of sugar beet root rot in the IMW, was shown to cause crown and brace root rot in corn (Brantner and Windels 2008; Führer Ithurrart et al. 2004). Recently, a bacterial root rot caused by *L. mesenteroides* was demonstrated to be widespread in the IMW (Strausbaugh and Gillen 2008) and potentially interacts with fungal root rots. With the changes in rotations, cultural practices, and occurrence of bacterial root rot in the IMW, an investigation was conducted to establish the fungal pathogens important in root rot and their association with bacterial root rot in sugar beet.

Materials and methods

Survey

An assessment was made of fungal root rot in recently (within 1 or 2 days of harvest) harvested roots delivered to piling grounds in Idaho and Oregon at the end of the 2004 and 2005 growing seasons. A total of 29 and 28 piling grounds were visited from the American Falls area (southeastern Idaho), Magic Valley (south-central Idaho), and Treasure Valley (southwestern Idaho to southeastern Oregon) in 2004 and 2005, respectively. A total of 225 roots in 2004 and 308 roots in 2005 with rot symptoms were collected. To ensure an even sampling distribution, no more than 15 symptomatic roots (deep or limited in penetration or area) were collected from a piling ground each year. The whole end of the pile was surveyed to ensure sampled roots came from multiple truck loads. The percentage of root area (space occupied by rot) involved with fungal (dry rot) and bacterial (wet rot) rot was visually assessed by cutting the root from crown to tail bisecting the affected area. Isolations were conducted by removing 10 mm × 10 mm pieces of internal root tissue from the margins between rotted tissue and white, healthy-appearing tissue. Isolations for fungi were made from an area with dry rot, and bacterial isolations were conducted from areas with a wet rot.

For fungal isolations, pieces of root tissue were surface disinfested in 0.6% sodium hypochlorite (NaOCl) for 60 s and then rinsed in sterilized reverse osmosis water for 60 s. The surface areas of each tissue piece were then removed and a 2 mm × 2 mm piece was placed on Difco potato dextrose agar (PDA; Becton Dickinson & Co., Sparks, Md.) and 2.0% Bacto agar (Becton Dickinson & Co.) an incubated at 22 °C. Both media were amended with 200 mg/L streptomycin sulfate. Representative colonies from each root were hyphal tipped onto streptomycin-amended PDA. Initial groupings and identifications were performed using a light microscope. The *Fusarium* spp. isolates were grown on synthetic nutrient-poor agar (Gerlach and Niremberg 1982) with pieces of sterile (autoclaved for 15 min at 121 °C) carnation (*Dianthus caryophyllus* L.) leaves placed on the agar surface prior to inoculation. The *Fusarium* isolates were grouped and identified based on techniques and descriptions by Nelson et al. (1983). A subset of 16 *Fusarium* isolates (*Fusarium acuminatum* Ellis & Everh., F109, F122, F213, F216, F242, and F260; *Fusarium culmorum* (Sm.) Sacc., F68, F72, and F225, and F263; *Fusarium equiseti* (Corda) Sacc., F110; *Fusarium oxysporum* Schindl., F94, F97, F271, F281, and F297) was sent to the Fusarium Research Center, University Park, Pennsylvania, for confirmation of identification. Selected isolates (F6, F39, F42, F321, F373, F389, F415, and F418) from each of the non-*Fusarium* fungal groups were submitted to Microbial ID (MIDI Labs, Newark, Del.) for sequencing of 28S ribosomal RNA (rRNA) variable D2 region (approximately 300 bp). The primers used corresponded to positions 3334 and 3630 in the *Schizosaccharomyces japonicus* Yukawa & Maki large subunit rRNA gene. Sequence analysis was performed using MicroSeq (Applied Biosystems, Foster City, Calif.) microbial analysis software and database. Sequence comparisons were also conducted using BLASTn version 2.2.18 (Altschul et al. 1997) to search the GenBank database via the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/blast). The methods and results for the identification of the bacterial and yeast isolates were reported previously (Strausbaugh and Gillen 2008). This paper relates the results from fungal isolations to the previously reported bacterial isolations.

