Evaluation of resistance to *Rhizoctonia solani* in soybean and assessment of fungicide sensitivity in isolates from sugar beet and soybean

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Abstract

Soybean and sugar beet are commonly grown in rotation in the Red River Valley of MN and ND and southern Minnesota. Both crops are highly susceptible to *Rhizoctonia solani*. Currently there are no Rhizoctonia resistant soybean cultivars available to the growers. Partially resistant commercial sugar beet cultivars are available, but they are susceptible to *R. solani* during the seedling stage. Disease management for both crops relies partially on the use of fungicides. The primary objectives of this study were (i) to determine the response of different soybean genotypes to *R. solani* in the field and growth chamber, and (ii) to determine the sensitivity of *R. solani* isolates from soybean and sugar beet to the fungicides sedaxane, penthiopyrad, fluxapyroxad, azoxystrobin, and pyraclostrobin. Soybean genotypes were evaluated for their response to *R. solani* at Waseca and Crookston, MN field locations. All genotypes evaluated at Crookston (n=20) in 2018 and 2019 had high losses in inoculated plots in plant population and yield compared to noninoculated control plots. Losses were also severe for all genotypes (n=36) at Waseca in 2018. Some soybean genotypes had relatively low losses in 2017 and 2019, however, their performance was not consistent across the years. MN 1613CN was the only genotype that performed well in 2017 and 2019, but it had high plant population and yield losses in 2018. Significant genotype by isolate interaction was detected when 16 soybean genotypes were evaluated for their response to four isolates of *R. solani* in the growth chamber. All genotypes were highly susceptible to isolate Rs 16WC3-2, but they differed in susceptibility to some other isolates. The soybean genotypes used in this study were not resistant to *R. solani,* but those with relatively low losses in plant population should be investigated further along with additional genotypes. The response of soybean genotypes to *R. solani* is strongly influenced by the isolate and environment, so future studies should continue to be conducted under different environmental conditions using multiple isolates. Sensitivity of *R. solani* AG 2-2 isolates (n=35) collected from soybean and sugar beet to the SDHI fungicides sedaxane, penthiopyrad, and fluxapyroxad, and to the QoI fungicides pyraclostrobin and azoxystrobin was determined using a mycelial growth inhibition method. The concentration of fungicide required to inhibit the radial growth of mycelium 50% (EC_{50}) compared to the growth on non-amended media was estimated for each isolate. The mean and range of EC_{50} values for sedaxane, penthiopyrad, fluxapyroxad and pyraclostrobin were 0.1 (0.03 to 0.3), 0.15 (0.05 to 0.27), 0.16 (0.08 to 0.3), and 0.24 (0.04 to 1.02) µg a.i./mL, respectively. The mean EC_{50} values of azoxystrobin for 22 isolates ranged from 0.76 to 2.36 µg a.i./mL. EC_{50} values for azoxystrobin could not be estimated for 13 isolates due to < 50% inhibition in growth, however, a pronounced decrease in mycelial density was observed as fungicide concentration increased. The SDHI fungicides and pyraclostrobin effectively inhibited the growth of the isolates of *R. solani* in vitro at low concentrations, but new methods are needed to determine in vitro sensitivity to

azoxystrobin. In summary, the different levels of partial resistance to *R. solani* detected in soybean genotypes suggest a need for improving resistance to *R. solani*, and fungicide sensitivity results indicate that currently labeled fungicides continue to be useful in managing seedling damping-off and root rot.

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Chapter 1: Review of Literature

Biology and life cycle of *Rhizoctonia solani*

DeCandolle described the genus *Rhizoctonia* and selected *Rhizoctonia crocorum* (Pers.) DC as the type species in 1815. The most important species of this genus, *R. solani,* was first characterized by a German agronomist Julius Kühn on potato tubers in 1858 (Kühn 1858). *Rhizoctonia solani* belongs to phylum Basidiomycota, but it lacks the formation of clamp connections, chlamydospores, conidia, and spermatia, and rarely produces basidiospores (Anderson 1982, Brown and McCarter 1976, Parmeter 1970). This fungus generally exists as vegetative mycelium or sclerotia with pale to dark brown, multinucleate, heterokaryotic, and septate hypha branched almost perpendicularly with a constricted base (Anderson 1982, Brown and McCarter 1976, Parmeter 1970). The sclerotia are primary survival structures that can survive in soil or plant residues for 3 to 5 years (Keijer 1996, Sumner and Bell 1994). The teleomorphic stage *Thanatephorus cucumeris (Frank) Donk* was first reported by Prillieux and Delacroix (1891) and later described on sugar beets by Kotila (1947).

Hyphal fusion in *R. solani* was first observed by Matsumoto et al. (1932) in the pathogen of rice sheath blight. They classified it as perfect fusion, imperfect fusion, and contact fusion, which later served as the basis for Schulz's (1936) classification of *R. solani* into five anastomosis groups (AG). Multinucleate strains of the pathogen are currently classified into 13 AG, i.e., AG 1 to AG 13 (Sneh et al. 1996, Carling et al. 1999 and 2002a, Ogoshi 1987). These AGs vary in their evolutionary traits, host range, and pathogenicity. Some AGs are further divided into subgroups based on traits other than hyphal fusion, except for AG 2, where the frequency of hyphal fusion serves as the basis for subgrouping (Ogoshi 1987).

Rhizoctonia solani has a necrotrophic or saprotrophic mode of nutrition and a broad host range, and some isolates are found in mycorrhizal associations with orchids (Ogoshi 1996, Masuhara et al. 1993). *R. solani* was reported to infect plants belonging to 35 orders, 52 families, 125 genera, and more than 142 species in Japan (Ogoshi 1996) although different AG can have

different host preference. Some of the economically important crops infected by *R. solani* include potato, rice, soybean, sugar beet, corn, cotton, onions, wheat, barley, and canola (Escande and Echandi 1991, Liu and Sinclair 1991, Carling et al. 1987, Ithurrart et al. 2004, Brown and McCarter 1976, Wiseman et al. 1996 Yitbarek et al. 1987, Ogoshi 1996, Windels and Brantner 2011, Ajayi-Oyetunde and Bradley 2017). High soil moisture (>75%) and warm temperature (20-30 ˚C) are favorable for disease development, but infection can occur over a wide range of temperatures (12 to 35 ˚C) and soil moisture levels as low as 25% (Bolton et al. 2010, Dorrance et al. 2003, Leach 1986, Khan 1993). The worldwide prevalence of *R. solani* is associated with its ability to survive in the soil for 3 to 5 years and infect a broad range of hosts in a variety of environmental conditions (Blazier and Conway 2004, Franc et al. 2001).

Infection of plants by *R. solani* can be initiated by sclerotia, basidiospores, or mycelium (Naito and Sugimoto 1978, Keijer 1996, Agrios 2005). When the environment is favorable, the sclerotia germinate to produce hyphae, which are attracted to hosts by chemical stimuli in root exudates (Gonzales Gracia et al. 2006, Keijer 1996). Once the hyphae come in contact with its host, it attaches to the host epidermis and produces short swollen hyphae, stroma, infection cushions, or appressoria, that give rise to penetration pegs (Keijer 1996, Weinhold and Sinclair 1996, Naito and Sugimoto 1978). The penetration peg enters the host through direct penetration, natural openings, or wounds on the surface. Direct penetration is achieved through the secretion of pectinolytic and cellulolytic enzymes that degrade the host's surface in the absence of natural openings or wounds (Weinhold and Sinclair 1996, Gonzales Gracia et al. 2006, Lisker et al. 1975). Successful infection is followed by the growth of fungal hyphae inter- and intracellularly and the colonization of endodermis which can result in pre and post-emergence damping-off, seed rot, hypocotyl rot, root rot, collar rot, crown rot, stem rot, foliar blight, and various other diseases (Weinhold and Sinclair 1996, Yang 2015, Ajayi-Oyetunde and Bradley 2017). Infected plants are usually observed in patches, and the symptoms are often more evident in low moisture conditions. Movement of infested soil (e.g., via irrigation, soil erosion, farm machinery, and equipment) and infected plant materials can spread the pathogen to new locations (Neher and Gallian 2011).

Soybean production

Soybean [Glycine max (L.) Merr] is one of the oldest crops of East Asia that can be traced back to 2838 B.C. in Chinese literature (Liu 1997). It was introduced to the United States in the mid-eighteenth century, but commercial cultivation did not begin until the early twentieth century (Liu 1997). Currently, soybean ranks among the top four crops produced worldwide by volume, and the U.S. is one of the leading producers and exporters of this commodity (Lee et al. 2016). From 1960 to 2018, the area under soybean production in the U.S. increased from 24 million acres to 89 million acres, and the production increased from 555 million bushels to 4.4 billion bushels, which is 90% of the total oilseed production in the nation. The top three soybean-producing states of Illinois, Iowa, and Minnesota shared 36% of the national soybean production in 2018 (USDA-ERS 2019b).

Only 15% of the total soybean harvest is used directly, while the rest is processed into meal and oil. Nearly 70% of the soybean produced in the U.S. is processed into animal feed, and the remaining is used for human consumption (15%), biodiesel production (5%), and industrial production (USDA-ERS 2019b, USDA 2015). Some of the processed soybean products include cooking oil, soymilk, soy sauce, tofu, miso, vegan meat substitutes, printing ink, paints, textiles, and cleaners.

Rhizoctonia root and hypocotyl rot in soybean

Seedling diseases (caused by *Rhizoctonia, Pythium, Fusarium,* and *Phomopsis)* were listed among the six most destructive diseases of soybeans in the U.S. from 1996 to 2014 and 2017 (Wrather and Koenning 2009, Koenning and Wrather 2010, Allen et al. 2017, Crop Protection Network 2019a). Rhizoctonia root and hypocotyl rot was the fourth leading soybean disease from 1996-2000 in Ontario, Canada (Anderson and Tenuta 2001). Epidemics of Rhizoctonia root rot were reported on soybeans in Iowa in 1967 (Tachibana 1968). Among the fungal taxa associated with soybean seedlings in Iowa, 27% were identified as *Rhizoctonia* in 1993 and 1994 (Rizvi and Yang 1996). Losses of 45% to 52% have been reported in soybean yield due to this disease in

inoculated experiments (Muyolo et al. 1993a, Chang et al. 2017). In 1994, *Rhizoctonia* and *Pythium* root rots were responsible for a combined loss of 108,000 metric tons in the top 10 soybean-producing countries (Wrather et al. 1997).

Symptoms of the disease can vary depending upon the AG of the casual organism. Anastomosis groups, 2-2 IIIB and IV, AG 3, AG 4, AG 5, and AG 11 can cause seedling damping-off and root and hypocotyl rot in soybeans (Ajayi-Oyetunde and Bradley 2017). Isolates of AG 2-2 IIIB and AG 4 are prevalent in the soybean growing areas, and AG 2-2 IIIB has been reported as being most aggressive to soybean (Liu & Sinclair 1991, Nelson et al. 1996, Ajayi-Oyetunde and Bradley 2018, Zhao et al. 2005a).

The pathogen can infect soybean at any growth stage, but the seedling stage is more susceptible to infection due to immature tissue and delayed expression of resistant genes (Windels and Brantner 2011). Infection can result in pre- and post-emergence damping-off, seed rot, hypocotyl rot, and root rot. Symptoms of post-emergence damping-off include soft, water-soaked stems that bend over due to the inability to support the seedling. Older seedlings may develop reddish-brown sunken lesions in the hypocotyl region, which can enlarge to girdle the stem (Agrios 2005). It may also be followed by stunting and wilting of foliage. Root rot, accompanied by reddish-brown cankers, and loss of lateral roots are usually observed later in the season as the plants progress to reproductive stages (Ajayi-Oyetunde and Bradley 2017).

Sugar beet production

The domestication (around 8500 B.C.) and selection of wild sea beet (*Beta maritima*) led to the development of red beets (around 100 B.C.) and fodder beets (around 1500 A.D.). The modern sugar beets (*Beta vulgaris L*.) originated from the germplasm of fodder beets through mass selection for sucrose content in Germany in the late seventeenth century (Stevanato and Panella 2013, Draycott 2006). Andreas Marggraf was the first person to obtain sucrose crystals from sugar beet in 1747. Commercial beet sugar extraction was pioneered by his student Franz Carl Achard

from his breeding line ‘White Silesian beet.’ He also opened the first beet sugar factory in Germany in 1801 (Draycott 2006).

In the U.S., sugar is processed from sugar beet and sugar cane. The first beet sugar factory in the U.S. was established in Massachusetts in 1838. Successful beet sugar production started in the U.S. in 1870 near a sugar beet processing factory established in California (Francis 2005). At present, the sugar beet industry is active in Michigan, Minnesota, North Dakota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, Oregan, and Washington in the U.S. The Red River valley of MN and ND along with southern MN, however, alone account for 50% of total sugar beet harvest in the nation. In 2019, 34.7 million tons of sugar beet was harvested from more than a million acres in the U.S. and processed into 5.17 million tons of sugar, accounting for 55% of the national sugar production (USDA-ERS, 2019c). The primary purpose of sugar beet cultivation is to produce white sugar, and the by-products are processed into pulp pellets, pulp shreds, molasses, and raffinate that are used as animal feeds (American Crystal sugar/Agri-products).

Rhizoctonia crown and root rot in sugar beet

According to sugar beet growers (n=313) surveyed in MN and ND, Rhizoctonia crown and root rot was the leading soilborne disease affecting sugar beet production in 2019 (Hakk et al. 2019). The proportion of field samples diagnosed with Rhizoctonia diseases was 49-66% in the Sugar beet Plant Pathology lab at the University of Minnesota between 2015 and 2018, which also highlights the importance of this disease (Brantner and Chanda 2017 and 2019b). *R. solani* AG 2-2, AG 3, AG 4, and AG 5 can cause seedling and root diseases in sugar beets. Isolates of AG 2-2 intraspecific subgroups (ISGs) IIIB and IV are the most aggressive and predominant AGs found on sugar beets in MN and ND (Brantner and Windels 2007, Windels and Brantner 2011, Nelson et al. 1996). In the intermountain west sugar beet growing region of ID and OR, AG 2-2 IIIB and AG 4 were predominant groups (Strausbaugh et al. 2011).

Seedling infection of sugar beet may result in pre- and post-emergence damping-off, seed rot, and hypocotyl rot. Infection in the adult root starts as small reddish-brown to black spots that

slowly coalesce to form ladder-like lesions on the surface and spread both internally and externally, creating cracks in severe conditions. Crown rot is observed when the pathogen infects the base of the petioles in the adult plants and expands to both above- and below-ground parts. Above-ground symptoms include wilting of foliage followed by chlorosis, formation of black necrotic lesions on the petioles, and dry rosette appearance of dead plants (Franc et al. 2001, Neher and Gallian 2011). This disease can reduce yield up to 100% in sugar beets in artificially inoculated field conditions (Windels and Brantner 2005; Chanda and Brantner 2016).

Management of *R. solani*

Management of soilborne diseases caused by *R. solani* is difficult by using a single method, so disease management typically involves a combination of cultural and chemical measures and the use of resistant cultivars where available (Henis et al. 1978).

Cultural practices

Crop rotation

Crop rotation with non-hosts of *R. solani* AGs (AG 2-2, AG 3 and AG 4) infecting soybean and sugar beet such as wheat and barley can help to reduce the inoculum load in the successive field season. Generally, a 3-year rotation is adopted by the growers in the Red river River valley Valley area of MN and ND and southern MN, but it could be increased up to 4 to 5 years in problematic areas (Khan et al. 2019, Neher and Gallian 2011). Potato serves as a host for AG 3 and AG 4, while soybean, dry beans, sugar beet, and corn can serve as a host for AG 2-2 (Nelson 1996, Windels and Brantner 2011, Neher and Gallian 2011). Rotation with these crops can increase the pathogen inoculum in the successive cropping season. Higher disease incidence has been reported on sugar beets when they were preceded by bean crops, so close rotation of these two crops is discouraged (Engelkes and Windels 1996).

Cultivation practices

R. solani is generally found in the upper 10 cm of the soil profile and rarely is found below a depth of 20-25 cm (Papavizaz et al. 1975). Deep tillage helps to reduce disease incidence by

burying the infested topsoil and infected crop debris below the root zone. It can also help to reduce weed hosts such as pigweed and Kochia. In addition, other practices adopted to manage the disease include early planting in cooler soils, shallow planting to reduce the germination period, drainage to improve soil aeration and water infiltration, proper spacing to reduce the humidity, sanitation to minimize the primary inoculum, and use of pathogen-free planting material (Chanda et al. 2019, Sserunkuma 2016). However, cultural practices such as hilling can increase infection by bringing the infested soil closer to the crown (Khan et al. 2019, Neher and Gallian 2011).

Resistant Cultivars

Disease resistance in sugar beet

None of the commercially available sugar beet cultivars exhibit complete resistance against the disease. All commercial cultivars, partially resistant to crown and root rot, are susceptible to *R. solani* early in the growing season (Engelkes and Windels 1994, Liu et al. 2019, Chanda et al. 2019). However, certain breeding lines are resistant to both Rhizoctonia seedling damping-off and Rhizoctonia crown and root rot (McGrath et al. 2015, Nagendran et al., 2009). Resistance to postemergence seedling damping-off was identified in sugar beet germplasm accession EL51 (Nagendran et al., 2009) which is also resistant to crown and root rot (Halloin et al. 2000). Seedling resistance was characterized as the inability of pathogen to colonize the stele tissues of hypocotyl due to the presence of barrier at the narrow endodermis (Nagendran et al., 2009). EL51 was crossed with a smooth root sugar beet line to produce germplasm SR98, which can be used to develop hybrids suitable to sugar beet growing regions (McGrath et al. 2015). Unfortunately, the compromise in sucrose yield has restricted the use of these resistant lines to breeding programs only.

Disease resistance in soybean

Rhizoctonia resistant soybean cultivars are not commercially available (Hartman et al. 2015, Ajayi-Oyetunde and Bradley 2017, Chang et al. 2017). For example, a significant decrease in stand count, nodule formation, and yield were observed in 21 soybean genotypes inoculated with

R. solani AG 4 in Alberta (Chang et al., 2017). Cardoso et al. (1978) found no significant differences in the disease reaction between 39 soybean cultivars and plant introductions (PIs), and the susceptible check to AG 4. In Belgium, all soybean varieties except one were highly susceptible to *R. solani* AG 2-2 IIIB (Pannecoucque et al. 2018). Several other studies have reported different responses of varieties to *R. solani*. Muyolo et al. (1993b) observed resistant to root rot and partially resistant hypocotyl rot responses in six (Asgrow 7986, Centennial, Hardee, Pella, RA 606, and Vickery) out of 15 soybean varieties inoculated with AG 2-2 and AG 4 isolates. Bradley et al. (2001) evaluated 90 commercial cultivars and 700 ancestral lines and reported a partially resistant reaction to AG 2-2 in 20 cultivars and 21 ancestral lines. In a study with 70 soybean cultivars in Canada, three were tolerant to *R. solani* (Zhang et al. 2013). The pathogenicity and aggressiveness of *R. solani* can differ both within and between the AG groups (Muyolo et al. 1993a; Nelson et al. 1996, Windels and Brantner 2011), and the level of disease resistance can vary accordingly (Zhao et al. 2005a). Soybean cultivar 'PI 442031', partially resistant to AG 4 and 5, was reported to be highly susceptible to AG 2-2 IIIB (Zhao et al. 2005a). The phenotypic assessment of F2 and F3 generations derived from a cross between partially resistant cultivar 'Savoy' and the susceptible cultivar 'Jack' for disease reaction and segregation, indicated that disease resistance to *R. solani* is inherited quantitatively (Bradley et al. 2001). This finding was supported by Zhao et al. (2005b) who identified three quantitative trait loci (QTLs) that explained 11%, 7% and 6.8 % of phenotypic variation in response to AG 4. Sserunkuma (2016) also identified one QTL that explained 43.1% variation in response to *R. solani* AG 2-2 IIIB, which could be useful in breeding programs.

