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INFLUENCE OF GLYPHOSATE ON RHIZOCTONIA CROWN AND ROOT ROT IN GLYPHOSATE-RESISTANT SUGARBEET

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INFLUENCE OF GLYPHOSATE ON RHIZOCTONIA CROWN AND ROOT ROT IN GLYPHOSATE-RESISTANT SUGARBEET

By

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ABSTRACT
INFLUENCE OF GLYPHOSATE ON RHIZOCTONIA CROWN AND ROOT ROT IN GLYPHOSATE-RESISTANT SUGARBEET

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Previous greenhouse studies on experimental lines of glyphosate-resistant sugarbeet indicated that tolerance to Rhizoctonia crown and root rot (*Rhizoctonia solani* Kühn) could be compromised after glyphosate was applied. In initial greenhouse experiments, exposure to glyphosate increased, did not affect, and decreased disease severity in three glyphosate-resistant sugarbeet varieties. A laboratory experiment indicated that *R. solani* mycelial growth did not increase in the presence of glyphosate, however, glyphosate applied at a 10X rate decreased growth when compared with the control. Additional greenhouse and field experiments on four commercial glyphosate-resistant sugarbeet varieties inoculated with *R. solani* indicated that herbicide did not affect disease severity, disease indices, or plant fresh weight, or the percent of sugarbeet considered harvestable or healthy. However, variety played a major role in differences of these parameters. An additional field experiment examining the effect of fungicide applications of azoxystrobin on *R. solani* and interactions with tank-mixtures of glyphosate and azoxystrobin indicated that herbicide treatments did not influence *R. solani* disease index or effectiveness of azoxystrobin. Foliar azoxystrobin application provided the greatest disease suppression when compared with in-furrow treatments and either fungicide treatment was better than no fungicide treatment. Choosing varieties with tolerance to Rhizoctonia crown and root and applying a foliar application of fungicide like azoxystrobin will be the key factors to help growers manage this disease.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

LITERATURE REVIEW

Introduction

Sugarbeet (*Beta vulgaris* L.) is a biennial crop that is treated like an annual when grown for sucrose production. In Michigan, sugarbeet is typically planted early in the spring as seed and roots are harvested in the fall (Asadi 2006). At harvest, leaf biomass is removed at the crown by specialized equipment that contains a series of blades (Smith 2001). Sugarbeet roots are then mechanically harvested from the soil and transported to sugar factories to be processed. Sugarbeet is a major source of sucrose, supplying 50 to 55% of the sucrose used in the United States and about 35% of the sucrose used worldwide (Harveson et al. 2009; Wilson 2001). Commercial production of sugarbeet began in the United States around 1870 in California, and followed only a few years later in Michigan (Harveson et al. 2009). Michigan is ranked the fourth highest state for sugarbeet production in the United States behind Minnesota, North Dakota, and Idaho (Harveson et al. 2009; NASS 2009). On average, 537,000 ha of sugarbeet were planted in the United States each year from 2000 to 2009, with approximately 66,000 ha per year grown in Michigan (NASS 2009). In 2008, the total production value of sugarbeet per year was over one billion dollars in the United States, with approximately 170 million dollars coming from Michigan alone (NASS 2009).

Glyphosate-resistant Crops

Glyphosate is the most widely used herbicide in the world because of its ability to control a broad spectrum of annual and perennial broadleaf and grass weed species (Duke and Powles 2008; Pline-Srnic 2005). With its introduction in the early 1970's, glyphosate quickly became a valuable tool (Baylis 2000). The use of glyphosate continued to increase with the introduction of glyphosate-resistant crops in 1996 (Gianessi 2008). Glyphosate use in glyphosate-resistant crops changed weed management tactics by making weed control easier and more effective with fewer herbicide applications and increasing profits (Baylis 2000; Green 2009).

Currently, there are six commercialized glyphosate-resistant crops: soybean [*Glycine max* (L.) Merr], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L.), alfalfa (*Medicago sativa* L.) and most recently (2008) glyphosate-resistant sugarbeet (*Beta vulgaris* L.) (Green 2009). Glyphosate-resistant sugarbeet varieties were quickly adopted by growers in Michigan. Approximately 98% of Michigan's sugarbeet hectares were planted with a glyphosate-resistant variety in 2009 (C. Guza, Agronomist, Michigan Sugar Company, Bay City, MI, personal communication). Competition from weeds is problematic for most sugarbeet growers and multiple conventional herbicide applications, in addition to cultivation and hand weeding, are the typical methods used to control weeds (Gianessi 2005). Weed control costs for conventional sugarbeet are estimated at approximately $336 per acre (Gianessi 2005) and nationwide net economic return for conventional sugarbeet was negative for 4 out of 6 years from 1995-2001 (Gianessi et al. 2002). The economic return for other glyphosate-resistant crops such as corn and soybean is similar or greater when compared

with conventional systems (Johnson et al. 2000; Nolte and Young 2002a, 2002b; Reddy and Whiting 2000).

The use of glyphosate in glyphosate-resistant sugarbeet provides growers the opportunity for excellent control of many weed species that can affect sugarbeet yield and quality (Kniss et al. 2004). Glyphosate applied to glyphosate-resistant sugarbeet provided similar or superior weed control when compared with a mixture of conventional herbicides including metamitron, phenmedipham plus desmedipham, and ethofumesate (Madsen and Jensen 1995). Two sequential applications of glyphosate applied to 10-cm weeds provided similar weed control when compared with a conventional herbicide combination of desmedipham plus phenmedipham, triflusulfuron, and clopyralid (Wilson et al. 2002). Additionally, two applications of glyphosate in glyphosate-resistant sugarbeet at a rate of 0.84 kg ae/ha provided 95% or greater weed control when applications were made starting at the 2-leaf stage (Dexter and Luecke 1999; Guza et al. 2002). Conventional postemergence (POST) herbicides do not effectively control weeds with more than two leaves, so many herbicide applications are necessary and seldom result in 100% control of weeds (Dale et al. 2006; Dale and Renner 2005). Wilson et. al. (2002) found that sucrose yields with a glyphosate herbicide program were as high as 10,000 kg/ha and that sucrose yield was reduced by as much as 15% where three sequential applications of phenmedipham plus desmedipham, triflusulfuron, and clopyralid were applied. In addition, Kemp et al. (2009) determined that when compared with conventional sugarbeet, fewer herbicide applications were required for improved weed control and higher yields in glyphosate-resistant sugarbeet varieties.

The introduction of glyphosate-resistant sugarbeet also provides growers the opportunity to adjust production practices. Narrowing row widths may be possible with reduced cultivation, to obtain higher yields, and as a result, greater economic return despite the additional seed costs associated with using glyphosate-resistant sugarbeet varieties (Armstrong 2009). Glyphosate is less expensive when compared with conventional sugarbeet weed control programs and the potential for greater economic returns is also possible with fewer herbicide applications resulting in improved weed control and increased yields (Kniss et al. 2004).

Glyphosate has a unique mode of action because it is the only herbicide that prevents production of the 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, resulting in inhibition of the shikimic acid pathway (Steinrucken and Amrhein 1980). Glyphosate competes with the substrate phosphoenolpyruvate (PEP), preventing the production of the EPSPS enzyme which is responsible for converting shikimate to chorismate (Amrhein et al. 1980; Bentley 1990; Dill 2005; Pline-Srnic 2005; Siehl 1997). This inhibition of EPSPS blocks the shikimic acid pathway, therefore preventing the production of the aromatic amino acids: tryptophan, tyrosine, and phenylalanine (Hanson and Gregory 2002; Siehl 1997). Glyphosate also reduces the production of secondary compounds including proteins, auxins, phytoalexins, folic acid, precursors of lignins, glavonoids, plastoquinone, and many more phenolic and alkaloid compounds (Bentley 1990). These secondary compounds are important for plant defense against pathogens, plant growth, and plant tolerance under stress (Pline-Srnic 2005). If these secondary compounds are inhibited, applications of glyphosate could lead to increased susceptibility to certain plant pathogens.

