

Soilborne pathogens/Agents pathogènes telluriques

Virulence, distribution and diversity of *Rhizoctonia solani* from sugar beet in Idaho and Oregon

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Abstract: *Rhizoctonia* root rot causes serious losses on sugar beet worldwide. In order to help explain why *Rhizoctonia* root rot management practices have not performed well in some areas of the Intermountain West (IMW), a survey was conducted. In the IMW from 2004 to 2006, 94 *Rhizoctonia solani* field isolates were collected from sugar beet roots. These field isolates were compared with 19 reference strains and 46 accessions from GenBank for genetic diversity based on sequencing of the ITS-5.8S rDNA region. Greenhouse pathogenicity tests on sugar beet and silage corn were conducted and plant damage was assessed using a randomized complete block design with at least four replications. The majority of the isolates had sequence identity with the AG-2-2 IIIB (47%) or AG-4 subgroups (44%). Most of the AG-2-2 isolates (87%) were associated with fields in the western portion of the production area, while 71% of the AG-4 isolates came from the eastern portion of the production area. Isolates from AG-2-2 IIIB were frequently more virulent on sugar beet and sequence of the ITS-5.8s region required cloning because of genetic diversity within isolates. Seven (all AG-2-2 IIIB) of 18 isolates tested could attack both sugar beet and corn, while two of the seven virulent isolates caused less root rot on corn. To reduce *Rhizoctonia* root rot on sugar beet and corn, crop rotations and the isolates utilized for selecting host resistance could be given further consideration.

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

Résumé: Le rhizoctone brun cause de graves pertes chez la betterave à sucre partout dans le monde. Afin d'expliquer pourquoi les pratiques de gestion du rhizoctone brun n'ont pas été très efficaces dans certaines des régions de l'Intermountain West (IMW) américain, une étude a été menée. Dans l'IMW, de 2004 à 2006, 94 isolats de *Rhizoctonia solani* ont été collectés, en champs, sur des racines de betteraves à sucre. Ces isolats ont été comparés à 19 souches de référence et à 46 obtentions de la GenBank afin d'établir la diversité génétique basée sur le séquençage de la région de l'ITS-5.8S de l'ADNr. Des tests de pathogénicité ont été effectués en serre sur des betteraves à sucre et sur du maïs à ensilage. Le dommage subi par les plants a été évalué au moyen d'un dispositif en blocs randomisés avec au moins quatre réplifications. La majorité des isolats possédait une identité de séquence avec les sous-groupes AG-2-2 IIIB (47 %) ou AG-4 (44 %). La plupart des isolats d'AG-2-2 (87 %) étaient associés à des champs de la partie occidentale de la région de production, tandis que 71 % des isolats d'AG-4 provenaient de la portion orientale. Les isolats d'AG-2-2 IIIB étaient très souvent plus virulents à l'égard de la betterave à sucre et la séquence de la région de l'ITS-5.8s a dû être clonée à cause de la diversité génétique trouvée chez les isolats. Sept (tous d'AG-2-2 IIIB) des 18 isolats testés pouvaient attaquer et la betterave à sucre et le maïs, tandis que deux des sept isolats virulents ont causé moins de pourridié sur le maïs. Il faudrait davantage porter attention aux rotations des cultures et au choix des isolats utilisés pour la sélection visant la résistance de l'hôte afin de réduire l'incidence du rhizoctone brun chez la betterave à sucre et le maïs.

Mots clés: *Beta vulgaris*, rhizoctone brun, *Rhizoctonia solani*, betterave à sucre, *Zea mays*

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Introduction

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is a widespread and important disease problem on many crops (Führer Ithurrart *et al.*, 2004). *Rhizoctonia solani* can cause disease on at least 200 different plant species (Anderson, 1982; Salazar *et al.*, 2000). In the irrigated region of the western USA, Rhizoctonia root and crown rot on sugar beet (*Beta vulgaris* L.) is important on 30–42% of the acreage with damage varying from 0 to at least 50%, depending on cropping history and environment (Schneider & Whitney, 1986; Kiewnick *et al.*, 2001; Büttner *et al.*, 2004). In Europe, Rhizoctonia root rot on sugar beet may be increasing in importance (Buddemeyer *et al.*, 2004; Führer Ithurrart *et al.*, 2004; Buhre *et al.*, 2009). In New York, damage caused by *R. solani* and *Rhizoctonia*-like fungi on beans, table beet, carrots and cabbage has increased steadily during the last 10 years (Ohkura *et al.*, 2009). Bacteria and other pathogens may also interact with *R. solani* to cause additional sugar beet root rot leading to lost tonnage, reduction in quality and storability (Martin, 2003; Strausbaugh & Gillen, 2008, 2009).

Current classification within the *R. solani* species complex relies largely on the grouping of isolates into anastomosis groups (AG) based on hyphal interactions (Ceresini *et al.*, 2007). At least 13 AG have been described within the *R. solani* species complex including AG-1 to -13 (Sneh *et al.*, 1991; Gonzalez *et al.*, 2001). AG-1 through -9 all have subgroups identified on the basis of characteristics including morphology, virulence, host range, nutritional requirements, biochemical characteristics, molecular characteristics and DNA sequence (Sneh *et al.*, 1991; Cubeta & Vilgalys, 1997; Gonzalez *et al.*, 2001; Carling *et al.*, 2002). Current knowledge suggests that AG represent independent evolutionary units within *R. solani* (Cubeta & Vilgalys, 1997; Kuninaga *et al.*, 1997; Gonzalez *et al.*, 2001; Ceresini *et al.*, 2007). Interpretation of anastomosis reactions has not always been straightforward, since the four hyphal interaction phenotypes can represent a continuum, and reproducibility of AG interactions can be affected by factors such as laboratory environment, nutritional conditions and genetic stability (Cubeta & Vilgalys, 1997; Carling *et al.*, 2002; Stodart *et al.*, 2007). Molecular approaches based on the analysis of ribosomal DNA (rDNA) sequences have added genetic support to the AG classification system and allowed the investigation of their evolutionary relationships (Guillemaut *et al.*, 2003). Phylogenetic studies based on sequence comparisons of the internal transcribed spacer (ITS) region established the relevance of this region to discriminate the different

AG (Gonzalez *et al.*, 2001). Sequence data may support genetic groups within *Rhizoctonia* species and their teleomorphs *Thanatephorous* and *Ceratobasidium* better than other characters used in the past such as number of nuclei, plant host or morphology (Gonzalez *et al.*, 2006).

On sugar beet, *R. solani* AG-1, -2-2, and -4 have been responsible for severely reduced stands, while seedlings have only been slightly susceptible to AG-5 (Windels & Nabben, 1989; Rush *et al.*, 1994; Nelson *et al.*, 1996; Carling *et al.*, 2002; Bolton *et al.*, 2010). On older roots, AG-2-2 has caused root and crown rot, while AG-4 has only produced superficial lesions (Windels & Nabben, 1989; Rush *et al.*, 1994). AG-2-2 was subdivided into intraspecific groups based on pathogenic specialization and temperature tolerance (Sneh *et al.*, 1991). AG-2-2 IIIB can grow at 35 °C and cause blight of mat rush and rice, while AG-2-2 IV does not grow at 35 °C (Sneh *et al.*, 1991). AG-2-2 IIIB and AG-2-2 IV are genetically divergent and have a wider host range than originally reported (Stevens Johnk & Jones, 1993; Engelkes & Windels, 1996; Nelson *et al.*, 1996). AG-2-2 IIIB strains have been shown to be pathogenic on a number of crops, including corn, dry bean, mustard, flax and sunflower (Nelson *et al.*, 1996). Other crops attacked by *R. solani* AG-2-2 include alfalfa, carrot, cucumber, lima bean, radish, rice, snap bean, sorghum, southern pea and soybean (Sumner & Bell, 1982; Grisham & Anderson, 1983; Liu & Sinclair, 1991; Engelkes & Windels, 1996; Ohkura *et al.*, 2009).

Rhizoctonia root rot on sugar beet results from initial infection by propagules, particularly sclerotia or mycelia (often associated with plant debris), with sclerotia able to survive for many years in soil (Cubeta & Vilgalys, 1997). To reduce inoculum in the soil, a minimum of three years rotation with a non-host crop is recommended, but since this fungus has a wide host range, rotation does not always reduce soil inoculum (Ruppel, 1985; Rush & Winter, 1990; Brantner & Windels, 2008). Application of fungicides such as azoxystrobin applied at planting can delay early infection and enhance establishment of vigorous stands, but does not completely prevent infections (Kiewnick *et al.*, 2001). Azoxystrobin applications just prior to cultivation in the two- to eight-leaf growth stage were most effective for crown and root rot control but optimum timing varies with growing area (Kiewnick *et al.*, 2001; Windels & Brantner, 2005; Kirk *et al.*, 2008). Resistant cultivars would be a preferred means of control (Gaskill, 1968; Panella, 2005; Nagendran *et al.*, 2008). However, 'specialty cultivars' with tolerance to Rhizoctonia root rot tend to suffer from reduced yield potential (Sneh *et al.*, 1991; Kiewnick *et al.*, 2001) and have not performed well in Idaho, the second largest sugar

beet producing state in the USA (USDA-NASS, 2009). Thus, to aid in the development of management options, the objectives of this study were to collect *R. solani* isolates from the Amalgamated Sugar Company production area in the IMW and compare them to the reference strains (particularly strain R9 which is used in several screening nurseries) through testing the following hypotheses: (1) through sequencing of the ITS-5.8S rDNA region isolates will be proven to vary for AG and genetic diversity; (2) AG distribution will vary across the production area; (3) virulence of isolates on sugar beet will vary within and between AG; and (4) virulence on field corn of a genetically diverse subset of isolates will vary within and between AG.

Materials and methods

Sampling and isolations

Sugar beet roots symptomatic for *Rhizoctonia* root rot were collected from 28 to 30 sites evenly distributed across the Amalgamated Sugar Company production area (southeastern Oregon to southeastern Idaho) in the Intermountain West from 2004 to 2006 (a total of 87 sites sampled). By sampling sites (piling ground and/or neighbouring fields) over a three-year period, most sugar beet fields in the piling ground's area would potentially be represented. Since the piling grounds are evenly distributed throughout the production system, sampling every other piling ground provided an easy way to maintain an even sampling distribution.

Four symptomatic roots per site and year were collected if they were present. A total of 279 roots were collected for a mean of 3.2 roots per site. Isolations were conducted by removing 10 × 10 mm pieces of internal root tissue from the margins between rotted tissue and white, healthy-appearing tissue. 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 × 2 mm piece was placed on potato dextrose agar (PDA; Becton Dickinson & Co., Sparks, Md.) amended with streptomycin sulfate (MP Biomedicals, Inc., Solon, OH) at 200 mg L⁻¹ and incubated at 22 °C. Representative colonies from each root which yielded *R. solani* were hyphal tip transferred onto streptomycin-amended PDA. Initial identifications were performed using a light microscope. The field isolates and reference strains were stored on sterile (autoclaved twice for 60 min at 121 °C on consecutive days) barley (*Hordeum vulgare* L.) kernels at -80 °C.

Sugar beet pathogenicity tests

A pathogenicity test with 113 *Rhizoctonia solani* isolates (94 field isolates and 19 reference strains representative of other sugar beet production areas in the USA and the different AG likely to be found; Table 1) and a non-inoculated check was conducted in the greenhouse on the commercial sugar beet cultivar 'Monohikari' (Seedex, Inc., Sheridan, WY). The experimental design was a randomized complete block with four replications. There was one plant per pot (experimental unit) used for each isolate/strain. Plants were grown from seed in 10.2 cm square plastic pots with Sunshine Mix No. 1 (Sun Gro Horticulture, Bellevue, WA) which contained 70–80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum and a 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 was set to hold 27 °C day and 20 °C night, with day length extended to 13 h with metal halide lamps (250 μmole s⁻¹ m⁻² measured at plant top). Inoculum for the fungal isolates was generated by placing sterile water-soaked barley kernels on a PDA plate near an inoculum plug. The kernels were colonized for three 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. Four weeks after inoculation, the percent foliar discoloration (mostly yellowing and some brown necrotic tissue) was visually determined. The top fresh weight was recorded and the roots were measured at the crown (widest part of root) and bisected through the infected area to visually estimate the percentage of root tissue rotted. A disease severity index (DSI = (top rating + root rot rating + (((crown size - largest crown size)/largest crown size) × -100) + (((top wt. - largest top wt.)/largest top wt.) × -100)/4) was established based on these four variables. Isolations as previously described were conducted on PDA amended with streptomycin (200 mg L⁻¹) with all roots with visible root rot symptoms. The experiment was repeated once.