Chemical fungicides

Ineffective genetic resistance and cultural disease management methods have supported the reliance on chemical fungicides to manage *Rhizoctonia* diseases, especially in the early stages of plant development. Fungicides can be used as seed treatment, in-furrow or postemergence applications. Most fungicides labeled for the management of *R solani* in soybeans and sugar beets are within the quinone outside inhibitor (QoIs), succinate dehydrogenase inhibitor (SDHI),

demethylation inhibitor (DMI), phenylpyrroles (PP) and aromatic hydrocarbon (AH) classes (Ajayi-Oyetunde and Bradley 2017, FRAC 2019).

In sugar beet, seed treatments such as penthiopyrad (Kabina ST), sedaxane (Vibrance), fluxapyroxad (Systiva), mixture of metconazole plus toclofos-methyl (Metlock + Rizolex) are effective in protecting sugar beets for 4 to 5 weeks after planting (Brantner and Chanda 2019a). Azoxystrobin (Quadris, Aframe, Satori, or Azteroid) or pyraclostrobin(Xanthion) can be applied in-furrow to protect the crops from planting to mid-season. Postemergence band application of azoxystrobin (Quadris, Aframe, Satori or Azteroid), the pre-mix of pyraclostrobin plus fluxapyroxad (Priaxor), and the pre-mix of fluatiafol plus azoxystrobin (Topguard EQ) or prothioconazole (Proline) are effective when applied at the four to eight leaf stage of the sugar beet for managing mid to early late season onset of root rot. Studies with these fungicides demonstrated a 15 to 33 % incidence of root rot compared to 75 % incidence in the untreated control (Brantner and Chanda 2018).

In soybean, sedaxane and fluxapyroxad are considered excellent for managing Rhizoctonia seed and seedling diseases; trifloxystrobin is fair to excellent; azoxystrobin is considered very good; carboxin, penflufen, fludioxonil, prothioconazole, and ipconazole are good; and pyraclostrobin is reported to be fair to good (Crop Protection Network 2019b).

Most of these fungicides provide broad-spectrum control by targeting a single-site in the metabolic pathway of the fungal pathogens. Increased reliance on such fungicides raises the risk of selection for resistance in the pathogen population (Gisi et al. 2002, Avenot and Michailides 2010, Ajayi-Oyetunde and Bradley 2017). The fungicide resistance action committee (FRAC) classified QoI fungicides as high risk, SDHI as a medium to high risk, DMI as medium risk, and PP and AH as low to medium risk of developing resistance (FRAC 2019). There are no reports of *R. solani* populations resistant to SDHI, DMI, PP, or AH fungicides, but QoI resistant AG 1A isolates were identified in Lousiana (Olaya et al. 2012). Monitoring the sensitivity of *R. solani* to fungicides, alternating or combining the fungicides with different modes-of-action, and employing integrated

disease management strategies could reduce the development of resistance and protect longevity of fungicides (Ajayi-Oyetunde and Bradley 2017, Arabiat and Khan 2016, Chanda et al. 2019).

Chapter 2: Response of soybean genotypes to *Rhizoctonia solani*

Synopsis

Soybean seedlings are highly susceptible to *Rhizoctonia solani* which causes pre- and postemergence damping-off, seed rot, hypocotyl and stem rot, and root rot. Commercial cultivars lack resistance against this disease, thus it is important to identify potential sources of resistance. The objectives of this study are, (i) to identify an effective method to inoculate soybeans with *R. solani* in the growth chamber, (ii) assess the pathogenicity of different *R. solani* AG 2-2 isolates collected from sugar beet and soybean on both crops, and (iii) determine the responses of different soybean varieties and breeding lines to *R. solani*. A mycelial suspension inoculation method resulted in high disease severity and the lowest standard deviation among the methods tested and was selected to conduct studies of resistance and virulence in the growth chamber. Isolates from soybean were more aggressive on soybean seedlings than isolates recovered from sugar beet. All isolates were highly virulent on sugar beet seedlings. A significant genotype-by-isolate interaction was observed when 16 soybean genotypes were evaluated for their response to four *R. solani* isolates in the growth chamber, indicating that the response of soybean genotypes can differ among isolates of *R. solani*. All 16 genotypes were highly susceptible to isolate Rs 16 WC3-2. Twenty soybean genotypes evaluated in field studies in Crookston were susceptible to the pathogen with a minimum of 51% loss in plant population and 43% loss in yield under conditions highly favorable for disease. Similarly, severe disease reaction was observed in Waseca in 2018, resulting in more than 75% and 60% mean losses in plant population and yield, respectively. Some genotypes had very low losses in plant stands and yield in 2017 and 2019; but a strong genotype-by-year interaction was observed and the same genotypes did not display the same results in 2018. Genotype MN1613CN was the only genotype to perform consistently well in both 2017 and 2019, but it was highly susceptible to *R. solani* in high temperature and moisture conditions in 2018. In summary, *R. solani* AG 2-2 isolates were pathogenic on both sugar beet and soybean irrespective

of the host of origin. Thus, close rotation of these crops could lead to significant economic loss due to *R. solani* in both crops. The response of soybean genotypes to *R. solani* can be strongly influenced by environment and isolate, therefore disease response should be evaluated against multiple isolates in different environmental conditions. Although none of the soybean genotypes used in this study were resistant to *R. solani*, some showed promising results with relatively low losses in plant population and yield.

Introduction

Soybean is a major agricultural commodity in the United States, contributing to 90% of the total oilseed production of the nation (USDA-ERS 2019b). Each year soybean is produced on approximately 7 million acres in Minnesota, making it the third leading state in soybean production in the nation (USDA-ERS 2019b). Seedling diseases as a group were consistently among the six most destructive soybean diseases in the U.S. from 1996 to 2014 and in 2017 (Wrather and Koenning 2009, Koenning and Wrather 2010, Allen et al. 2017, Crop Protection Network 2019).

Rhizoctonia root and stem rot is one of the most important seedling diseases of soybean (Chang et al. 2017). It can cause pre- and post-emergence damping-off, hypocotyl and stem rot, and root rot resulting in decreased stand and yield of soybean and increased weed infestation (Keijer 1996, Sumner and Bell 1994, Ajayi-Oyetunde and Bradley 2017, Chang et al., 2017). *Rhizoctonia solani* is classified into 13 anastomosis groups (AG 1-13) based on hyphal anastomosis (Sneh et al. 1996, Carling et al. 1999 and 2002a, Ogoshi 1987). The AG groups that infect soybean are AG 1-IA, AG 1-IB and AG 2-3 that cause foliar blight; AG 2-2 IIIB, AG 2-2 IV, AG 4, and AG 5 that cause seedling and root rot; and AG 3 and AG 7 that cause small lesions on roots (Nelson et al. 1996, Zhao et al. 2005a, Braid et al. 1996, Yang et al 1990, Ajayi-Oyetunde and Bradley 2017). The virulence of *R. solani* can vary within and between the AGs. Isolates of AG 2-2 IIIB were reportedly the most pathogenic groups on soybean seedlings in some studies (Liu and Sinclair 1991, Ajayi-Oyetunde and Bradley 2018; Zhao et al. 2005a), whereas isolates of AG 4 were similar in virulence to AG 2-2 in other studies (Nelson et al. 1996).

R. solani can survive in the soil for more than 3 years in the form of sclerotia and infect a wide range of crops. The hosts include corn and sugar beet, which are cultivated in rotation with soybean in Minnesota and North Dakota (Nelson et al., 1996). Close rotation of soybean with host crops is discouraged to avoid an increase of inoculum in successive cropping seasons (Engelkes and Windels 1996, Windels and Brantner 2011). Understanding the host range and pathogenicity of *R. solani* isolates infecting rotation crops is crucial to improve disease management.

Disease management approaches for *R. solani* in soybean includes a combination of tillage, early planting, crop rotation, and seed treatments. Due to the lack of effective cultural measures, and the risk of resistance associated with chemical fungicides, resistant soybean varieties are desirable for disease management (Chang et al., 2017). However, breeding for Rhizoctonia resistance in soybean has received minimal attention, and hence Rhizoctonia resistant commercial soybean varieties are not available (Hartman et al. 2015, Ajayi-Oyetunde and Bradley 2017, Chang et al. 2017).

Several studies have investigated host plant resistance to *R. solani* in soybean. For example, a significant decrease in stand count, nodule formation, and yield were observed in all genotypes inoculated with *R. solani* in Alberta (Chang et al., 2017). Although partially resistant lines have been reported in some studies (Bradley et al. 2001, Muyolo et al. 1993b, Zhao et al. 2005a) the level of genetic resistance can vary according to the AG and pathotypes. For example, germplasm accession ‘PI 442031’, was partially resistant to AG 4 and AG 5 but highly susceptible to AG 2-2 IIIB (Zhao et al. 2005a). In addition, the quantitative inheritance of Rhizoctonia resistance can pose significant constraints to breeding programs (Sserunkuma 2016, Bradley et al. 2005; Zhao et al. 2005b). Partial resistance to *R. solani* has been evaluated phenotypically using inoculated field and greenhouse studies (Bradley et al. 2001 and 2005). Simple sequence repeat (SSR) markers linked to four quantitative trait loci (QTLs) associated with partial resistance to *R. solani* in soybean have been identified, which may be useful in breeding for resistance (Sserunkuma 2016, Zhao et al. 2005b).

There were three main objectives in this study. i) Identify an effective and consistent method to inoculate soybeans with *R. solani* in controlled conditions. ii) Determine the pathogenicity of different *R. solani* isolates from sugar beet on soybean and vice versa. iii) Determine the responses of multiple genotypes of soybean to *R. solani* in field and growth chamber environments.

Materials and Methods

Evaluation of methods to inoculate with *R. solani* in controlled environments. Soybean cultivar 'NS2031' (Northstar Genetics, Wanamingo, MN) was selected for growth chamber studies due to its susceptibility to *R. solani* AG 2-2 IIIB in our 2017 field studies. All experiments used *R. solani* 2-2 IIIB isolate, Rs 6-222. The experiments were arranged in a completely randomized block design with three inoculation methods and a non-inoculated control. Each inoculated treatment was replicated six times, controls were replicated three times, and the experiment was repeated once.

The first inoculation method used mycelial suspensions of *R. solani* grown on 9 cm diameter Potato Dextrose Agar (PDA) (Difco™, BD Diagnostic Systems, Sparks, MD) plates for 1 week. Five plates were macerated in 1 L of deionized water using a blender for 1 min (modified from Bradley et al. 2001). Non-inoculated PDA suspension was prepared in the same way for mock inoculation. 10 cm square pots were filled 75% with a 50:50 mixture of Pro-mix BX (Premier Horticulture INC., Selon SIMDUT, Canada, #10380) and field soil (autoclaved 2 hr), followed by the addition of four seeds per pot. Then 1 ml of mycelial or PDA suspension was placed directly on each seed at planting and covered with 2 cm of soil mix (Fig. 2.1A).

The second inoculation method used a layer of infested sorghum inoculum in each pot. Sorghum grains were soaked in water overnight, and the soaked grain (@1 L) was placed in spawn bags (22.5" x 8 x 4.7, 2.25 mils) with filters (Fungi perfecti LLC, Olympia, WA) and autoclaved twice for 70 min each on consecutive days. Each sterilized bag was inoculated with 16 mycelial plugs (1cm diameter) from 1-week-old cultures of Rs 6-222 grown on PDA, and the bags were sealed with a heat sealer. After 3 wk of incubation at approx.25 °C, the colonized sorghum grains were placed into paper bags and dried at 35° for 48 hr. Non-infested sorghum grains were prepared in

the same way without the pathogen to use in mock inoculation. Pots were half-filled with soil mix followed by a layer of 10 cc of infested or non-infested sorghum (Fig. 2.1B). Sorghum grains were covered with 2 cm of soil before sowing four seeds per pot, which were covered with 2 cm of soil.

The third inoculation method used ground barley inoculum (modified from Windels and Brantner 2011). Barley grains were prepared and infested with *R. solani* using the same procedure described above. Both infested and non-infested barley grains were ground with a coffee grinder after drying. Three-quarters of 10 cm square pots were filled with a potting soil mix, then a layer of 5 g of ground barley inoculum was spread over the surface, and four seeds were placed directly on top of the inoculum and covered with 2 cm of soil (Fig. 2.1C).

For all inoculation methods, 1.5 g Osmocote 14-14-14 smart-release® fertilizer (The Scotts Company, Marysville, Ohio, #LB3376) was added to each pot, and the soil mix was covered with a layer of 0.32 to 0.54 cm of sand to reduce fungal gnats. All pots were kept in a growth chamber at 26 °C with 14-hr light . After 3 weeks, soybean roots were washed thoroughly and rated for root damage on a scale of 0-4 (modified from Nelson et al. 1996). Healthy roots were rated 0, roots with lesions up to 3 mm or with less than 25% girdling of the stem were rated 1, roots with lesions 3-10 mm or 25 - 50 % girdling of the stem were rated 2, roots with lesions greater than 10 mm lesions or more than 50% stem girdling were rated 3, and dead seedlings were rated 4. Disease severity index was estimated for each treatment as follows: DSI = [Σ(disease score category x number of plants in the disease score category)] / [(total number of plants) × (maximum disease score)] × 100. Controls were excluded from the analysis as their DSI was close to zero. The study was analyzed using a linear model including experiment, block(exp), inoculation method and experiment x inoculation method interaction. The variances in the DSI of plants inoculated with *R. solani* using different methods were compared using analysis of variance (ANOVA), and Fisher's Least significant Difference (LSD) test with was used to separate significantly different means using package Agricolae' in R (version 3.6.2).

Pathogenicity of different *R. solani* isolates on soybean. Nine *R. solani* AG 2-2 isolates collected from soybean and ten AG 2-2 isolates from sugar beet were selected for the study. The

AG of the isolates was confirmed based on PCR amplification with AG 2-2 specific primer sets developed by Carling et al. (2002b) (data not shown). Mycelial suspensions were prepared for each isolate as described above. Non-inoculated PDA plates were used to prepare mock suspensions. Three soybean genotypes, M07-209037 (advanced breeding line), M09-285032 (advanced breeding line), and MN1701CN (released public cultivar), from the soybean breeding program at the University of Minnesota were included. The experiment was conducted in a completely randomized block design with four replications. The entire experiment was repeated once. Three seeds were sown per pot and inoculated at planting with mycelial suspensions of one isolate per pot as described above. This study was conducted in a growth chamber set at a constant temperature of 26 °C with 14-hr photoperiod. Plants were rated for root rot after 3 weeks and a disease severity index for each pot was determined as described above The total fresh biomass of plants (including roots) in each experimental unit was estimated as a proportion of biomass of inoculated plants to the biomass of respective control plants within each block. The DSI and relative biomass were first analyzed using analysis of variance (ANOVA) on a linear model including experiment, block(exp), source of isolate, isolate(source), soybean genotype, soybean genotype x source, soybean genotype x isolate(source), experiment x source, experiment x genotype, experiment x isolate(source), experiment x genotype x isolate(source) and experiment x genotype x source. Due to a significant experiment by isolate interaction, the experiments were further analyzed separately using a model with block, source of isolate, isolate(source), soybean genotype, soybean genotype x isolate(source) and soybean genotype x source. The DSI of controls were close to zero and excluded from the analysis. The Fisher's LSD test was used to separate the means at $p = 0.05$, and Kendall's Tau-b test was used to determine correlations between DSI and biomass.

Determination of the pathogenicity of *R. solani* isolates on sugar beet. The experiment was conducted in a completely randomized block design with four replications and repeated once. Ground barley inoculum was prepared as described above for nine soybean isolates and 10 sugar beet isolates, and non-infested ground barley was used for mock

inoculations. Soil mix was prepared by mixing equal volumes of Pro-mix BX potting mix (Premier Horticulture INC., Selon SIMDUT, Canada, #10380) and autoclaved field soil. Ground barley inoculum of each isolate was added individually to the soil mix at the ratio of 1:500 mass by volume. 10 cm square pots were filled 75% with the soil-inoculum mix and 25 sugar beet seeds of the Rhizoctonia-susceptible cultivar 'Crystal 101' were placed in each pot and covered by 2 cm of soil mix. This study was conducted in a growth chamber set at a constant temperature of 26 °C with 14-hour photoperiod. Plants were rated for disease severity 3 wk post inoculation on a 0 - 3 scale modified from Fink and Buchholtz (1954), where 0 = healthy plant; 1 = light brown root with water-soaked appearance; 2 = live plants with brown root and slightly constricted hypocotyl; and 3 = dead plants. Then relative fresh plant biomass and DSI were determined as described above for each experimental unit. ANOVA was used to determine the effects of experiment, block(exp), source of isolate, isolate (source), experiment x source, experiment x isolate (source) on DSI and biomass and Fisher's LSD test was used to separate the means. The two experiments were analyzed separately due to the significant experiment by isolate interaction. The correlation between DSI and biomass were determined by Kendall's Tau test.

Response of soybean genotypes to *R. solani* in field studies. Multiple soybean genotypes were studied for their response to *R. solani* in three field seasons from 2017-2019 at the University of Minnesota Southern Research and Outreach Center in Waseca, MN and two field seasons from 2018-2019 at the Northwest Research and Outreach Center in Crookston, MN. In Waseca, four arbitrarily chosen commercial cultivars and 24 breeding lines provided by the soybean breeding program at the University of Minnesota of relative maturity 1.2 – 2.0 were used in the field study in 2017. Three commercial soybean cultivars and 33 breeding lines of relative maturity 1.2 - 2 were used in 2018, and five commercial soybean cultivars and 23 breeding lines were used in 2019. A total of 40 genotypes were evaluated during the 3 years in Waseca. Fifteen of the genotypes were used in all three field seasons, 20 were included in two seasons, and 5 genotypes were used only once due to limited seed availability. In Crookston, 10 soybean breeding lines and 10 public cultivars in maturity groups 0 - 0.5 were used in the studies in 2018 and 2019.

The studies were planted in Waseca on June 1, 2017, May 31, 2018, and May 28, 2019, and in Crookston on May 24, 2018 and May 28, 2019. Soybean seeds were mixed with infested sorghum prepared as described above and sown using cone planters in plots with two rows three m in length spaced 0.6 m apart. Eighty seeds were planted per row (140949 seeds/ha) with 30 cc of sorghum inoculum (21406 cc /ha) in Waseca and 20 cc in Crookston (14308 cc /ha). Soybean seeds were mixed with non-infested sorghum for the control plots. A split-plot experimental design was used with three blocks (replications), where soybean genotypes were the main plot factors and inoculation treatment (inoculated versus non-inoculated) was the split plot factor. Soybean genotypes were randomized within each block. Plant population per plot was determined at emergence (VE) and full maturity (R8) stages. The soybean plots were machine harvested by late October to early November, seed weights were adjusted to 13% moisture and the yield was expressed in tons per ha. Losses in plant population and yield in the inoculated plots compared to the non-inoculated plots in each block were determined in percentage for each soybean genotype.

The Wilcoxon rank-sum test was used to compare final stands and yields between inoculated and non-inoculated treatments (Table 2.11). The percentage loss in plant population and yield of soybean genotypes evaluated in Crookston were analyzed using ANOVA on a linear model including year, block (year), soybean genotype and year x soybean genotype interaction. For the soybean genotypes evaluated in Waseca, the percentage loss in plant population and yield were analyzed separately for each year using a linear model including block and soybean genotype. The 15 soybean genotypes evaluated across all 3 years in Waseca were further analyzed using a linear model including year, block (year), soybean genotype, and soybean genotype x year interaction. ANOVA and Fisher's LSD test were used to compare the variances and means of different soybean genotypes, respectively.