Glyphosate-resistant crops contain a CP4-EPSPS gene that was isolated from *Agrobacterium* sp. and glyphosate-resistant crops expressing this enzyme exhibit a high level of resistance to glyphosate (Dill 2005; Pline-Srnic 2005). While glyphosate-resistant crops have a form of EPSPS that is not affected by glyphosate, the resistant EPSPS may not be as efficient as native EPSPS when exposed to glyphosate (Pline-Srnic 2005). The reduced efficiency of this non-native EPSPS enzyme may result in the decreased production of secondary compounds that help protect the plant from pathogens (Pline-Srnic 2005). Despite the ability of glyphosate-resistant crops to exhibit resistance to glyphosate, applications of glyphosate may still have an effect on the synthesis of plant defense compounds (Pline-Srnic 2005). This may be important especially for diseases caused by soil-borne pathogens, such as *Rhizoctonia solani* Kühn (Altman and Campbell 1977). Limited resistance is available in commercial cultivars, therefore increasing the importance of using cultural control methods to reduce the impact of these diseases (Johal and Huber 2009).

Glyphosate and Disease Interactions

Prior to the introduction of glyphosate-resistant crops, studies on glyphosate disease interactions have indicated that glyphosate may influence disease severity and susceptibility to certain pathogens in non-glyphosate-resistant crops. Keen et al. (1982) determined that by inhibiting phytoallexin production, soybean were more susceptible to root rot (caused by the pathogen *Phytophthora megasperma* Drechsler f. sp. *glycines* Kuan & Erwin) after glyphosate applications. Johal and Rahe (1984) determined that dry bean grown in autoclaved soil or vermiculite survived a 10-µg dose of glyphosate while

dry bean grown in an unsterile soil (with *Pythium* and *Fusarium* spp. present) or autoclaved soil infested with *Pythium* spp. did not survive. This indicated that glyphosate applications in the presence of *Pythium* or *Fusarium* spp. increase the efficacy of glyphosate. Additional studies in dry bean demonstrated that glyphosate applications reduced the production of phytoallexins and these plants were more susceptible to anthracnose [*Colletotrichum lindemuthanium* (Sacc. & Magn.) Briosi & Cavara] (Johal and Rahe 1988; Johal and Rahe 1990). In a *Fusarium*-susceptible tomato (*Solanum lycopersicum* L.) cultivar, glyphosate increased the growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* Synder and Hans when compared with tomatoes of the same cultivar that did not receive glyphosate applications (Brammal and Higgins 1988).

More recent studies in glyphosate-resistant crops, including glyphosate-resistant sugarbeet, have indicated a potential for increased susceptibility to some soil-borne pathogens after glyphosate was applied (Larson et al. 2006; Sanogo et al. 2000; Sanogo et al. 2001). In the late 1990's after the introduction of glyphosate-resistant soybean, growers raised concern about increased disease prevalence of sudden death syndrome (caused by the pathogen *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*) (Sanogo et al. 2000; Sanogo et al. 2001). Growth chamber and greenhouse experiments were conducted to determine the effect of glyphosate on the development of sudden death syndrome in glyphosate-resistant soybean (Sanogo et al. 2000). *In vitro* studies indicated that conidial germination, mycelial growth, and sporulation were reduced by glyphosate. However, there was a significant increase in sudden death syndrome disease severity and the frequency of isolation of *F. solani* from soybean roots in plants treated with glyphosate when compared with plants with no herbicide application. Field studies supported

findings in the greenhouse and demonstrated that glyphosate-resistant soybean was more susceptible to sudden death syndrome after glyphosate was applied (Sanogo et al. 2001).

Larson et al. (2006) determined that two experimental varieties of glyphosate-resistant sugarbeet, B4RR and H16, were more susceptible to certain isolates of *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht. f. sp. *betae* Snyd. & Hans. after glyphosate was applied. The variety B4RR demonstrated excellent tolerance to *R. solani* AG-2-2-IIIB when a surfactant control treatment was applied. However, B4RR plants treated with glyphosate had a significant increase in disease severity when compared with a no herbicide control. This indicated that in a variety tolerant to *R. solani*, resistance may be lost after glyphosate was applied. The second variety, H16, was more susceptible to *R. solani* and thus had a significantly higher disease severity rating than the B4RR variety, when treated with a surfactant control. After glyphosate applications, disease severity was not statistically different between varieties, further demonstrating the loss of resistance in B4RR. However, glyphosate had no significant effect on fungal growth of *R. solani* and the production of overwintering structures when compared with the control. Additional studies were conducted to determine the effect of glyphosate on the production of shikimic acid. It was determined that for both glyphosate-resistant varieties and at all growth stages, the rate of shikimic acid accumulation was greater after glyphosate was applied compared with the surfactant control. Although no differences in fungal growth or production of overwintering structures were detected, it appears that glyphosate applications can increase disease severity and the production of shikimic acid in at least some varieties of glyphosate-resistant sugarbeet.

However, other studies demonstrated that in glyphosate-resistant crops, glyphosate applications had no effect or decreased the severity of diseases caused by soil-borne pathogens (Njiti et al. 2003; Pankey et al. 2005). Field studies conducted in glyphosate-resistant soybean determined that there were no significant effects of glyphosate on sudden death syndrome (*F. solani*) disease severity or soybean yield, and that selecting cultivars with tolerance to sudden death syndrome was the best way to manage this disease (Njiti et al. 2003). These results were in contrast to greenhouse and field results reported by Sanogo et al. (2000) and (2001). Varietal differences as well as environmental factors such as planting date, genotype, and other soil factors, may explain why glyphosate has no effect on *F. solani* disease severity in certain varieties, but increases disease severity in others. In greenhouse studies on glyphosate-resistant cotton, applications of glyphosate had no effect on susceptibility to *Rhizoctonia solani* Kühn AG-2-2-IV (Pankey et al. 2005). In fact, field studies indicated that glyphosate applications actually reduced disease severity when compared with other preemergence herbicides and the non-treated control.

Field studies also have been conducted to determine if glyphosate influenced severity of foliar diseases in glyphosate-resistant crops. In glyphosate-resistant soybean, glyphosate applications had no effect on the disease severity of white mold (Sclerotinia stem rot), caused by the fungus *Sclerotinia sclerotiorum* (Lib) de Bary (Lee et al. 2000; Nelson et al. 2002). Nelson et al. (2002) determined that glyphosate applications to glyphosate-resistant soybean did not affect soybean response, reproductive development, canopy development, flower number, *S. sclerotiorum* lesion size, or phytoalexin production, and that disease severity and grain yield were impacted by cultivar selection

rather than herbicide treatment. Lee et al. (2000) further demonstrated that neither the glyphosate-resistant trait in glyphosate-resistant soybean nor glyphosate application influenced soybean yield, disease severity, or *S. sclerotiorum* growth, and did not increase soybean susceptibility to white mold.

Studies with glyphosate-resistant wheat (*Triticum aestivum* L.) indicated that glyphosate actually decreased disease severity of leaf rust (caused by the pathogen *Puccinia triticina* Eriks) and stem rust fungus (cause by the pathogen *Puccinia graminis* f. sp. *tritici* Eriks) when exposed to glyphosate 21 d to 35 d after inoculation (Anderson and Kolmer 2005). Additional studies by Feng et al. (2005) determined that glyphosate also reduced the disease severity of leaf rust (caused by the pathogen *P. triticina*) and stripe rust (caused by the pathogen *Puccinia striiformis* f. sp. *tritici* Westend) in glyphosate-resistant wheat. Baley et al. (2008) found that glyphosate-resistant wheat cultivars were not more susceptible than glyphosate-susceptible cultivars to the pathogens *Rhizoctonia solani*, *R. oryzae* Ryker & Gooch, *Gaeumannomyces graminis* (Sacc.) v. Arx & J. Olivier var. *tritici* J. Walker, and *Pythium ultimum* Trow.