Corn pathogenicity tests

A pathogenicity test with 18 *Rhizoctonia solani* isolates (selected based on genetic diversity) and a non-inoculated check were conducted in the greenhouse on the silage corn (*Zea mays* L.) hybrid, PHI 1 (contact Pioneer Hi-Bred, Johnston, IA for uncoded name). The experimental design was a randomized complete block with five replications per treatment. Plant growth conditions and inoculum preparation was as described for the sugar beet

Table 1. Anastomosis group, source, and collection date of 113 *Rhizoctonia solani* isolates (94 field isolates from sugar beet and 19 reference strains) used in sequence analysis of the internal transcribe spacer regions (ITS) and 5.8S rDNA region.

| AG* | Isolate [†] | Identity (%) with Accession [‡] | Origin | Year [§] | GenBank |
|----------|----------------------|--|---------------------------|-------------------|-----------------------|
| A | F9 | 98% AY927358 | Cassia Co, Idaho, USA | 2004 | FJ492068 |
| A | F18 | 99% AY927358 | Gooding Co, Idaho, USA | 2004 | FJ492077 |
| A | F179 | 100% AY927341 | Malheur Co, Oregon, USA | 2004 | FJ492097 |
| A | F184 | 100% AY927358 | Canyon Co, Idaho, USA | 2004 | FJ492098 |
| A | F320 | 100% AY927349 | Malheur Co, Oregon, USA | 2005 | FJ492126 |
| E | F14 | 99% AB290019 | Cassia Co, Idaho, USA | 2004 | FJ492073 |
| E | F15 | 96% AB290019 | Minidoka Co, Idaho, USA | 2004 | FJ492074 |
| K | F523 | 99% AB286932 | Elmore Co, Idaho, USA | 2006 | FJ492158 |
| 1 1A | CS-2 | 99% AF354097 | Japan | ND | FJ492099 |
| 1 1B | Shiba-2 | 100% AB122137 | Japan | ND | FJ492100 |
| 1 1C | BV-7 | 99% AF354058 | Japan | ND | FJ492101 |
| 2 BI | TE2-4 | 99% AB054873 | Japan | ND | FJ492108 |
| 2-1 | FC-2 | 99% AB122124 | Japan | ND | FJ492102 |
| 2-2 IIIB | F16 | 99% AB054864 | Minidoka Co, Idaho, USA | 2004 | FJ492075 |
| 2-2 IIIB | F22 | 99% AB054858 | Owyhee Co, Idaho, USA | 2004 | FJ492081 |
| 2-2 IIIB | F30 | 99% AB054858 | Canyon Co, Idaho, USA | 2004 | FJ492089 |
| 2-2 IIIB | F36 | 98% AB054855 | Malheur Co, Oregon, USA | 2004 | FJ492095 |
| 2-2 IIIB | F303 | 99% AB054863 | Canyon Co, Idaho, USA | 2005 | FJ492112 |
| 2-2 IIIB | F304 | 99% AB054863 | Canyon Co, Idaho, USA | 2005 | FJ492113 |
| 2-2 IIIB | F305 | 98% AB054864 | Canyon Co, Idaho, USA | 2005 | FJ492114 |
| 2-2 IIIB | F313 | 98% AB054864 | Cassia Co, Idaho, USA | 2005 | FJ492121 |
| 2-2 IIIB | F314 | 98% AB054855 | Canyon Co, Idaho, USA | 2005 | FJ492122 |
| 2-2 IIIB | F315 | 99% AB054858 | Canyon Co, Idaho, USA | 2005 | FJ492123 |
| 2-2 IIIB | F316 | 99% AB054858 | Canyon Co, Idaho, USA | 2005 | FJ492124 |
| 2-2 IIIB | F321 | 99% AB054858 | Elmore Co, Idaho, USA | 2005 | FJ492127 |
| 2-2 IIIB | F322 | 98% AB054858 | Elmore Co, Idaho, USA | 2005 | FJ492128 |
| 2-2 IIIB | F325 | 98% AB054863 | Cassia Co, Idaho, USA | 2004 | FJ492131 |
| 2-2 IIIB | F330 | 98% AB054858 | Elmore Co, Idaho, USA | 2005 | FJ492133 |
| 2-2 IIIB | F500 | 99% AB054858 | Canyon Co, Idaho, USA | 2006 | FJ492136 |
| 2-2 IIIB | F501 | 99% AB054858 | Canyon Co, Idaho, USA | 2006 | FJ492137 |
| 2-2 IIIB | F502 | 98% AB054858 | Canyon Co, Idaho, USA | 2006 | FJ492138 |
| 2-2 IIIB | F503 | 98% AB054858 | Canyon Co, Idaho, USA | 2006 | FJ492139 |
| 2-2 IIIB | F504 | 99% AB054857 | Canyon Co, Idaho, USA | 2006 | FJ492140 |
| 2-2 IIIB | F505 | 99% AB054857 | Canyon Co, Idaho, USA | 2006 | FJ492141 |
| 2-2 IIIB | F506 | 98% AB054855 | Jerome Co, Idaho, USA | 2006 | FJ492142 |
| 2-2 IIIB | F507 | 98% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492143 |
| 2-2 IIIB | F508 | 98% AB054863 | Cassia Co, Idaho, USA | 2006 | FJ492144 |
| 2-2 IIIB | F509 | 98% AB054855 | Owyhee Co, Idaho, USA | 2006 | FJ492145 |
| 2-2 IIIB | F510 | 98% AB054855 | Owyhee Co, Idaho, USA | 2006 | FJ492146 |
| 2-2 IIIB | F511 | 96% AB054858 | Owyhee Co, Idaho, USA | 2006 | FJ492147 |
| 2-2 IIIB | F512 | 98% AB054855 | Owyhee Co, Idaho, USA | 2006 | FJ492148 |
| 2-2 IIIB | F513 | 97% AB054858 | Owyhee Co, Idaho, USA | 2006 | FJ492149 |
| 2-2 IIIB | F514 | 99% AB054863 | Elmore Co, Idaho, USA | 2006 | FJ492150 |
| 2-2 IIIB | F515 | 99% AB054858 | Elmore Co, Idaho, USA | 2006 | FJ492151 |
| 2-2 IIIB | F516 | 98% AB054863 | Elmore Co, Idaho, USA | 2006 | FJ492152 |
| 2-2 IIIB | F517 | 99% AB054858 | Owyhee Co, Idaho, USA | 2006 | FJ492153 |
| 2-2 IIIB | F518 | 99% AB054858 | Owyhee Co, Idaho, USA | 2006 | FJ492154 |
| 2-2 IIIB | F519 | 98% AB054855 | Owyhee Co, Idaho, USA | 2006 | FJ492155 |
| 2-2 IIIB | F520 | 99% AB054863 | Elmore Co, Idaho, USA | 2006 | FJ492156 |
| 2-2 IIIB | F521 | 99% AB054863 | Twin Falls Co, Idaho, USA | 2006 | FJ492157 |
| 2-2 IIIB | F524 | 98% AB054863 | Cassia Co, Idaho, USA | 2006 | FJ492159 |
| 2-2 IIIB | F548 | 99% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492160 |
| 2-2 IIIB | F549 | 99% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492161 |
| 2-2 IIIB | F550 | 99% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492162 |
| 2-2 IIIB | F551 | 99% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492163 |
| 2-2 IIIB | F552 | 98% AB054855 | Jerome Co, Idaho, USA | 2006 | FJ492164 |
| 2-2 IIIB | F553 | 98% AB054858 | Jerome Co, Idaho, USA | 2006 | FJ492165 |
| 2-2 IIIB | 87-36-2 | 99% AB054858 | North Dakota, USA | ND | FJ492171 |

(Continued)

Table 1. (Continued.)

| AG* | Isolate [†] | Identity (%) with Accession [‡] | Origin | Year [§] | GenBank |
|----------|----------------------|--|---------------------------|-------------------|-----------------------|
| 2-2 IIIB | C-116 | 99% AB054854 | Japan | ND | FJ492103 |
| 2-2 IIIB | R4 | 98% AB054863 | Texas, USA | ND | FJ492175 |
| 2-2 IIIB | R9 | 98% AB054863 | Colorado, USA | ND | FJ492110 |
| 2-2 IIIB | W-22 | 99% AB054864 | Wisconsin, USA | ND | FJ492170 |
| 2-2 IV | F24 | 99% AB054865 | Canyon Co, Idaho, USA | 2004 | FJ492083 |
| 2-2 IV | RI-64 | 98% AB054865 | Japan | ND | FJ492104 |
| 2-2 IV | H502 | 99% AB054862 | USA | ND | FJ492169 |
| 2-2 IV | H-3-77 | 99% AB054859 | Minnesota, USA | ND | FJ492172 |
| 2-2 IV | 5E13 | 98% AB054865 | Minnesota, USA | ND | FJ492173 |
| 2-2 IV | 2C13 | 100% AB054859 | Montana, USA | ND | FJ492174 |
| 3 PT | ST-11-6 | 99% AB019009 | Japan | ND | FJ492105 |
| 4 HG-I | F20 | 100% AB000012 | Gooding Co, Idaho, USA | 2004 | FJ492079 |
| 4 HG-I | F31 | 100% AB000012 | Canyon Co, Idaho, USA | 2004 | FJ492090 |
| 4 HG-I | F32 | 100% AB000012 | Canyon Co, Idaho, USA | 2004 | FJ492091 |
| 4 HG-I | F37 | 100% AB000012 | Malheur Co, Oregon, USA | 2004 | FJ492096 |
| 4 HG-I | R101 | 98% AB000018 | Japan | ND | FJ492106 |
| 4 HG-II | F1 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492060 |
| 4 HG-II | F2 | 100% AF354074 | Twin Falls Co, Idaho, USA | 2004 | FJ492061 |
| 4 HG-II | F3 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492062 |
| 4 HG-II | F4 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492063 |
| 4 HG-II | F5 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492064 |
| 4 HG-II | F6 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492065 |
| 4 HG-II | F7 | 99% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492066 |
| 4 HG-II | F8 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492067 |
| 4 HG-II | F10 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492069 |
| 4 HG-II | F11 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492070 |
| 4 HG-II | F12 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492071 |
| 4 HG-II | F13 | 100% AF354074 | Cassia Co, Idaho, USA | 2004 | FJ492072 |
| 4 HG-II | F17 | 100% AF354074 | Gooding Co, Idaho, USA | 2004 | FJ492076 |
| 4 HG-II | F19 | 100% AF354074 | Gooding Co, Idaho, USA | 2004 | FJ492078 |
| 4 HG-II | F21 | 100% AF354074 | Gooding Co, Idaho, USA | 2004 | FJ492080 |
| 4 HG-II | F23 | 99% AF354074 | Owyhee Co, Idaho, USA | 2004 | FJ492082 |
| 4 HG-II | F25 | 99% AF354074 | Owyhee Co, Idaho, USA | 2004 | FJ492084 |
| 4 HG-II | F26 | 96% AF354074 | Canyon Co, Idaho, USA | 2004 | FJ492085 |
| 4 HG-II | F27 | 100% AF354074 | Canyon Co, Idaho, USA | 2004 | FJ492086 |
| 4 HG-II | F28 | 99% AF354074 | Canyon Co, Idaho, USA | 2004 | FJ492087 |
| 4 HG-II | F29 | 100% AF354074 | Owyhee Co, Idaho, USA | 2004 | FJ492088 |
| 4 HG-II | F33 | 98% AF354074 | Canyon Co, Idaho, USA | 2004 | FJ492092 |
| 4 HG-II | F34 | 100% AF354074 | Canyon Co, Idaho, USA | 2004 | FJ492093 |
| 4 HG-II | F35 | 100% AF354074 | Malheur Co, Oregon, USA | 2004 | FJ492094 |
| 4 HG-II | F302 | 99% AF354074 | Bingham Co, Idaho, USA | 2005 | FJ492111 |
| 4 HG-II | F306 | 100% AF354074 | Elmore Co, Idaho, USA | 2005 | FJ492115 |
| 4 HG-II | F307 | 98% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492116 |
| 4 HG-II | F309 | 100% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492117 |
| 4 HG-II | F310 | 99% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492118 |
| 4 HG-II | F311 | 100% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492119 |
| 4 HG-II | F312 | 100% AF354074 | Cassia Co, Idaho, USA | 2005 | FJ492120 |
| 4 HG-II | F319 | 99% AF354074 | Malheur Co, Oregon, USA | 2005 | FJ492125 |
| 4 HG-II | F323 | 100% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492129 |
| 4 HG-II | F324 | 100% AF354074 | Gooding Co, Idaho, USA | 2005 | FJ492130 |
| 4 HG-II | F329 | 100% AF354074 | Cassia Co, Idaho, USA | 2005 | FJ492132 |
| 4 HG-II | F498 | 100% AF354074 | Jerome Co, Idaho, USA | 2006 | FJ492134 |
| 4 HG-II | F499 | 99% AF354074 | Jerome Co, Idaho, USA | 2006 | FJ492135 |
| 5 | ST-6-1 | 99% DQ355140 | Japan | ND | FJ492107 |
| 8 | 72 | 99% AF354068 | Australia | ND | FJ492109 |

Notes: *AG = anastomosis group.