Response of different soybean varieties and breeding lines to *R. solani* in controlled environments. Sixteen soybean genotypes were arbitrarily chosen for this study, among which 12 were included in the field study in Waseca and four were in the field study in Crookston. Four isolates of *R. solani* AG 2-2 were also selected and mycelium suspensions were prepared for each

isolate as described above. The experiment was conducted twice using a completely randomized block design with four replications. Each experimental unit (pot) with three seeds was inoculated with the mycelial suspension of one isolate or mock inoculum, and plants were kept in the growth chamber as described above. After 3 weeks, the relative biomass was estimated for each experimental unit and the seedlings were rated for stem and root rot to determine the DSI on each pot as described above. The DSI and relative biomass were analyzed using a linear model including experiment, block (exp), isolate, soybean genotype, isolate x soybean genotype, experiment x isolate, experiment x soybean genotype, and experiment x soybean genotype x isolate. As the DSI index of controls were close to zero, they were excluded from the analysis. Significant experiment by isolate interactions were observed on both DSI and relative biomass, thus the two experiments were analyzed separately, and means were separated using Fisher's LSD. Kendall's tau b test was used to determine the correlation between DSI and biomass.

Results

Evaluation of methods to inoculate with *R. solani* in growth chamber studies. The disease severity index (DSI) of soybean plants inoculated with infested sorghum and the mycelial suspension were significantly higher (P= 0.0015) than those inoculated with ground barley inoculum (Table 2.1). The mycelial suspension method resulted in the lowest standard deviation for the DSI, and thus was selected for use in further studies with *R. solani* in the growth chamber.

Pathogenicity of *R. solani* isolates on soybean. The DSI and fresh biomass of inoculated soybean genotypes varied significantly among the 19 *R. solani* isolates in both experiments (Tables 2.2, 4.4, 4.5, 4.7, 4.8). Soybean isolates resulted in significantly higher mean DSI values and low mean relative biomass on soybean compared to the sugar beet isolates in both experiments (Tables 2.2, 4.4, 4.5, 4.7, 4.8). Inoculation with isolate Rs 16WC3-2 resulted in the numerically highest mean DSI in both trials (97.4% in experiment 1 and 91.7% in experiment 2), which was significantly higher than the DSI of plants inoculated with 11 of the 19 isolates in experiment 1 and 10 isolates in experiment 2 (Table 2.2). In contrast, inoculation with Rs 13-52-2

(mean DSI 42.7%) and Rs 06-77-4 (mean DSI 33.9%) resulted in the significantly lowest mean DSI experiments 1 and 2, respectively. In experiment 1, plants inoculated with Rs 89-29-3A had the numerically highest mean relative biomass (102.6 %), which was significantly higher than relative biomass resulted from the inoculation with 11 isolates, whereas plants inoculated with Rs 16 WC 1 had the numerically lowest mean relative biomass (4%) which was significantly lower than relative biomass resulted from inoculation with 16 isolates. Similarly, in experiment 2, inoculation with Rs 13-52-2 resulted in the numerically highest relative biomass (90.1%) which was significantly higher than that resulting from inoculation with 10 isolates, while inoculation with Rs soy 18-1 resulted in the numerically lowest relative biomass (19.7) that was significantly lower than the biomass resulting from inoculation with 12 other isolates.

The virulence of Rs 06-77-4 was inconsistent across the two experiments. This isolate resulted in a high mean DSI (89.1) and low mean relative biomass (41.4%) in experiment 1, which contrasted with the lowest mean DSI (33.9) and high mean relative biomass (86.2%) value with this isolate in experiment 2 (Table 2.2). The effect of soybean genotype following inoculation was significantly different for DSI in experiment 2, but not in experiment 1 (Tables 2.2, 4.4 and 4.5). However, the three varieties differed significantly for relative biomass in both experiments (Tables 4.7 and 4.8). However, the mean DSI of the three individual soybean genotypes inoculated with the highly aggressive isolate (Rs 16WC3-2) were greater than 90 in both experiments, which indicated that all lines were highly susceptible to this isolate. There were no genotype-by-isolate interactions for either DSI or biomass for inoculated soybean. DSI and biomass were negatively correlated in both experiments (exp. 1: Tau= -0.49, P< 2.2e-16; exp. 2: Tau= -0.37, P= 6.721e-15).

Pathogenicity of *R. solani* isolates on sugar beet. The DSI caused by the *R. solani* isolates was not significantly different on inoculated sugar beets in experiment 1 (Tables 2.3 and 4.10). In contrast, significant differences were observed among the isolates in experiment 2 (Tables 2.3 and 4.11). The mean DSI caused by all individual *R. solani* isolates was higher than 92 in both experiments, indicating that all isolates were highly aggressive on sugar beet seedlings (Table 2.3).

The *R. solani* isolates also differed significantly for their effects on relative fresh plant biomass in both experiments (Tables 2.3, 4.13 and 4.14), but the means of biomass were low and near zero for almost half of the isolates because most plants were dead at the end of the experiment (Table 2.3). DSI and relative biomass were negatively correlated in both experiments (experiment 1: Tau= -0.43, P= 4.886e-05; experiment 2: Tau= -0.725454, P= 1.929e-13).

Response of soybean genotypes to *R. solani* in field studies. At the Crookston field location, the two years were significantly different for loss in plant population and yield, but no genotype-by-year interactions were detected (Tables 2.4 and 4.15). The mean loss in plant population was higher in 2018, but the mean loss in yield was higher in 2019. Due to the significant differences in the two years, data from each year is presented separately. Percentage loss in plant population for inoculated vs. control plots was not significantly different between the soybean genotypes in Crookston in either year. The percentage loss in yield was significantly different among soybean genotypes in 2018 with Traill having the numerically lowest mean yield loss of 43.4% which was significantly lower than the mean yield loss of 11 genotypes(Table 2.4). Overall, >51% mean losses in plant population and 43% mean losses in yield were measured for all individual soybean genotypes in both years, suggesting that all genotypes evaluated in Crookston were susceptible to the pathogen.

At the Waseca field location, soybean genotypes differed significantly for yield loss in 2017 and 2019, whereas significant difference in plant population loss between the genotypes was detected only in 2019 (Tables 2.5, 4.2 -4.26). In 2017, M12-454049 had a mean yield loss (7.3%) that was significantly lower than 14 other genotypes (Table 2.5). However in 2018, the mean loss in plant population for M12-454049 was >75%. Further, the mean loss in yield was > 60% for all soybean genotypes and the effect of genotype was not significant for yield or population (Table 2.5). In 2019, M09-343025 had a low mean loss in plant population (8.8%) that was significantly different from eight other genotypes. M09-343025 also had mean yield loss of 2.4%, which was significantly lower than 17 other genotypes (Table 2.5). The low losses in plant population and yield of M09-343025 in response to *R. solani* in 2019 differed from its high losses in 2018.

Significant year-by-genotype interactions in Waseca indicated that the losses in plant populations and yield were not consistent between the years for the 15 soybean genotypes evaluated from 2017 to 2019 (Tables 2.6 and 4.27). The year x genotype interaction was significant and the data from each year are presented separately (Table 2.6). All the genotypes were highly susceptible to the pathogen in 2018. Among the 15 varieties studied across all three years, MN1613CN, MN1701CN, MN1806CN, and MN1312CN performed well in 2017 with less than 21% yield loss, whereas M09-343025, M09-285149 and M09-285032 performed well in 2019 with less than 10% yield loss. Only MN1613CN had less than 21% yield loss in both years (Table 2.6).

The inoculated and non-inoculated treatments had significantly different plant populations and yield in all field experiments (Table 2.7). The soil temperature at 10 cm depth and precipitation during the seedling stages (late May to June) were higher in 2018 than 2019 at Crookston. At Waseca, 2017 had the highest the mean soil temperature among the 3 years, 2018 had the highest precipitation in June, and 2019 had the coolest soil temperature and lowest precipitation. High rainfall from mid to late field season in 2019 at both locations delayed harvest of the crop (Fig 2.2).

Response of different soybean varieties and breeding lines to *R. solani* in controlled conditions. The effect of genotype was significant for both DSI and relative biomass in both experiments (Table 2.8, 2.9, 4.30, 4.31, 4.33 and 4.34). The performances of soybean genotypes were not consistent across the two trials (Table 2.8, 2,9, 4.29). In the combined analysis of DSI for the four isolates, M09-343025 had the numerically lowest mean DSI (52.6) in experiment 1 that was significantly lower than the DSI of 8 other genotypes. MSC10-558061 had the numerically lowest mean DSI (53.1) in experiment 2 that was significantly lower than the DSI of 12 genotypes (Table 2.8). In experiment 1, M09-285032 had a mean relative biomass (86%) that was similar to MN1701CN and significantly higher than 14 genotypes. In experiment 2, M11-358032 had the significantly highest mean relative biomass (84.1%) (Table 2.9). Isolate Rs 16WC3-2 resulted in the highest mean DSI and the lowest fresh plant biomass, while Rs 16-48-1 resulted in the lowest mean DSI and the highest biomass in both trials (Table 2.8 and 2.9). Isolate Rs 16-48-1 did not have consistent virulence across the two experiments, with very low DSI in Experiment 1 (mean

DSI= 6.5) and moderate DSI in Experiment 2 (mean DSI= 52) (Table 2.8). Significant genotype-by-isolate interaction was also detected for relative biomass in both trials and for DSI in the first trial (Tables 2.8, 4.30, 4.33 and 4.34), suggesting that genotypes may respond differently to different isolates. There was no significant variation in DSI among the soybean genotypes inoculated with Rs 16WC3-2 and Rs 6-222 in either experiment, but significant differences were observed among the genotypes on inoculation with Rs 13-52-2. The mean DSI for individual soybean genotypes inoculated with the most aggressive isolate (Rs 16 WC 3-2) was greater than 83, and the relative biomass was less than 53% in both experiments, indicating all genotypes studied are susceptible to this isolate. There was a highly significant negative correlation between the DSI and relative fresh plant biomass in both experiments (experiment 1: Tau= -0.705, P< 2.2e-16; Experiment 2: Tau= -0.62, P< 2.2e-16).

Discussion

Rhizoctonia solani can significantly affect stand establishment and yield in soybeans (Chang et al. 2017, Cardoso et al. 1978, Pannecoucque et al. 2018). Commercial soybean cultivars are not available that provide resistance against this pathogen, and little effort has been dedicated to breeding for *Rhizoctonia* resistance in soybean (Hartman et al. 2015, Chang et al. 2017). Partially resistant sources were reported in several studies by 2005, but not much progress has been observed in the development of resistant cultivars (Muyolo et al. 1993b, Bradley et al. 2001, Zhao et al. 2005a). It is therefore essential to assess the responses of additional soybean genotypes to *R. solani* to identify potential sources of disease resistance. In this study, we evaluated the response of 60 soybean genotypes to *R. solani* 2-2 IIIB isolate in the field. This is the first study to compare methods used to inoculate *R. solani* in a growth chamber and to determine the response of northern soybean genotypes to multiple AG 2-2 isolates collected from infected soybean and sugar beet samples in the growth field and/or growth chamber conditions.

The methods used to evaluate the soybean genotypes in for resistance to *R. solani* in this study included phenotypic assessments in both field and greenhouse environments. The accuracy and relevancy of phenotypic disease assessment is determined in part by the severity and

uniformity of the disease across all experimental units in a study (Vaghela et al. 2014). Previous studies used various substrates such as infested wheat grains (Chang et al. 2017), wheat bran (Zhang 2014), barley (Vagher et al. 2014), sorghum (Schneider et al. 1976), and dent corn (Engelkes and Windels 1996) for infesting field experiments with *R solani*. However, field efficiency might not translate to greenhouse or growth chamber conditions due to the controlled environment, limited root zone, alterations in soil composition and microbiome, and vice versa. Zhang et al (2013) reported the variation in resistance expression of soybean genotypes to *Fusarium* between greenhouse and field experiments and emphasized the need for standardized protocols.

Some of the material and methods used previously to inoculate soybeans with *R. solani* in controlled conditions include a mycelial suspension (Bradley et al. 2001), colonized mix of soil and potato dextrose broth (PDB) (Penă et al. 2013, Sserunkuma 2016), infested wheat (Pannecoucque, 2018), infested oats (Dorrance et al. 2003), and infested ground and whole barley grains (Windels and Brantner 2011, Vagher et al. 2014). However, efficiency of these methods has not been compared in a single study. Windels and Brantner (2011) observed severe symptoms on soybean seedlings planted in soil mixed with ground barley inoculum containing *R. solani* AG 2-2 IIIB isolates. In our study, ground barley inoculum with one isolate of a *R. solani* IIIB isolate applied in a layer produced moderate symptoms on soybean seedlings. Infested dry sorghum grain inoculum is commonly used in the field studies due to its small, uniform size and consistency in field distribution (Schneider et al. 1976). A mycelial suspension method was used in the post-emergence evaluation of partial resistance to *R. solani* in different soybean genotypes by Bradley et al. in 2001 and 2005. In this present study, inoculations with both sorghum and mycelial suspension methods had similar disease severity, but the mycelial suspension method was more consistent across replications. In addition, sorghum grains in the growth chamber pots sometimes resulted in odor issues, stunting, and water-soaked to black lesions in the root tips of the soybean seedlings. This could have resulted from the colonization and decomposition of sorghum grains by the saprobes present in the soil. Therefore, the mycelial suspension method was selected as the best of these methods studied to inoculate soybean plants with *R. solani* for further experiments.

We evaluated 60 soybean genotypes for reacting to *R. solani* in inoculated field experiments at locations from 2017 to 2019. *Rhizoctonia solani* significantly reduced the plant population and yield in the inoculated plots compared to the non-inoculated plots at both locations in all field seasons, which is aligns with observations in previous studies. (Muyolo 1993a, Chang et al. (2017). For example, 39 genotypes evaluated by Cardoso et al (1978) were all similar to the susceptible check. Disease resistant reactions were not observed in any genotypes evaluated by Pannecoucque et al. (2018), but one had lower susceptibility. Chang et al. (2017) reported some genotypes with lower susceptibility, but they were not consistent across experiments in different years. Muyolo et al. (1993b) and Bradley et al. (2001) reported partial disease resistance in six and 20 soybean genotypes, respectively. Similarly, Zhang et al. (2013) reported three soybean cultivars tolerant to the pathogen. However, we did not observe much variation among the soybean genotypes in their responses to *R. solani*. The differences in plant population loss were not significant between the genotypes except for one trial in Waseca in 2019. Even though the differences in yield losses were significant with very low losses in the inoculated plots of some genotypes, their performances were not consistent across years. Inconsistency in the responses of soybean genotypes across different years were also observed by Chang et. al (2017).

Disease development by *R. solani* is favored by high temperature and moist soil conditions (Bolton et al. 2010, Dorrance et al. 2003, Leach, 1986), and soybean seedlings are more susceptible to the pathogen than adult plants (Windels and Brantner 2011). In a previous study, early season rainfall was accompanied by large reductions in plant population and yield in plots inoculated with *R. solani* (Chang et al. 2017). We also observed higher reductions in plant population in the year with the highest early season rainfall (2018). Plant stand loss was the lowest in the year (2019) with low early season rainfall and cool soil temperature at both locations. The reduction in yield also followed similar pattern across the years in Waseca. Yield loss was higher in 2019 in Crookston despite the significantly lower loss in in plant population compared to 2018. The overall mean yield of control plots was also lower in 2019 in Crookston. The presence of natural

populations of *R. solani* in the soil, high late season precipitation in September and October, and delayed harvest may have contributed to the lower yields in 2019.

Soybean genotypes also differed in their responses to *R. solani* in a controlled environment in a growth chamber. The significant genotype-by-isolate interaction in the controlled environment studies suggests that soybean genotypes respond differently to different *R. solani* isolates within the same AG. This finding should be explored further with larger sets of isolates. The DSI and relative plant biomass were negatively correlated, suggesting that *R. solani* can significantly reduce the biomass of infected plants. Similar results were observed by Pannecoucque et al. (2017) for effects of *R. solani* on stem and leaf biomass of plants. In contrast, Bradley et al. (2001) did not observe a correlation between disease severity and the dry root weight in greenhouse studies.

In the growth chamber studies with multiple isolates, the virulence of some isolates were not consistent between the experiments. Similar results were also observed by Zhao et al. (2005a) and Nelson et al. (1996). The inconsistency in the virulence of some isolates across the experiments could be associated with difference in environment,soil nutrents, inoculum levels, and production and activity of hydrolytic enzymes (Tachibana 1968, Lewis and Papavizas 1977, Khan 1993, Kousik et al. 1995, Baker and Walker 1962, Geypens 1978, van Bruggen et al. 1986, Keinath 1995). Although we tried to maintain uniform environmental conditions (temperature, photoperiod, humidity, and soil moisture) across all the experiments, variability could have from the fact that each batch of soil and inoculum can be slightly different.

Rhizoctonia solani is a common pathogen of sugar beet that is often rotated with soybeans in MN and ND. It is therefore important to understand the disease dynamics of *R. solani* related to these two cropping systems. In our study, *R. solani* AG 2-2 isolates were pathogenic to both sugar beet and soybean, which is supported by previous studies (Windels and Brantner 2011, Engelkes and Windels 1996, Nelson et al. 1996). All isolates in our study were highly aggressive on sugar beet seedlings, resulting in greater than 90 mean DSI for individual isolates. The *R. solani* Isolates recovered from soybeans were significantly more aggressive on soybeans than the isolates from sugar beets. These results suggest that host preference may have allowed the soybean isolates to

infect soybean more aggressively compared to the sugar beet isolates. The rotation of these crops could increase inoculum in fields and disease risk in the successive season.

In summary, the mycelial suspension method was determined to be the most effective method to inoculate soybean with *R. solani* in the growth chamber. Our growth chamber studies suggest that all AG 2-2 isolates from soybean and sugar beet were highly pathogenic on sugar beet but isolates from soybean had higher virulence on soybean compared to those from sugar beet. Cultivation of these two crops in succession can lead to significant loss in the production of both crops. *R. solani* significantly decreased the biomass, plant population, and seed yield in soybean. Although soybean genotypes differed in their reaction to *R. solani* in the field, their response was greatly influenced by the environmental conditions. None of the soybean genotypes included in this study were resistant to the disease in high temperature and moisture conditions. However, some soybean genotypes showed promising results with relatively low losses in stand establishment and yield when the environmental parameters were less favorable for *R. solani*, and these should be investigated further. In the growth chamber studies, soybean genotypes responded similarly to some isolates, while their response varied for other isolates suggesting that variety x isolate interaction can influence disease development. Future research should evaluate additional soybean genotypes for their response to multiple *R. solani* isolates under different environmental conditions.

Table 2.1. Mean disease severity index (DSI) of soybean plants inoculated with *R. solani* (Rs 6-222) using three different inoculation methods in the growth chamber.