Rhizoctonia Crown and Root Rot

Rhizoctonia crown and root rot, caused by the soil-borne pathogen *Rhizoctonia solani*, is a problematic disease in many crops throughout Michigan, including sugarbeet (Windels et al. 2009; Kirk et al. 2008). Rhizoctonia crown and root rot reduces economic returns for sugarbeet by as much as 24% in the United States and causes up to 50% yield loss, depending on disease severity (Franc et al. 2001; Windels et al. 2009). Although AG-2-2-IIIB is the most common and virulent subgroup causing Rhizoctonia crown and

root rot in sugarbeet, another subgroup, AG-2-2-IV, is also found in Michigan (Engelkes and Windels 1996; Kirk et al. 2008). The first symptoms that are observed with Rhizoctonia crown and root rot are foliar (Franc et al. 2001; Windels et al. 2009). Leaves permanently wilt and dark lesions form at the base of the petiole or on the crown of the beet. Leaves then become dry and collapse, but remain attached to the crown and form a dry, dark rosette. Root symptoms include black lesions that begin anywhere on the root, but may coalesce and cover the entire root surface as the disease progresses. Root tissue is typically firm underneath these lesions. However, root tissue begins to soften underneath these lesions and cracks may also develop in advanced stages of the disease.

Rhizoctonia solani has many host crops in addition to sugarbeet, which makes it difficult to control with crop rotation alone (Rush and Winter 1990; Schuster and Harris 1960). Soybean, dry bean (*Phaseolus vulgaris* L.), corn, and cucumber (*Cucumia sativus* L.), as well as many weed species, can act as alternate hosts for *R. solani* (Sneh et al. 1998; Windels et al. 2009). Many of these crops are commonly used in a rotation with sugarbeet in Michigan and many potential weed hosts are common species found in sugarbeet fields, further increasing the buildup of disease inoculum (Windels et al. 2009).

Varieties bred for tolerance to Rhizoctonia crown and root rot provide additional options for managing this disease, and varieties with varying levels of tolerance are readily available to Michigan sugarbeet growers. Although these varieties do not completely prohibit infection, they certainly limit fungal colonization and disease severity (Ruppel 1973).

Additional methods for controlling Rhizoctonia crown and root rot in sugarbeet include applications of strobilurin fungicides, such as azoxystrobin (Jacobsen et al. 1998;

Kirk et al. 2008). Applications of azoxystrobin in-furrow at sugarbeet planting can reduce infection early in the season, but may not prevent later infections (Jacobsen et. al. 1998; Karaoglanidis and Karadimos 2006; Kiewnick et al. 2001; Windels and Brantner 2000). Single fungicide applications are typically made either in-furrow at planting or postemergence (POST) to sugarbeet between the 4- to 8-leaf stage (Karaoglanidis and Karadimos 2006; Whitney and Duffus 1986). If glyphosate-resistant sugarbeet are more susceptible to plant pathogens after glyphosate is applied, then fungicide applications may be important in controlling sugarbeet diseases such as Rhizoctonia crown and root rot.

Potential interactions between fungicide and glyphosate applications could influence the efficacy of fungicide treatments used to manage Rhizoctonia crown and root rot. Kataria and Gisi (1990) found that DNOC, dicamba, ioxynil, and bromoxynil when used in combination with the fungicide cyproconazole were synergistic in reducing disease severity of *Rhizoctonia ceralis* Van der Hoeven and *Pseudocercosporella herpotrichoides* (Fron) Deighton in wheat. However, Jacobsen et al. (1998) determined that there was no effect on *Rhizoctonia solani* control efficacy, when azoxystrobin was applied in a tank-mix of desmedipham plus phenmedipham and clopyralid. Additional field studies using a tank-mix of these same herbicides with triflusulfuron and sethoxydim again indicated no reduction in *R. solani* control or sugarbeet yield when combined with azoxystrobin (Jacobsen et al. 1998). These results are similar to earlier studies which showed that preemergence (PRE) applications of diclofop methyl and ethofumesate followed by POST applications of desmedipham plus phenmedipham, EPTC, trifluralin, and metolachlor did not increase disease severity of Rhizoctonia crown

and root rot (Ruppel et al. 1982). However, other studies have reported antagonistic effects of glyphosate when tank-mixed with fungicide applications. *In vitro* studies conducted by Hill and Stratton (1991) determined that metribuzin when used in combination with the fungicide chlorothalonil were antagonistic and reduced control of *Alternaria solani* (Ell. and Mart.) Jones and Grout. Ward (1984) also reported that in soybean, tank-mixed applications of metalaxyl and glyphosate resulted in reduced control of *Phytophthora megasperma* Drechs f. sp. *glycinea* (Hildeb.) Kuan and Erwin. In sugarbeet, Sprague et al. (2005) reported an increase in sugarbeet injury when azoxystrobin was applied within 3 days prior to or after micro-rate herbicide applications. Therefore, potential interactions between glyphosate and applications of azoxystrobin may have an effect on disease severity if *R. solani* is present. Additionally, if glyphosate-resistant crops are more susceptible to soil-borne pathogens such as *R. solani*, fungicide applications may be more important in controlling Rhizoctonia crown and root rot in glyphosate-resistant sugarbeet.

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CHAPTER 2

INFLUENCE OF GLYPHOSATE ON RHIZOCTONIA CROWN AND ROOT ROT IN GLYPHOSATE-RESISTANT SUGARBEET

Abstract: Greenhouse experiments were conducted in 2008 to determine if glyphosate had an effect on disease severity when compared with a conventional standard-split herbicide treatment or no herbicide treatment. Three potential commercially-available varieties of glyphosate-resistant sugarbeet were used for this experiment. Hilleshög 9027RR exhibited the most tolerance to Rhizoctonia crown and root rot when no herbicide was applied. However, after exposure to either a 0.84 or 1.68 kg ae/ha rate of glyphosate, this variety exhibited an increase in disease severity. There were no significant differences between herbicide treatments in the Hilleshög 9028RR variety, and glyphosate decreased disease severity in Hilleshög 9032RR when compared with the no herbicide treatment. Experiments conducted to determine if glyphosate had an effect on *Rhizoctonia solani* Kühn growth *in vitro*, indicated that glyphosate did not increase the rate of radial growth. A 10x rate of glyphosate plus ammonium sulfate (AMS) actually decreased the rate of radial growth of *R. solani.* Field and additional greenhouse experiments were conducted in 2008 and 2009 to determine if glyphosate influenced the disease severity of *R. solani* in four commercial varieties of glyphosate-resistant sugarbeet. Differences in disease severity and the percent of harvestable sugarbeet in the field were observed when comparing the four varieties, but glyphosate did not significantly influence the disease severity when compared with the standard-split treatment or no herbicide treatment. Despite the first greenhouse experiment that

indicated that glyphosate may increase disease severity in some varieties, results from additional experiments indicate that herbicide treatment, including glyphosate applications, did not affect disease severity. Choosing a variety with tolerance to Rhizoctonia crown and root rot is the most important factor in reducing disease severity in commercial varieties of glyphosate-resistant sugarbeet.

Nomenclature: Glyphosate; Standard-split; Rhizoctonia crown and root rot, *Rhizoctonia solani* Kühn; sugarbeet, *Beta vulgaris* L.

Key words: Glyphosate-resistant crops; disease severity; fresh weight; dry weight; disease index; harvestable sugarbeet; healthy sugarbeet

Introduction

For decades, glyphosate has played an important role in weed management because of its broad spectrum control of annual and perennial broadleaf and grass weed species (Duke and Powles 2008; Pline-Srnic 2005). Glyphosate continues to be a valuable weed management tool for growers with the introduction of glyphosate-resistant crops. Currently, there are six commercialized glyphosate-resistant crops: soybean [*Glycine max* (L.) Merr], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L.), alfalfa (*Medicago sativa* L.), and sugarbeet (*Beta vulgaris* L.) (Green 2009). The newest commercialized glyphosate-resistant crop is sugarbeet, introduced in 2008. Since commercialization, glyphosate-resistant sugarbeet have quickly been adopted, with almost 98% of Michigan's sugarbeet acres planted to glyphosate-resistant varieties in 2009 (C. Guza, Agronomist, Michigan Sugar Company, Bay City, MI, personal communication).