[†]Reference strains in bold.[‡]Identity (%) with Accession = percent identity found with BLASTn to first GenBank accession associated with a refereed publication.[§]ND = no date available.^{||}GenBank accession number for the sequence data associated with this isolate.

pathogenicity tests. The plants were inoculated at the one- to two-leaf growth stage by placing an infested kernel 10 mm down into the potting mix next to the seed. Three weeks after inoculation at the five- to six-leaf growth stage, the top fresh weight was determined and the roots were evaluated visually for root lesion number and area (percentage of root area affected by lesions). Isolations as previously described were conducted (except root lesions and not 10 × 10 mm pieces were removed) on PDA amended with streptomycin (200 mg L⁻¹) for all roots with lesions. The experiment was repeated once.

DNA extraction and polymerase chain reaction

The 113 *Rhizoctonia solani* isolates were grown in potato dextrose broth (PDB; Becton Dickinson & Co., Sparks, Md.) at 21 °C until approximately a 10-mm diameter ball of tissue was generated from a hyphal plug. The PDB was poured off and the tissue was rinsed with sterile reverse osmosis water. A portion of the tissue was placed in a sterile 2 mL micro centrifuge tube (filled tube to the 0.5 mL mark) and stored at -80 °C. Frozen tissue in individual tubes was freeze-dried and then pulverized using a Retsch MM 301 mixer mill (Retsch Inc., Newton, PA) with 5 mm stainless steel beads. DNA was extracted using a DNeasy Plant Mini Isolation kit (Qiagen Inc., Valencia, CA) following standard protocols suggested by the manufacturer. The DNA was stored at -20 °C.

Polymerase chain reactions (PCR) were performed in volumes of 30 µL using the AmpliTaq Gold (Applied Biosystems Inc., Foster City, CA) *Taq* polymerase in accordance with the manufacturer's instructions supplemented with 2.4 µL of 25 mM MgCl₂, 0.75 µL of 10 µM each ITS primer and 60 ng of target DNA. Amplification consisted of 5 min at 95 °C followed by 40 cycles of 95 °C for 35 s, 62 °C for 50 s, and 72 °C for 90 s. After the last cycle, the reaction was held at 72 °C for 10 min and then 4 °C.

Samples were amplified using primers ITS1 and ITS4 (White *et al.*, 1990). Amplification products were electrophoresed through agarose gels (1.8% wt/vol) supplemented with ethidium bromide (0.01 mg mL⁻¹) in Tris borate EDTA buffer (TBE, 89 mM Tris base, 89 mM boric acid and 2 mM EDTA).

Amplicons produced from the 113 *Rhizoctonia solani* isolates using the ITS primer set were sent to TACGen (Richmond, CA) for sequencing in both directions. Isolates that had sequences with double peaks in the chromatograms were cloned using the pGEM-T Easy Vector System (Promega Corp., Madison, WI) and sequenced again in both directions using the SP6 5'-TATTTAGGTGACACTATAG-3' and T7

5'-TAATACGACTCACTATAGGG-3' plasmid primers. Sequence identity between clones was calculated using BioEdit version 7.0.9.0 (Hall, 1999). Results were compared with accessions in GenBank to confirm species identity and anastomosis group using BLASTn 2.2.17 (Altschul *et al.*, 1997). DNA sequences were aligned using ClustalX Ver. 2.0 (Conway Institute, UCD, Dublin). Prior to phylogenetic analysis, the ends of the sequences were trimmed using BioEdit so only the ITS1, 5.8S, and ITS2 regions (White *et al.*, 1990) were compared. For isolates with multiple clones (Supplemental Table A1, online), only the most common sequence (FJ492 accession series) was included in the phylogenetic analysis. GenBank accessions for 46 *R. solani* strains representing the extent of genetic diversity identified previously in the USA and internationally were also included in the phylogenetic analysis. Phylogenetic analysis was performed using PAUP 4.0 Beta Version 10 (Sinauer Associates, Inc., Sunderland, MA). Maximum parsimony analysis was performed with the heuristic search with simple taxon addition sequences, tree bisection-reconnection (TBR) branch swapping and MaxTrees = 100. Confidence intervals in tree topologies were estimated by bootstrap analysis with 1000 replicates. Only nodes with bootstrap values over 50% were considered significant. The resulting tree was visualized using the program TreeView X Version 0.5.0 (Rodrick D. M. Page, University of Glasgow, Scotland). Distance matrix analysis was conducted with the neighbour-joining (NJ) algorithm and the Jukes-Cantor genetic distance model (calculates divergence time; 0 = isolate sequences were the same).

Data analysis

The SAS Univariate procedure (SAS Institute Inc., Cary, NC) was used to test for 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 linear methods procedure, and Fisher's protected least significant difference was used for mean comparisons. For analysis of unbalanced data sets (group comparisons), the SAS generalized linear mixed models procedure was utilized and variance between trials was evaluated through residual log likelihoods. For regression analysis, the SAS Linear regression procedure was used.

Results

AG group and genetic diversity

Sequences from the ITS-5.8S rDNA region for 113 *R. solani* isolates/strains were deposited as GenBank

accessions FJ492060–FJ492175 (Table 1). BLASTn analysis indicated that all 19 reference strains had the same AG group identity as established previously, except for 5E13. 5E13 had 98% identity with an AG-2-2 IV strain and should have had close identity with AG-2-1 (Table 1).

The majority (91%) of the field isolates had identity with either the AG-2-2 IIIB or one of the AG-4 subgroups (Table 1). Most of the isolates (74%) came from the western portion (sites west of and including Twin Falls, ID) of the Amalgamated Sugar Company production area.

The AG-2-2 IIIB subgroup was comprised of 44 field isolates (47% of all field isolates) of which 87% came from the western portion of the Amalgamated Sugar Company production area. All AG-2-2 IIIB isolates or reference strains required cloning because the initial sequencing was not homogeneous (double peaks in DNA chromatograms) except for the following 13 isolates or strains (C-116, F22, F303, F305, F313, F321, F322, F504, F507, F511, F513, F524 and R4). The AG for all

isolates was the same as that identified before cloning. Among the six AG-2-2 IIIB field isolates cloned multiple times, 98 to 100% sequence identity was evident among clones within an isolate (Supplemental Table A1, online). Among the 57 clones from these six isolates, there were a total of 44 variable sites (Supplemental Table A2, online) and 30 unique sequences (Supplemental Table A1, online). Depending on the isolate, the number of variable sites could range from 6 to 16 (Supplemental Table A1, online). In the phylogram (Fig. 1), isolates in the AG-2-2 IIIB subgroup formed two groups. The field isolates that formed a smaller group ranging from isolate F16 to F511 in Fig. 1, had little diversity. The genetic distance from F16 to F511 was only 0.0474. Two reference strains and seven accessions considered to be AG-2-2 IIIB fell in this smaller group. The AG-2-2 IIIB reference strains and accessions confirm the BLASTn results which showed the field isolates in this group should be considered AG-2-2 IIIB strains. The field isolates in the

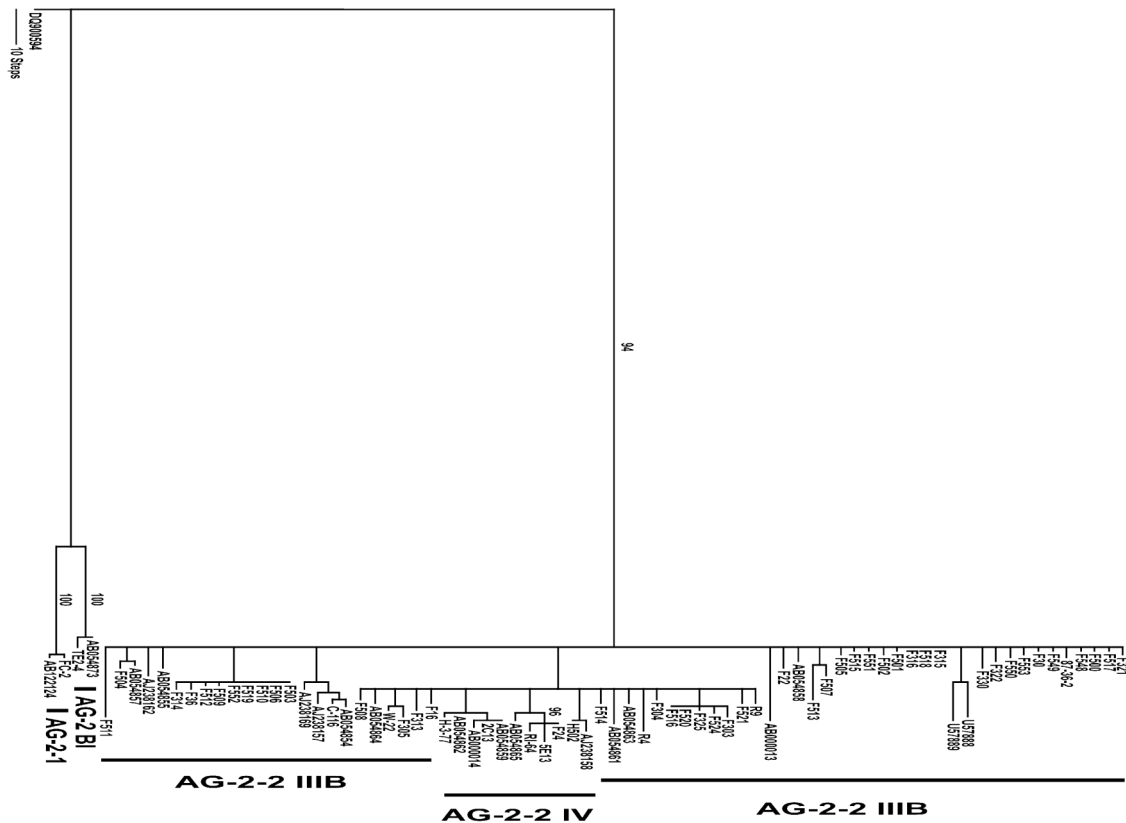


Fig. 1. Phylogenetic comparison of sequences with PAUP of the ITS region and 5.8S rDNA of 77 *Rhizoctonia solani* AG-2 isolates in a bootstrap 50% majority-rule consensus tree. The tree length was 585 steps with 173 parsimony-informative characters along with consistency index (CI) = 0.6547 and retention index (RI) = 0.7233. The relative support for each clade is indicated by bootstrap values on branches. The F series numbers indicate isolates sequenced in the present study. Reference strains sequenced in the present study included: AG-2-1 = FC-2; AG-2 BI = TE2-4; AG-2-2 IIIB = C-116, R9, 87-36-2, R4, and W-22; AG-2-2 IV = RI-64, H502, H-3-77, 5E13, and 2C13. For strains designated with GenBank accession numbers, the sequencing had been conducted in other studies. The out-group was *Rhizoctonia zeae* accession DQ900594.

larger group of AG-2-2 IIIB isolates (range from F321 to F514 in Fig. 1) had a genetic distance of 0.0181, which is even smaller than that of the smaller group. Three reference strains and six accessions considered to be AG-2-2 IIIB fell in this larger group confirming the BLASTn results for the isolates in this group. The reference strain R9 frequently used to screen germplasm fell towards the centre of this phylogram along with Idaho isolate F521. The largest genetic distance from R9 to any of the AG-2-2 IIIB field isolates was 0.0379 to F513. For comparison, the genetic distances from R9 to the AG-2 BI strain TE2-4, AG-2-1 strain FC-2, and the outgroup were 0.0988, 0.0966 and 0.3654, respectively.