Inoculation method	Mean DSI[y]	SD[z]
Mycelial suspension	78.1a	12.9
Sorghum	79.7a	17.3
Ground barley	57.8b	13.9

[y] Data represent the means from two experiments, each with six replications. Means followed by the same letters do not differ significantly based on Fisher's least significant difference (LSD) test at the 5% level of significance.
[z] Data represent the standard deviation from two experiments, each with six replications

Table 2.2. Disease severity index (DSI) and fresh plant biomass of three soybean genotypes inoculated in two experiments of growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.[v]

Isolate	DSI[w]				Relative fresh biomass (%) [x]			
	M07-209037	**M09-285032**	**MN1701CN**	**Mean[y]**	**M07-209037**	**M09-285032**	**MN1701CN**	**Mean[y]**
Experiment 1								
Soybean isolates								
Rh Mcleod	67.2	70.3	65.6	67.7ef	102.7	87.5	87.7	92.6ab
Rh Waseca 114B	84.4	92.2	93.8	90.1ab	56.6	40.6	24.6	40.6ef
Rs 16WC1	95.3	95.3	96.9	95.8a	6.6	1.8	3.6	4g
Rs 16WC1-2	96.9	93.8	98.4	96.4a	7	16.6	3.5	9g
Rs 16WC2-2	84.4	85.9	85.9	85.4bc	67.9	54.9	49.6	57.4de
Rs 16WC3-1	87.5	76.6	82.8	82.3bc	52.9	78.2	75.7	68.9cd
Rs 16WC3-2	98.4	100	93.8	97.4a	8.7	0	16	8.2g
Rs 6-222	92.2	85.9	85.9	88abc	33.7	51	58.2	47.6ef
Rs soy 18-1	90.6	85.9	93.8	90.1ab	25.7	42.2	24.6	30.8f
Mean	88.5	87.3	88.5	88.1	40.2	41.4	38.2	39.9
Sugar beet isolates								
Rs 86-42-4	73.4	68.8	59.4	67.2ef	114.6	86.8	99	100.1ab
Rs 89-29-3A	65.6	54.7	60.9	60.4f	125.5	94.3	87.9	102.6a
Rs 95-16-2	56.3	70.3	62.5	63ef	124.9	82.1	83.4	96.8ab
Rs 06-77-4	87.5	93.8	85.9	89.1ab	50.6	23.8	49.8	41.4ef
Rs 10-102-3	65.6	64.1	54.7	61.5f	104.6	85.8	80.1	90.2ab
Rs 11-88-2	75	60.9	53.1	63ef	102.3	73.6	91.6	89.2ab
Rs 13-52-2	50	39.1	39.1	42.7g	107.2	83.3	87.5	92.7ab
Rs 16-48-1	87.5	90.6	85.9	88abc	46.3	35	40.9	40.7ef
Rs 17-43-2	85.9	84.4	67.2	79.2cd	72.8	69.4	104	82.1bc
Rs 18-11	68.8	71.9	73.4	71.4de	89.5	82.4	89.5	87.1abc
Mean	71.6	69.8	64.2	68.5	93.8	71.6	81.4	82.3
Experiment 1 mean[z]	**79.6**	**78.1**	**75.7**	**77.8**	**68.4**	**57.3**	**60.9**	**62.2**
Experiment 2								
Soybean isolates								
Rh Mcleod	73.4	70.3	71.9	71.9e	85.7	88	94.4	89.3a
Rh Waseca 114B	82.8	81.3	82.8	82.3abcd	19.5	47.5	37.4	34.8bcde
Rs 16WC1	87.5	93.8	85.9	89.1a	36.2	14.7	41.6	30.8cde
Rs 16WC1-2	87.5	96.9	90.6	91.7a	27.4	3.8	36.1	22.4de
Rs 16WC2-2	78.1	96.9	75	83.3abcd	68.8	12.5	39.3	40.2bcd
Rs 16WC3-1	85.9	87.5	84.4	85.9abc	34.3	42.8	49.6	42.2bc
Rs 16WC3-2	92.2	92.2	90.6	91.7a	20.5	9.6	33.4	21.2e

Rs 6-222	76.6	70.3	75	74de	48.6	58	51.9	52.8b
Rs soy 18-1	90.6	93.8	89.1	91.1a	19.9	15.8	23.3	19.7e
Mean	83.9	87	82.8	84.5	40.1	32.5	45.2	39.3
Sugarbeet isolates								
Rs 86-42-4	62.5	68.8	71.9	67.7e	95.3	80.6	87.5	87.8a
Rs 89-29-3A	71.9	73.4	64.1	69.8e	92.9	85.3	89.2	89.1a
Rs 95-16-2	67.2	76.6	68.8	70.8e	92.2	91.7	85.9	89.9a
Rs 06-77-4	42.2	50	9.4	33.9g	78	73.7	107	86.2a
Rs 10-102-3	71.9	82.8	73.4	76cde	89.7	58.2	91.7	79.8a
Rs 11-88-2	76.6	79.7	75	77.1bcde	78.3	59.7	89.7	75.9a
Rs 13-52-2	32.8	48.4	56.3	45.8f	95.9	88.4	85.9	90.1a
Rs 16-48-1	90.6	93.8	87.5	90.6a	30.2	22.6	46	32.9cde
Rs 17-43-2	89.1	84.4	87.5	87ab	15.2	39.7	40.9	31.9cde
Rs 18-11	82.8	68.8	71.9	74.5de	65	70.7	91.1	75.6a
Mean	68.8	72.7	66.6	69.3	73.3	67	81.5	73.9
Experiment 2 mean[z]	**75.9**	**79.4**	**74.3**	**76.5**	**57.6**	**50.7**	**64.3**	**57.5**

[v] Data represent the mean of four replications

[w] Disease severity index (DSI)= 0-100, 0= no disease, 100= all plants dead at 3 weeks after planting

[x] Relative fresh biomass determined as proportion of fresh biomass of inoculated plants to the biomass of control expressed in percentage

[y] Data represent the mean of three soybean genotypes and four replications. Means followed by the same letters in the columns within same experiment do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

[z] Data represent the mean of 19 isolates and four replications.

Table 2.3. Disease severity index (DSI) and fresh plant biomass of seedlings of sugar beet cultivar "Crystal 101" inoculated in two growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.

Isolate	DSI[y]		Fresh biomass/plant (g)[z]	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Soybean isolates				
Rh Mcleod	100a	98.7a	24.2abc	9.9de
Rh Waseca 114B	100a	100a	0e	0e
Rs 16WC1	100a	100a	0e	0e
Rs 16WC1-2	100a	100a	0e	0e
Rs 16WC2-2	99.7a	99a	27.4ab	37.2b
Rs 16WC3-1	99.3a	98ab	19.5abcde	33.8bc
Rs 16WC3-2	100a	100a	0e	0e
Rs 6-222	100a	94.7bc	11.8bcde	25.7bcd
Rs soy 18-1	100a	100a	0e	0e
Mean	99.9	98.9	9.2	11.8
Sugar beet isolates				
Rs 86-42-4	100a	98.7a	21.9abcd	31.8bc
Rs 89-29-3A	97.7a	97.3ab	36.5a	7.8de
Rs 95-16-2	100a	99a	7.8cde	12.7cde
Rs 06-77-4	100a	100a	0e	2.9e
Rs 10-102-3	99.3a	92.3c	23.2abcd	63.3a
Rs 11-88-2	100a	100a	0e	0e
Rs 13-52-2	100a	100a	0e	0e
Rs 16-48-1	99a	100a	3.8de	3.8e
Rs 17-43-2	100a	100a	0e	0e
Rs 18-11	100a	94.3bc	5.1cde	44.7ab
Mean	99.6	98	9.8	16.7

[y] Disease severity index (DSI)= 0-100, 0= no disease, 100= all plants dead at 3 weeks after planting. Data represent the mean of three replications. Means followed by the same letters in the columns do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

[z] Relative fresh biomass determined as proportion of fresh biomass of inoculated plants to the biomass of control expressed in percentage. Data represent the mean of three replications. Means followed by the same letters in the columns do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

Table 2.4. Losses in plant population and yield of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2018 and 2019.[y]

Genotype	Plant population loss (%)			Yield loss (%)		
	2018	2019	Mean	2018	2019	Mean
Dawson	89.8a	67.2a	78.5a	59.7abc	80.8a	70.3ab
Lambert	89.8a	62.6a	76.2a	60.4abc	88.6a	74.5ab
M08-362045L	81.2a	70.2a	75.7a	72.3ab	80.7a	76.5ab
M09-251081	73.3a	54.2a	63.7a	62.8abc	84.8a	73.8ab
M09-269045	72.7a	73.2a	73a	78.4a	85.9a	82.1a
M12-357057	84.4a	72a	78.2a	74.5ab	83.1a	78.8ab
M12-366065	86.6a	68a	77.3a	70.1ab	69.2a	69.7ab
M12-439036	94.9a	65.4a	80.2a	74.9ab	81.7a	78.3ab
M12-440084	74.5a	58.3a	66.4a	56.6bc	76a	66.3ab
MCH13-108037	77.6a	69.4a	73.5a	46.7c	73.6a	60.1ab
MCH13-109062	88.3a	62.2a	75.3a	58.7abc	70.2a	64.4ab
MN0071	77.1a	68.5a	72.8a	62.1abc	82.6a	72.3ab
MN0083	84.6a	51.4a	68a	61.7abc	73.4a	67.6ab
MN0310CN	91.2a	84.1a	87.6a	70.6ab	88.4a	79.5ab
MN0404CN	88.7a	55.7a	72.2a	78.1a	70.5a	74.3ab
MN0606CN	94.5a	64.2a	79.4a	77.9a	86.7a	82.3a
MN0810CN	88.7a	66.1a	77.4a	74.8ab	68.3a	71.6ab
MN0902CN	92.8a	69.4a	81.1a	79.1a	89.7a	84.4a
Sheyenne	94a	57.8a	75.9a	74.9ab	83.9a	79.4ab
Traill	69.4a	54.7a	62a	43.4c	58.3a	50.8b
Mean[z]	84.7	64.7	74.72	66.9	77.2	72.85

[y] Data represent the mean of three replications. Means followed by the same letters in the columns do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

[z] Data represent the mean of 20 genotypes and three replications.

Table 2.5. Losses in plant population and yield of 40 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN during 2017- 2019.[z]

Genotypes	Plant population loss (%)			Yield loss (%)		
	2017	2018	2019	2017	2018	2019
AG1733		89.3a	63.1abc		87.9a	52.8abcd
IA1019		93.3a	43.3cdefg		94.5a	33.3cdefghijk
M07-209037	79.8a	84.7a	31.6defgh	78.6a	78.0a	31.7defghijk
M08-365100		94.6a	19.7gh		95.2a	15.7ghijkl
M09-278026	62.0a	93.8a	22.5efgh	51.3bc	100a	19.2fghijkl
M09-285032	64.7a	95.0a	22.5efgh	74.4ab	96.1a	9.2ijkl
M09-285149	48.0a	86.3a	10.7h	37.8cdefgh	78.6a	7.2kl
M09-343025	36.1a	91.9a	8.7h	31.8cdefghi	87.3a	2.4l
M10-236-2007		92.4a	27defgh		95a	20.3fghijkl
M10-238-2036		78.3a	23.6efgh		71.2a	11.2hijkl
M11-241015		88.4a	64abc		89.5a	37.6bcdefgh
M11-315-1026		94.2a	50.4bcd		96.4a	48.3abcde
M11-358032	31.5a	84.4a	31defgh	24.8defghi	88.1a	24.5efghijkl
M11-377069	20.5a	94.3a		15.8hi	92.6a	
M12-420037	37.0a	80.6a	47.8bcde	47.1cde	75.7a	68.8a
M12-437041	50.0a			52.4bc		
M12-437045		91.7a	21.6fgh		88.2a	8.6jkl
M12-453032	33.0a	89.0a		17.7hi	85.5a	
M12-454049	34.3a	83.6a		7.2i	71.4a	
MBC11-404-010	32.8a	88.0a		35.1cdefgh	91.5a	
MCH13-104100	58.6a	90.4a		29.5cdefghi	91.0a	
MCH13-110052		85.6a			81.8a	
MCH13-112071	45.2a	86.4a		24.3efghi	82.8a	
MN 1311	29.9a			20.2ghi		
MN1312CN	41.7a	95.0a	63.2abc	14.2hi	94.9a	35.1cdefghij
MN1410	40.9a	91.2a	64.6abc	43.6cdefg	95.6a	51abcde
MN1606R		92.7a			96.5a	
MN1613CN	35.6a	85.1a	40cdefg	20.8fghi	76.7a	18.1fghijkl
MN1701CN	22.0a	87.2a	46.9bcdef	20.6fghi	76.6a	35.5cdefghi
MN1806CN	47.0a	93.0a	63.3abc	17hi	97.6a	38.2bcdefg
MSC09-774089	37.6a	80.3a	58.1abc	32cdefghi	62.1a	39.4bcdefg
MSC09-777143	56.3a	87.0a	38.8cdefg	45.4cdef	80.1a	31.7defghijk
MSC10-559061	47.4a	88.2a	71ab	28.1cdefghi	87.9a	53.3abcd
MSC10-578025	30.6a	80.5a		33.7cdefgh	73.1a	
NS 1661NR2	49.1a		71.6ab	50bc		58.8abc
NS1916NR2	51.4a	76.7a		49.7bcd	73.9a	
NS 2031NR2	45.2a		59.1abc	35cdefgh		43.9abcdef

NS61882R2X			79a			64.1ab
P15T46R2		75.5a	57.6abc		60.0a	37.4cdefgh
Viking 1234R2N	54.1a	79.2a		44.1cdefg	78.7a	
Mean[z]	43.7	87.6	44.4	35.1	84.9	33.2

[z] Data represent the mean of three replications. Means followed by the same letters in the columns do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

Table 2.6. Losses in final plant stand and yield of 15 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated plots at Waseca, MN during 2017-2019.[z]

Genotypes	Plant population loss (%)				Yield loss (%)			
	2017	**2018**	**2019**	**Mean**	**2017**	**2018**	**2019**	**Mean**
M07-209037	79.8a	84.7a	31.6ab	65.3abc	78.6a	78.0a	31.7ab	62.8a
M09-278026	62.0a	93.8a	22.5ab	59.5abcde	51.3abc	100a	19.2ab	56.8abc
M09-285032	64.9a	95.0a	22.5ab	60.7abcde	74.4ab	96.1a	9.2b	59.9ab
M09-285149	48.0a	86.3a	10.7b	48.3de	37.8abc	78.6a	7.2b	41.2d
M09-343025	36.1a	91.9a	8.8b	45.6e	31.8abc	87.3a	2.4b	40.5d
M11-358032	31.5a	84.4a	31.0ab	49de	24.8bc	88.1a	24.5ab	45.8bcd
M12-420037	37.1a	80.6a	47.8ab	55.1abcde	47.1abc	75.7a	68.8a	63.9a
MN1312CN	41.7a	95.0a	63.2ab	66.7abc	14.2c	94.9a	35.1ab	48bcd
MN1410	40.9a	91.2a	64.6ab	65.5abc	43.6abc	95.6a	51.0ab	63.4a
MN1613CN	35.6a	85.1a	40.0ab	53.6bcde	20.8c	76.7a	18.1ab	38.5d
MN1701CN	22.0a	87.2a	46.9ab	52cde	20.7c	76.6a	35.5ab	44.3cd
MN1806CN	47.0a	93.0a	63.3ab	67.7ab	17.0c	97.6a	38.2ab	50.9abcd
MSC09-774089	37.6a	80.3a	58.1ab	58.7abcde	32.1abc	62.1a	39.4ab	44.5cd
MSC09-777143	56.3a	87.0a	38.8ab	60.7abcd	45.4abc	80.1a	31.7ab	52.4abcd
MSC10-559061	47.4a	88.2a	71.0a	68.8a	28.1abc	87.9a	53.3ab	56.4abc
Mean	45.8	88.2	41.4	58.5	37.8	85	31	51.3

[z] Data represent the mean of three replications. Means followed by the same letters in the columns do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

Table 2.7. Mean plant population and yield of soybean in inoculated and control plots at Waseca from 2017-2019 and Crookston from 2018-2019.[y]

Treatment	Waseca						Crookston			
	Plant population (Plants m^{-2})			Yield (t ha^{-1})			Plant population (Plants m^{-2})		Yield (t ha^{-1})	
	2017	2018	2019	2017	2018	2019	2018	2019	2018	2019
Control	44.9	53.1	50.8	3.3	3.7	3.9	43.4	60.2	5.4	2.9
Inoculated	24.8	6.6	28.8	2.1	0.6	2.7	6.6	21.0	1.8	0.7
p-value[z]	< 2.20 e-16	<2.20 e-16	<2.20 e-16	<2.20 e-16	<2.20 e-16	1.08 e-14	<2.20e-16	<2.20e-16	<2.20 e-16	<2.20 e-16

[y] Data represent means of 28, 36 and 28 soybean genotypes and three replications evaluated respectively in 2017, 2018 and 2019 at Waseca and 20 genotypes and three replications in Crookston.

[z] probability of control and inoculated treatments having identical distribution based on Wilcoxon rank-sum test at ($P<0.05$)

Table 2.8. Disease severity index (DSI) of 16 soybean genotypes inoculated with four *R. solani* isolates in two replicated growth chamber experiments.

Genotype	*R. solani* isolates[x]				Mean[y]
	Rs 16 WC 3-2	Rs 13-52-2	Rs 6-222	Rs 16-48-1	
Experiment 1					
Lambert	93.8a	79.2abcdef	70.8a	0.1c	61bcde
M07-209037	93.8a	91.7ab	79.2a	0.1c	66.2abcd
M09-278026	85.4a	91.7ab	70.8a	16.7b	66.2abcd
M09-285032	79.2a	72.9def	72.9a	16.7b	60.4cde
M09-343025	83.3a	89.6abc	37.5a	0.1c	52.6e
M11-358032	81.3a	75cdef	75a	8.4bc	59.9cde
MCH13-109062	91.7a	64.6f	68.8a	6.3bc	57.8de
MN0310CN	97.9a	77.1bcdef	70.8a	2.1bc	62bcd
MN0902CN	95.8a	72.9def	75a	0.1c	61bcde
MN1312CN	95.8a	87.5abcd	85.4a	0.1c	67.2abc
MN1410	85.4a	81.3abcde	75a	0.1c	60.4cde
MN1701CN	85.4a	70.8ef	81.3a	0.1c	59.4cde
MN1806CN	89.6a	87.5abcd	68.8a	0.1c	61.5bcd
MSC09-774089	95.8a	79.2abcdef	77.1a	8.4bc	65.1abcd
MSC09-777143	93.8a	93.8a	79.2a	10.5bc	69.3ab
MSC10-558061	97.9a	83.3abcde	72.9a	33.3a	71.9a
Mean[z]	90.4a	81.1b	72.5c	6.5d	62.2
Experiment 2					
Lambert	100a	60.4bcd	58.3a	35.4a	63.5cdef
M07-209037	83.3a	77.1ab	70.8a	72.9a	76ab
M09-278026	87.5a	79.2ab	70.8a	64.6a	75.5ab
M09-285032	89.6a	66.7bc	66.7a	43.8a	66.7bcde
M09-343025	100a	60.4bcd	45.8a	33.3a	59.9def
M11-358032	85.4a	58.3bcd	56.3a	33.3a	58.3ef
MCH13-109062	87.5a	47.9cd	77.1a	47.9a	65.1bcde
MN0310CN	100a	79.2ab	87.5a	54.2a	80.2a
MN0902CN	100a	64.6bc	72.9a	54.2a	72.9abc
MN1312CN	93.8a	77.1ab	66.7a	62.5a	75abc
MN1410	93.8a	68.8bc	81.3a	60.4a	76ab
MN1701CN	93.8a	66.7bc	50a	66.7a	69.3abcde
MN1806CN	100a	68.8bc	70.8a	60.4a	75abc
MSC09-774089	95.8a	72.9ab	56.3a	54.2a	69.8abcde
MSC09-777143	85.4a	93.8a	52.1a	54.2a	71.4abcd
MSC10-558061	89.6a	39.6d	50a	33.3a	53.1f
Mean[z]	92.8a	67.6b	64.6b	52.0c	69.2

[x] Data represent mean of Disease severity index (DSI) of four replications. DSI= 0-100, 0= no disease, 100= all plants dead at 3 weeks after planting.
[y] Data represent mean of four isolates and four replications. Means followed by the same letters in the columns within the same experiment do not differ significantly based on Fisher's least significant difference ($p < 0.05$)
[z] Data represent mean of 16 genotypes and four replications. Means followed by the same letters in the rows do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

Table 2.9. Relative fresh plant biomass(%) of 16 soybean genotypes inoculated with four *R. solani* isolates in two growth chamber experiments.