Competition from weeds is problematic for most sugarbeet growers. Traditionally, multiple herbicide applications, in addition to cultivation and hand weeding, were necessary to manage weeds (Gianessi 2005). Also, conventional postemergence (POST) herbicides do not effectively control weeds with more than two leaves, so many herbicide applications are necessary and seldom result in 100% control (Dale et al. 2006; Dale and Renner 2005). However, with the introduction of glyphosate-resistant sugarbeet, growers can achieve excellent control of many weed species that affect sugar quality and yield (Kemp et al. 2009; Kniss et al. 2004). When compared with conventional herbicide treatments, glyphosate is less expensive and fewer applications are needed to control weeds with greater economic returns (Dexter and Luecke 1999; Guza et al. 2002; Kemp et al. 2009; Kniss et al. 2004).

However, concerns have been raised about potential increases in disease pressure after glyphosate is applied, due to physiological effects of the herbicide on plants. In plants, glyphosate inhibits the shikimic acid pathway, preventing the production of aromatic amino acids, as well as secondary compounds, including phytoalexins (Bentley 1990; Hanson and Gregory 2002; Siehl 1997). Some of these secondary compounds are important for plant defense against pathogens, plant growth, and plant tolerance under stress (Pline-Srnic 2005). If these secondary compounds are inhibited, applications of glyphosate could lead to increased susceptibility to certain plant pathogens. Glyphosate-resistant crops are not injured by glyphosate applications because they contain a CP4-EPSPS gene that exhibits a high level of resistance to glyphosate. However, this enzyme may not be as efficient as native EPSPS when exposed to glyphosate and may result in reduced production of secondary compounds that help protect the plant from pathogens.

Previous studies in glyphosate-resistant crops, including glyphosate-resistant sugarbeet, demonstrated an increased susceptibility to soil-borne pathogens after glyphosate was applied (Larson et al. 2006; Sanogo et al. 2000; Sanogo et al. 2001). In greenhouse and field experiments, glyphosate-resistant soybean were more susceptible to sudden death syndrome, caused by the pathogen *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*, after glyphosate was applied (Sanogo et al. 2000; Sanogo et al. 2001). In addition, Larson et al. (2006) determined that two non-commercial varieties of glyphosate-resistant sugarbeet were more susceptible to certain isolates of both *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht. f. sp. *betae* Snyd. & Hans after glyphosate was applied.

In contrast, other studies demonstrated that glyphosate applications had no effect on, or even decreased the severity of, diseases caused by soil-borne pathogens (Njiti et al. 2003; Pankey et al. 2005). In glyphosate-resistant soybean, Njiti et al. (2003) determined that glyphosate had no effect on soybean yield or disease severity of sudden death syndrome. These results conflicted with greenhouse and field results reported by Sanogo et al. (2000) and (2001). There were differences between these studies concerning variety selection and varietal response to the disease. In addition, there were differences in environmental factors such as planting date, genotype, and other soil factors. This may explain why glyphosate has no effect on *F. solani* disease severity in certain varieties, but increases disease severity in others. In glyphosate-resistant cotton, greenhouse experiments conducted by Pankey et al. (2005) showed that glyphosate had no effect on damping off or soreshin (caused by the pathogen *Rhizoctonia solani*). Furthermore, in the field, glyphosate actually reduced *R. solani* induced disease severity.

Rhizoctonia solani is a soil-borne pathogen that can induce root disease in many crops throughout Michigan, including Rhizoctonia crown and root rot in sugarbeet (Kirk et al. 2008; Windels et al. 2009). Depending on disease pressure, Rhizoctonia crown and root rot reduces economic returns for sugarbeet by as much as 24% and results in up to 50% yield loss (Franc et al. 2001; Windels et al. 2009). The greenhouse study by Larson et. al (2006), indicating that applications of glyphosate to glyphosate-resistant sugarbeet increased Rhizoctonia disease severity, raised sugarbeet grower concerns about this potential interaction with the 2008 commercialization of glyphosate-resistant sugarbeet. To address these concerns, the objectives of this research were to: 1) investigate the effect of glyphosate on the disease severity of Rhizoctonia crown and root rot in glyphosate-resistant sugarbeet varieties in the greenhouse and the field, and 2) determine if glyphosate has an effect on mycelial growth of *Rhizoctonia solani in vitro.*

Materials and methods

Response of three sugarbeet varieties in the greenhouse *(Experiment 1).* Glyphosate-resistant sugarbeet varieties, Hilleshög 9027RR,[1] Hilleshög 9028RR, and Hilleshög 9032RR, were planted 2.54 cm deep in a pasteurized sandy loam soil with a soil pH of 7.1. Plants were grown in the greenhouse where temperature was maintained at 25 $\pm$ 5 C with a 16-h photoperiod of natural sunlight and supplemental lighting was provided at 1,000 $\mu mol/m^2/s$ photosynthetic photon flux. Plants were watered daily to maintain adequate soil moisture for plant growth. One week after planting, seedlings were thinned to one plant per pot. At 14 d after planting, sugarbeet were fertilized weekly with 50 ml of a solution containing 6.61 g/L of 20:20:20 ($N:P_2O5:K_2O$).

The experiment was arranged in a three-factor completely randomized design with five replications, and repeated in time. Factors included *Rhizoctonia solani* inoculation (inoculated or non-inoculated), sugarbeet variety (Hilleshög 9027RR, Hilleshög 9028RR, and Hilleshög 9032RR), and herbicide treatment. Herbicide treatments consisted of two rates of glyphosate[2] (0.84 and 1.68 kg ae/ha) plus ammonium sulfate at 2% v/v, a standard conventional sugarbeet herbicide mixture (phenmedipham at 270 g/ha plus desmedipham[3] at 270 g/ha, triflusulfuron[4] at 9 g/ha, and clopyralid[5] at 104 g/ha), and a no-herbicide control. Herbicide applications were made when sugarbeet were at the 6- to 8-leaf growth stage using a single tip track-sprayer with a Teejet 8001E[6] flat-fan nozzle. The sprayer was calibrated to deliver 187 L/ha at a pressure of 234 kPa at a speed of 1.6 km/h.

Within 24 hours after herbicide application, treatments that were slated to be inoculated were inoculated with *R. solani* AG-2-2-IIIB, the most common and virulent *R. solani* subgroup found in Michigan (Kirk et al. 2008). Rhizoctonia inoculum was prepared by growing *R. solani* AG-2-2-IIB on moist autoclaved millet (*Panicum miliaceum* L.). Autoclaved millet seeds were spread over a water agar plate on which a 7 mm plug of the pathogen (*Rhizoctonia solani*) had been placed at the approximate center. The millet was colonized as the fungus grew, and after 7 to 10 d, the plate was completely covered with visible fungal growth. The millet was removed from the plate, air dried in a biological safety cabinet for 2 to 3 d, and stored in a sterile closed container at 4 to 7 C until it was ready to be used. Pots were inoculated by burying one millet seed approximately 1 cm deep adjacent to the sugarbeet crown. Sterile-autoclaved millet seed

was used in the non-*Rhizoctonia* inoculated control pots. After inoculation, inoculum was watered in.

Sugarbeet were harvested approximately 21 d after treatment (DAT) by removing the whole plant from the pot and washing roots to remove any excess soil. Each sugarbeet root was rated for disease severity using the 0 to 7 Rhizoctonia crown and root rot rating scale as follows: 0 = no visible signs of disease; 1 = inactive lesions; 2 = less than 5 % active lesions; 3 = 6 to 25 % of the root rotted; 4 = 26 to 50% of the root rotted; 5 = 51 to 75 % of the root rotted; 6 = greater than 75 % of the root rotted, but still some living tissue; 7 = roots completely rotted and dead (Ruppel et al. 1979). Sugarbeet fresh weights were recorded. One replication of sugarbeet roots was sliced into approximately 1 cm sections, surface disinfected for 60 s in 0.5 % sodium hypochlorite, and plated on potato dextrose agar[7] (PDA) to confirm the presence of *R. solani.* The remaining samples were air dried for one week at 28 C and dry weights were recorded. Dry weight results followed similar trends as fresh weight results, therefore only plant fresh weight data are presented.