The one AG-2-2 IV isolate, F24, fell with the 13 AG-2-2 IV reference strains or accessions on the phylogram confirming the BLASTn results. All the AG-2-2 IV isolates/strains fell between the two AG-2-2 IIIB groups on the phylogram.

The AG-2 BI and AG-2-1 reference strains and accessions were placed into distinct clades separate from the other isolates, strains and accessions. Reference strain 5E13 should have fallen with this group but fell in with the AG-2-2 IV strains instead. The BLASTn results also indicated it belonged with the AG-2-2 IV strains. The largest genetic distance, 0.1265, between AG-2 isolates was between AG-2 BI strain TE2-4 and AG-2-2 IIIB isolate F513. The genetic distance from the out-group to all the AG-2 isolates ranged from 0.3453 to 0.3734. These data support the separation seen on the phylogram.

The AG-4 HG-II subgroup had 37 field isolates (39% of all field isolates) and only two (F28 and F34) isolates required cloning because the initial sequencing was not homogeneous. The HG-II isolates formed one uniform clade on the phylogram (Fig. 2). Isolate F26 appears separate from the other HG-II isolates on the phylogram, but bootstrap values do not support separating it into its own clade. An additional four isolates were associated with the AG-4 HG-I subgroup and also fell into a separate clade. If the AG-4 subgroups were combined, these isolates represent 44% of all field isolates of which 63% originated in the western portion of the Amalgamated Sugar Company production area. The 14 reference strains or accessions all fell with the appropriate AG-4 subgroups confirming the BLASTn results. The largest genetic distance between any of the AG-4 isolates was 0.0379 between F26 and F35. The genetic distance from the out-group (*R. zea*) to all the AG-4 isolates ranged from 0.3546 to 0.3848. These data support the separation seen on the phylogram.

Five field isolates had 98–100% identity with AG-A, two isolates had 96 and 99% identity with AG-E, and one isolate had 99% identity with AG-K. The phylogenetic relationship among these isolates, diverse isolates

from the AG-2-2 and AG-4 groups, reference strains and accessions was established in Fig. 3. The different AG form distinct clades as expected. Within *R. solani* isolates and strains the largest genetic distance, 0.2157, was between AG-2-2 IIIB isolate F511 and AG-1 1B accession AB122137. The largest genetic distance between *R. solani* included in Fig. 3 and the *R. zea* out-group, DQ900594, was 0.4310 with AG-2-2 IIIB isolate F511.

Sugar beet top discolouration

For the top discolouration in the sugar beet pathogenicity tests, trials 1 and 2 were not significantly different ($P = 0.2987$) and variances were homogeneous ($P = 0.5461$), so data were analyzed together. Compared with the non-inoculated check, 52 isolates [46 AG-2-2 IIIB (discolouration ranged from 39 to 100%), 4 AG-2-2 IV (31 to 53%), and 2 AG-4 HG-I (20 to 29%)] had significantly ($P < 0.05$) more top discolouration (Supplemental Table A3, online). The AG-2-2 IIIB and IV isolates appeared to be randomly distributed on the phylogram. Compared with reference strain R9, 28 isolates (all AG-2-2 IIIB) caused more top discolouration and were distributed across AG-2-2 IIIB groups on the phylogram. When analyzed by AG, trials 1 and 2 were not significantly different ($P = 0.6843$) and a comparison of residual log likelihoods indicated variances were similar ($\chi^2 = 0.72$, $P > 0.50$), so data were analyzed together. AG-2-2 IIIB (83%) and AG-2-2 IV (30%) were different from each other and had more top discolouration than other AG and the non-inoculated check (Table 2).

Sugar beet root rot

For root rot in the sugar beet pathogenicity tests, trials 1 and 2 were not significantly different ($P = 0.5057$) and variances were homogeneous ($P = 0.5545$), so data were analyzed together. Compared with the non-inoculated check, 57 isolates [47 AG-2-2 IIIB (rot ranged from 19 to 100%), 4 AG-2-2 IV (34 to 71%), 3 AG-4 HG-I (17 to 25%), and 3 AG-4 HG-II (14 to 29%)] had significantly more root rot than the control (Supplemental Table A3, online). The 3 AG-4 HG-I isolates fell together on the phylogram, while isolates from the other groups were more widely distributed. Compared with reference strain R9, 36 isolates (all AG-2-2 IIIB) caused more root rot and were distributed across AG-2-2 IIIB groups on the phylogram. When analyzed by AG, trials 1 and 2 were not significantly different ($P = 0.7991$) and a comparison of residual log likelihoods indicated variances were similar ($\chi^2 = 1.48$, $P > 0.10$), so data were analyzed together. AG-2-2 IIIB (85% rot) and AG-2-2 IV (36%) were different from each other and had more root rot than the

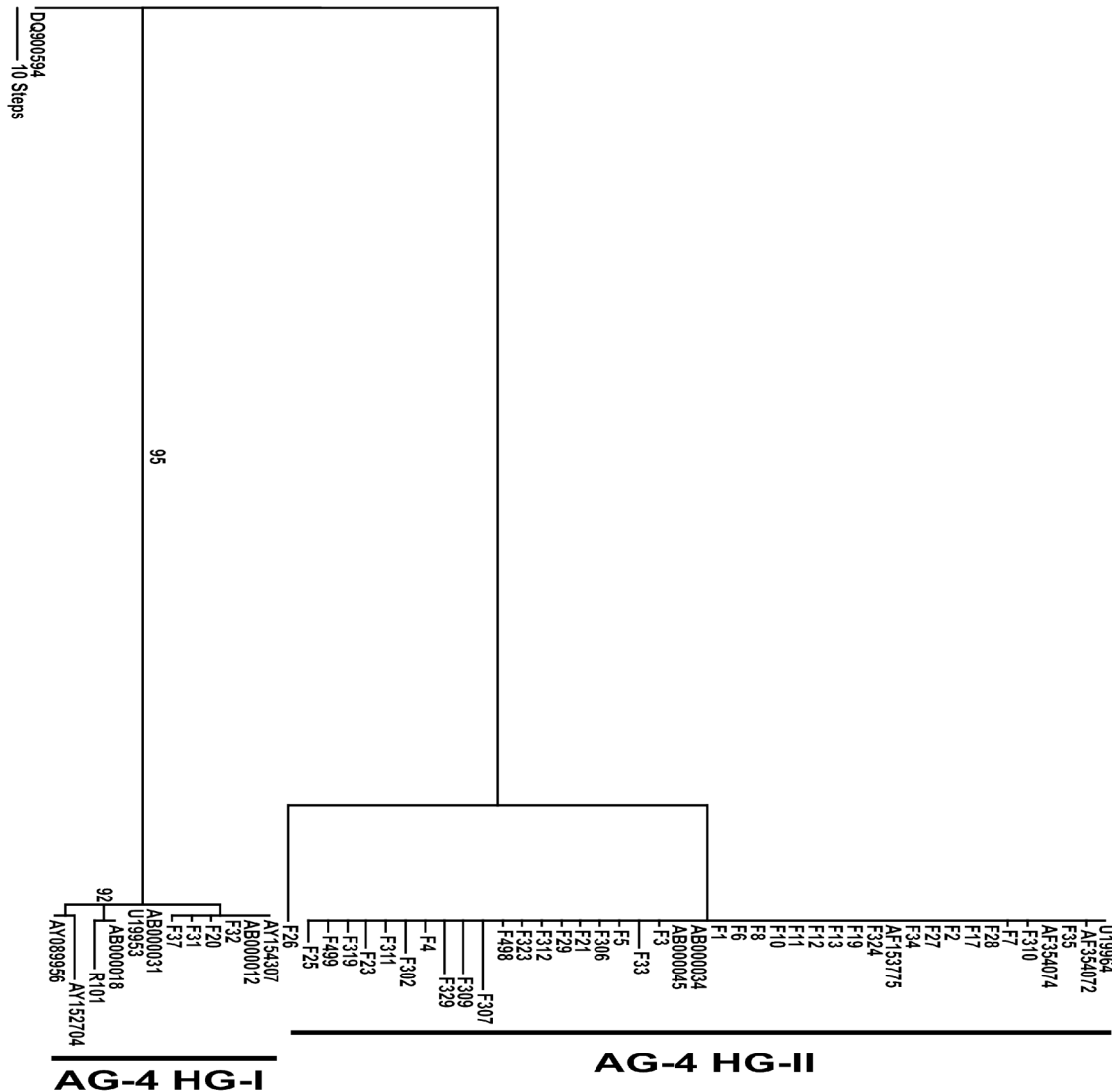


Fig. 2. Phylogenetic comparison of sequences with PAUP of the ITS region and 5.8S rDNA of 55 *Rhizoctonia solani* AG-4 isolates in a bootstrap 50% majority-rule consensus tree. The tree length was 306 steps with 192 parsimony-informative characters along with consistency index (CI) = 0.8562 and retention index (RI) = 0.8333. The relative support for each clade is indicated by bootstrap values on branches. The F series numbers indicate isolates sequenced in the present study along with the AG-4 HG-I reference strain R101. For strains designated with GenBank accession numbers, the sequencing had been conducted in other studies. The out-group was *Rhizoctonia zae* accession DQ900594.

other AG and non-inoculated check (Table 2). Isolations from roots with rot revealed *R. solani* was always present. The regression of root rot versus top discoloration was a positive relationship ($r^2 = 0.93$, $P < 0.0001$).

Sugar beet crown size

With root crown size in the sugar beet pathogenicity tests, trials 1 and 2 were not significantly different ($P = 0.7360$), but the variances were not homogeneous ($P = 0.0341$), so data were analyzed separately. In trial 1, compared with the non-inoculated check, 52 isolates [43 AG-2-2

IIIB (size ranged from 0 to 39 mm), 4 AG-4 HG-II (21 to 44 mm), 3 AG-4 HG-I (24 to 33 mm), and 2 AG-2-2 IV (23 to 32 mm)] had smaller crowns (Supplemental Table A3, online). In trial 2, compared with the non-inoculated check, 47 isolates [44 AG-2-2 IIIB (size ranged from 6 to 26 mm) and 3 AG-2-2 IV (23 to 26 mm)] had smaller crowns. In trial 1, compared with reference strain R9, 26 isolates (all AG-2-2 IIIB) had smaller crowns. In trial 2, compared with reference strain R9, six isolates (all AG-2-2 IIIB) had smaller crowns. When analyzed by AG, trials 1 and 2 were not significantly different ($P = 0.4694$) but a comparison of residual log likelihoods indicated