Pathogenicity tests

A pathogenicity test with 21 treatments (18 fungal isolates, two fungal check strains (R9, *Rhizoctonia solani* AG-2-2IIIB; FC216C, *Fusarium oxysporum*), and a noninoculated check) was conducted in the greenhouse on the commercial sugar beet cultivar, Monohikari (susceptible to fungal root rots). The experimental design was a randomized complete block with six replications per treatment. The experiment was repeated once. Plants were grown from seed in 10.2 cm plastic pots with Sunshine Mix No. 2 (Sun

Gro Horticulture, Bellevue, Wash.) which contained 70%–80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, and wetting agent. The potting mix was steam pasteurized at 71 °C for 30 min. The plants were fertilized once a week with 20:10:20 (N:P:K) general-purpose fertilizer at 200 ppm N. The greenhouse temperature was set at 23 °C day and 18 °C night, with daylength extended to 13 h with metal halide lamps (250 $\mu mol \cdot s^{-1} \cdot m^{-2}$ measured at plant top). Inoculum for the fungal isolates was generated by placing sterile (autoclaved twice for 60 min at 121 °C on consecutive days) barley (*Hordeum vulgare* L.) kernels on a PDA plate near an inoculum plug. The kernels were colonized for 3 weeks. The plants were inoculated at the eight-leaf growth stage by placing an infested kernel 10 mm down into the potting mix next to the root of a plant. Four weeks after inoculation, the percentage of foliage discolored was determined. The top fresh mass was determined, and the roots were rated for percent rot by bisecting the roots from crown to tail. Isolations (as described earlier) on PDA amended with streptomycin (200 mg/L) were conducted from all roots with a rating significantly different from the noninoculated check.

Field trials

To assess the influence of rhizoctonia root rot and manure on the development of bacterial root rot, a field study was conducted for 2 years in a field that had been planted with wheat (*Triticum aestivum* L.) the previous year and fall plowed. A split-plot design was used where the main plot treatments were manure or no manure and arranged in a randomized complete block design with eight replications. Within the main plots, there was a noninoculated plot and a plot inoculated with *R. solani*. The manure treatment consisted of spreading dairy manure with a wet mass of 246 t/ha (dry mass 108 t/ha) on 11 April 2007 with a small plot manure spreader Frontier MS1117 (H&S Manufacturing, Rowlett, Tex.). The spreader was weighted before and after applications with load cells. Analysis of manure was performed by Stukenholtz Laboratory, Twin Falls, Idaho. Following the manure application, the field was disked, harrowed, and marked. In 2007, the seed of commercial sugar beet cultivar HH005 (contact Holly Hybrids, Sheridan, Wyoming, for uncoded cultivar name) were planted on 1 May to a density of 352 272 seed/ha and thinned to 117 424 plants/ha on 29 May. The seed were treated with fungicides Allegiance FL (15.6 g a.i. metalaxyl/100 kg seed) and Thiram 42S (250 g a.i. thiram/100 kg seed) to limit the influence of damping-off pathogens and allow for good stand establishment. The plots were four rows wide (56 cm row spacing) and 10.4 m long. The crop was managed according to standard cultural practices. Irrigation was applied through hand lines as needed. The *R. solani* treatment was applied on 13 June at the eight-leaf growth stage by applying 0.6 g/plant of ground barley kernels infested with *R. solani* isolate F321 (AG-2-2IIIB) followed by cultivation and irrigation as described by Ruppel et al. (1979). A trace amount of curly top was present in the fields both years, but other disease problems were not evident. Disease data were collected by hand digging 10 roots at random from an outside row on 4 September. The leaves were evaluated visually for discoloration, and the roots were bisected to establish the percentage of root tissue with dry fungal rot and wet bacterial rot. Isolations were conducted to prove the presence of *R. solani* in the dry type rot with PDA (using the previously described technique) and *Leuconostoc mesenteroides* in the wet type rot with GYP amended with tetracycline (0.2 mg/L) and vancomycin (0.03 g/L) as described by Benkerroum et al. (1993). The center two rows were harvested on 9 October with the aid of a mechanical topper and small plot harvester. Two samples collected from each plot at harvest were submitted to the Amalgamated Tare Laboratory, Paul, Idaho. Percent sugar was determined using an Autopol 880 polarimeter (Rudolph Research Analytical, Hackettstown, N.J.) and a half-normal mass sample dilution and aluminum sulfate clarification method (ICUMSA Method GS6-3 1994; Bartens 2005). Conductivity was measured using a Foxboro conductivity meter model 871EC (Foxboro, Foxboro, Mass.), and nitrate was measured using a multimeter Model 250 (Denver Instruments, Denver, Colo.) with Orion probes 900200 and 9300 BNWP (Krackler Scientific, Inc., Albany, N.Y.).