Rhizoctonia solani* growth *in vitro. A laboratory experiment measured the fungal growth of *Rhizoctonia solani* AG-2-2-IIIB in the presence of glyphosate. The methods used in this experiment were described by Harikrishnan and Yang (2001) and Larson et al. (2006). Petri plates (100 x 15 mm) were filled with 25 ml of herbicide-amended water agar[8] (1.5 % weight to water ratio). Herbicide rates were calculated based on the area of the plate (56.5 cm^2). All herbicide and additive aqueous stock solutions were filter-

sterilized (0.2 μm) before being added to autoclaved PDA. Herbicide treatments included the following: glyphosate alone at 0, 9.5, 19, 38, or 190 μg ae/ml (0, 0.5, 1, 2, and 10X the recommended use rate); glyphosate at the same rates plus ammonium sulfate at 0, 41, 82, 164, or 818 μg/ml; ammonium sulfate alone at 82 μg/ml; and the standard conventional sugarbeet herbicide mixture of phenmedipham plus desmedipham, triflusulfuron, and clopyralid at 6, 6, 0.2, and 2.4 μg/ml, respectively. Mycelial plugs (7 mm diameter) of *R. solani* AG-2-2-IIIB were removed from three wk old stock cultures and transferred to the center of each plate. Plates were parafilmed and incubated in the dark at 27 ± 2 C. Radial growth was measured daily for 5 d until mycelia reached the edge of the plate. Each treatment was replicated five times and the experiment was repeated in time.

Response of four sugarbeet varieties in the field. A field experiment was conducted in 2008 and 2009 in the Saginaw Valley region of Michigan. The 2008 experiment was located in St. Charles, Michigan on a Misteguay silty clay (fine, mixed, semiactive, calcareous, mesic Aeric Endoaquepts) with a soil pH of 7.8 and 3.0 % organic matter. The 2009 experiment was located in Frankenmuth, Michigan on a Tappan-Londo complex (fine-loamy, mixed, active, calcareous, mesic Typic Endoaquolls) with a soil pH of 7.7 and 2.4 % organic matter. Following dry bean (*Phaseolus vulgaris* L.) harvest, fields were fall-chisel plowed and in the spring, fields were cultivated twice prior to planting. Fertilizer applications were standard for sugarbeet production in Michigan. The glyphosate-resistant sugarbeet varieties Hilleshög 9027RR, Hilleshög 9028RR, Hilleshög 9029RR, and Crystal RR827[9] were planted 2.5-cm deep in 76-cm rows at a

population of 122,000 seeds/ha on April 25, 2008 and April 16, 2009. Hilleshög 9032RR was removed from these experiments, since this variety was not being commercially grown in Michigan. Plots were six rows wide by 9.1 m in length. Each variety was planted, one per row, in rows two through five. Rows one and six served as border rows. Commercial sugarbeet varieties selected for this experiment were approved by Michigan Sugar Company and were thought to have varying degrees of Rhizoctonia crown and root rot tolerance.

The experimental design was a split-strip-plot with all treatments replicated four times. Herbicide treatment was the main-plot factor, *R. solani* inoculation was the sub-plot factor, and variety was the strip-plot factor. When sugarbeet were at the 6- to 8-leaf stage, plots were inoculated with *R. solani* AG-2-2-IIIB. Rhizoctonia inoculum was grown on a barley medium. Pans of barley, saturated with water, were autoclaved and 9 (7 mm) plugs of *R. solani* grown on potato dextrose agar were placed into the pans. Parafilm-sealed pans were incubated at 25 C $\pm$ 2 for 3 wk. Once the barley was colonized, it was air dried and ground into a fine flour. Inoculum was applied directly over each sugarbeet row at 2 g/m of row using a modified drop spreader.[10] The inoculum rate was confirmed by determining the amount of leftover inoculum and calculating the kg applied per m of row. Plots that were $_{\text{non-inoculated}}$ served as a control. All plots were cultivated following inoculation to put soil and inoculum in the crown for increased disease severity (Ruppel et al. 1979).

Herbicide treatments included 1) a glyphosate herbicide program, 2) a standard-split program (standard herbicide program used in conventional sugarbeet), and 3) a hand-weeded control (no herbicide). The glyphosate program consisted of glyphosate at

0.84 kg ae/ha plus ammonium sulfate at 2% v/v, applied three times at 2- to 4-leaf, 4- to 6-leaf, and 6- to 8-leaf sugarbeet. The standard-split program consisted of a combination of desmedipham at 180 g ai/ha plus phenmedipham at 180 g ai/ha, triflusulfuron at 9 g ai/ha, clopyralid at 104 g ai/ha, and non-ionic surfactant at 0.25% v/v, applied twice at the cotyledon to 2-leaf and 2- to 4-leaf stage sugarbeet. The rates of desmedipham plus phenmedipham were increased to 270 g ai/ha in the second standard-split application. All plots were maintained weed-free by hand-weeding throughout the growing season. Herbicide treatments were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 178 L/ha at 207 kPa through 10003 AirMix[11] nozzles, spaced 51 cm apart at approximately 56 cm above the canopy. Plots were rated for herbicide injury 14 d after the last herbicide application timing.

Sugarbeet stand counts were recorded for each variety four weeks after planting and at harvest. Approximately 8 wk after inoculation, sugarbeet were lifted from the soil using a modified lift harvester.[12] Individual sugarbeet roots were evaluated for disease severity using the 0 to 7 scale described previously (Ruppel et al. 1979). Stand counts were used to determine how many sugarbeet were missing from each plot due to advanced disease severity. Values were adjusted by assigning each of the missing sugarbeet a disease severity rating of 7. An average disease index was determined for each variety in each plot. The disease index was calculated as a weighted average based on the number of sugarbeet in each of the eight disease classes (Ruppel et al. 1979). The percent of healthy sugarbeet were determined by calculating the percent of sugarbeet that had a disease severity rating of 0 or 1. Harvestable sugarbeet were determined by calculating the percent of sugarbeet with a disease severity rating 3 or less.

Precipitation data was recorded by weather stations operated by the Michigan Automated Weather Network[13] (Table 1) which were located within 3 km of the experimental locations.

Response of four sugarbeet varieties in the greenhouse *(Experiment 2).* This greenhouse experiment evaluated the four commercial sugarbeet varieties that were used in the field experiments in 2008 and 2009: Hilleshög 9027RR, Hilleshög 9028RR, Hilleshög 9029RR, and Crystal RR827. Two of these varieties, Hilleshög 9027RR and Hilleshög 9028RR, were also evaluated in greenhouse *Experiment 1.* Methods for this experiment were similar to *Experiment 1*, with certain exceptions. Sugarbeet were planted in a professional potting mix[14] with a soil pH of 5.9. At the 4-leaf stage, sugarbeet were fertilized once with a micronutrient[15] solution containing boron and other micronutrients. Similar procedures were used for Rhizoctonia inoculation, except the inoculum was produced on barley (*Hordeum vulgare* L. subsp. *vulgare*). After colonization, barley was air-dried and ground into a fine flour. Pots were inoculated by spreading 0.5 ml of the barley inoculum around the sugarbeet crown. The non-inoculated pots received 0.5 ml of sterile-autoclaved barley flour. The experiment was arranged in a three-factor completely randomized design with four replications, and repeated in time. All other procedures and measurements were similar to *Experiment 1.*

Statistical Analysis. All data were analyzed using the PROC MIXED procedure in SAS 9.1.[16] An analysis of variance was performed to test for significant interactions and main

effects. Data were combined over experiments and/or years and main effects when appropriate interactions were not significant. Interactions between main effects were analyzed using the SLICE option in the LSMEANS statement. Mean separation for treatment differences was performed using Fisher's Protected LSD at the $p \leq 0.05$ significance level. In the laboratory experiment, radial fungal growth of the different treatments was compared by determining the slope of each replication with TableCurve 2D 5.01[17] and analyzing this data in SAS, as described previously.

Results and Discussion

Response of three sugarbeet varieties in the greenhouse *(Experiment 1)*. Two experimental replications of greenhouse *Experiment 1* were conducted in early 2008, prior to the full-commercial release of glyphosate-resistant sugarbeet. Experimental replication was not significant; therefore the data were combined for analysis. Inoculation with *R. solani* AG-2-2-IIIB was significant and the average disease severity for plants that were inoculated was 4.2 (Table 2). Rhizoctonia crown and root rot was not present on any of the sugarbeet that were non-inoculated, indicating that the pathogen was not present in the soil used in the greenhouse experiments. Therefore, the non-inoculated treatments were dropped from further analysis. However, the non-inoculated plants were used to standardize sugarbeet fresh weight among the varieties. Fresh weight data is presented as a percent of the non-inoculated treatments.