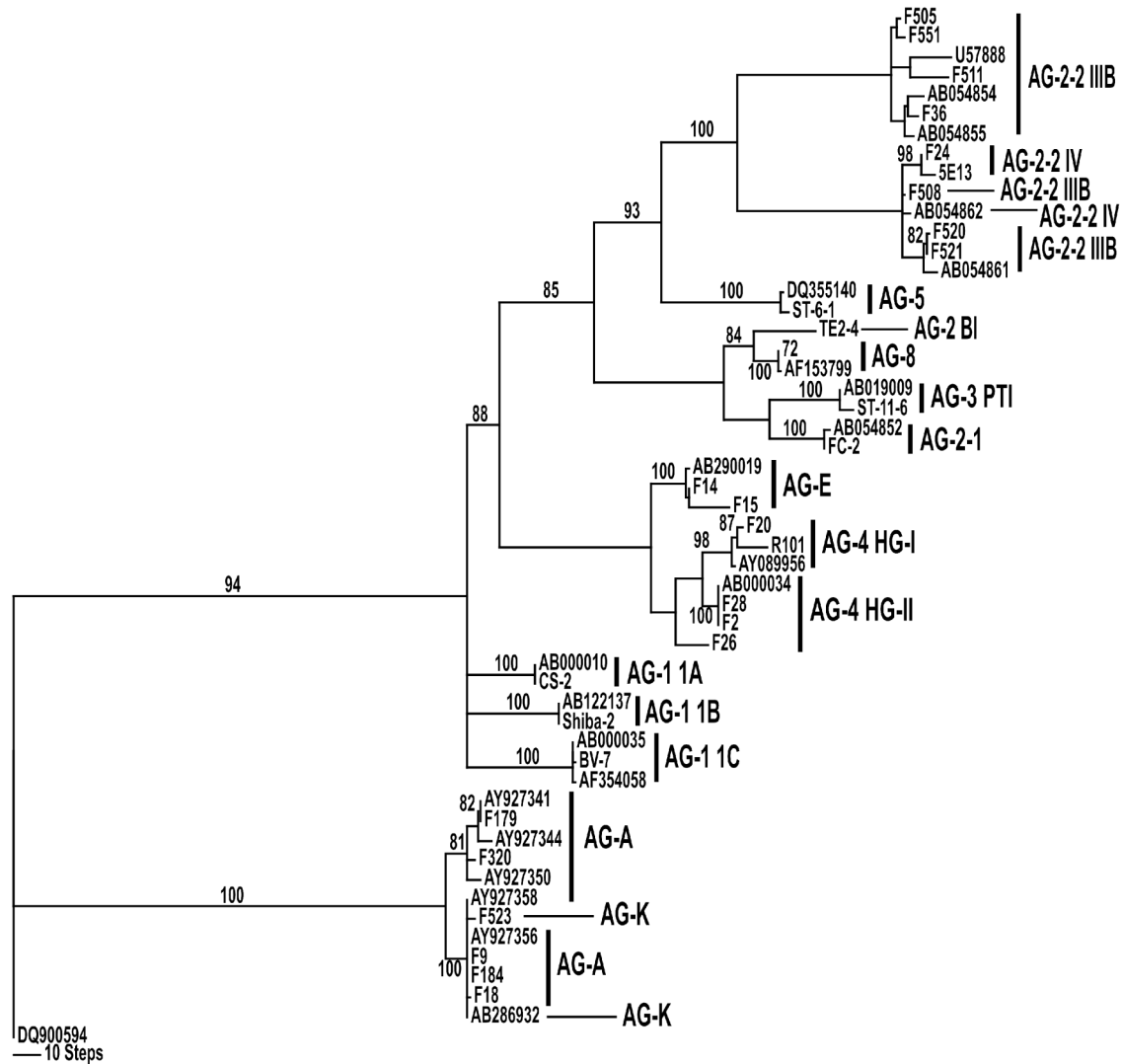


Fig. 3. Phylogenetic comparison of sequences with PAUP of the ITS region and 5.8S rDNA of 52 *Rhizoctonia solani* isolates in a bootstrap 50% majority-rule consensus tree. The tree length was 856 steps with 117 parsimony-informative characters along with consistency index (CI) = 0.6297 and retention index (RI) = 0.8776. The relative support for each clade is indicated by bootstrap values on branches. The F series numbers indicate isolates sequenced in the present study. Reference strains sequenced in the present study included: AG-1 1A = CS-2; AG-1 1B = Shiba-2; AG-1 1C = BV-7; AG-2-1 = FC-2; AG-2 BI = TE2-4; AG-2-2 IV = 5E13; AG-3 PT = ST-11-6; AG-4 HG-I = R101; AG-5 = ST-6-1; AG-8 = 72. For strains designated with GenBank accession numbers, the sequencing had been conducted in other studies. The out-group was *Rhizoctonia zeae* accession DQ900594.

variances were different ($\chi^2 = 10.64$, $P < 0.005$), so data were analyzed separately. In both trials AG-2-2 IIIB had smaller crowns than other AG and the non-inoculated check (Table 2).

Sugar beet top weight

With top fresh weight in the sugar beet pathogenicity tests, trials 1 and 2 were not significantly different ($P = 0.3232$), but the variances were not homogeneous ($P = 0.0045$), so data were analyzed separately. In trial 1, compared with

the non-inoculated check, 44 isolates [43 AG-2-2 IIIB (weight ranged from 4 to 42 g) and 1 AG-2-2 IV (32 g)] had smaller tops (Supplemental Table A3, online). In trial 2, compared with the non-inoculated check, 52 isolates [46 AG-2-2 IIIB (weight ranged from 3 to 61 g), 3 AG-2-2 IV (26 to 34 g), 2 AG-4 HG-I (55 to 58 g), and 1 AG-4 HG-II (60 g)] had smaller tops (Supplemental Table A3, online). In trial 1, compared with reference strain R9, 14 isolates (all AG-2-2 IIIB) had smaller tops. In trial 2, compared with reference strain R9, 32 isolates (all AG-2-2 IIIB) had smaller tops. When analyzed by AG, trials 1 and

Table 2. Greenhouse pathogenicity tests for 113 *Rhizoctonia solani* isolates representing different anastomosis groups on the sugar beet cultivar 'Monohikari'.

| AG* | Top (%) [†] | Root rot [‡] (%) | Crown size (mm) | | Top fresh weight (g) | | DSI [§] |
|--------------|----------------------|---------------------------|-----------------|----------|----------------------|----------|------------------|
| | | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | |
| 2-2 IIIB | 83 a | 85 a | 16 d | 17 c | 19 c | 17 c | 79 a |
| 2-2 IV | 30 b | 36 b | 34 bc | 32 b | 54 b | 48 b | 38 b |
| 4 HG-I | 13 c | 14 c | 32 c | 36 ab | 65 ab | 67 a | 24 c |
| 5 | 4 c | 0 c | 38 a-c | 30 b | 57 ab | 73 a | 18 c |
| 4 HG-II | 5 c | 3 c | 38 a-c | 39 ab | 66 ab | 73 a | 16 c |
| 1 IC | 2 c | 0 c | 38 a-c | 39 ab | 64 ab | 63 ab | 16 c |
| A | 6 c | 2 c | 41 a-c | 39 ab | 68 ab | 73 a | 15 c |
| K | 3 c | 0 c | 43 a-c | 36 ab | 65 ab | 69 a | 15 c |
| E | 4 c | 0 c | 39 a-c | 40 ab | 65 ab | 79 a | 14 c |
| 2 BI | 1 c | 0 c | 37 a-c | 42 ab | 62 ab | 73 a | 14 c |
| 2-1 | 2 c | 0 c | 38 a-c | 38 ab | 65 ab | 79 a | 14 c |
| 1 IA | 4 c | 0 c | 42 a-c | 41 ab | 70 ab | 75 a | 13 c |
| 1 IB | 2 c | 0 c | 44 a | 42 a | 63 ab | 72 a | 13 c |
| Check | 1 c | 0 c | 43 ab | 38 ab | 60 ab | 82 a | 13 c |
| 8 | 4 c | 0 c | 40 a-c | 41 ab | 76 a | 76 a | 12 c |
| 3 PT | 1 c | 0 c | 42 ab | 41 ab | 68 ab | 76 a | 12 c |
| $P > F^{ }$ | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

Notes: *AG = anastomosis group. Check = non-inoculated check. The means and standard deviations were established based on all isolates within an AG.

[†]Top = percent foliar discoloration.

[‡]Root rot = percentage of root with discoloration.

[§]DSI = top rating + root rot rating + (((crown size - largest crown size)/largest crown size) × -100) + (((top wt. - largest top wt.)/largest top wt.) × -100)/4.

^{||} $P > F$ was the probability associated with the F value. Means within a column followed by the same letter are not significantly different based on Fisher's protected least significant difference ($P \leq 0.05$).

2 were significantly different ($P = 0.0097$) and a comparison of residual log likelihoods indicated variances were different ($\chi^2 = 3.98$, $P < 0.05$), so data were analyzed separately. In both trials, AG-2-2 IIIB had less top fresh weight than the other AG and the non-inoculated check (Table 2). AG-2-2 IV had less top fresh weight than the non-inoculated check, but not all other AG. The regression of root rot versus crown size or top weight were negative relationships ($r^2 = 0.79$, $P < 0.0001$ and $r^2 = 0.87$, $P < 0.0001$, respectively).

Sugar beet disease severity index

With disease severity index in the sugar beet pathogenicity tests, trials 1 and 2 were not significantly different ($P = 0.8089$) and variances were homogeneous ($P = 0.4707$), so data were analyzed together. Compared with the non-inoculated check, 55 isolates/strains [47 AG-2-2 IIIB (index ranged from 31 to 96), 4 AG-2-2 IV (38 to 57), 2 AG-4 HG-I (30 to 36), and 2 AG-4 HG-II (27 to 30)] could cause disease (Supplemental Table A3, online). Compared with reference strain R9, 35 isolates (all AG-2-2 IIIB) were significantly more virulent and six AG-2-2 IIIB isolates were significantly less virulent.

When analyzed by AG, trials 1 and 2 were not significantly different ($P = 0.8204$) and a comparison of residual log likelihoods indicated variances were similar ($\chi^2 = 0.48$, $P > 0.25$), so data were analyzed together. AG-2-2 IIIB (index of 79) and AG-2-2 IV (38) were significantly different from each other (Table 2). These two AG were also the only AG different from the non-inoculated check (Table 2). The 20 most virulent isolates based on disease severity index appeared to be randomly distributed (no clustering evident) on the phylogram in Fig. 1.

Corn top weight

The two trials differed for top fresh weight ($P = 0.0029$) and thus they were analyzed separately. In trial 1, the treatments did not differ for top fresh weight (Table 3). In trial 2, plants treated with six isolates (F27, F32, F320, F508, F512 and F552) had significantly less top weight than the non-inoculated check.

Corn root lesion number

The two trials differed for lesion number ($P = 0.0192$) and thus they were analyzed separately. Seven isolates (F303, F304, F512, F517, F548, F551 and F552; all AG-2-2 IIIB)

Table 3. Greenhouse pathogenicity tests for *Rhizoctonia solani* isolates on Pioneer silage corn hybrid PHI 1.

| Isolate* | AG [†] | Lesion number [‡] | | | | | | | |
|--------------|-----------------|----------------------------|----------|----------------------------|-----|---------|-----|------------------------------|---------|
| | | Top fresh weight (g) | | Lesion number [‡] | | | | Lesion area (%) [§] | |
| | | Trial 1 | Trial 2 | Trial 1 | | Trial 2 | | Trans | Untrans |
| F304 | 2-2 IIIB | 16.4 | 20.3 a-e | 3.1 a | 9.4 | 2.4 a | 5.8 | 4.9 a | 26 |
| F552 | 2-2 IIIB | 17.6 | 19.0 c-e | 3.0 a | 8.8 | 2.4 a | 5.8 | 4.8 ab | 25 |
| F551 | 2-2 IIIB | 17.6 | 20.6 a-e | 2.8 ab | 7.8 | 2.3 a | 5.2 | 4.0 bc | 19 |
| F512 | 2-2 IIIB | 16.8 | 18.4 de | 2.7 ab | 6.8 | 1.6 bc | 2.4 | 3.6 c | 16 |
| F548 | 2-2 IIIB | 17.6 | 19.9 a-e | 2.5 b | 6.0 | 2.1 ab | 4.2 | 3.5 c | 15 |
| F303 | 2-2 IIIB | 14.6 | 23.8 a | 1.6 c | 2.2 | 1.7 b | 2.6 | 1.8 d | 3 |
| F517 | 2-2 IIIB | 18.5 | 20.5 a-e | 1.5 c | 2.4 | 1.1 cd | 1.0 | 1.6 d | 3 |
| F521 | 2-2 IIIB | 20.1 | 21.6 a-e | 1.0 d | 0.8 | 1.1 d | 0.8 | 1.1 de | 1 |
| F15 | E | 18.3 | 23.0 a-c | 0.8 d | 0.2 | 0.8 d | 0.2 | 0.8 e | 0 |
| F307 | 4 HG-II | 17.1 | 23.5 ab | 0.9 d | 0.4 | 0.7 d | 0.0 | 0.7 e | 0 |
| CS-2 | 1 1A | 18.2 | 22.4 a-d | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| F18 | A | 17.4 | 23.8 a | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| F27 | 4 HG-II | 18.0 | 18.1 de | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| F320 | A | 18.4 | 17.6 e | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| F508 | 2-2 IIIB | 17.3 | 19.2 b-e | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| ST-6-1 | 5 | 14.7 | 22.3 a-d | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| F32 | 4 HG-I | 15.1 | 17.5 e | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| 2C13 | 2-2 IV | 18.2 | 21.8 a-e | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| Check | | 14.7 | 24.4 a | 0.7 d | 0.0 | 0.7 d | 0.0 | 0.7 e | 0 |
| $P > F^{ }$ | | 0.1340 | 0.0124 | <0.0001 | | <0.0001 | | <0.0001 | |

Notes: *Check = non-inoculated check.