The experiment was repeated in 2008 with the same methods except the amount of manure varied. Although an attempt was made to apply a similar volume of manure as in 2007, the manure was less moist. Thus, the wet mass of the manure applied on the 22 April 2008 was 125 t/ha (dry mass was 72 t/ha). The trial was planted on 6 May and thinned on 3 June. The *R. solani* treatment was applied on 24 June at the eight-leaf growth stage. The plots were evaluated for root rot and foliar symptoms on 8 September and harvested for yield on 20 October.

Data analysis

The SAS Univariate procedure (SAS Institue Inc., Cary, N.C.) was used to test the normality of the data. Bartlett's test was utilized to test for homogeneity of variance among experiments. Data from multiple repetitions of an experiment were pooled when possible and analyzed using the SAS general linear methods procedure, and Fisher's protected least significant difference was used for mean comparisons. Analysis with transformed data in the field trials was evaluated.

Results

Root isolations

A total of 430 fungal isolates were obtained from rotting sugar beet roots at harvest that were considered to potentially be pathogenic. The primary fungi isolated were *F. oxysporum* (24% of isolates), *Geotrichum* spp. (16%), *R. solani* (15%), *F. acuminatum* (15%), *Mucor* spp. (14%), *Phoma betae* (6%), oomycetes (3%), *F. culmorum* (3%), and *F. equiseti* (2%). In support of the microscopic observations, the following isolate identifications were also confirmed with sequencing and submitted to GenBank (accession numbers are given in parentheses): *Geotrichum* sp. F373 (FJ031013); *R. solani* F6 (FJ031009) and F321 (FJ031012); *Mucor* spp. F39 (FJ031010) and F42 (FJ031011); *Phoma betae* F415 (FJ031015) and F418 (FJ031016); and *Pythium* spp. F389 (FJ031014). Based on partial sequencing of 28S rRNA gene, *Geotrichum* sp. F373

had a 1% difference from the *Geotrichum candidum* Link reference strain in the MicroSeq database. *Geotrichum* sp. F373 also had 100% identity with the *Geotrichum klebahnii* (Stautz) Morenz GenBank accession AY235029. Based on partial sequencing of 28S rRNA gene, *R. solani* isolate F6 had 1% difference with the *R. solani* reference strains M8679 and M8994 in the MicroSeq database. *R. solani* F6 also had 97%–100% identity with 30 *Thanatephorus cucumeris* (A.B. Frank) Donk GenBank accessions, but the AG group varied among these accessions (sequencing and phylogenetic analysis from another study suggests this isolate belongs in AG-4 HG-II; data not shown). Based on partial sequencing of 28S rRNA gene, *R. solani* F321 had 1%–2% difference with the *R. solani* reference strains M8679 and M8994 in the MicroSeq database. *Rhizoctonia solani* F321 also had 97%–99% identity with 20 *T. cucumeris* GenBank accessions, but the AG group varied among these accessions (sequencing and phylogenetic analysis from another study suggests this isolate belongs in AG-2-2IIIB; data not shown). Based on partial sequencing of 28S rRNA gene, *Mucor* sp. F39 and 42 had 0.4%–0.5% difference with the *Mucor circinelloides* Tiegh. reference strain in the MicroSeq database. *Mucor* sp. F39 and 42 also had 99%–100% identity with the *M. circinelloides* GenBank accessions AY213710 and AB363774 and other *Mucor* accessions. Based on partial sequencing of 28S rRNA gene, *Phoma betae* F415 and F418 were identical to the *Phoma betae* reference strain in the MicroSeq database. *Phoma betae* F415 and F418 also had 97%–99% identity with a number of fungal strains on GenBank including *Phoma herbarum* Westend accession AY293788. Based on partial sequencing of 28S rRNA gene, *Pythium* sp. F389 had <1% difference with a number of *Pythium* reference strains in the MicroSeq database. *Pythium* sp. F389 also had 97%–99% identity with 10 *Pythium* GenBank accessions, but the species in these accessions varied. Also, 129 fungal isolates were obtained that were a mixture of different genera considered saprophytes or secondary invaders.