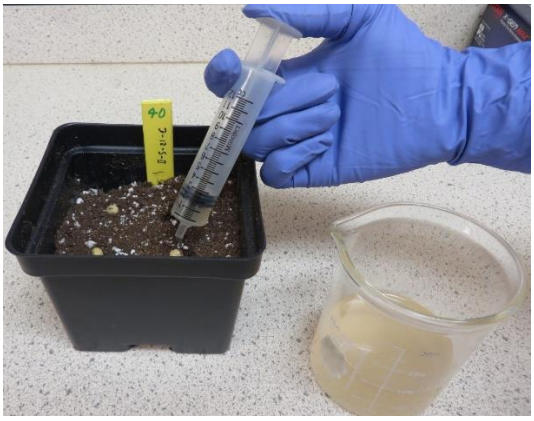
Genotype	*R. solani* isolates[x]				Mean[y]
	Rs 16 WC 3-2	Rs 13-52-2	Rs 6-222	Rs 16-48-1	
Experiment 1					
Lambert	18.8bcde	61.8ab	67.9abc	96.2a	61.2bcde
M07-209037	24.1bcde	23.3cd	83.2a	124.6a	63.8bcd
M09-278026	24.2bcde	30bcd	37.1c	87.6a	44.7ef
M09-285032	72a	64.6ab	85.3a	122.2a	86a
M09-343025	48.9abc	22.6cd	79.8a	104.7a	64bcd
M11-358032	52.7ab	58.4abc	67.5abc	92.3a	67.7bc
MCH13-109062	16.4bcde	85.4a	52.6abc	92.9a	61.8bcde
MN0310CN	2.6e	32.4bcd	50abc	99.7a	46.1ef
MN0902CN	3.6de	43.1bcd	30.3c	103.2a	45.1ef
MN1312CN	7.1de	36.6bcd	54.8abc	102.9a	50.4def
MN1410	29.4bcde	54.4abc	40.2bc	93.6a	54.4cdef
MN1701CN	41.2abcd	82.1a	76.2ab	92a	72.9ab
MN1806CN	32.8bcde	43.1bcd	67.4abc	99.1a	60.6bcdef
MSC09-774089	12.2cde	61.1ab	59.3abc	94.8a	56.8bcdef
MSC09-777143	13.3cde	17.3d	55.4abc	89.2a	43.8f
MSC10-558061	8.2de	50.3abcd	53.1abc	80.1a	47.9def
Mean[z]	25.5d	47.9c	60b	98.4a	57.1
Experiment 2					
Lambert	0c	84.9ab	65.3abcd	100.4a	62.6bc
M07-209037	31.9ab	53.1bcd	47.2bcde	84.9a	54.3bcd
M09-278026	19.3abc	84.8ab	67abcd	83.9a	63.7bc
M09-285032	5.2c	78.3abc	56abcde	100.7a	60bc
M09-343025	0c	79.6abc	76.6abc	93.1a	62.3bc
M11-358032	44.7a	93.7a	97.6a	100.3a	84.1a
MCH13-109062	17bc	48.5cd	24.5de	75.4a	41.3de
MN0310CN	0c	30.7d	20.9e	88.2a	34.9e
MN0902CN	0c	71abc	38.2cde	95.3a	51.1bcde
MN1312CN	0c	76.1abc	51.5bcde	95.8a	55.8bcd
MN1410	16.9bc	68.5abc	44.7bcde	67a	49.3cde
MN1701CN	11.1bc	48.9cd	75.9abc	77.5a	53.4bcd
MN1806CN	0c	79.4abc	57.5abcde	78.6a	53.8bcd
MSC09-774089	11.9bc	89.5a	65.7abcd	98.6a	66.4b
MSC09-777143	25.7abc	24.8d	87ab	87.7a	56.3bcd
MSC10-558061	11.6bc	94.1a	55.7abcde	95.3a	64.2bc

Mean[z]	12.2c	69.1b	58.2b	88.9a	57.1

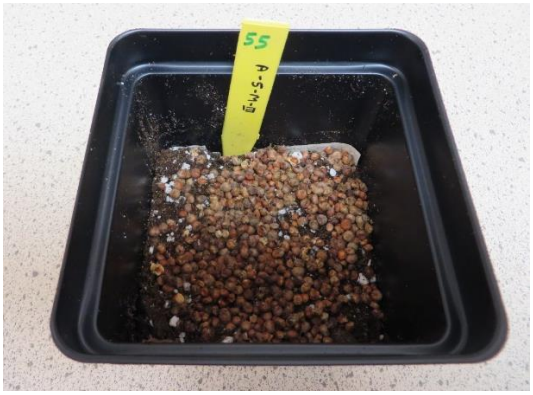
[x] Relative fresh biomass determined as proportion of fresh biomass of inoculated plants to the biomass of control expressed in percentage. Data represent the mean of four replications.

[y] Data represent the mean of four isolates and four replications. Means followed by the same letters in the columns within the same experiment do not differ significantly based on Fisher's least significant difference ($p < 0.05$)

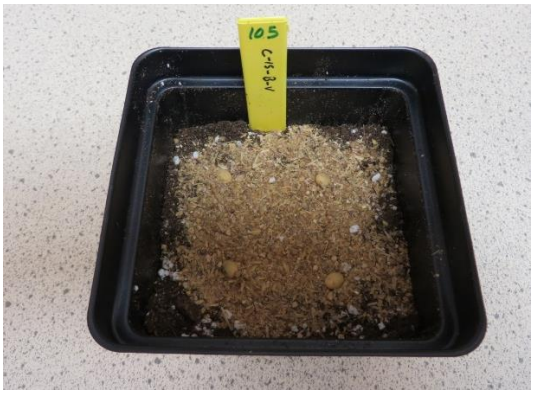
[z] Data represent mean of 16 genotypes and four replications. Means followed by the same letters in the rows do not differ significantly based on Fisher's least significant difference ($p < 0.05$).



(A)

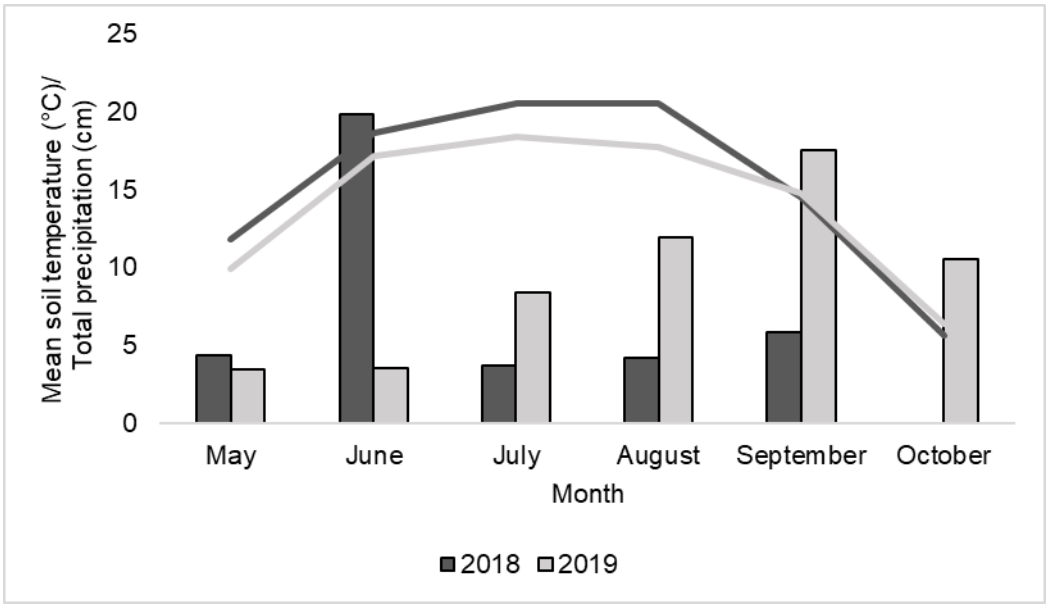


(B)

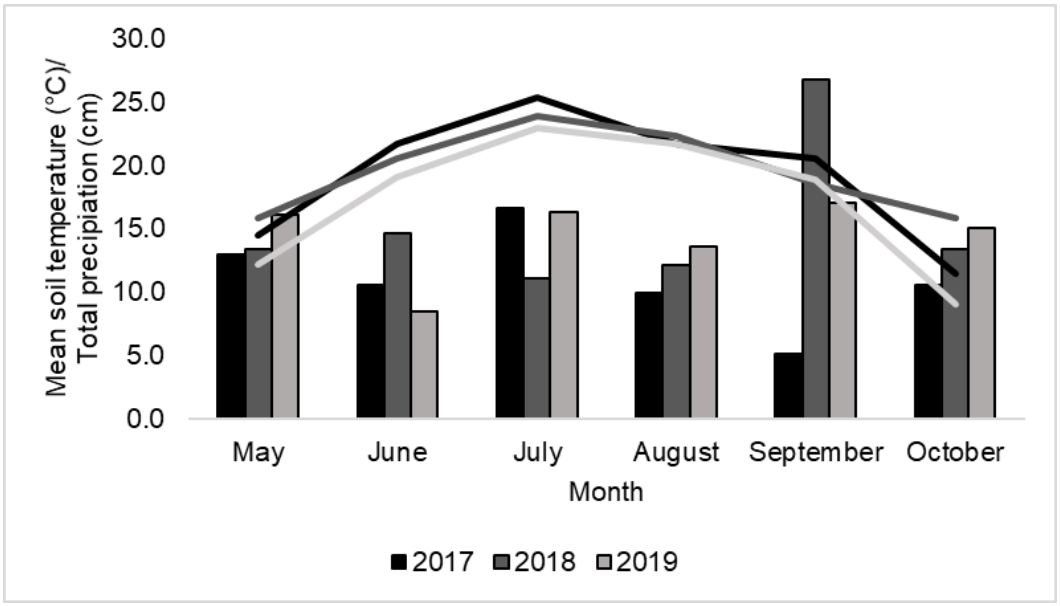


(C)

Fig.2.1. Inoculation of soybean at planting with 1 ml of mycelial suspension of *R. solani* per seed (A), a layer of 5cc of sorghum grain infested with the pathogen (B), and a layer of 5g pathogen infested ground barley inoculum (C).



(A)



(B)

Fig.2.2. Mean soil temperature at 10 cm depth and total precipitation from during 2018 and 2019 growing seasons from the Northwest Research and Outreach Center at Crookston, MN (A) and during 2017, 2018, and 2019 growing seasons from Southern Research and Outreach center at Waseca, MN (B). Lines represent the mean soil temperature and bars represent the total precipitation

Chapter 3: Sensitivity of *Rhizoctonia solani* AG 2-2 isolates from soybean and sugar beet to selected SDHI and QoI fungicides

Synopsis

Rhizoctonia solani causes root and stem diseases on soybean and sugar beet, and fungicides are commonly used to manage these diseases. Monitoring fungicide sensitivity in the pathogen population is needed to understand how the pathogen is responding to fungicides and to help develop fungicide resistant management strategies. Isolates (n=35) of *R. solani* AG 2-2 collected from soybean and sugar beet before (baseline) and after (non-baseline) exposure to selected fungicides were evaluated for their sensitivity to the SDHI fungicides sedaxane, penthiopyrad, and fluxapyroxad, and to the QoI fungicides pyraclostrobin, and azoxystrobin using a mycelial growth inhibition method. The fungicide concentration required to suppress the radial growth of mycelium 50% compared to the growth on non-amended plates (EC_{50}) was determined for each isolate. The average and range of EC_{50} values for sedaxane, penthiopyrad, fluxapyroxad and pyraclostrobin were 0.1 (0.03 to 0.3), 0.15 (0.05 to 0.27), 0.16 (0.08 to 0.3), and 0.24 (0.04 to 1.02) µg a.i./mL, respectively. The EC_{50} values of QOI fungicides did not differ significantly between the baseline and non-baseline isolates. All isolates were considered to be baseline for the SDHI fungicides. EC_{50} values for SDHI fungicides were significantly higher for isolates from soybean than sugar beet, but EC50 values did not differ between soybean and sugar beet isolates for the QoI fungicides. The mean EC_{50} values of azoxystrobin for 22 isolates ranged from 0.76 to 2.36 µg/m; however, the EC_{50} values could not be estimated for 13 isolates due < 50% inhibition in growth despite a visible decrease in mycelial density as fungicide concentration increased. In summary, the three SDHI fungicides and pyraclostrobin effectively suppressed *R. solani* in-vitro at low concentrations, and other methods are needed to assess the sensitivity of isolates to azoxystrobin.

Introduction

Soybean and sugar beet are among the major agricultural crops in Minnesota and North Dakota and are often cultivated in close rotation (USDA-ERS 2019a, Windels and Brantner 2005).

Seedling and root diseases caused by soilborne pathogens are significant threats to the profitable production of these crops. *Rhizoctonia solani* can cause seed decay and pre- and post-emergence damping-off in seedlings in both crops. Symptoms in adult plants include stem and root rot in soybean, and crown and root rot in sugar beet (Ajayi-Oyetunde and Bradley 2017; Neher and Gallian 2011). *Rhizoctonia solani* can reduce the yields of sugar beet by 40-100% and soybean up to 52% (Windels and Brantner 2005; Chanda and Brantner 2016; Chang et al. 2017). Engelkes and Windels (1996) suggested avoiding close rotation of sugar beet and bean crops due to their common susceptibility to *R. solani*. Based on a survey of sugar beet growers (n=313) in 2019, Rhizoctonia was the major soilborne pathogen affecting sugar beet production in MN and ND (Hakk et al. 2019). Rhizoctonia was also common in field samples received by the sugar beet pathology lab at the University of Minnesota during 2015 - 2018 (Brantner and Chanda 2017 and 2019b).

The isolate type of *R. solani* and environment can influence disease development. There are 14 anastomosis groups (AG) known of multinucleate *R. solani* (AG 1-13 and AG-BI) based on hyphal fusion reactions (Carling 1996; Carling et al. 1999; Ogoshi 1987). AG 2-2, AG 3, AG 4, and AG 5 can cause seedling and root diseases on both soybean and sugar beet (Windels and Brantner 2011; Windels and Nabben 1989; Nelson et al. 1996; Liu and Sinclair 1991). In addition, AG 1 and AG 2 can infect sugar beet (Windels and Nabben 1989), and AG 7 and AG 11 can infect soybean (Baird et al. 1996; Carling et al. 1994). Isolates of AG 2-2 intraspecific groups IIIB and IV are prevalent in Minnesota and North Dakota and they cause root rot of sugar beet and soybean (Brantner and Windels 2007, Nelson et al. 1996). This pathogen can survive in the soil for up to 3 years as sclerotia and infect a wide range of hosts, including rotation crops and weeds (Sumner and Bell 1994). *Rhizoctonia solani* can infect when soil temperatures are from 12 to 35 ˚C with soil moisture as low as 25%, but high soil moisture and warm temperatures (>20 ˚C) increase disease development (Bolton et al. 2010, Dorrance et al. 2003, Leach 1986, Neher and Gallian 2011).

There are no commercial soybean varieties known that are resistant to Rhizoctonia stem and root rot (Hartman et al. 2015, Ajayi-Oyetunde and Bradley 2017, Chang et al. 2017)). Although

partially resistant sugar beet varieties are available, resistance is not expressed until plants reach the 6-8 leaf stage, and fungicides are needed for disease management (Engelkes and Windels 1994; Liu et al. 2019; Chanda et al. 2019). Seed treatment against *R. solani* has been mandated by the sugar beet cooperatives for all commercial sugar beet cultivars since 2017 in MN and ND. The fungicides azoxystrobin, pyraclostrobin, sedaxane, fluxapyroxad, metconazole+toclofos-methyl are commonly used for seed treatments, in-furrow, and postemergence application against *R. solani* in soybeans and/or sugar beets (Khan et al. 2019, Crop Protection Network 2019, Pierson et al. 2018). Penthiopyrad is also used for seed treatment in sugar beet (Khan et al. 2019)

Azoxystrobin and pyraclostrobin belonging to Quinone outside inhibitors (QoI) class of fungicides are the active ingredients in Quadris® (azoxystrobin, 22.9%; Syngenta) and Headline® (pyraclostrobin, 23.6%; BASF). Azoxystrobin was first registered for the control of *R. solani* in 1997, and by 1999 it was labeled for more than 50 crops, including soybeans and sugar beets (US EPA, 1997). Pyraclostrobin was labeled for use in sugar beets in 2002 and soybeans in 2007 (US EPA 2002 and 2007). These fungicides inhibit ATP production by binding to the quinone oxidizing (Qo) site of cytochrome bc_1 complex I and restrict the transfer of electrons from cytochrome b to cytochrome c (Balba 2007; Gisi et al. 2002).

Penthiopyrad, sedaxane, and fluxapyroxad belonging to the succinate dehydrogenase inhibitor (SDHI) class of fungicides and are active ingredients in the broad-spectrum fungicides, Kabina ST (Penthiopyrad, 40%; Mitsui chemicals), Vibrance® (Sedaxane, 43.7%; Syngenta) and Systiva® (Fluxapyroxad, 33.3%; BASF), respectively. Sedaxane was labeled for use in soybeans in 2012 and for sugar beet in 2015 (US EPA 2012a, Jones 2015). Penthiopyrad and fluxapyroxad were registered for both crops in 2012, but they were not commercially available then (US EPA 2012b and 2012c). Kabina ST, Vibrance®, and Systiva® were first recommended as seed treatments to the growers of MN and ND in 2014, 2016, and 2017 respectively (Khan et al. 2016, and 2017). The SDHI group of fungicides inhibit the reduction of ubiquinone by binding to the active site of complex II (SDHB, C, or D), which halts ATP production (Avenot and Michailides 2010).

Extensive use of chemical fungicides with a single-site mode-of-action favors the development of resistance in fungal populations. According to the Fungicide Resistance Action Committee (FRAC), QoI and SDHI fungicides are respectively at high and medium to high risk of developing resistance, respectively (FRAC 2019). The single point mutations in the SDHB, SDHC, and SDHD regions have independently conferred resistance to various SDHI fungicides in multiple fungal species (Avenot and Michailides 2010). Similarly, the mutation in the *cytochrome b* gene (G143A) has introduced resistance to the QoI's (Gisi et al. 2002).

Fungicide resistance management tactics involve applying active ingredients with different modes-of-action to reduce selection for resistance and retain fungicide efficacy (FRAC 2019). Estimation of the baseline sensitivity to fungicides in fungal populations before fungicide exposure and evaluation of sensitivity to established fungicides after potential exposure will help to detect shifts in sensitivity over time. The sensitivity to fungicides is often determined in-vitro by estimating the effective concentration (dose) required to reduce radial mycelial growth by 50% (EC_{50}) in fungicide amended vs. non-amended media (Martin et al. 1984, Carling et al. 1990, (https://www.frac.info/resistance-overview)).