None of the glyphosate-resistant sugarbeet varieties used in *Experiment 1* showed visible signs of damage from the herbicide treatments (data not shown). However, there were differences in disease severity and ultimately plant fresh weight, with the different

herbicide treatment-variety combinations. The most *Rhizoctonia*-tolerant variety of the three glyphosate-resistant varieties evaluated when no herbicide was applied was Hilleshög 9027RR, with a disease severity rating of 2.8 (Table 2). The other glyphosate-resistant varieties, Hilleshög 9028RR and Hilleshög 9032RR, were more susceptible to *R. solani*, with disease severity ratings of 4.8 and 4.9, respectively, in the no herbicide controls (Table 2).

Applications of glyphosate at 0.84 and 1.68 kg/ha to Hilleshög 9027RR increased the disease severity rating from 2.8 to 4.7 and 5.9, respectively (Table 2). Increased disease severity was also reflected with reduced plant fresh weight (Table 3). There was a 39 and 61% reduction in plant fresh weight when glyphosate was applied at 0.84 and 1.68 kg/ha, respectively, as compared with the no herbicide control (Table 3). This response was similar to results observed by Larson et al. (2006), where an increase in Rhizoctonia crown and root rot disease severity occurred when glyphosate was applied to a Rhizoctonia-tolerant glyphosate-resistant sugarbeet variety.

Although the glyphosate-resistant sugarbeet varieties Hilleshög 9028RR and Hilleshög 9032RR had similar disease severity ratings for the no herbicide control, they responded differently to the herbicide treatments. None of the herbicide treatments significantly changed the disease severity rating or plant fresh weight for Hilleshög 9028RR (Tables 2 and 3). However, there was a significant reduction in disease severity when Hilleshög 9032RR was exposed to the standard herbicide program or glyphosate at 0.84 kg when compared with the no herbicide control (Table 2). Sugarbeet fresh weight also was higher with the standard herbicide program as compared with the no herbicide control (Table 3). This may indicate that certain herbicides could decrease disease

severity in certain varieties. Sanogo (2000) and (2001) demonstrated that glyphosate applications influenced sudden death syndrome disease severity in some varieties of glyphosate-resistant soybean, but this response was variety dependent. Differing results in our experiment could also vary based on environmental differences. Pankey et al. (2005) showed that in glyphosate-resistant cotton, glyphosate applications reduced Rhizoctonia disease severity in the field, even though there was no effect in the greenhouse.

***Rhizoctonia solani* growth *in vitro*.** In our initial greenhouse experiment, we observed contrasting results among the three varieties evaluated. An increase in disease severity was observed when glyphosate was applied to Hilleshög 9027RR and a decrease in disease severity was found when glyphosate at 0.84 kg/ha or the standard conventional herbicide mixture was applied to Hilleshög 9032RR. A laboratory experiment was conducted to determine if these differences were explained by the rate of mycelial growth of *Rhizoctonia solani* in the presence of glyphosate. The addition of ammonium sulfate to glyphosate did not have a significant effect on the rate of mycelial growth. Therefore data are combined over the glyphosate alone and the glyphosate plus ammonium sulfate treatments.

There were significant differences in mycelial growth for the different rates of glyphosate (Table 4). The highest rate of glyphosate (190 µg/ml), equivalent to 10X the normal use rate of glyphosate, inhibited mycelial growth when compared with the control. However, lower rates of glyphosate (0.5, 1, or 2X) and the standard conventional herbicide mixture of phenmedipham plus desmedipham, triflusulfuron and

clopyralid treatment did not significantly influence the growth rate of *R. solani*. Thus an increase in the rate of mycelial growth of *R. solani* cannot explain the increased disease severity after glyphosate was applied in Hilleshög 9027RR. Larson et al. (2006) also concluded that fungal growth at varying rates of glyphosate were not significantly different from the control, except at the highest glyphosate concentration (40 µg/ml). The reduction in the rate of mycelial growth at the highest rate of glyphosate may be due to the adjuvants in the glyphosate formulation. Lee et al. (2000) found that *Sclerotinia sclerotiorum* mycelia were inhibited by a formulation blank with proprietary adjuvants at 100 mM ae glyphosate. The formulated glyphosate without an adjuvant did not inhibit mycelial growth on herbicide amended PDA. It also is possible that glyphosate may have anti-fungal activity and inhibit growth of *R. solani*. Feng et al. (2005) determined that in glyphosate-resistant wheat, glyphosate decreased the disease severity of *P. triticina* and *P. striiformis*.

Response of four sugarbeet varieties in the field. Field experiments were conducted using four commercial varieties of glyphosate-resistant sugarbeet to confirm earlier greenhouse results. Interactions between years were not significant. Therefore, all data are presented as a combination of the 2008 and 2009 experiments. The two-way interaction of variety x herbicide was not significant (Table 5) for any of the parameters evaluated. Therefore, data are discussed as the main effects of variety and herbicide for all parameters.

Rhizoctonia inoculation. Inoculation of *R. solani* subgroup AG-2-2-IIIB was highly effective. The combination of cultivation and precipitation (Table 1) following Rhizoctonia inoculation resulted in an average disease index of 5.9 in the field (Table 6). This provided a good basis for treatment separation. The natural *R. solani* infestations in the field were low each year based on the disease indices, 2 or less (data not shown). Therefore, the non-inoculated treatments were dropped from further analysis.

Herbicide injury. The glyphosate-resistant sugarbeet varieties did not show visible signs of damage from glyphosate treatments. However, applications of the standard-split herbicide program (two applications) uniformly caused 13% injury to each of the four glyphosate-resistant sugarbeet varieties evaluated (data not shown). Injury symptoms consisted of yellowing and stunting compared with the non-treated control and are consistent with what others have observed with this program (Wilson 1994, 1995). Approximately 2 wks after this evaluation, sugarbeet recovered from this damage.

Variety. The main effect of variety was significant for Rhizoctonia disease indices and the percentage of harvestable sugarbeet (Table 5). Sugarbeet that are considered harvestable have a disease severity rating of 3 or less. The percentage of healthy sugarbeet was not significant. Sugarbeet that are considered healthy have a disease severity rating of 0 or 1. Averaged across all herbicide treatments, Hilleshög 9027RR and Hilleshög 9029RR were the most tolerant to *R. solani* infection, with disease index ratings of 5.5 and 5.7, respectively (Table 6). The disease index rating for Hilleshög 9028RR was significantly higher than Hilleshög 9027RR, but was not significantly

different than Hilleshög 9029RR. Crystal RR827 was the most susceptible variety to *R. solani* infection, with a disease severity index of 6.6. The percentage of harvestable sugarbeet followed the same trend as the disease index ratings (Table 6). However, regardless of variety, 15% or fewer of the sugarbeet were considered harvestable. Fewer than 3% of the sugarbeet were considered healthy (Table 6).

Herbicide. The main effect of herbicide was not significant (Table 5). These results indicate that glyphosate had no effect on the development of Rhizoctonia crown and root rot when compared with the standard conventional herbicide treatments or no herbicide controls. This is in contrast to our *Experiment 1* results and to the Larson et al. (2006) findings.

In *Experiment 1*, applications of glyphosate increased disease severity for Hilleshög 9027RR. However, the field experiment did not support these findings. One potential explanation for the contrasting results is the difference in inoculation media. In the first set of experiments, the Rhizoctonia inoculum was grown on millet, however, the field experiment used a ground barley media. Overall disease severity could have been affected by the different soil types used in each of these experiments. The presence of additional soil pathogens, as well as additional environmental factors, could have resulted in differences between these experiments. In addition, other studies have indicated that time of herbicide application in relation to disease infection may influence the susceptibility of plants to pathogens. In the greenhouse, sugarbeet were inoculated within 24 h of herbicide treatment. However, in the field, sugarbeet were inoculated days after the last herbicide application. Studies with glyphosate-resistant wheat (*Triticum aestivum*

L.) have indicated that glyphosate actually decreased disease severity of leaf rust (caused by the pathogen *Puccinia triticina*) and stem rust fungus (cause by the pathogen *Puccinia graminis* f. sp. *tritici* Eriks) when exposed to glyphosate 21 d to 35 d after inoculation (Anderson and Kolmer 2005).