The root rot fungal isolates from Treasure Valley and Magic Valley comprised 96% of the isolates, and the remaining 4% came from the American Falls growing area. *Fusarium* spp. (*F. oxysporum*, *F. acuminatum*, and *F. culmorum*) were predominantly (67% of the *Fusarium* isolates) found in the Treasure Valley, except for *F. equiseti*. Eight of the 10 *F. equiseti* isolates were from the Magic Valley. *Geotrichum* spp. and *Rhizoctonia solani* isolates were more evenly distributed between Treasure Valley (51% and 48% of these isolates, respectively) and Magic Valley (43% and 50%, respectively). *Mucor* spp. (71% of isolates), *Phoma betae* (85%), and oomycetes (55%) were predominantly found in the Magic Valley. Bacteria and yeast associated with wet rot symptoms had been reported previously (Strausbaugh and Gillen 2008).

Root symptoms

The mean percentage of root tissue with fungal rot symptoms was only 1%–4% regardless of the fungal organism isolated (Table 1). The mean total rot when only fungi were isolated was 6%. The root area rotted when only bacteria were isolated ranged from 56% to 72% with an overall mean total rot of 71%. When multiple organisms were isolated, rot ranged from 37% to 79% with an overall mean total rot of 68%. Cavities created by root rots involved a mean of 8%–11% of the total root area. The lactic acid bacteria dominated (81% of isolates) roots with bacteria isolated individually. The lactic acid bacteria were present 90% of the time when other bacteria and yeast were isolated.

Pathogenicity tests

The percentages of discolored foliage and top fresh masses were not significantly different ($P = 0.6716$ and 0.6456, respectively) in the two experiments, and the variances were homogeneous ($P = 0.3914$ and 0.1910, respectively). Therefore, these data were analyzed together (Table 2). Root discoloration differed ($P = 0.0144$) among experiments. Therefore, these data were analyzed separately. *Rhizoctonia* isolates R9 and F321 caused significant foliar discoloration, reduction in top fresh mass, and root discoloration. Damage caused by other fungal and *Rhizoctonia* isolates was not significantly different from the noninoculated check, except for *Geotrichum* isolate F373. In the first study, F373 resulted in root discoloration that was significantly different from the noninoculated check but caused no significant damage in the second test. In plants with significant root discoloration, isolations confirmed the presence of the inoculated pathogen completing Koch's postulates.

Field trials

For some variables, the *Rhizoctonia* × manure treatment interaction was significant ($P \leq 0.05$), so the *Rhizoctonia* and uninoculated check treatments were analyzed and presented separately for the manure and no manure treatments (Table 3 and 4). In field trial 1, data were analyzed without transformation, because transformation did not improve analysis. In field trial 2, the data were square root transformed to improve the analysis. Transformation did not improve normality but did cut the coefficient of variation nearly in half. There was no fungal or bacterial root rot in the noninoculated check plots for either trial regardless of whether manure was applied or not (Table 3). When *R. solani* was inoculated, there was always fungal and bacterial rot regardless of whether manure was applied or not (Table 3). When *R. solani* was inoculated, mean fungal rot ranged from 3% to 5% in both trials regardless of whether manure was applied or not. Isolations showed *R. solani* was present in all roots with fungal rot. In trial 1, bacterial root rot ranged from 75% to 78% on average, whereas the range was 6%–28% in trial 2. Isolations indicated *Leuconostoc* was present in all roots with bacterial rot, although other bacteria and yeast were also present at times. Significant differences were present between the fungal treatment and the noninoculated check for all yield variables except sucrose content for the manure treatment (Table 4). The fungal treatment had enough influence to significantly reduce tonnage and sucrose yield compared with the noninoculated check in both trials regardless of whether manure was applied or not.