R. solani isolates from soybean had low EC_{50} values for two SDHI (penflufen, sedaxane), and low to moderate EC_{50} values for two DMI (ipconazole, and prothioconazole) fungicides (Ajayi-Oyetunde et al. 2017). *R. solani* isolates from sugar beet had low EC_{50} values for one SDHI (penthiopyrad), one DMI (prothioconazole), and one QoI (pyraclostrobin) fungicides and low to high EC_{50} values for two QoI fungicides (azoxystrobin, and trifloxystrobin). Chen et al. (2014) reported low EC_{50} values of fluxapyroxad for *R. solani* AG 1A isolates, causing sheath blight in rice. Although in-vivo resistance of *R. solani* AG 2-2 from soybean and sugar beet to these classes of fungicides has not been reported, the development of resistance to azoxystrobin in *R. solani* AG 1A, has raised concerns (Olaya et al. 2012). Evaluation of the levels of fungicide sensitivity in a recently collected *R. solani* populations from sugar beet and soybeans to the fungicides currently in use is

crucial for monitoring the shifts in fungicide sensitivity and developing strategies to retain fungicide longevity.

The objectives of this study were i) to determine the baseline sensitivity of *R. solani* AG 2-2 isolates from soybean and sugar beet in Minnesota and North Dakota to sedaxane, penthiopyrad, and fluxapyroxad, and ii) to determine the sensitivity (non-baseline) of *R. solani* AG 2-2 isolates to azoxystrobin and pyraclostrobin.

Materials and Methods

Source of *R. solani* AG 2-2 isolates and storage

Thirty-five isolates of *R. solani* AG 2-2 obtained from diseased sugar beet or soybean plants in Minnesota and North Dakota between 1986 and 2018 were selected for this study (Table 3.1). All Isolates were hyphal tipped and stored on colonized barley grain at -80 ˚C. Five isolates collected between 1986 and 1995 and never exposed to QoI fungicides were considered as baseline isolates for those fungicides. All 35 isolates were considered as baseline isolates for the three SDHI fungicides. All isolates were confirmed to be AG 2-2 based on PCR amplification with AG 2-2 specific primer sets developed by Carling et al. (2002b) (data not shown).

In vitro sensitivity assays

The range of fungicide concentrations and the type of agar medium used in the study were determined by conducting pilot experiments with four isolates: Rs 95-16-2, Rs 13-52-2, Rs 15-74, and Rs 16WC2-2 (data not shown). Technical grades (active ingredient (a.i.) >99%) of sedaxane (Syngenta AG, Basel, Switzerland), penthiopyrad (Sumitomo Chemical C., Ltd., Tokyo, Japan), fluxapyroxad (BASF SE, Ludwigshafen, Germany), and pyraclostrobin (BASF SE, Ludwigshafen, Germany) were dissolved in acetone (LR grade >99% purity) to make stock solutions of 10 mg a.i./mL These were serially diluted to prepare stock fungicide concentrations of 0.01, 0.1, and 1 mg a.i./mL. Potato Dextrose Agar (PDA) (Difco™, BD Diagnostic Systems, Sparks, MD) was amended with each dilution to prepare final fungicide concentrations of 0.01, 0.1, 1, and 10 µg a.i./mL and poured into 100 by 15mm plates. An equivalent amount of acetone was added to non-amended

control plates. Due to the low solubility of technical grade azoxystrobin in water, formulated Quadris® (Syngenta AG) fungicide was used to amend 0.5X clarified V8 agar prepared by adding 80 ml of clarified V8 juice and 16g of agar to 1L water (personal communication, J. Willbur, Michigan State University) at concentrations of 0, 0.01, 0.1, 1, 10, 100 and 200 µg a.i./mL. Salicylhydroxamic acid (SHAM) was dissolved in methanol and added to both fungicide-amended and non-amended media at 100 µg a.i./mL (Arabiat and Khan, 2016; Wise et al. 2008) in experiments with QoI fungicides. The EC_{50} values of azoxystrobin were estimated for 22 of the 35 isolates. The EC_{50} values could not be determined for the remaining 13 isolates, including three baseline isolates, because radial growth was inhibited <50% at the highest concentration (200 µg a.i./mL) of the fungicide in at least one experiment.

Mycelial plugs 5 mm diameter were taken from the edge of actively growing 3-day-old cultures using a sterile cork borer, placed inverted at the center of the plates, and then incubated in the dark at 25 ˚C in a growth chamber. After 48 hr of growth, the diameter of the fungal colonies was measured in two perpendicular axes, and the mean diameter was calculated for each colony. The diameter of the agar plug (i.e., 5mm) was subtracted from the mean diameter of the fungal colonies. Percentage relative growth was calculated for each plate as follows: [(mean diameter of the fungal colony on the amended plate / mean diameter of fungal colony on the non-amended plate) *100]. These values were fitted to a four-parameter log-logistic model to estimate the absolute EC_{50} values using the ‘drc’ package in R (Noel et al. 2018; Ritz et al. 2015).

Each experiment was arranged as a completely randomized design (CRD) with three replications, and the entire experiment was repeated once for each fungicide. In the two experiments with fluxapyroxad and azoxystrobin and the first experiment with sedaxane and penthiopyrad, all 35 isolates were tested concurrently in a single group. In other experiments, isolates were tested in two groups due to limited growth chamber space. Isolate Rs 15-74 was used as an internal check in all experiments. Only the experiments where the mean EC_{50} value of the internal check was within the 95% confidence interval were used for analysis (Wise et al. 2008).

Levene's test of homogeneity of variance was used to determine whether the two experiments could be pooled for analysis. Variances from the two experiments were homogenous for all fungicides except azoxystrobin. Thus, the two experiments for azoxystrobin were analyzed separately, and the two experiments for all other fungicides were combined for analysis. Shapiro-Wilk's test ($\alpha = 0.05$) was used to check the assumption of normality of residuals, and a non-parametric Wilcoxon rank-sum test was used to compare EC_{50} values of (i) baseline isolates with non-baseline isolates, and (ii) soybean isolates with sugar beet isolates. The correlation between the fungicides EC_{50} values was determined by Spearman's correlation analysis at a 5% significance level. The analyses were performed in R Studio version 3.6.1.

Results

The mean EC_{50} values for the SDHI fungicides sedaxane, penthiopyrad and fluxapyroxad were 0.1, 0.15 and 0.16 µg a.i./mL, with ranges of 0.03 to 0.3, 0.05 to 0.27, and 0.08 to 0.3 µg/mL, respectively (Table 3.2). The EC_{50} values of 74% of isolates were less than 0.1 µg/mL for sedaxane, while 88% and 91% of isolates had EC_{50} values between 0.1-1 µg/mL for penthiopyrad and fluxapyroxad, respectively (Fig.3.1A). The isolates with EC_{50} values below 1 µg/mL were considered extremely sensitive, those with EC_{50} values 1 to 10 µg/mL to be moderately sensitive, and those greater than 10 µg/mLs as tolerant to a fungicide (Martin et al. 1984). The EC_{50} values for all three SDHI isolates were significantly higher for soybean isolates compared to sugar beet isolates (Table 3.3).

The EC_{50} values for the QoI fungicide pyraclostrobin were slightly higher than they were for the SDHI fungicides with the mean and range of 0.24 µg/mL and 0.04 to 1.02 µg/mL, respectively (Table 3.2). Most isolates were extremely sensitive to pyraclostrobin with EC_{50} values between 0.1-1 µg/mL (Fig.3.1B). For pyraclostrobin, the EC_{50} values were similar for soybean and sugar beet isolates (Table 3.3).

Among the five fungicides, the QoI fungicide azoxystrobin had the highest EC_{50} values and the widest range for the isolates tested. The reduction in mycelial density was more pronounced

than the reduction in radial growth with increasing concentration of this fungicide. The mean EC_{50} values of azoxystrobin in the two experiments were 0.76 and 2.36 µg/mL, and they ranged from 0.13 to 3.01 and 0.15 to 18.33 µg/mL, respectively (Table 3.4). Most isolates were extremely sensitive to azoxystrobin with EC_{50} values between 0.1-1 µg/mL in both experiments. In experiment 1, six isolates had moderately sensitive EC_{50} values between 1-10 µg/mL, among which two isolates were tolerant with EC_{50} values between 10-50 µg/mL in experiment 2 (Fig.3.1B). The EC_{50} values of both QoI fungicides were similar for baseline and non-baseline isolates (Table 3.5).

A significant positive correlation (P = 0.0001, r = 0.53) between the sensitivity to penthiopyrad and fluxapyroxad was observed. There was also significant positive correlation between sensitivity to sedaxane and penthiopyrad (P = 0.0068, r = 0.32), and between sedaxane and fluxapyroxad (P = 0.0045, r = 0.34). Sensitivity to pyraclostrobin and azoxystrobin also had a highly significant positive correlation (P = 0.007, r = 0.4).

Discussion

Rhizoctonia solani AG 2-2 is a common pathogen infecting sugar beet and soybean in MN and ND (Brantner and Windels 2007). Given that both crops are hosts for *R. solani* AG 2-2, the inoculum can carry over from sugar beet to soybean and vice versa where both crops are grown. Consequently, the fungicides that are used for managing Rhizoctonia diseases in sugar beet can also influence disease development in soybean fields and vice versa. *Rhizoctonia solani* AG 2-2 can cause significant seedling loss in sugar beet and soybean fields. Sugar beet and soybean seedlings are very susceptible because of a lack of genetic resistance at this stage. Fungicide seed treatments offer protection during these early growth stages. To our knowledge, this is the first study to evaluate baseline sensitivity of *R. solani* AG 2-2 isolates to fluxapyroxad in vitro, to study fungicide sensitivity in isolates of *R. solani* collected after 1986 in MN, and to compare in vitro sensitivity of isolates recovered from soybean and sugar beet to pyraclostrobin and azoxystrobin.

During 2014 – 2017, three SDHI fungicides- sedaxane, penthiopyrad, and fluxapyroxad were labeled and mandated by sugar beet cooperatives as seed treatments for sugar beet. Thus,

they are widely and frequently used where sugar beet is grown. In addition, most of these fungicides are also widely used in soybean production. Two QoI fungicides used in this study have also been commonly used by sugar beet growers in this bi-state area as in-furrow or postemergence applications since 2000 (Hakk et al. 2019).

All *R. solani* isolates were extremely sensitive to the three SDHI fungicides evaluated, which is consistent with previous studies on these fungicides with isolates collected from other states and/or at earlier dates (Ajayi-Oyetunde et al. 2017; Arabiat and Khan, 2016; Chen et al. 2014; Goll et al. 2014). *Rhizoctonia solani* also showed a similar response to flutionil, carboxin, and penflufen belonging to the same fungicide chemistry (Li et al. 2014; Zhao et al. 2019; Kataria et al. 1991; Ajayi-Oyetunde et al. 2017). The high sensitivity of *R. solani* isolates to sedaxane, penthiopyrad, and fluxapyroxad could result from the fact that these fungicides recently became available for commercial use between 2014 to 2017, and isolates had no previous exposure to them. Several field and greenhouse studies also demonstrated high efficacy of these fungicides in controlling *Rhizoctonia* seedling diseases (Ajayi-Oyetunde et al. 2017; Arabiat and Khan 2016; Chen et al. 2014; Liu and Khan 2013). The EC_{50} values of sedaxane, penthiopyrad and fluxapyroxad reported in this study can be used as a baseline for *R. solani* AG 2-2 isolates from soybean and sugar beet in MN and ND and are useful for monitoring potential shifts the sensitivity to these SDHI fungicides over time.

Among all the fungicides tested, *R. solani* isolates were least sensitive to azoxystrobin. This study included three baseline isolates of *R. solani* that had never been exposed to azoxystrobin. Several other studies have also reported high, and wide-ranging EC_{50} values for azoxystrobin for isolates of *R. solani* (Arabiat and Khan 2016; LaMondia 2012). The high EC_{50} values for azoxystrobin are not surprising since the fungicide has been widely used to control the disease for two decades. However, high EC_{50} values for azoxystrobin has also been attributed to other common respiration pathways that were not inhibited by SHAM (Arabiat and Khan 2016; LaMondia 2012; Olaya, G., Syngenta AG, personal communication).

In our study, the variances in the reduction of radial growth with azoxystrobin over the two experiments were not homogenous. Noel et al. (2018) suggested at least two data points are required above and below the 50% relative growth for accurate estimation of absolute EC_{50} values. In our study, the radial growth of 13 isolates was not inhibited by 50% at any concentration of azoxystrobin used (up to 200 µg a.i./mL) in at least one experiment, so EC_{50} values of azoxystrobin were not estimated for those isolates. We observed that the mycelial density of the fungal colonies exposed to azoxystrobin decreased with increasing concentrations of this fungicide, but we had no effective method to measure the density.

Several researchers suggested that the mycelial inhibition method is inappropriate to assess the in vitro sensitivity of *R. solani* to azoxystrobin (N. Gambhir and S. Everhart, University of Nebraska; G. Olaya, Syngenta AG; and J. Wilbur, Michigan State University, personal communication). Wilbur et al. (2019) reported that the inhibition in mycelial density was more meaningful than the inhibition of the radial growth for the *R. solani* isolates from sugar beet in Michigan, and that the mycelial inhibition method might overestimate EC_{50} values of azoxystrobin for *R. solani*. Arabiat and Khan (2016) and LaMondia (2012) found that the recommended dose of azoxystrobin (672 ml ha^{-1}) was effective for controlling isolates with high EC_{50} values (as high as 597 µg a.i./mL) determined with the radial growth inhibition method. This suggests that all isolates tested in our study were sensitive to that recommended dose of azoxystrobin used in-vivo. Wise et al. (2008) suggested that a spore germination method provides a better estimation of in vitro sensitivity to QoI fungicides compared to the mycelial growth inhibition method for fungi that produce spores, such as *Ascochyta* species. However, *R. solani* isolates rarely produce spores, suggesting that new methods that measure mass or density of fungal colonies could be valuable to estimate in vitro sensitivity to azoxystrobin. In addition, in vitro findings should be validated by in vivo assays, which is supported by several studies (FRAC 2019; Arabiat and Khan 2016; Avenot and Michailides 2010; Gisi et al. 2002).

Resistance to one fungicide with single-site mode of action can confer resistance to other fungicides within the same FRAC class. For example, *Erysiphe graminis* f.sp. *tritici* and *Podosphaera fusca* have developed cross- resistance to multiple QoI fungicides (Chin et al. 2001, Fernández-Ortuño 2006). Similarly, Boscalid resistant isolates of *Alternaria alternata* expressed cross-resistance to carboxin (Avenot 2008). We observed a positive correlation (P = 0.007, r = 0.4) between EC_{50} values for pyraclostrobin and azoxystrobin, suggesting a moderate level of cross-resistance. Similar results were observed by Arabiat and Khan in 2016 in *R. solani* (P= 0.01, r=0.3) and Wise et al. (2008) in *Aschochyta rabei* (P=0.0001, r = 0.53). Weak to moderately positive correlation among three SDHI also indicate the potential for cross-resistance in the future.

R. solani isolates collected from sugar beets were more sensitive to the SDHI fungicides than the isolates from soybeans based on EC_{50} values. However, because of very low EC_{50} values for both soybean and sugar beet, the difference in EC_{50} between soybean and sugar beet isolates is not biologically meaningful and likely has no influence on the efficacy of the fungicides for managing *R. solani* in these crops.

In summary, *R. solani* isolates were sensitive to sedaxane, penthiopyrad, fluxapyroxad, and pyraclostrobin. While the EC_{50} values of azoxystrobin were higher than other fungicides, results were inconclusive, and we suggest a need to identify a different method to assess the in-vitro sensitivity of *R. solani* to azoxystrobin. Studies on fungicide sensitivity should be conducted routinely with a wider range of isolates and AGs to detect potential changes in the sensitivity to the active ingredients. Any shift in the fungicide sensitivity should be validated with in vivo studies to test the efficacy of the recommended dose. In addition, fungicides with higher EC_{50} values and single-site modes of action should be rotated with other fungicide chemistries to retain their efficacy for longer periods.

Table 3.1. Year and state of isolation, and host of origin for the isolates of *R. solani* AG 2-2 used in this study.

Isolate	Year	State	Origin
Baseline[a]			
Rs 86-42-4	1986	MN	Sugar beet
Rs 87-40-5	1987	ND	Sugar beet
Rs 89-29-3A	1989	MN	Sugar beet
Rs 93-29-6	1993	MN	Sugar beet
Rs 95-16-2	1995	ND	Sugar beet
Non-baseline[b]			
Rs 06-77-4	2006	MN	Sugar beet
Rs 10-102-3	2010	MN	Sugar beet
Rs 11-8-5	2011	MN	Sugar beet
Rs 11-88-2	2011	MN	Sugar beet
Rs 12-23-2	2012	MN	Sugar beet
Rs 12-109-1	2012	MN	Sugar beet
Rs 13-52-2	2013	MN	Sugar beet
Rs 13-118-1	2013	MN	Sugar beet
Rs 15-48	2015	MN	Sugar beet
Rs 15-74	2015	ND	Sugar beet
Rs 16-48-1	2016	MN	Sugar beet
Rs 16-82-3	2016	MN	Sugar beet
Rs 17-15-4	2017	MN	Sugar beet
Rs 17-43-2	2017	MN	Sugar beet
Rs 17-29-6	2017	MN	Sugar beet
Rs 18-14-4	2018	MN	Sugar beet
Rs 18-11	2018	MN	Sugar beet
Rh Waseca114B	2006	MN	Soybean
Rh McLeod	2009	MN	Soybean
Rs 16WC1	2016	MN	Soybean
Rs 16WC1-2	2016	MN	Soybean
Rs 16WC1-3	2016	MN	Soybean
Rs 16WC2-1	2016	MN	Soybean
Rs 16WC2-2	2016	MN	Soybean
Rs 16WC2-3	2016	MN	Soybean
Rs 16WC3-1	2016	MN	Soybean
Rs 16WC3-2	2016	MN	Soybean
Rs 16WC3-3	2016	MN	Soybean
Rs 6-222	2006	MN	Soybean
Rs soy 18-1	2018	MN	Soybean

[a] Isolates collected prior to labeled use of azoxystrobin or pyraclostrobin
[b] Isolates collected after labeled use of azoxystrobin or pyraclostrobin considered as non-baseline isolates for the QoI fungicides

Table 3.2. EC_{50} values of sedaxane, penthiopyrad, fluxapyroxad, and pyraclostrobin for 35 *R. solani* isolates tested in radial growth assays.[a]

Isolate	EC50 (µg/mL)			
	Sedaxane	**Penthiopyrad**	**Fluxapyroxad**	**Pyraclostrobin**
Rh McLeod	0.07	0.12	0.16	0.59
RhWaseca114B	0.12	0.27	0.25	0.83
Rs 06-77-4	0.08	0.14	0.17	0.07
Rs 10-102-3	0.26	0.1	0.1	0.23
Rs 11-8-5	0.07	0.21	0.15	0.14
Rs 11-88-2	0.08	0.13	0.13	0.19
Rs 12-109-1	0.08	0.13	0.14	0.33
Rs 12-23-2	0.06	0.16	0.18	0.07
Rs 13-118-1	0.07	0.12	0.16	0.04
Rs 13-52-2	0.07	0.1	0.16	0.07
Rs 15-48	0.1	0.14	0.24	0.81
Rs 15-74	0.06	0.05	0.08	0.16
Rs 16-48-1	0.08	0.1	0.14	0.06
Rs 16-82-3	0.09	0.14	0.22	0.1
Rs 17-15-4	0.06	0.09	0.12	0.06
Rs 17-29-6	0.07	0.1	0.12	0.12
Rs 17-43-2	0.09	0.13	0.17	0.16
Rs 18-11	0.3	0.16	0.14	1.02
Rs 18-14-4	0.15	0.13	0.15	0.39
Rs 6-222	0.3	0.27	0.3	0.53
Rs 86-42-4	0.08	0.13	0.11	0.08
Rs 87-40-5	0.03	0.13	0.1	0.19
Rs 89-29-3A	0.07	0.18	0.13	0.35
Rs 93-29-6	0.05	0.09	0.1	0.13
Rs 95-16-2	0.05	0.11	0.12	0.42
Rs soy 18-1	0.09	0.15	0.16	0.09
Rs16WC1	0.08	0.14	0.16	0.08
Rs16WC1-2	0.09	0.17	0.17	0.13
Rs16WC1-3	0.1	0.21	0.22	0.06
Rs16WC2-1	0.13	0.18	0.17	0.05
Rs16WC2-2	0.13	0.21	0.19	0.28
Rs16WC2-3	0.1	0.13	0.2	0.15
Rs16WC3-1	0.1	0.21	0.25	0.38
Rs16WC3-2	0.09	0.13	0.14	0.09
Rs16WC3-3	0.1	0.2	0.13	0.14

[a] Data are means of three replications and two experiments.