[†]AG = anastomosis group.

[‡]Lesion number = number of lesions evident on the root system. Trans = square root transformation since most data points were less than 20%. Untrans = the untransformed means.

[§]Lesion area = percentage of root system with discoloration.

^{||} $P > F$ was the probability associated with the F value. Means within a column followed by the same letter are not significantly different based on Fisher's protected least significant difference ($P \leq 0.05$).

caused significantly more lesions than the non-inoculated check (no lesions) in trial 1 (Table 3). In trial 2, six of the same isolates (F303, F304, F512, F548, F551 and F552; all AG-2-2 IIIB) caused significantly more lesions than the non-inoculated check (no lesions). Isolations from roots with lesions (35 plants in the first experiment and 31 plants in the second experiment) revealed *R. solani* was always present.

Corn root lesion area

With lesion area, the trials were similar ($P = 0.2412$) and their variances were homogeneous ($P = 0.0645$), so the trials were analyzed together. The lesions caused by seven isolates (F303, F304, F512, F517, F548, F551 and F552; all AG-2-2 IIIB) were significantly larger than the non-inoculated check (Table 3). The lesion area associated with five isolates (F304, F552, F551, F512 and F548) was greater than that of F303 and F517. Isolates F521 and F508, both AG-2-2 IIIB isolates, were non-pathogenic on corn.

Discussion

The majority of the *R. solani* isolates (91%) from sugar beet in the Amalgamated Sugar Company production area of the Intermountain West were from AG-2-2 IIIB (47%) or AG-4 subgroups (44%). Most of the AG-2-2 IIIB isolates (87%) were associated with fields in the western portion (Twin Falls, ID and areas west into southeastern Oregon) of the production area and sequencing of the ITS-5.8S rDNA region revealed not much diversity within and among the AG-2-2 IIIB isolates. The eastern portion (east of Twin Falls, ID to Blackfoot, ID) of the production area had primarily AG-4 isolates (71%) with little diversity in the ITS sequence. Most AG-2-2 IIIB isolates/strains (98%) could cause root rot on sugar beet in greenhouse assays based on the observed disease severity index. Of the 18 *R. solani* isolates tested on corn, seven AG-2-2 IIIB isolates could cause greater root lesion area than was observed on the non-inoculated check and differences between virulent isolates existed as well. Root rot problems identified in the past in the Amalgamated

Sugar Company production area (Strausbaugh & Gillen, 2008, 2009) have been associated with the western portion. Additional research will be required to explain why the root rot problems and AG-2-2 IIIB isolates predominate in the western portion of the production area and not the eastern portion.

In New York, AG-2-2 and AG-4 isolates from naturally infected vegetables predominated (Ohkura *et al.*, 2009), which was similar to the results reported in the present study. In addition to AG-2-2 IIIB, isolates of AG-4 and AG-2-2 IV were found that caused significantly more disease on sugar beet than was observed on the non-inoculated control, as has been reported by other researchers (Windels & Nabben, 1989; Engelkes & Windels, 1996). Isolates of AG-2-2 IIIB were as a group more virulent than other AG on sugar beet and corn, which is consistent with previous results (Engelkes & Windels, 1996; Carling *et al.*, 2002; Ohkura *et al.*, 2009; Bolton *et al.*, 2010).

Within AG-2 there are three previously recognized intraspecific subgroups, namely AG-2-1, AG-2-2 (with three cultural types IIIB, IV, and LP) and AG-2-3 (Hyakumachi *et al.*, 1998; Godoy-Lutz *et al.*, 2003; Toda *et al.*, 2004). A newly described type AG-2t also has been differentiated (Hyakumachi *et al.*, 1998; Godoy-Lutz *et al.*, 2003; Toda *et al.*, 2004). AG-2-2 had been divided into AG-2-2 IIIB (rush type) and AG-2-2 IV (root rot type) by Ogoshi 1987 (Salazar *et al.*, 2000). There was no distinct clustering when comparing the location of 2-2 IIIB isolates on the phylogram and virulence on sugar beet and corn. For a more rigorous investigation into genetic diversity, the sequencing of additional genes should be considered. However, establishing the AG for the isolates, primary objective of sequencing and phylogenetics, was achieved nicely.

Although the AG-4 isolates caused much less root and crown rot than the AG-2-2 IIIB isolates on sugar beet, some isolates were pathogenic, and strains from AG-4 have been noted to cause more severe stem and stolon cankers on potato than the more common AG-3 isolates (Balali *et al.*, 1995; Ceresini *et al.*, 2007). Given the importance of *Rhizoctonia* on potato (Ceresini *et al.*, 2007) and the large acreage of potato production in Idaho, the AG-4 data may also prove useful to the potato industry. In addition, while the AG-4 isolates traditionally only produce a superficial rot on mature sugar beet roots, they can severely reduce stands (Windels & Nabben, 1989; Rush *et al.*, 1994), thus they may be a concern for damping-off.

The isolates from different AG formed distinct clades as noted in previous studies (Kuninaga *et al.*, 1997; Gonzalez *et al.*, 2001; Carling *et al.*, 2002). The AG-1 strains

formed a distinct cluster in the phylogram (Fig. 3) as expected from previous studies (Kuninaga *et al.*, 1997; Gonzalez *et al.*, 2001). The AG-2-2 isolates were distinctly separated from the AG-2-1 reference strain as expected based on previous studies (Kuninaga *et al.*, 1997; Carling *et al.*, 2002). In a previous study (Carling *et al.*, 2002), AG-2-2 IIIB and IV isolates were closely related and spread out together on the phylogram with the IV strains surrounding the IIIB strains. In other studies (Salazar *et al.*, 2000; Fenille *et al.*, 2003; Toda *et al.*, 2004), the LP, IV and IIIB clades formed distinct clusters with the IV and IIIB clusters next to one another. In the present study, the IIIB isolates surrounded the IV isolates in the phylograms (Figs. 1 and 3). Also the AG-4 HGI and HGII isolates and strains used in the current study fell into distinct groups as noted previously (Kuninaga *et al.*, 1997; Gonzalez *et al.*, 2001; Fenille *et al.*, 2003). The AG-A and K isolates were closely related and the AG-E isolates fell into a more distant clade as expected based on previous work (Sharon *et al.*, 2008). Although isolated from sugar beet, none of these binucleate isolates were pathogenic on sugar beet or corn. Some binucleate *Rhizoctonia* have been shown to have the potential to provide some bio-control of *R. solani* (Herr, 1988; Escande & Ehandi, 1991). These isolates could be of interest to examine further for their potential use on sugar beet for disease management.

The disparity in homogeneous sequencing within an isolate between AG-2-2 IIIB and AG-4 isolates was unexpected. While 70% of AG-2-2 IIIB isolates had heterogeneous ITS sequence, only 5% of AG-4 isolates had heterogeneous sequence. The number of variable sites among 57 clones from six AG-2-2 IIIB isolates ranged from 6 to 16 and 30 unique sequences were identified. These data are the first for AG-2-2 and are similar to when six AG-2-1 isolates had been investigated (Pannecoucq & Höfte, 2009). In the AG-2-1 study, the number of variable sites per isolate ranged from 1 to 12 and 32 unique sequences were detected (Pannecoucq & Höfte, 2009). When AG-3 isolates were investigated, variability was confined to 10 and four positions in the ITS1 and ITS2, respectively (Ahvenniemi *et al.*, 2009). With these AG-3 isolates, the compensatory base changes found in the secondary structure of ITS2 were correlated with genetic incompatibility (Ahvenniemi *et al.*, 2009). An explanation for the variation found within clones from a single isolate from numbered AG is the multinucleate nature of the cells which can contain from 3 to 28 nuclei (Sneh *et al.*, 1991). Despite the variation found within AG-2-2 IIIB isolates, all clones from a single isolate shared 98 to 100% sequence identity and would be assigned to the same AG subgroup.

Sampling every other piling ground provided an easy way to maintain an even sampling distribution, since the piling grounds are evenly distributed throughout the production system. The pile sampling does require that roots make it through mechanical harvesting which would be unlikely for some completely rotted roots. This should not have hindered us from finding virulent isolates since more virulent isolates typically spread to neighbouring roots up and down a row (Harveson, 2008). Thus although the primary infected roots may be completely rotted and unharvestable, the roots with secondary infection have varying levels of rot and will still be harvestable. Therefore sampling piling grounds should not have diminished our chances of finding virulent isolates. If roots with Rhizoctonia root rot become completely rotted and unharvestable by mechanical means, the majority of the rot is typically related to bacteria and yeast (Strausbaugh & Gillen, 2008, 2009). Thus even if roots unharvestable by mechanical means would have been dug in a field collection, isolating *Rhizoctonia* would have been problematic. Therefore even with field collections, isolations would likely have required using neighbouring harvestable roots.

Breeding for resistance to *R. solani* in sugar beet has been and will continue to be an important goal. The current source of resistance to *R. solani* in sugar beet can trace its ancestry to a narrow germplasm base (Panella, 2005). Three quantitative trait loci (QTL) for *R. solani* resistance in sugar beet were found on chromosomes 4, 5 and 7 collectively explaining 71% of the total phenotypic variation (Lein *et al.*, 2008). Resistance to *R. solani* damping-off also has been identified (Nagendran *et al.*, 2008). These sources of resistance and additional sources can hopefully be incorporated into sugar beet germplasm without loss of yield potential. Selection for resistance has been based around *R. solani* strain R9 although some other strains also have been used (Nagendran *et al.*, 2008). Sugar beet germplasm with resistance to R9, an AG-2-2 IIIB strain, has resistance to other AG-2-2 IIIB strains and AG-2-2 IV strains as well (Engelkes & Windels, 1996). However, given the range of virulence and the numerous isolates with higher virulence ratings than R9, the stability of screening with only the R9 strain should be revisited. If variability is observed for responses, as different sources of resistance are identified, they should be screened with 2-2 IIIB isolates exhibiting a range of virulence and genetic diversity as well as AG-2-2 IV isolates.

When selecting for Rhizoctonia resistant germplasm, sugar beet roots have traditionally been sorted into disease classes (0–7 or 1–9) based on the percentage of root surface covered by rot (Ruppel *et al.*, 1979; Büttner *et al.*, 2004). Once the number of roots in a class has been established, a disease index or Rhizoctonia index were

calculated (Ruppel *et al.*, 1979; Büttner *et al.*, 2004). This approach has served the industry well for selecting resistant germplasm but becomes problematic for comparing *R. solani* isolates on one cultivar. The classes established in the scales favour separation on the resistant end of the scale, while for isolate comparison, separation on the susceptible end would be preferable. To solve this problem, percentage data were utilized in this research. The percentage of rot was not just surface rot but an estimate of the volume of root rotted since the roots were bisected to allow for internal assessment. The percentage data were continuous and not categorical allowing for standard statistical analysis and this has allowed for easy assessment of a related disease problem, bacterial root rot (Strausbaugh & Gillen, 2008, 2009), at the same time in other studies. The root rot data collected had a strong relationship with foliar discoloration, crown size and top weight ($r^2 = 0.93, 0.79, \text{ and } 0.87$, respectively with $P < 0.0001$) in sugar beet cultivars examined. However, there were isolates which seemed to affect some variables a little more strongly than other isolates, so a disease severity index (DSI) was established to compare isolates based on all variables. Some variables such as crown size and top weight vary between cultivars, so these variables would be problematic if utilized to try and identify resistant germplasm. Thus, this approach with four variables and DSI should be utilized only for comparing isolates of *R. solani* on a single cultivar and not for identifying resistant germplasm.