Table 1. Percentages of rotted root area associated with fungi and bacteria in sugar beet roots collected from piles in southern Idaho and southeastern Oregon in 2004 and 2005.

Organism isolated	No. of roots	Root area with rot symptoms (%)*			Cavity (%)*
		Fungal	Bacterial	Total	
Fungi isolated individually					
Fusarium oxysporum	85	2±1	3±8	5±8	13±9
Fusarium acuminatum	57	2±1	6±7	8±7	11±6
Geotrichum spp.	51	4±2	6±11	10±12	6±2
Rhizoctonia solani	50	2±3	4±12	6±13	8±9
Mucor spp.	33	2±2	0±0	2±2	14±9
Phoma betae	24	2±1	3±12	5±12	14±9
Overall mean		2	4	6	11
Bacteria isolated individually†					
Bacterial group A	90	1±1	72±28	73±28	7±6
Bacterial group B	15	2±1	61±22	63±21	13±8
Bacterial group C	6	2±0	56±10	58±10	12±3
Overall mean		1	70	71	8
Multiple organisms‡					
Bacterial groups A + B	24	1±1	64±27	65±27	9±7
Bacterial groups A + C	7	2±2	62±25	64±26	18±10
Bacterial groups A + D	24	1±1	79±26	80±26	5±9
Bacterial groups A + B + D	11	2±1	64±41	66±40	10±7
Bacterial groups B + D	8	1±1	69±32	70±32	23±16
Geotrichum spp. + A	8	4±1	37±42	41±43	6±2
Overall mean		2	66	68	10

*Fungal, dry rot; bacterial, wet rot; total, all types of rot combined. Cavity, cavities formed in root that appeared to be caused by rotting organisms. Values are means ± SDs.

†Group A, *Leuconostoc* and *Lactobacillus* spp. (lactic acid bacteria); group B, *Gluconobacter* and *Acetobacter* spp. (acetic acid bacteria); group C, *Enterobacter*, *Escherichia*, *Pectobacterium*, and *Serratia* spp. (enteric bacteria); group D, *Pichia* spp. (yeast). These groupings and identifications are according to Strausbaugh and Gillen (2008).

‡Combinations represented by five roots or fewer were not presented.

Discussion

Although fungi were associated with root rot, bacteria were associated with a higher percentage of root rot in both the field survey and field trials. Root rot only involved a mean of 6% of the root tissue when fungi were isolated individually, whereas 71% and 68% of the tissue had rotted when bacteria were isolated individually or with other organisms, respectively. In field trials, roots inoculated with *Rhizoconia solani* isolate F321 (AG-2-2IIIB) had 3%–5% fungal root rot, whereas 6%–78% of the root mass was associated with bacterial rot, which supports survey observations. Manure did not lead to root rots in the field and had little if any influence when F321 was inoculated. A range of fungi were associated with root rot in sugar beet in the survey, but only *Rhizoconia solani* isolate F321 consistently resulted in rot symptoms during greenhouse pathogenicity tests. Some fungi found to be nonpathogenic in the greenhouse assay may possibly be pathogenic in the field under different environmental conditions.