Table 3.3. EC_{50} values (µg/mL) of different SDHI and QoI fungicides for *R. solani* isolates collected from sugar beet and soybean.

Crop	Sedaxane		Penthiopyrad		Fluxapyroxad		Pyraclostrobin	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Soybean	0.11	0.07-0.3	0.18	0.12-0.27	0.19	0.13-0.3	0.26	0.05-0.83
Sugar beet	0.09	0.03-0.3	0.13	0.05-0.21	0.14	0.08-0.24	0.24	0.04-1.02
p-value[a]	5.113e-09***		2.133e-08***		1.135e-08***		0.266	

[a] probability of identical distribution between the EC_{50} values for *R. solani* isolates collected from sugar beets, and the EC_{50} values for *R. solani* isolates collected from soybeans based on Wilcoxon rank-sum test at ($p < 0.05$).

Table 3.4. EC_{50} values of azoxystrobin for 22 *R. solani* AG 2-2 isolates.

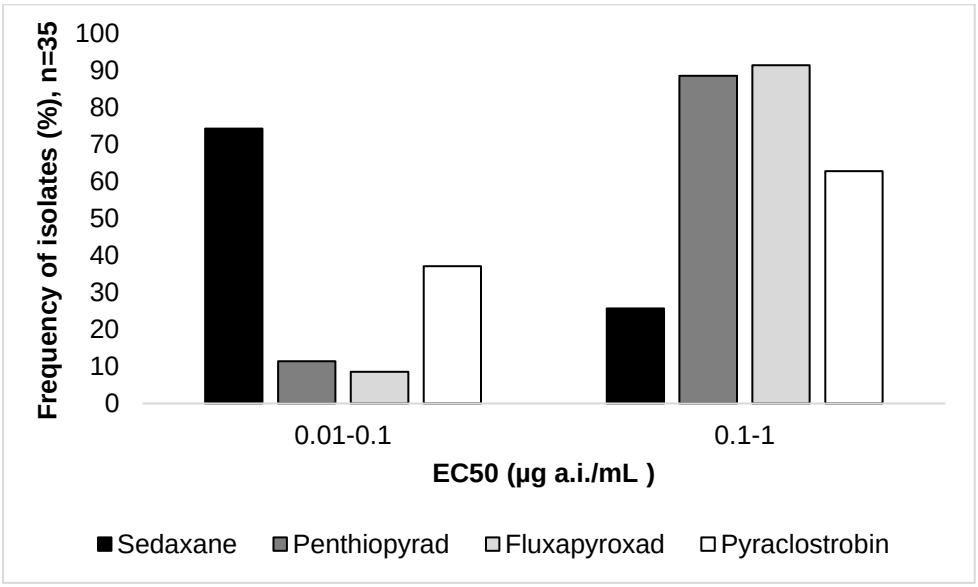
Isolate	EC50 (µg/mL)	
	Experiment 1	**Experiment 2**
Rs 06-77-4	0.36	0.17
Rs 11-8-5	0.38	0.3
Rs 12-23-2	0.24	0.41
Rs 13-118-1	0.13	0.21
Rs 13-52-2	0.35	0.15
Rs 15-74	1.04	0.58
Rs 16-48-1	0.5	0.29
Rs 16-82-3	0.86	0.23
Rs 17-15-4	3.01	18.33
Rs 17-29-6	0.18	0.42
Rs 17-43-2	1.41	1.09
Rs 93-29-6	1.1	0.2
Rs 95-16-2	0.43	0.2
Rs soy 18-1	0.38	0.42
Rs16WC1	0.19	0.45
Rs16WC1-2	0.36	0.21
Rs16WC2-1	0.39	0.58
Rs16WC2-2	1.14	6.51
Rs16WC2-3	0.86	2.94
Rs16WC3-1	2.64	16.83
Rs16WC3-2	0.43	0.86
Rs16WC3-3	0.37	0.47

[a] Data are means of three replications.

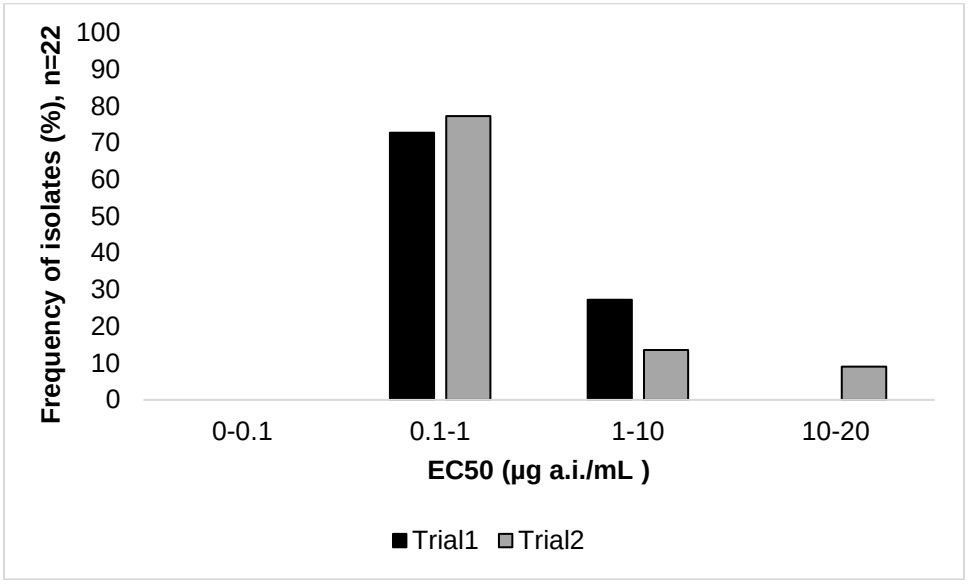
Table 3.5. EC_{50} (µg/mL) values of pyraclostrobin and azoxystrobin for baseline and non-baseline isolates of *R. solani.*

Isolates	Pyraclostrobin		Azoxystrobin			
			Experiment 1		Experiment 2	
	Mean	Range	Mean	Range	Mean	Range
Baseline	0.23	0.08-0.42	0.77	0.43-1.1	0.2	0.2-0.201
Non-baseline	0.25	0.04-1.02	0.76	0.13-3.01	2.6	0.15-18.33
p-value[a]	0.935		0.458		0.077	

[a] probability of identical distribution beetween EC_{50} values of baseline and non-baseline *R. solani* isolates having based on Wilcoxon rank-sum test ($p < 0.05$).



(A)



(B)

Fig.3.1. Frequency distribution of EC_{50} values for sedaxane, penthiopyrad, fluxapyroxad, and pyraclostrobin for 35 isolates (A) and azoxystrobin for 22 isolates of *R. solani AG 2-2*(B).

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Appendices

Appendix 1: Determination of Intra Specific Group (ISG) of the *R. solani* isolates within AG 2-2 using PCR and temperature assays

A. PCR based assay. The objective of this study was to confirm the intraspecific sub-group (ISG) of 35 *Rhizoctonia solani* AG 2-2 isolates (Chapter 3, Table 3.1) based on polymerase chain reaction (PCR) assays using AG 2-2 IIIB and IV specific primer sets.

Methods. DNA was extracted from 5-day-old cultures of 35 *R. solani* isolates grown in Difco™ Potato Dextrose Broth (PDB) using a Fast DNA prep kit (Qiagen, Hilden, Germany). PCR assays were conducted using the P22-IIIB and P22-IV primer set described by Carling et al. (2002b), and the MDB-034/035 primer set described by Bolton et al. (2010). Reactions and thermal cycling parameters were set up and conducted as described by the authors in an Eppendorf Mastercycler Pro thermal cycler (Eppendorf, Hauppauge, NY). Isolates Rs 6-222 from soybean and Rs 17-29-6 from sugar beet, previously identified using Sanger sequencing as AG 2-2 IIIB and AG 2-2 IV, respectively, were used as positive controls for the respective anastomosis groups. PCR products were visualized on 1.5% agarose gel stained with GelRed® staining dye.

Results and Discussion: The P22-IV primer set amplified 15 isolates out of 35 isolates, including the positive control AG 2-2 IV isolate Rs 17-29-6. The AG 2-2 IIIB control isolate Rs 6-222 was not amplified. The P22-IIIB primer set, however, amplified all 35 isolates, including AG 2-2 IIIB and AG 2-2 IV positive control isolates, suggesting a lack of specificity for this primer set.

Similar results were observed by Bolton et al. (2010) and Misawa and Kurose (2019). Bolton et al. (2010) developed the MDB-034/035 primers based on modifications of the P22-IIIB primer set and further optimized the PCR conditions to improve specificity to AG 2-2 IIIB isolates. The isolates used in this study had four types of PCR amplification results (Table 4.1): (i) no amplification with P22-IV and strong amplification with MDB-034/035 (ii) amplification with P22-IV and no amplification with MDB-034/035 (iii) amplification with P22-IV and strong amplification with MDB-034/035 and (iv) amplification with P22-IV and weak amplification (faint band) with MDB-

034/035. Categories (iii) and (iv) were designated as AG 2-2 hybrid subgroup (h.s.) IIIB + IV. Overall, 21 out of 35 isolates were grouped as AG 2-2 IIIB, 6 as AG 2-2 IV and 8 as AG 2-2 hybrid subgroup (h.s.) IIIB + IV.

B. Temperature growth study. Based on the description by Sneh et al. (1991), AG 2-2 IIIB isolates grow at 35 °C and AG 2-2 IV isolates do not. confirm the intraspecific sub-group (ISG) of 35 *Rhizoctonia solani* AG 2-2 isolates (Chapter 3, Table 3.1) based on their growth at 35 °C.

Methods: Thirty-five AG 2-2 isolates (Table 3.1) were evaluated for their ability to grow at 35 °C to confirm their intraspecific groups using a method described by Brantner and Windels (2007). Two additional isolates Rs 86-72-7 and Rs 87-36-4, previously verified as AG 2-2 IV and AG 2-2 IIIB based on their growth at 35 ˚C by Brantner and Windels (2007), were used as positive controls for the respective AG ISGs. Mycelial plugs (5 mm diameter) were taken from the edge of 3-day-old colonies of each isolate and transferred to 100 by 15 mm plates containing 20 mL Potato Dextrose Agar (PDA) (Difco™, BD Diagnostic Systems, Sparks, MD). Each isolate was transferred to eight plates, and four plates were incubated at 25 ˚C and four at 35 ˚C. A baseline was drawn at the edge of the fungal colony after 24 hr of incubation, and radial growth was measured from the baseline after 48 hr and 72 hr of incubation. After 72 hr of incubation, most of the isolates at 25 °C had reached the edge of the plates, so only the measurements taken at 48 hr were used for the evaluation. Percentage relative growth was estimated for colonies on individual plates of each isolate at 35 °C in comparison to the average growth at 25 °C as [(mean radial growth of fungal colony at 35 °C / mean radial growth of the fungal colony at 25 °C) *100]. Isolates with no growth at 35 °C were considered as AG 2-2 IV, and those with growth were considered as AG 2-2 IIIB.

Results and Discussion: Ten isolates, including AG 2-2 IV control isolate Rs 86-72-7, had no growth at 35 °C, so they were designated as AG 2-2 IV ISG based on the temperature growth assay (Table 4.1). Twenty-seven isolates, including the Ag 2-2 IIIB control isolate Rs 87-36-4, were designated as AG 2-2 IIIB.

Determination of ISG within AG 2-2 isolates based on temperature and PCR assays was not in complete agreement. Isolate Rs 13-52-2 with no growth at 35 °C, had no amplification with P22-IV and strong amplification with MDB-034/035, while three isolates Rs 89-29-3A, Rs 93-29-6 and Rs 15-74 (h.s. IIIB+IV based on PCR) had no growth at 35 °C and had weak amplification with MDB-034/035 along with strong amplification with P22-IV. Isolate Rs 18-14-4 had strong amplification with P22-IV and was able to grow (15.6%) at 35 °C. Isolates 17-29-6, Rs 10-102-3 and Rs 11-88-2 (h.s. IIIB+IV based on PCR) had strong amplification with P22-IV and weak amplification with MDB-034/035 and were able to grow (5.08 – 11.82%) at 35 °C. Isolates Rs 15-48 and Rs 17-15-4 (h.s. IIIB+IV based on PCR) had strong amplification with both P22-IV and MDB-034/035 primer sets and had 55.14% and 74.31% growth at 35 °C, respectively.

Intersubgroup mating has been reported in *R. solani* AG 2-1, AG 4, and AG 2-2 (Misawa et al. 2017; Misawa and Kurose 2019). According to Misawa and Kurose (2019), the hyphal anastomosis of AG 2-2 IIIB and IV subgroups could establish heterogenic mycelia with nuclei of both IIIB and IV strains. We speculate that the proportion of nuclei from IIIB and IV subgroup can ultimately influence the relative ability of these isolates to grow at 35 °C. In the hybrid subspecies (h.s) IIIB + IV groups observed by Misawa and Kurose (2019), PCR amplification with P22-IV primer set was faint and IIIB primer set was strong and these hybrid groups were morphologically similar to IIIB cultures indicating the presence of higher proportion of nuclei from 2-2 IIIB. However, in our study, IIIB PCR products varied in intensity indicating some AG 2-2 ISG IV isolates acquired IIIB type nuclei during natural hyphal anastomosis.

Table. 4.1. Amplification of 35 *R. solani* isolates using AG 2-2 IIIB and IV specific primer sets and their mean growth at 35 °C.

Isolate	PCR Amplification assay			Temperature assay	
	P22 IV[a] (AG 2-2 IV)	MDB-034/035[b] (AG 2-2 IIIB)	AG	Mean growth (%)[c]	AG
Rs 86-72-7[d]	Yes	No	IV	0	IV
Rs 86-42-4	Yes	No	IV	0	IV
Rs 87-40-5	Yes	No	IV	0	IV
Rs 89-29-3A	Yes	Faint	h.s	0	IV
Rs 93-29-6	Yes	Faint	h.s	0	IV
Rs 95-16-2	Yes	No	IV	0	IV
Rs 12-109-1	Yes	No	IV	0	IV
Rs 13-52-2	No	Yes	IIIB	0	IV
Rs 15-74	Yes	Faint	h.s	0	IV
Rs 18-11	Yes	No	IV	0	IV
Rs 17-29-6[e]	Yes	Faint	h.s	5.1	IIIB
Rs 10-102-3	Yes	Faint	h.s	7.3	IIIB
Rs 11-88-2	Yes	Faint	h.s	11.8	IIIB
Rs 11-8-5	No	Yes	IIIB	14.2	IIIB
Rs 18-14-4	Yes	No	IV	15.6	IIIB
Rs 12-23-2	No	Yes	IIIB	23	IIIB
Rs 16-48-1	No	Yes	IIIB	41.7	IIIB
Rh Waseca 114B	No	Yes	IIIB	45.7	IIIB
Rs 13-118-1	No	Yes	IIIB	52.4	IIIB
Rs soy 18-1	No	Yes	IIIB	53.7	IIIB
Rs 15-48	Yes	Yes	h.s	55.1	IIIB
Rs 06-77-4	No	Yes	IIIB	60	IIIB
Rs 16WC3-2	No	Yes	IIIB	63.9	IIIB
Rs16WC1-2	No	Yes	IIIB	68.2	IIIB
Rs16WC2-3	No	Yes	IIIB	71.4	IIIB
Rs 17-15-4	Yes	Yes	h.s	74.3	IIIB
Rs 6-222[f]	No	Yes	IIIB	75	IIIB
Rs 16-82-3	No	Yes	IIIB	80.2	IIIB
Rs16WC1-3	No	Yes	IIIB	81.4	IIIB
Rs 87-36-4[g]	No	Yes	IIIB	83.3	IIIB
Rs16WC1	No	Yes	IIIB	87.1	IIIB
Rs 17-43-2	No	Yes	IIIB	88.5	IIIB
Rh Mcleod	No	Yes	IIIB	90.1	IIIB
Rs16WC2-1	No	Yes	IIIB	91.8	IIIB
Rs16WC3-3	No	Yes	IIIB	96.1	IIIB

Rs16WC2-2	No	Yes	IIIB	99	IIIB
Rs 16WC3-1	No	Yes	IIIB	109.4	IIIB

[a] PCR primers and conditions based on Carling et al. (2002b)

[b] PCR primers and conditions based on Bolton et al. (2010)

[c] Based on method from Brantner and Windels (2007), mean growth at 35 °C expressed in percentage relative to growth at 25 °C

[d] AG 2-2 IV 35 ˚C positive control

[e] AG 2-2 IV PCR positive control

[f] AG 2-2 IIIB PCR positive control

[g] AG 2-2 IIIB 35 ˚C positive control

Appendix 2: Determination of interaction of *R. solani* AG 5 and AG 2-2 isolates on sugar beets

In 2017, three *Rhizoctonia* like fungi were isolated from adult sugar beet roots from northwest MN (Polk County) with internal dry rot symptoms by the sugar beet pathology lab at the University of Minnesota. Two of the isolates (Rs 17-29-1 and Rs 17-29-6) were identified as AG 2-2 IV and one (Rs 17-29-3) as AG 5 through Sanger sequencing. AG 2-2 is the primary anastomosis group causing seedling and root diseases of sugar beet in MN (Brantner and Windels 2007). AG 5 causes stem canker and black scurf of potato (Bandy et al. 1984) and has low to moderate virulence on sugar beet seedlings (Windels and Nabben 1989). AG 5 is often found associated with sugar beet seedlings, but its interaction with other *R. solani* AGs and potential impacts on disease development has not been explored. The objectives of this study were to determine the interaction of AG 5 with AG 2-2 IIIB and 2-2 IV in causing seedling damping-off and adult root rot.

Sugar beet seedling assay in the growth chamber. One isolate of *R. solani* belonging to each intraspecific group (ISG) of AG 2-2 IIIB (Rs 385), AG 2-2 IV (Rs 17-29-6), and AG 5 (Rs 17-29-3), recovered from infected sugar beet samples, were selected for the study. Seedling assays were conducted in three separate experiments, each with six treatments and four replications arranged in a completely randomized block design. The six treatments included ground barley inoculums of three individual isolates- AG 2-2 IIIB, AG 2-2 IV, and AG 5; 1:1 mixture (by weight) of AG 5 and AG 2-2 IIIB; 1:1 mixture (by weight) of AG 5 and AG 2-2 IV; and a non-infested control. The infested ground barley inoculum was mixed into Sungro Sunshine #8 potting mix at ratios of 1:500 (1g inoculum per 500 cm^3 soil). Non-infested ground barley was used for the control. Rhizoctonia susceptible ‘Crystal 101’ sugar beet variety was planted in 10 cm square pots and kept in a growth chamber at 25°C and 14 hr light. After 3 weeks, sugar beet seedlings were rated for root rot on a 0 - 3 scale modified from Fink and Buchholtz (1954), and a disease severity index was calculated as, DSI= [Σ(disease score category x number of plants in the disease score category)] / [(total number of plants) × (maximum disease score)] × 100.