When 18 of the *R. solani* isolates were tested for root rot on corn, seven AG-2-2 IIIB isolates could cause significant lesion area, while isolates from AG-A, AG-E, AG-1 1A, AG-2-2 IV, AG-4 HG-I, AG-4 HG-II, and AG-5 were not significantly different from the non-inoculated check. One AG-2-2 IIIB isolate, F508, appeared to also cause no damage to corn. Previous studies show corn yields can be reduced up to 47% with *R. solani* AG-2-2 (Sumner & Minton, 1989), particularly on sprinkler irrigated corn (Sumner & Bell, 1982). Others report that some isolates of AG-2-2, AG-4 and AG-5 could cause root discoloration in corn, but were not highly virulent (Rush *et al.*, 1994; Nelson *et al.*, 1996). In Minnesota, corn was affected more by AG-2-2 IIIB than AG-2-2 IV (Brantner & Windels, 2008). In Europe, all AG-2-2 IIIB isolates caused severe damage in sugar beet and corn with distinct root rot symptoms, whereas isolates AG-2-2 IV, AG-2-1 and AG-4 caused weak symptoms on sugar beet and even weaker symptoms on corn (Führer Ithurrart *et al.*, 2004). In New York, *R. solani* isolates from AG-2-2, AG-5 and AG-11 were more aggressive on sweet corn than isolates from AG-2-1, CAG-2 and CAG-6, while isolates from AG-1 and AG-4 were intermediate

(Ohkura *et al.*, 2009). In Idaho, the silage corn acreage has increased from 29,947 ha in 1970 to 80,937 ha in 2007, which is likely a reflection of the increase in milking cows from 144,000 head in 1970 to 555,000 head in 2008 (USDA-NASS, 2008). This increase in silage corn acreage has led to more corn in rotation with sugar beet. Breeding resistance to *Rhizoctonia* root rot in corn could be promising, since considerable differences in disease severity exist in corn genotypes (Buddemeyer *et al.*, 2004; Pfähler & Peterson, 2004). Perhaps establishing a level of *Rhizoctonia* root rot resistance in corn hybrids produced in Idaho and other areas of the USA should be considered to reduce disease and inoculum buildup. However, recent data from Europe indicate that corn preceding sugar beet can influence the sugar beet crop performance but the effect was not related to the susceptibility of the corn genotype (Kluth & Varrelmann, 2010).

Although efforts were made to sample all of the Amalgamated Sugar Company production areas equally, 77% of the isolates came from the western half of the production area, which is where sugar beet root rot is more commonly encountered. In previous research, the eastern end of the production area had very little fungal or bacterial root rot (Strausbaugh & Gillen, 2008, 2009), which mirrors results from the collection of *R. solani* isolates. The eastern portion of the production area does not use furrow irrigation which might explain part of the disparity in root rot distribution, since fields that require more than 24 h to get water across them frequently have root rot problems. Also, the silage corn acreage, potentially susceptible to *R. solani*, has impacted the western production area (70% of silage corn acreage) to a greater extent (USDA-NASS, 2008). Another influence could be that the eastern portion of the production area has 80% of the potato production (USDA-NASS, 2008) in their rotation which is produced on ground frequently fumigated with metam-sodium to control *Verticillium*. Metam-sodium will also influence other soil fungal populations including *R. solani* (Fravel & Lewis, 2004; Candole *et al.*, 2007a, 2007b). These observations may prove helpful in guiding future research and management options. Host resistance in sugar beet and corn should be increased and potential interactions between isolates from diverse highly virulent AG-2-2 IIIB strains and host resistance in various host crops should be investigated to improve management options.

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Supplemental Table A1. Differences in the internal transcribed spacer (ITS) 1-5.8S ribosomal DNA-ITS2 region among 57 clones from six *Rhizoctonia solani*2-2 IIIB isolates from sugar beet roots and accession numbers for 30 unique sequences detected.

| Isolate | Number of | | Identity (%) [†] | Accession numbers [‡] |
|---------|-----------|-----------------|---------------------------|---|
| | Clones | Variable sites* | | |
| F508 | 8 | 16 | 98–100 | FJ492144 (2X), GU811659, GU811660, GU811661, GU811662, GU811663, GU811664 |
| F517 | 10 | 16 | 98–100 | FJ492153 (3X), GU811665, GU811666, GU811667, GU811668, GU811669, GU811670, GU811671 |
| F521 | 10 | 6 | 99–100 | FJ492157 (3X), GU811672 (2X), GU811673 (2X), GU811674 (2X), GU811675 |
| F548 | 9 | 12 | 98–100 | FJ492160 (4X), GU811676, GU811677, GU811678, GU811679, GU811680 |
| F551 | 10 | 13 | 98–100 | FJ492163 (3X), GU811681 (2X), GU811682 (2X), GU811683, GU811684, GU811685 |
| F552 | 10 | 9 | 99–100 | FJ492164 (5X), GU811686 (2X), GU811687, GU811688, GU811689 |

*Number of nucleotide sites that were variable between clones within an isolate.

[†]Sequence identity between clones within an isolate.

[‡]Only 30 unique sequences were detected since the sequences for some clones within (frequency noted in parentheses) and between isolates were the same. Clones with similar sequences between isolates were: GU811665, GU811676, and GU811682 same as FJ492164; GU811677 same as FJ492163; GU811681 same as FJ492160; and GU811683 and GU811689 same as GU811678.

Supplemental Table A2. Location (null position indicated across top) of polymorphic sites in the internal transcribed spacer (ITS) 1-5.8S ribosomal DNA-ITS 2 region for 30 unique sequences obtained from 57 clones selected from six Rhizoctonia solani 2-2 IIIB isolates.

Table with columns for GenBank accession, ITS 1 (positions 1-11), 5.8S (positions 1-33), and ITS 2 (positions 1-66). Each row represents a unique sequence, showing the nucleotide sequence and polymorphic sites indicated by numbers above the letters.

Supplemental Table A3. *Rhizoctonia solani* isolated from sugar beet roots (94 field isolates and 19 reference strains) were inoculated onto roots of the sugar beet cultivar Monohikari in greenhouse assays.

| Isolate* | AG [†] | Top [‡] (%) | Root rot (%) [§] | Crown (mm) | | Top fresh wt. (g) | | DSI |
|-----------|-----------------|----------------------|---------------------------|---------------|---------------|-------------------|---------------|-------------------|
| | | | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | |
| F551 | 2-2 IIIB | 100 a | 100 a | 6 f-i | 8 yz | 5 m | 4 st | 96 a |
| F552 | 2-2 IIIB | 100 a | 100 a | 1 hi | 12 t-z | 5 m | 4 st | 96 a |
| F501 | 2-2 IIIB | 100 a | 100 a | 4 g-i | 12 s-z | 5 m | 6 r-t | 95 a |
| F30 | 2-2 IIIB | 100 a | 99 a | 9 z-i | 8 x-z | 7 lm | 5 st | 94 ab |
| F314 | 2-2 IIIB | 100 a | 100 a | 7 b-i | 13 s-z | 5 m | 5 st | 94 a-c |
| F505 | 2-2 IIIB | 100 a | 100 a | 7 c-i | 11 u-z | 6 m | 5 r-t | 94 ab |
| F513 | 2-2 IIIB | 100 a | 100 a | 6 d-i | 12 s-z | 6 m | 5 st | 94 ab |
| F304 | 2-2 IIIB | 100 a | 98 ab | 11 x-g | 10 v-z | 7 lm | 8 n-t | 93 a-e |
| F315 | 2-2 IIIB | 100 a | 100 a | 6 f-i | 14 q-z | 6 m | 10 n-t | 93 a-e |
| F500 | 2-2 IIIB | 100 a | 100 a | 6 e-i | 16 o-y | 5 m | 6 r-t | 93 a-d |
| F508 | 2-2 IIIB | 100 a | 100 a | 4 g-i | 18 n-w | 5 m | 7 o-t | 93 a-e |
| F521 | 2-2 IIIB | 99 ab | 100 a | 9 y-i | 11 u-z | 7 lm | 6 q-t | 93 a-e |
| F548 | 2-2 IIIB | 100 a | 98 ab | 15 u-f | 8 x-z | 7 lm | 3 t | 93 a-e |
| F553 | 2-2 IIIB | 94 a-c | 99 a | 0 i | 12 t-z | 5 m | 11 m-t | 93 a-e |
| F502 | 2-2 IIIB | 100 a | 100 a | 12 w-g | 16 o-y | 6 m | 6 r-t | 92 a-f |
| F509 | 2-2 IIIB | 100 a | 100 a | 10 x-h | 18 n-w | 4 m | 6 r-t | 92 a-f |
| F511 | 2-2 IIIB | 100 a | 96 ab | 10 x-h | 10 w-z | 10 lm | 6 r-t | 92 a-e |
| F303 | 2-2 IIIB | 100 a | 96 ab | 17 s-a | 11 u-z | 8 lm | 6 p-t | 91 a-f |
| F36 | 2-2 IIIB | 100 a | 99 a | 15 u-f | 15 p-z | 8 lm | 7 o-t | 91 a-f |
| F549 | 2-2 IIIB | 100 a | 100 a | 16 u-d | 12 t-z | 11 lm | 4 st | 91 a-f |
| F512 | 2-2 IIIB | 100 a | 96 ab | 11 x-g | 17 n-y | 8 lm | 7 o-t | 90 a-f |
| F520 | 2-2 IIIB | 100 a | 99 ab | 22 o-v | 12 t-z | 10 lm | 4 st | 90 a-f |
| F514 | 2-2 IIIB | 100 a | 99 a | 23 n-u | 17 o-y | 13 k-m | 8 n-t | 88 a-g |
| F22 | 2-2 IIIB | 88 a-e | 94 a-c | 18 r-z | 6 z | 13 k-m | 6 r-t | 87 a-g |
| F517 | 2-2 IIIB | 98 ab | 90 a-e | 16 t-b | 14 q-z | 10 lm | 6 p-t | 87 a-g |
| 87-36-2 | 2-2 IIIB | 90 a-d | 90 a-e | 6 d-i | 14 r-z | 4 m | 21 k-s | 87 a-g |
| F321 | 2-2 IIIB | 88 a-e | 91 a-d | 8 a-i | 12 t-z | 8 lm | 21 k-t | 86 a-g |
| F313 | 2-2 IIIB | 98 ab | 93 a-c | 20 q-x | 21 l-t | 8 lm | 12 m-t | 85 a-g |
| F316 | 2-2 IIIB | 90 a-d | 92 a-c | 17 s-a | 12 s-z | 19 i-m | 13 m-t | 85 a-g |
| F516 | 2-2 IIIB | 94 a-c | 96 ab | 18 r-z | 21 l-t | 15 j-m | 9 n-t | 85 a-g |
| F305 | 2-2 IIIB | 93 a-c | 90 a-e | 22 p-u | 20 m-v | 16 j-m | 8 n-t | 83 b-h |
| F330 | 2-2 IIIB | 88 a-e | 89 b-e | 8 a-i | 26 h-o | 8 lm | 25 k-r | 82 c-i |
| F550 | 2-2 IIIB | 80 c-e | 91 a-d | 9 y-i | 17 n-y | 5 m | 31 j-m | 82 d-i |
| F507 | 2-2 IIIB | 86 a-e | 93 a-c | 18 r-z | 18 n-x | 21 i-m | 17 l-t | 81 e-i |
| F506 | 2-2 IIIB | 78 c-e | 89 a-e | 13 v-g | 11 u-z | 35 g-i | 11 m-t | 80 f-i |
| F518 | 2-2 IIIB | 82 b-e | 85 b-f | 18 r-y | 22 l-s | 10 lm | 26 k-p | 78 g-j |
| F504 | 2-2 IIIB | 76 d-f | 78 d-h | 19 q-x | 25 i-p | 25 h-l | 24 k-s | 72 h-k |
| R4 | 2-2 IIIB | 75 d-f | 80 c-g | 34 c-l | 19 n-w | 40 g-i | 7 o-t | 71 i-k |
| R9 | 2-2 IIIB | 74 d-f | 69 g-i | 26 l-t | 20 m-u | 24 i-l | 35 j-k | 68 j-l |
| F325 | 2-2 IIIB | 73 ef | 78 d-h | 28 i-q | 24 j-q | 35 g-i | 21 k-t | 68 j-l |
| F322 | 2-2 IIIB | 61 fg | 69 g-i | 27 j-r | 14 r-z | 42 f-h | 21 k-s | 65 k-m |
| F519 | 2-2 IIIB | 59 f-h | 76 e-h | 16 t-c | 26 g-n | 29 h-j | 49 h-j | 64 k-m |
| F510 | 2-2 IIIB | 52 g-i | 59 i-k | 39 a-h | 12 t-z | 56 b-f | 5 r-t | 58 l-n |
| H-3-77 | 2-2 IV | 53 g-i | 71 f-i | 32 e-n | 26 h-o | 54 d-f | 27 k-n | 57 l-n |
| 2C13 | 2-2 IV | 54 g-i | 58 i-k | 34 c-l | 23 k-r | 56 c-f | 26 k-q | 54 m-o |
| F16 | 2-2 IIIB | 44 h-j | 66 h-j | 34 c-l | 26 h-o | 68 a-d | 26 k-o | 51 no |
| F503 | 2-2 IIIB | 42 ij | 46 k | 15 u-e | 36 a-g | 20 i-m | 79 a-e | 47 n-p |
| F524 | 2-2 IIIB | 39 ij | 54 jk | 34 c-l | 29 e-m | 56 c-f | 39 i-k | 46 n-p |
| F24 | 2-2 IV | 31 jk | 46 k | 23 n-u | 39 a-d | 32 g-j | 62 c-h | 43 o-q |
| H502 | 2-2 IV | 31 jk | 34 l | 41 a-d | 25 i-p | 62 a-e | 34 j-k | 38 p-r |
| F32 | 4 HG-I | 29 j-l | 25 l-n | 24 m-u | 33 a-j | 54 d-f | 55 g-i | 36 p-r |
| F515 | 2-2 IIIB | 17 k-o | 19 l-p | 26 k-s | 32 a-k | 48 e-g | 61 d-h | 31 q-s |
| F20 | 4 HG-I | 20 k-m | 24 l-o | 33 d-m | 30 d-l | 65 a-e | 58 f-i | 30 r-t |
| F27 | 4 HG-II | 14 l-o | 29 lm | 21 p-v | 33 a-j | 66 a-e | 76 a-f | 30 r-t |
| F319 | 4 HG-II | 18 k-n | 19 l-q | 30 h-p | 37 a-f | 56 b-f | 74 a-g | 27 r-u |
| F311 | 4 HG-II | 15 k-o | 14 n-s | 44 ab | 29 f-m | 68 a-d | 60 e-h | 24 s-v |
| F31 | 4 HG-I | 8 m-o | 17 m-r | 27 j-r | 36 a-g | 72 a-c | 83 ab | 22 s-v |