Recently, lactic acid bacteria have been shown to be the primary bacteria associated with bacterial root rot in sugar beet grown in the IMW (Strausbaugh and Gillen 2008). *Leuconostoc mesenteroides* subsp. *dextranicum* was the most aggressive organism in pathogenicity tests, but *Lactobacillus plantarum* (Hammes and Hertel) Bergey et al. also caused rot symptoms (Strausbaugh and Gillen 2008). These lactic acid bacteria can be found on plant surfaces and in soil, fermenting vegetables, dairy products, manure, wine, and sugar factories (Chen et al. 2005; Cogan and Jordan 1994; Conn et al. 1995; Holt et al. 1994; Mundt and Hammer 1968; Server-Busson et al. 1999). Given the widespread distribution of lactic acid bacteria in nature and the numerous wounds inflicted on sugar beet roots at harvest, it is surprising that there is not more bacterial root rot in storage piles. In the field, root rot in general has been observed to be more frequent in furrow irrigated fields, particularly if irrigation times are extended to 24 h and beyond (data not shown). Given these observations, perhaps moisture availability plays a role in establishing bacterial root rot. Storage piles filled with roots harvested or stored under wet conditions were more troublesome to manage (Bugbee 1982).

The majority of the fungal rot was found on roots in Treasure Valley and Magic Valley, whereas roots from the American Falls region had very little fungal rot. A similar pattern was established in a previous study that focused on bacterial root rot in the IMW (Strausbaugh and Gillen 2008). Most roots in this study with bacterial root rot also had some fungal rot. Perhaps fungal infection or a combination of fungal infection and moisture play a role in facilitating bacterial root rot. In the field trials, bacterial root rot was only present when *R. solani* had been inoculated.

Manure was another variable under consideration in the field studies. *Leuconostoc* and *Lactobacillus* are commonly found as natural microflora in humans and animals, in dairy products, and on some plant surfaces (Brashears et al. 2003;

Table 2. Disease parameters and fresh mass for fungal isolates inoculated onto the sugar beet cultivar Monohikari at the eight-leaf growth stage in the greenhouse.

Fungal isolate identification	Isolate identication No.	Foliar discoloration (%)	Top fresh mass (g)	Root discoloration (%)	
				Test 1	Test 2
Rhizoctonia solani check	R9	83 a	19 b	80 b	69 a
Rhizoctonia solani	F321	89 a	14 b	97 a	68 a
Fusarium oxysporum	F152	3 b	70 a	2 d	5 b
Rhizoctonia solani	F302	3 b	72 a	3 d	4 b
Rhizoctonia solani	F6	1 b	69 a	2 d	4 b
Geotrichum sp.	F389	3 b	66 a	2 d	2 b
Geotrichum sp.	F359	3 b	69 a	8 cd	2 b
Geotrichum sp.	F373	2 b	71 a	17 c	2 b
Fusarium oxysporum	F143	3 b	73 a	6 d	2 b
Phoma sp.	F413	2 b	73 a	1 d	2 b
Fusarium acuminatum	F260	2 b	70 a	7 cd	2 b
Fusarium oxysporum	F281	3 b	73 a	6 d	1 b
Fusarium acuminatum	F100	5 b	74 a	7 cd	1 b
Phoma sp.	F418	2 b	70 a	4 d	1 b
Mucor sp.	F62	3 b	70 a	5 d	1 b
Phoma sp.	F415	4 b	71 a	9 cd	1 b
Mucor sp.	F39	2 b	72 a	6 d	1 b
Fusarium acuminatum	F109	3 b	69 a	4 d	1 b
Fusarium oxysporum check	FC216C	2 b	70 a	5 d	1 b
Mucor sp.	F42	2 b	72 a	1 d	0 b
Noninoculated check		1 b	69 a	2 d	0 b
$P > F$*		<0.0001	<0.0001	<0.0001	<0.0001
LSD ($P \leq 0.05$)		9	9	10	13

*$P > F$ is the probability associated with the F value when data were analyzed using the SAS general linear methods procedure (SAS Institute Inc. 2008). The percentages of discolored foliage and top fresh masses were not significantly different ($P = 0.6716$ and 0.6456, respectively) in the two experiments, and the variances were homogeneous ($P = 0.3914$ and 0.1910, respectively). Therefore, these data were analyzed together. Root discoloration differed ($P = 0.0144$) between experiments. Therefore, these data were analyzed separately. Means followed by the same letter within a column did not differ significantly based on Fisher's protected least significant difference (LSD) value with $P \leq 0.05$.