Adult sugar beet assay. An adult sugar beet study was also conducted in the field with susceptible sugar beet varieties ‘Crystal 101’ in 2018 and ‘Crystal 572 RR’ in 2019. The study followed a completely randomized block design with the same six treatments used in the seedling assay and two replications in both years. Sugar beet seeds were sown in two 7.62 m long rows spaced 0.3 m apart in mid-May. After 2 months in mid-July, 12 rows with approximately 50 adult plants were arbitrarily selected. The soil next to each plant was removed to a depth of 2.5 cm, 2.5 cc one of the six ground barley inoculum treatments was added and covered with soil. The plants were manually irrigated on the day of inoculation and 1 week after inoculation with 8 gallons of water per row. Three weeks after inoculation, 10 plants were randomly dug out from each row in the first year and 15 plants in the second year. Then the beets were rated for Rhizoctonia root and crown rot on a 0-7 scale where, 0=no symptoms, 1= scurfy, superficial, inactive lesions in <2.5% area, 2-6= active black lesions extending up to 5, 25, 50, 75, and 100% root area and 7= completely dead beet (Scholten et al. 2001). Adult root rot scores were converted to the midpoint of disease severity range for analysis. The experiments in each study were combined for the analysis. Due to the lack of normality and heteroscedasticity, data from both seedling and adult assays were analyzed using a non-parametric Kruskal-Wallis test followed by Dunn’s test for multiple comparisons between the treatments in R (version 3.6.2).

Results and discussion. Both AG 2-2 isolates were highly virulent on sugar beet seedlings, the mean DSI of sugar beet plants inoculated with was 91.3 with the IIIB isolate and 90.3 for the IV isolate (Fig 4.1). The IIIB isolate was more aggressive on adult beets than the IV isolate. The mean DSI of sugar beet plants inoculated with AG 2-2 IIIB was 79.7, and with the AG 2-2 IV isolate was 56.8 (Fig 4.2). This is similar to the results reported by Bolton et al. (2010), and Panella (2005) and Windels and Brantner (2011). However, Windels and Brantner (2011) also reported some AG 2-2 IV isolates with higher virulence than some AG 2-2 IIIB isolates. AG 5 isolate was the least aggressive on sugar beet seedlings (mean DSI= 42.7), but no symptoms were observed on adult beets inoculated with AG 5 isolate. Windels and Nabben (1989) also reported AG 5 to be slightly to moderately pathogenic in sugar beet seedlings and non-pathogenic to adult beets.

Several studies reported a reduction in disease severity caused by *R. solani* in beans, kidney beans, poinsettia, cabbage, Arabidopsis and soybean in the presence of binucleate *Rhizoctonia* species (BNR) (Wen et al. 2005, Xue et al. 1998, Tohid and Taheri 2014, Ross et al. 1998, Hwang and Benson 2002, Sharon et al. 2011, Poromarto et al. 1998). We also observed significant reductions in the disease severity of sugar beet plants inoculated with AG 2-2 isolates in both seedling and adult plants in the presence of AG 5. Mixture of AG 2-2IIIB and AG 5 (mean DSI= 78.4) was similar to AG 2-2 IIIB alone in infecting sugar beets seedlings (Fig 4.2), but the mixture was less aggressive than AG 2-2 IIIB isolate on adult sugar beets (mean DSI= 27) (Fig 4.1). However, the virulence of AG 2-2 IV and AG5 mixture was significantly lower than AG 2-2 IV on both seedlings and adult beets (mean DSI of seedlings= 56.8, adult= 2.7) (Figs. 4.1 and 4.2). The disease severity of adult sugar beets inoculated with AG 2-2IV and AG 5 mixture was not significantly different from the non-inoculated plants (Fig 4.2). This indicates that the interaction of two intraspecific groups, AG 5 and AG 2-2, might play a role in reducing the disease severity. The decrease in disease severity could be a result of competition (Cook and Baker 1983) for space and nutrition in the soil, or the activation of plants defenses (Sharon et al. 2011, Tohid and Taheri 2014).

In summary, *R. solani* AG 5 was moderately pathogenic to sugar beets seedlings, and the symptoms were similar to those caused by AG 2-2. The virulence of both AG 2-2 IIIB and IV isolates on adult plants was significantly reduced in the presence of AG 5, while virulence on seedlings was only reduced for AG 2-2 IV. The reduction in disease severity could result from the interaction of AG 5 with the AG 2-2 isolates. The findings from this study should be confirmed with a larger set of isolates, and the mechanism of the interactions should be investigated.

Figures

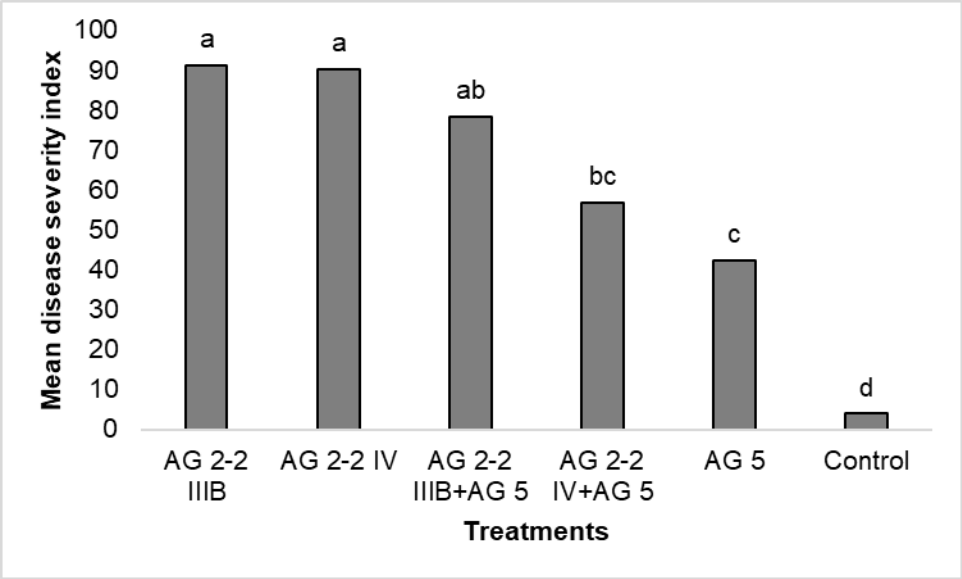

Fig. 4.1. Mean disease severity index resulting from inoculation of sugar beet seedlings with different intraspecific groups of *R. solani* and their combinations for three experiments. Ranks of treatments with the same letters do not differ significantly based on Dunn's test ($p < 0.05$).

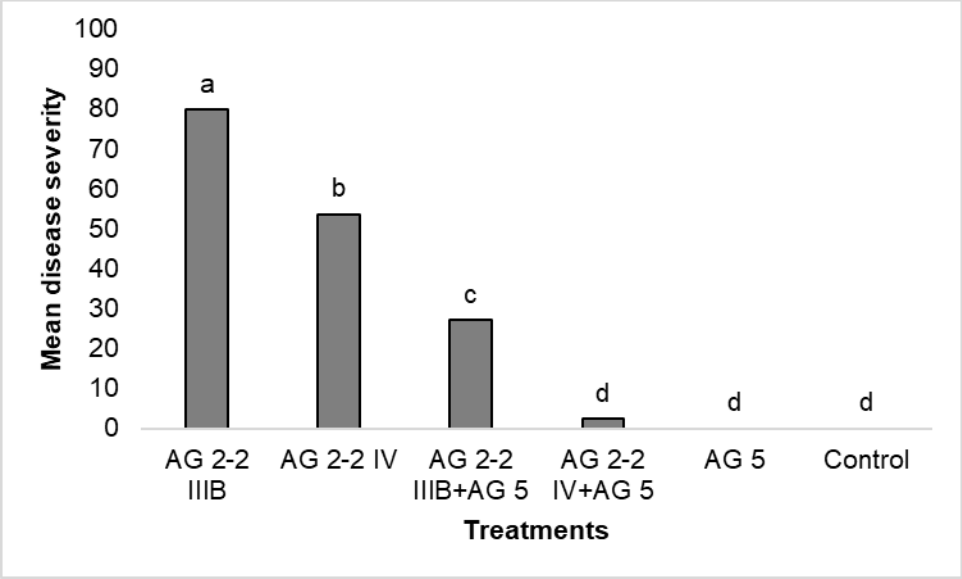

Fig. 4.2. Mean disease severity index resulting from inoculation of adult sugar beets with different intraspecific groups of *R. solani* and their combinations over two years. Treatments with the same letters do not differ significantly based on Dunn's test ($p < 0.05$).

Appendix 3: Statistical analysis of response of soybean genotypes to *R. solani* isolates

Table 4.2. Combined analysis of variance for disease severity index (DSI) of soybean plants inoculated with *R. solani* in two trials with three inoculation methods.

Source of variation	Df	Mean square	P
Inoculation method	2	1787.11	0.001
Block	2	138.49	0.43
Experiment	2	77.53	0.55
Experiment x Inoculation method	2	372.18	0.2
Residuals	29	216.75	

Table 4.3. Combined analysis of variance for disease severity index (DSI) of three soybean genotypes inoculated in two experiments of growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	189	0.03
Block (Exp)	6	245	0.04
Soybean genotype	2	582	0.001
Source of isolate	1	34460	< 2.2e-16
Isolate (source)	17	2556	< 2.2e-16
Source x genotype	2	235	0.11
Isolate (source) x genotype	34	132	0.13
Experiment x source	1	543	0.08
Experiment x isolate (source)	17	1401	1.28E-08
Experiment x genotype	2	240	0.02
Experiment x genotype x source	2	101	0.07
Experiment x genotype x isolate(source)	34	168	0.92
Residuals	336	149	

Table 4.4. Analysis of variance for disease severity index (DSI) of three soybean genotypes inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	50	0.79
Soybean genotype	2	289	0.14
Source of isolate	1	21825.1	<2e-16
Isolate (source)	17	1727.2	<2e-16
Source x genotype	2	318.9	0.11
Isolate (source) x genotype	34	99	0.9
Residuals	168	144.4	

Table 4.5. Analysis of variance for disease severity index (DSI) of three soybean genotypes inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	440.4	0.04
Soybean genotype	2	532.6	0.03
Source of isolate	1	13177.1	<2e-16
Isolate (source)	17	2229.8	<2e-16
Source x genotype	2	17.8	0.89
Isolate (source) x genotype	34	201.4	0.13
Residuals	168	153.3	

Table 4.6. Combined analysis of variance for relative fresh biomass of three soybean genotypes inoculated in two experiments of growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	2522	0.03
Block (Exp)	6	1233	0.04
Soybean genotype	2	3922	0.0008
Source of isolate	1	168642	$< 2.2e^{-16}$
Isolate (source)	17	11850	$< 2.2e^{-16}$
Source x genotype	2	1197	0.10
Isolate (source) x genotype	34	700	0.13
Experiment x source	1	1681	0.08
Experiment x isolate (source)	17	2457	$1.28e^{-08}$
Experiment x genotype	2	2044	0.02
Experiment x genotype x crop	2	1456	0.07
Experiment x genotype x isolate(source)	34	359	0.92
Residuals	336	539	

Table 4.7. Analysis of variance for relative fresh biomass of three soybean genotypes inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	1965	0.013
Soybean genotype	2	2443	0.01
Source of isolate	1	101999	<2e-16
Isolate (source)	17	8401	<2e-16
Source x genotype	2	2607	0.009
Isolate (source) x genotype	34	494	0.59
Residuals	168	533	

Table 4.8. Analysis of variance for relative fresh biomass of three soybean genotypes inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	501	0.43
Soybean genotype	2	3523	0.002
Source of isolate	1	68324	<2e-16
Isolate (source)	17	5906	<2e-16
Source x genotype	2	45	0.92
Isolate (source) x genotype	34	565	0.9
Residuals	168	544	

Table 4.9. Combined analysis of variance for disease severity index (DSI) of sugar beet cultivar 'Crystal 101' inoculated in two experiments of growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	55.68	0.0003
Block(experiment)	6	7	0.11
Source of isolate	1	10.4	0.1
Isolate(source)	17	12.58	0.0001
Experiment x source	1	2.096	0.47
Experiment x isolate(source)	17	10.42	0.001
Residuals	108	3.96	

Table 4.10. Analysis of variance for disease severity index (DSI) of sugar beet cultivar 'Crystal 101' inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	1.21	0.39
Source of isolate	1	1.58	0.26
Isolate (source)	17	1.35	0.35
Residuals	54	1.19	

Table 4.11. Analysis of variance for disease severity index (DSI) of sugar beet cultivar 'Crystal 101' inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	12.79	0.14
Source of isolate	1	10.92	0.2
Isolate (source)	17	21.65	0.0006
Residuals	54	6.73	

Table 4.12. Combined analysis of variance for relative fresh biomass of sugar beet cultivar 'Crystal 101' inoculated in two experiments of growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	900.41	0.04
Block(experiment)	6	78.32	0.9
Source of isolate	1	285.1	0.25
Isolate(source)	17	1629.11	$3.857e^{-12}$
Experiment x source	1	169.53	0.37
Experiment x isolate(source)	17	505.87	0.004
Residuals	108	212.01	

Table 4.13. Analysis of variance for relative fresh biomass of sugar beet cultivar 'Crystal 101' inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	47.27	0.86
Source of isolate	1	7.46	0.84
Isolate (source)	17	606.43	0.0006
Residuals	54	189.65	

Table 4.14. Analysis of variance for relative fresh biomass of sugar beet cultivar 'Crystal 101' inoculated in growth chamber studies with 19 *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	109.38	0.7
Source of isolate	1	447.13	0.17
Isolate (source)	17	1528.56	5.43E-08
Residuals	54	234.36	

Table 4.15. Combined analysis of variance for losses in plant population of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2018 and 2019.

Source of variation	Df	Mean square	P
Year	1	11975.5	$2.86e^{-11}$
Block(Year)	4	953.9	0.002
Soybean genotype	19	225.6	0.33
Year x Soybean genotype	19	154.6	0.72
Residual	76	197.7	

Table 4.16. Analysis of variance for losses in plant population of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2018.

Source of variation	Df	Mean square	P
Block	2	1159.4	0.001436
Soybean genotype	19	194.3	0.233824
Residual	38	148.35	

Table 4.17. Analysis of variance for losses in plant population of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2019.

Source of variation	Df	Mean square	P
Block	2	748.49	0.06013
Soybean genotype	19	185.96	0.74276
Residual	38	247.04	

Table 4.18. Combined analysis of variance for losses in yield of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2018 and 2019.

Source of variation	Df	Mean square	P
Year	1	4278.6	$8.27e^{-06}$
Block(Year)	4	1853.5	$1.65e^{-06}$
Soybean genotype	19	406.1	0.01
Year x Soybean genotype	19	144	0.73
Residual	76	187	

Table 4.19. Analysis of variance for losses in yield of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2018.

Source of variation	Df	Mean square	P
Block	2	1858.49	8.83E-05
Soybean genotype	19	335.37	0.02
Residual	38	154.16	

Table 4.20. Analysis of variance for losses in yield of 20 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Crookston, MN in 2019.

Source of variation	Df	Mean square	P
Block	2	1848.46	0.000946
Soybean genotype	19	214.81	0.505014
Residual	38	219.78	

Table 4.21. Analysis of variance for losses in plant population of 28 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2017.

Source of variation	Df	Mean square	P
Block(Year)	2	1383.36	0.03
Soybean genotype	27	533.34	0.13
Residual	54	376.57	

Table 4.22. Analysis of variance for losses in yield of 28 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2017.

Source of variation	Df	Mean square	P
Block(Year)	2	16.03	0.93
Soybean genotype	27	891.82	1.50E-05***
Residual	54	234.87	

Table 4.23. Analysis of variance for losses in plant population of 36 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2018.

Source of variation	Df	Mean square	P
Block(Year)	2	540.04	0.0007
Soybean genotype	34	95.47	0.11
Residual	68	67.29	

Table 4.24. Analysis of variance for losses in yield of 36 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2018.

Source of variation	Df	Mean square	P
Block(Year)	2	266.18	0.23
Soybean genotype	34	322.52	0.02
Residual	68	180.41	

Table 4.25. Analysis of variance for losses in plant population of 27 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2019.

Source of variation	Df	Mean square	P
Block(Year)	2	1130.78	0.02
Soybean genotype	26	1241.99	$5.36e^{-07}$
Residual	52	252.65	

Table 4.26. Analysis of variance for losses in yield of 27 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN in 2019.

Source of variation	Df	Mean square	P
Block(Year)	2	916.34	0.04
Soybean genotype	26	1004.18	$2.12e^{-05}$
Residual	52	264.67	

Table 4.27. Combined analysis of variance for losses in plant population of 15 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN during 2017- 2019.

Source of variation	Df	Mean square	P
Year	2	30103.7	$< 2.2e^{-16}$
Block(Year)	6	433.9	0.03
Soybean genotype	14	517	0.14
Year x Soybean genotype	28	709.4	0.0002
Residual	84	259.8	

Table 4.28. Combined analysis of variance for losses in yield of 15 soybean genotypes inoculated with *R. solani* IIIB in comparison to paired non-inoculated field plots at Waseca, MN during 2017-2019.

Source of variation	Df	Mean square	P
Year	2	38915	$< 2.2e^{-16}$
Block(Year)	6	252	0.42
Soybean genotype	14	698	0.002
Year x Soybean genotype	28	890	$2.967e^{-06}$
Residual	84	247	

Table 4.29. Combined analysis of variance for disease severity index (DSI) of 16 soybean genotypes inoculated in two experiments of growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	5617	$5.40e^{-07}$
Block (Exp)	6	131	0.72
Isolate	3	88996	$< 2.2e^{-16}$
Soybean genotype	15	706	$3.84e^{-05}$
Isolate x genotype	45	382	0.002
Experiment x isolate	3	22912	$< 2.2e^{-16}$
Experiment x genotype	15	573	0.001
Experiment x genotype x isolate	45	299	0.06
Residuals	378	216	

Table 4.30. Analysis of variance for disease severity index (DSI) of 16 soybean genotypes inoculated in growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	89	0.6
Soybean genotype	15	363	0.002
Isolate	3	93133	< 2.2e-16
Isolate x genotype	45	290	0.000654
Residuals	189	144	

Table 4.31. Analysis of variance for disease severity index (DSI) of 16 soybean genotypes inoculated in growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	173.9	0.61
Soybean genotype	15	916.3	0.0001
Isolate	3	18776.1	< 2.2e-16
Isolate x genotype	45	390.8	0.081
Residuals	189	287.6	

Table 4.32. Combined analysis of variance for relative fresh biomass of 16 soybean genotypes inoculated in two experiments of growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet.

Source of variation	Df	Mean square	P
Experiment	1	91	0.69
Block (Exp)	6	2371	0.0005
Isolate	3	119770	$< 2.2e^{-16}$
Soybean genotype	15	2590	$8.227e^{-8}$
Isolate x genotype	45	1109	0.0006
Experiment x isolate	3	702	$3.283\ e^{-8}$
Experiment x genotype	15	1580	0.0005
Experiment x genotype x isolate	45	702	0.17
Residuals	378	579	

Table 4.33. Analysis of variance for relative biomass of 16 soybean genotypes inoculated in growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet in Experiment 1.

Source of variation	Df	Mean square	P
Block	3	4294	0.0002
Soybean genotype	15	2200	1.62E-05
Isolate	3	59709	< 2.2e-16
Isolate x genotype	45	888	0.04
Residuals	189	609	

Table 4.34. Analysis of variance for relative biomass of 16 soybean genotypes inoculated in growth chamber studies with four *R. solani* isolates recovered from soybean and sugar beet in Experiment 2.

Source of variation	Df	Mean square	P
Block	3	447	0.48
Soybean genotype	15	1971	1.76E-05
Isolate	3	67700	< 2.2e-16
Isolate x genotype	45	923	0.009
Residuals	189	549	