(Continued)

Supplemental Table A3. (Continued.)

| Isolate* | AG [†] | Top [‡] (%) | Root rot (%) [§] | Crown (mm) | | Top fresh wt. (g) | | DSI |
|----------|-----------------|----------------------|---------------------------|------------|---------|-------------------|---------|-------------------|
| | | | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | |
| F35 | 4 HG-II | 14 l-o | 11 o-t | 31 g-p | 34 a-i | 75 a | 72 a-g | 22 s-v |
| F320 | A | 18 k-n | 12 o-t | 40 a-h | 40 ab | 73 a-c | 77 a-f | 20 s-v |
| 5E13 | 2-2 IV | 8 m-o | 10 o-t | 38 a-h | 37 a-f | 64 a-e | 69 a-g | 20 s-v |
| F13 | 4 HG-II | 10 m-o | 7 p-t | 34 c-l | 36 a-f | 65 a-e | 83 a | 19 t-v |
| F26 | 4 HG-II | 5 m-o | 5 q-t | 31 f-o | 39 a-d | 58 b-f | 73 a-g | 19 s-v |
| ST-6-1 | 5 | 4 m-o | 0 t | 39 a-h | 30 c-l | 57 b-f | 73 a-g | 18 t-v |
| C-116 | 2-2 IIIB | 7 m-o | 4 r-t | 41 a-d | 36 a-g | 68 a-d | 68 a-h | 17 uv |
| RI-64 | 2-2 IV | 4 m-o | 0 t | 37 a-j | 40 ab | 56 b-f | 68 a-h | 17 uv |
| R101 | 4 HG-I | 5 m-o | 0 t | 36 a-k | 38 a-e | 64 a-e | 70 a-g | 17 uv |
| F306 | 4 HG-II | 3 m-o | 4 r-t | 34 c-l | 36 a-f | 64 a-e | 75 a-g | 17 uv |
| F8 | 4 HG-II | 6 m-o | 1 st | 38 a-i | 34 a-h | 65 a-e | 73 a-g | 17 uv |
| F17 | 4 HG-II | 4 m-o | 1 st | 39 a-h | 39 a-d | 59 a-f | 74 a-g | 16 uv |
| F19 | 4 HG-II | 3 m-o | 2 st | 37 a-j | 40 a-c | 60 a-e | 74 a-g | 16 uv |
| BV-7 | 1 IC | 2 no | 0 t | 38 a-h | 40 a-c | 64 a-e | 63 b-h | 16 uv |
| F2 | 4 HG-II | 5 m-o | 3 st | 36 a-j | 41 ab | 62 a-e | 73 a-g | 16 uv |
| F28 | 4 HG-II | 4 m-o | 4 r-t | 37 a-j | 40 a-c | 67 a-d | 77 a-f | 16 uv |
| F3 | 4 HG-II | 8 m-o | 2 st | 39 a-h | 40 a-c | 64 a-e | 76 a-f | 16 uv |
| F307 | 4 HG-II | 3 m-o | 2 st | 38 a-h | 34 a-i | 72 a-c | 70 a-g | 16 uv |
| F323 | 4 HG-II | 3 m-o | 4 r-t | 35 a-l | 37 a-f | 67 a-d | 79 a-e | 16 uv |
| F33 | 4 HG-II | 2 no | 2 st | 38 a-i | 42 a | 65 a-e | 66 a-h | 16 uv |
| F34 | 4 HG-II | 2 no | 4 r-t | 34 b-l | 39 a-d | 69 a-d | 75 a-f | 16 uv |
| F18 | A | 2 no | 0 t | 41 a-f | 38 a-f | 65 a-e | 68 a-h | 15 v |
| F21 | 4 HG-II | 6 m-o | 0 t | 37 a-j | 40 ab | 61 a-e | 79 a-e | 15 uv |
| F23 | 4 HG-II | 4 m-o | 2 st | 40 a-g | 42 a | 63 a-e | 74 a-g | 15 v |
| F29 | 4 HG-II | 3 m-o | 0 t | 40 a-h | 41 ab | 62 a-e | 71 a-g | 15 v |
| F37 | 4 HG-I | 3 m-o | 4 r-t | 38 a-i | 41 ab | 70 a-d | 70 a-g | 15 uv |
| F4 | 4 HG-II | 6 m-o | 2 st | 39 a-h | 41 ab | 68 a-d | 68 a-h | 15 uv |
| F498 | 4 HG-II | 2 no | 0 t | 41 a-f | 38 a-e | 65 a-e | 66 a-h | 15 v |
| F499 | 4 HG-II | 3 m-o | 2 st | 36 a-j | 38 a-f | 71 a-d | 71 a-g | 15 uv |
| F523 | K | 3 m-o | 0 t | 43 a-d | 36 a-g | 65 a-e | 69 a-g | 15 uv |
| W-22 | 2-2 IIIB | 6 m-o | 0 t | 40 a-g | 37 a-f | 68 a-d | 73 a-g | 15 uv |
| F7 | 4 HG-II | 5 m-o | 0 t | 42 a-d | 37 a-f | 69 a-d | 67 a-h | 15 uv |
| F1 | 4 HG-II | 3 m-o | 1 st | 43 a-c | 37 a-f | 69 a-d | 68 a-h | 14 v |
| F10 | 4 HG-II | 4 m-o | 0 t | 40 a-f | 38 a-f | 62 a-e | 80 a-d | 14 v |
| F11 | 4 HG-II | 3 m-o | 1 st | 42 a-d | 38 a-e | 66 a-d | 73 a-g | 14 v |
| F12 | 4 HG-II | 2 no | 1 st | 40 a-g | 38 a-f | 71 a-d | 72 a-g | 14 v |
| F14 | E | 6 m-o | 0 t | 39 a-h | 39 a-d | 73 ab | 77 a-f | 14 v |
| F15 | E | 2 no | 0 t | 39 a-h | 40 ab | 56 c-f | 82 ab | 14 v |
| F184 | A | 2 no | 0 t | 42 a-d | 37 a-f | 65 a-e | 70 a-g | 14 v |
| FC-2 | 2-1 | 2 no | 0 t | 39 a-h | 38 a-f | 65 a-e | 79 a-e | 14 v |
| TE2-4 | 2 BI | 1 o | 0 t | 37 a-j | 42 a | 62 a-e | 73 a-g | 14 v |
| F25 | 4 HG-II | 4 m-o | 2 st | 44 a | 40 ab | 63 a-e | 72 a-g | 14 v |
| F302 | 4 HG-II | 2 no | 1 st | 39 a-h | 42 a | 67 a-d | 69 a-h | 14 v |
| F5 | 4 HG-II | 6 m-o | 0 t | 40 a-f | 42 ab | 68 a-d | 71 a-g | 14 v |
| F6 | 4 HG-II | 3 m-o | 0 t | 41 a-d | 40 ab | 69 a-d | 62 c-h | 14 v |
| F9 | A | 4 m-o | 0 t | 40 a-h | 40 a-c | 68 a-d | 74 a-g | 14 v |
| F179 | A | 3 m-o | 0 t | 41 a-e | 41 ab | 67 a-d | 78 a-f | 13 v |
| CS-2 | 1 IA | 4 m-o | 0 t | 42 a-d | 41 ab | 70 a-d | 75 a-g | 13 v |
| Shiba-2 | 1 IB | 2 no | 0 t | 44 a | 42 a | 63 a-e | 72 a-g | 13 v |
| F312 | 4 HG-II | 4 m-o | 0 t | 40 a-g | 40 ab | 70 a-d | 79 a-e | 13 v |
| F324 | 4 HG-II | 2 no | 0 t | 39 a-h | 42 ab | 70 a-d | 75 a-g | 13 v |
| F329 | 4 HG-II | 2 no | 0 t | 43 a-c | 40 ab | 69 a-d | 69 a-h | 13 v |
| Check | | 1 no | 0 t | 43 a-c | 38 a-f | 60 a-e | 82 a-c | 13 v |

(Continued)

Supplemental Table A3. (Continued.)

| Isolate* | AG [†] | Top [‡] (%) | Root rot (%) [§] | Crown (mm) | | Top fresh wt. (g) | | DSI |
|--------------------|-----------------|----------------------|---------------------------|------------|----------|-------------------|----------|-------------------|
| | | | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | |
| ST-11-6 | 3 PT | 1 o | 0 t | 42 a-d | 41 ab | 68 a-d | 76 a-f | 12 v |
| 72 | 8 | 4 m-o | 0 t | 40 a-h | 41 ab | 76 a | 76 a-f | 12 v |
| F309 | 4 HG-II | 2 no | 0 t | 42 a-d | 41 ab | 69 a-d | 76 a-f | 12 v |
| F310 | 4 HG-II | 2 no | 0 t | 43 a-d | 39 a-d | 70 a-d | 79 a-e | 12 v |
| P > F [¶] | | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

*Check = non-inoculated check. Strain in bold is reference strain used to screen for resistance.

[†]AG = anastomosis group.

[‡]Top = percentage of foliage with discoloration.

[§]Root rot = percentage of root with discoloration.

^{||}DSI = (top rating + root rot rating + (((crown size - largest crown size)/largest crown size) × -100) + (((top wt. - largest top wt.)/largest top wt.) × -100)/4.

[¶]P > F was the probability associated with the F value. Means within a column followed by the same letter are not significantly different based on Fisher's protected least significant difference ($P \leq 0.05$).