Table 3. Fungal and bacterial root rot and foliar discoloration on plants inoculated with and without *Rhizocotonia solani* and manure on the sugar beet cultivar HH005 in Kimberly, Idaho, in 2007 (trial 1) and 2008 (trial 2).

Variable	Fungal rot (%)		Bacterial rot (%)		Top discoloration (%)	
	No manure	Manure	No manure	Manure	No manure	Manure
Trial 1						
Rhizocotonia solani	5	5	78	75	87	81
Noninoculated check	0	0	0	0	4	3
$P > F$*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Trial 2						
Rhizocotonia solani	3	5	6	28	13	37
Noninoculated check	0	0	0	0	0	0
$P > F$*	0	<0.0001	0.07	<0.0001	0.003	<0.0001

Note: *Rhizoctonia solani* isolate F321 (AG-2-2IIIB) was used in the expreiments. Manure was applied in the spring prior to planting Noninoculated check, no fungal inoculation; fungal rot, percentage of root area with dry rot; bacterial rot, percentage of root area with wet rot; top discoloration, percentage of foliage with discoloration.

*Probability associated with the F value when data were analyzed using the SAS general linear methods procedure (SAS Institute Inc. 2008). In trial 2, the data were square root transformed to improve homogenity of variance.

Zarazaga et al. 1999). In the large intestine of cattle, lactobacilli can range from 10^8 to 10^9/g dry matter (Brashears et al. 2003). Isolations from cattle fecal material revealed 85% of isolates were gram positive (72% rods, which were lactobacilli and 27% cocci, which were *Leuconostoc* spp.; Brashears et al. 2003). In the ecology of feedlot pad manure, gram-positive clostridial and lactic acid producing bacteria tended to predominate (Ouvverkerk and Klieve 2001). Thus, we had to consider the possibility that manure applications to fields may be contributing to bacte-

Table 4. Yield data for plants inoculated with and without *Rhizocotonia solani* and manure application on the sugar beet cultivar HH005 in Kimberly, Idaho, in 2007 (trial 1) and 2008 (trial 2).

	Nitrate (ppm)		Conductivity (mmhos)		Sucrose content (%)		Root yield (t/ha)		ERS (kg/ha)	
Variable	No manure	Manure	No manure	Manure	No manure	Manure	No manure	Manure	No manure	Manure
Trial 1										
Rhizocotonia solani	1082	946	1.318	1.299	12.47	13.34	13.9	17.0	1 317	1756
Noninoculated check	873	1116	0.959	1.279	13.67	13.15	89.9	84.1	10 006	8533
$P > F$*	0.0256	0.043	0.01	0.8337	<0.0001	0.5895	<0.0001	<0.0001	<0.0001	<0.0001
Trial 2										
Rhizocotonia solani	594	816	1.099	1.327	13.72	13.19	54.4	43.8	5 943	4433
Noninoculated check	426	640	0.936	1.154	14.55	13.86	87.9	85.2	10 487	9292
$P > F$*	0.0074	0.0032	0.0025	0.0116	0.0218	0.1289	<0.0001	<0.0001	<0.0001	<0.0001

Note: *Rhizoctonia solani* isolate F321 (AG-2-2IIIB) was used in the trials. Manure was applied in the spring prior to planting. Noninoculated check, no fungal inoculation; ERS, estimated recoverable sucrose.

*Probability associated with the *F* value when data were analyzed using the general linear methods procedure (SAS Institute Inc. 2008).

rial root rot in sugar beet. In the field studies, no bacterial root rot occurred with or without manure when fungal infection was not present. When *R. solani* was inoculated into the crown area, fungal and bacterial root rot developed. The amount on bacterial root rot with and without manure was similar. Thus, fungal infection appeared to be more important to the development of bacterial root rot than manure applications.