Response of four sugarbeet varieties in the greenhouse *(Experiment 2)*. An additional greenhouse experiment *(Experiment 2)* was conducted using the four commercial varieties of glyphosate-resistant sugarbeet used in the field to confirm earlier field and greenhouse results. Experimental replications for the greenhouse studies were not significant, so data were combined for analysis. The two-way interaction of variety x herbicide was not significant in the greenhouse (Table 7) for any of the parameters evaluated. Therefore, data are discussed as the main effects of variety and herbicide for disease severity and fresh plant weight.

Rhizoctonia inoculation. Inoculation of *R. solani* subgroup AG-2-2-IIIB was highly effective in the greenhouse. Adequate moisture in the greenhouse following Rhizoctonia inoculation resulted in an average disease severity rating of 5.9 (Table 8). Rhizoctonia crown and root rot was not present on any of the non-inoculated sugarbeet, indicating that the pathogen was not present in the potting mix used in the experiment. Therefore, the non-inoculated treatments were dropped from further analysis. However, the non-inoculated plants were used to standardize sugarbeet fresh weight among the varieties. Fresh weight data is presented as a percent of the non-inoculated treatments.

Variety. The main effect variety was significant for Rhizoctonia disease severity and sugarbeet fresh weight (Table 7). The order of Rhizoctonia tolerance of the varieties was different in the greenhouse compared to the field. In the greenhouse, Hilleshög 9028RR had the lowest disease severity rating (4.8) (Table 8). Hilleshög 9027RR and Hilleshög 9029RR had similar disease severity ratings of 5.9 and 6.1, respectively. Again Crystal RR827 was the most susceptible variety with a disease severity rating of 6.7; however this was not significantly different from Hilleshög 9029RR. The fresh weight of Rhizoctonia-inoculated sugarbeet was reduced by 61% or more when compared with the non-inoculated control (Table 8). The fresh weight of Hilleshög 9028RR was significantly higher than fresh weights of the other varieties.

Herbicide. The glyphosate-resistant sugarbeet varieties did not show visible signs of damage from glyphosate treatments or the standard conventional herbicide mixture. In addition, the main effect of herbicide was not significant for disease severity or sugarbeet fresh weight in the greenhouse (Table 7). These results indicate that glyphosate had no effect on the development of Rhizoctonia crown and root rot when compared with the standard conventional herbicide treatments or no herbicide controls. This is in contrast to our *Experiment 1* results and to the Larson et al. (2006) findings.

In *Experiment 1*, applications of glyphosate increased disease severity for Hilleshög 9027RR. However, field and additional greenhouse experiments did not support these findings. One potential explanation for the contrasting results is the difference in inoculation media. In *Experiment 1*, the Rhizoctonia inoculum was grown on millet, however, the field and additional greenhouse experiment (*Experiment 2*) used a

ground barley media. The overall Rhizoctonia disease severity was lower for the inoculum grown on millet (average disease severity rating = 4.2) when compared with the barley source (average disease severity rating = 5.9). Overall disease severity could have been affected by the different soil types used in each of these experiments. Issues with other soil pathogens, such as *Fusarium* spp., resulted in the switch from a pasteurized field soil in *Experiment 1* to a professional potting mix in *Experiment 2*. The presence of additional soil pathogens in these soil media sources could have resulted in differences between these experiments. In addition, sugarbeet in *Experiment 2* were fertilized with a micronutrient solution and this may explain why herbicide had an influence on disease severity in *Experiment 1*, but not in *Experiment 2*. Previous studies have demonstrated that some glyphosate-resistant soybean varieties exhibit an increase in manganese (Mn) deficiency symptomalogy than conventional varieties (Dodds et al. 2001, 2002; Loecker et al. 2010). Although this appears to be variety specific and more problematic in severe Mn-deficient soils, possible interactions between micronutrient applications to glyphosate-resistant in *Experiment 2* could explain differences in the level of disease severity and response to herbicides when compared with *Experiment 1*.

We also observed a difference in the ranking of Rhizoctonia tolerance among the varieties when comparing the greenhouse and field experiments. Although Hilleshög 9027RR was the most Rhizoctonia tolerant variety in two of the three experiments, it appears there may not be vast differences in the tolerance levels within the three Hilleshög varieties (9027RR, 9028RR, and 9029RR). However, Crystal RR827 was always the most susceptible variety to Rhizoctonia crown and root rot. In addition, the micronutrient solution may also have resulted in differing results among the varieties. A

micronutrient solution was added to sugarbeet in *Experiment 2* because sugarbeet showed boron deficiency. Hilleshög 9027RR appeared to demonstrate the most severe deficiency symptoms of the four varieties and this may explain the difference in ranking of Rhizoctonia tolerance for *Experiment 2* when compared with *Experiment 1* and the field experiment.

Our results indicate that glyphosate does not influence disease severity of Rhizoctonia crown and root rot in four commercially-available varieties of glyphosate-resistant sugarbeet. Growers can make several glyphosate applications to glyphosate-resistant sugarbeet varieties without increasing susceptibility to Rhizoctonia crown and root rot. Although greenhouse *Experiment 1* indicated that glyphosate may increase disease severity, glyphosate applications did not influence disease severity in additional field and greenhouse experiments. Variety selection is the most important factor in reducing disease severity of Rhizoctonia crown and root rot in glyphosate-resistant sugarbeet. To prevent yield and sugar quality loss, using a variety with excellent tolerance to *R. solani* is recommended.

SOURCES OF MATERIALS

[1] Syngenta Seeds Inc., 1020 Sugarmill Rd., Longmont, CO 80501.

[2] Roundup WeatherMAX, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[3] Betamix, Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein, Germany.

[4] UpBeet, E.I. du Pont de Nemours and Co., Crop Protection, 1007 Market St., Wilmington, DE 19898.

[5] Stinger, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268.

[6] Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187.

[7] Becton & Dickinson, and Co., 7 Loveton Circle, Sparks, MD 21152.

[8] Sigma Chemical Co., 6050 Spruce St., St. Louis, MO 63103.

[9] BetaSeed, Inc., 1788 Marschall Road, Shakopee, MN 55379.

[10] Gandy Company, 528 Gandrud Road, Owatonna, MN 55060.

[11] AirMix 11003, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

[12] Tractor Supply Company, 200 Powell Place, Brentwood, TN 37027.

[13] Michigan Automated Weather Network, Web site:

http://www.agweather.geo.msu.edu/

[14] Baccto Professional Potting Mix, Michigan Peat Company, P.O. Box 980129, Houston, TX 77098.

[15] MicroMax, Grace-Sierra, 1001 Yosemite Dr., Milpitas, CA 95035.

[16] The SAS System for Windows, Version 9.1, SAS Institute, Inc., 100 SAS Campus Dr., Cary NC 27513.

[17] TableCurve 2D 5.01, Systat Software Inc., 501 Canal Blvd., Richmond, CA 94804-2028.

Table 1. Monthly precipitation and the 30-year average for experiments located in the Saginaw Valley region of Michigan in 2008 and 2009.

	Precipitation (mm)		
	2008	2009	30 yr.
April	51	119	72
May	29	31	71
June	99	122	83
July	100	69	70
August	53	88	96
Total	332	429	392

[a] Precipitation data was collected from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/).

Table 2. Response of three glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in the presence and absence of herbicides.

Herbicide treatment	H 9027RR	H 9028RR	H 9032RR
	disease severity (0 - 7 scale)[c]		
No herbicide	2.8 ab[d]	4.8 cde	4.9 de
Standard conventional program[b]	4.0 abcd	4.7 cde	2.5 a
Glyphosate (0.84 kg ae/ha)	4.7 cde	4.4 cde	3.0 abc
Glyphosate (1.68 kg ae/ha)	5.9 e	4.7 cde	4.0 abcd

[a] *Rhizoctonia solani* inoculum was prepared with a millet medium.