Numerous fungi, such as *R. solani*, *F. oxysporum*, and *Phoma betae* were isolated and have been previously established as causing root rots on sugar beet (Harveson 2006; Harveson and Rush 1998; Schneider and Whitney 1986). However, in pathogenicity tests only *Rhizoctonia* isolate F321 was pathogenic, whereas isolates F6 and F302 were not pathogenic. Subsequent internal transcribed spacer 5.8*S* rDNA sequencing and phylogenetic analysis has shown isolate F321 was similar to AG-2-2IIIB testers, whereas F6 and F302 were similar to AG-4 HG-II (data not shown). Rhizoctonia root rot on mature roots is normally attributed to isolates that belong in the AG-2-2IIIB subgroup, whereas AG-4 isolates are normally considered to be more important at emergence (Führer Ithurrart et al. 2004; Kirk et al. 2008; Rush et al. 1994).

The *F. oxysporum* isolates including the pathogenic check FC216C were not pathogenic in the greenhouse assays but may have been compromised by our pH 8.0 water. The southern Idaho production area has alkaline pH 8.0 soil and irrigation water (McDole and Maxwell 1987), so the greenhouse assay conditions were not unusual. Recently, fusarium wilt in spinach (*Spinacia oleracea* L.) was shown to be greatly reduced by raising the pH of acid soil (du Toit et al. 2008). Also, inoculating with a spore suspension rather than infested kernels may have led to different results. Other *Fusarium* spp., such as *F. acuminatum*, *F. culmorum*, and *F. equiseti*, were associated with rotted tissue in the field but did not prove to be pathogenic in the greenhouse assay. In previous work, *F. acuminatum* has been shown to be associated with fusarium yellows symptoms (Hansen and Hill 2004; Ruppel 1991).

Mucor spp. were not pathogenic in the greenhouse assays and have not been mentioned previously on sugar beet in association with root rot. Another zygomycete, *Rhizopus stolonifer*, (Ehrenb.:Fr.) Vuill. has been isolated from rotted tissue (Schneider and Whitney 1986). *Phoma betae* isolates were not pathogenic, but this fungus is considered to be more important on post harvest roots (Bugbee 1982; Bugbee and Cole 1976).

Geotrichum isolate F373 was pathogenic only in one test but not in a subsequent assay. Both pathogenic and nonpathogenic isolates occur on citrus fruit (Nakamura et al. 2008). If more isolates had been tested on sugar beet, perhaps pathogenic isolates could have been found. *Geotrichum* has not been documented on sugar beet previously (Schneider and Whitney 1986) and was the most commonly isolated fungus found in combination with lactic acid bacteria (Table 1). In decay of tomato (*Solanum lycopersicum* L.) fruit, *Leuconostoc* was rarely the sole organism isolated (Conn et al. 1995). *Geotrichum candidum* was frequently isolated along with *Leuconostoc* from decaying tomato tissue (Conn et al. 1995). *Geotrichum* is a filamentous yeast-like fungus found in a wide range of

habitats, such as plant tissues, silage, soil, milk, air, and water (Pottier et al. 2008). Finding *G. candidum* in association with lactic acid bacteria is not unusual because it has the ability to catabolize lactic acid produced by lactic acid bacteria (Greenberg and Ledford 1979). On citrus fruits, *G. candidum* is considered to be a plant pathogen that leads to postharvest losses because of sour rot (Nakamura et al. 2008).

Oomycetes were isolated in low frequency compared with most other fungi on the mature roots. Had isolations been conducted earlier in the season, perhaps more oomycetes would have been obtained. *Aphanomyces cochlioides* is difficult to isolate unless tissue is recently infected (Windels 2000). In the Red River Valley, *Aphanomyces* is of considerable importance in the root rot complex (Dyer et al. 2004).

As crop rotations and production practices continue to evolve in the IMW, root rots will likely continue to be a challenge to deal with in sugar beet production. Bacterial root rot was shown to be an important part of the root rot complex in sugar beet (Strausbaugh and Gillen 2008). The industry has focused primarily on fungal root rots in breeding for root rot resistance (Büttner et al. 2004; Ruppel et al. 1979). However, given the bacterial root rot documented in this study, root rot by bacteria should also be addressed in root rot resistance breeding efforts.

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