[b] The standard conventional herbicide program included phenmedipham at 270 g ai/ha plus desmedipham at 270 g ai/ha, triflusulfuron at 9 g ai/ha, and clopyralid at 104 g ai/ha.

[c] Sugarbeet roots were rated for disease severity on a 0 to 7 scale (0 = no disease and 7 = completely rotted).

[d] Means followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 3. Fresh weights of three glyphosate-resistant sugarbeet varieties exposed to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in the presence and absence of herbicides.

Herbicide treatment	H 9027RR	H 9028RR	H 9032RR
	% of non-inoculated[c]		
No herbicide	83 ab[d]	54 bc	33 cd
Standard conventional program[b]	59 abc	41 cd	91 a
Glyphosate (0.84 kg ae/ha)	44 cd	62 abc	55 bc
Glyphosate (1.68 kg ae/ha)	22 d	46 cd	63 abc

[a] *Rhizoctonia solani* inoculum was prepared with a millet medium.

[b] The standard conventional herbicide program included phenmedipham at 270 g ai/ha plus desmedipham at 270 g ai/ha, triflusulfuron at 9 g ai/ha, and clopyralid at 104 g ai/ha.

[c] Fresh weights were determined by dividing the fresh weight of the Rhizoctonia-inoculated plants by the fresh weight of non-inoculated plants for each treatment.

[d] Means followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 4. Mycelial growth of *Rhizoctonia solani*[a] isolate AG-2-2-IIIB *in vitro* in the presence of varying rates of glyphosate and a standard sugarbeet herbicide mixture. Glyphosate data are combined over treatments with and without ammonium sulfate since there was not a significant difference in the rate of mycelial growth for these treatments.

Herbicide treatment	Rate	Mycelial growth rate[b]
	— µg/ml —	———— cm/d ————
Control	—	1.05 b[c]
Glyphosate (0.5X)	9.5	1.05 b
Glyphosate (1X)	19	1.03 b
Glyphosate (2X)	38	1.02 ab
Glyphosate (10X)	190	0.96 a
Phenmedipham + desmedipham + triflusulfuron + clopyralid	6 + 6 + 0.2 + 2.4	1.04 b

[a] *Rhizoctonia solani* inoculum was prepared with a millet medium.

[b] Growth rate was determined by the slope for mycelial growth from days 1 to 4 (cm/day).

[c] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 5. P-values for main effects and interactions of herbicide treatments and four *Rhizoctonia solani* inoculated glyphosate-resistant sugarbeet varieties for field experiments conducted in 2008 and 2009.

Effects[a]	Disease index[b]	Harvestable[c]	Healthy[d]
	p-value		
Herbicide	0.8762	0.9714	0.5835
Variety	<0.0001	<0.0001	0.5152
Variety x herbicide	0.9904	0.9991	0.7081

[a] Inoculation was removed from further analysis since it was highly significant and non-inoculated plants had a disease severity rating of less than 2.

[b] Disease is rated based on a 0 to 7 scale (0 = no disease and 7 = completely rotted) and the disease index is calculated by determining a weighted average based on the number of sugarbeet in each of the eight disease classes.

[c] Harvestable sugarbeet is the percentage of sugarbeet in the plot with a disease severity rating of 3 or less.

[d] Healthy sugarbeet is the percentage of sugarbeet in the plot with a disease severity rating of 1 or less.

Table 6. Response of four glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in field experiments conducted in 2008 and 2009. Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

Variety	Disease index[b]	Harvestable[c]	Healthy[d]
	0 - 7 scale	%	%
Hilleshog 9027RR	5.5 a[e]	15 a	2 a
Hilleshog 9028RR	5.9 b	9 b	1 a
Hilleshog 9029RR	5.7 ab	12 ab	1 a
Crystal RR827	6.6 c	2 c	0 a

[a] Inoculation was removed from further analysis since it was highly significant and non-inoculated plants had a disease severity rating of less than 2.

[b] Disease is rated based on a 0 to 7 scale (0 = no disease and 7 = completely rotted) and the disease index is calculated by determining a weighted average based on the number of sugarbeet in each of the eight disease classes.

[c] Harvestable sugarbeet is the percent of sugarbeet in the plot with a disease severity rating of 3 or less.

[d] Healthy sugarbeet is the percent of sugarbeet in the plot with a disease severity rating of 1 or less.

[e] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 7. P-values for main effects and interactions of herbicide treatments on *Rhizoctonia solani*[a] isolate AG-2-2-IIIB disease severity and plant fresh weight of four glyphosate-resistant sugarbeet varieties for greenhouse *Experiment 2.*

Effects[b]	Disease severity	Fresh weight
	p-value	
Herbicide	0.3672	0.2024
Variety	< 0.0001	0.0012
Variety x herbicide	0.2330	0.1667

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Inoculation was removed from further analysis since it was highly significant and non-inoculated plants had a disease severity rating of less than 1.

Table 8. Response of four glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in greenhouse *Experiment 2*. Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

Variety	Disease severity[b]	Fresh weight[c]
	0 - 7 scale	% of non-inoculated
Hilleshog 9027RR	5.9 b[d]	22 b
Hilleshog 9028RR	4.8 a	39 a
Hilleshog 9029RR	6.1 bc	25 b
Crystal RR827	6.7 c	13 b

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Sugarbeet roots were rated for disease severity on a 0 to 7 scale (0 = no disease and 7 = completely rotted).

[c] Fresh whole weight is determined by weighing the whole plant and dividing that weight by the weight of the same non-inoculated treatment.

[d] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

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CHAPTER 3

INFLUENCE OF GLYPHOSATE AND FUNGICIDE TREATMENTS ON RHIZOCTONIA CROWN AND ROOT ROT IN GLYPHOSATE-RESISTANT SUGARBEET

Abstract: A field experiment was conducted in 2008 and 2009 in the Saginaw Valley region of Michigan to determine if there were potential interactions between applications of glyphosate and the fungicide azoxystrobin and to determine the effectiveness of foliar and in-furrow azoxystrobin applications when *Rhizoctonia solani* is present. Significant differences in disease indices, percentage of harvestable sugarbeet, and percentage of healthy sugarbeet were evident among the different varieties and fungicide treatments of azoxystrobin, but herbicide treatment did not significantly affect these parameters. Hilleshög 9027RR and Hilleshög 9029RR had the lowest disease indices and highest percentage of healthy sugarbeet when compared with Crystal RR827 and Hilleshög 9028RR. When compared with the in-furrow application or no fungicide treatment, foliar fungicide applications of azoxystrobin resulted in the lowest disease index (2.0) and highest percentage of healthy sugarbeet (42 %). In-furrow fungicide application of azoxystrobin reduced the disease index when compared with no fungicide application. Similar trends were observed for harvestable sugarbeet, except for Crystal RR827 where there was not a significant difference between the in-furrow azoxystrobin application and no fungicide treatment. Hilleshög 9027RR and Hilleshög 9029RR exhibited the most tolerance to Rhizoctonia crown and root rot. Hilleshög 9028RR appeared to be moderately tolerant and Crystal RR827 was the most susceptible of the four glyphosate-

resistant sugarbeet varieties. Foliar fungicide applications of azoxystrobin resulted in the lowest disease index and highest percentage of healthy and harvestable sugarbeet when compared with the in-furrow application or no fungicide treatment. Glyphosate did not affect the efficacy of fungicide treatments, but choosing a Rhizoctonia-tolerant variety and applying foliar fungicide applications appear to the best methods for managing Rhizoctonia crown and root in glyphosate-resistant sugarbeet.

Nomenclature: Glyphosate; standard-split; azoxystrobin; Rhizoctonia crown and root rot, *Rhizoctonia solani* Kühn; sugarbeet, *Beta vulgaris* L.

Key words: Glyphosate-resistant crops; disease index; healthy sugarbeet; harvestable sugarbeet

Introduction

Glyphosate is the most widely used herbicide in the world due to its ability to control a broad spectrum of annual and perennial broadleaf and grass weed species (Duke and Powles 2008; Pline-Srnic 2005). The introduction of glyphosate-resistant crops in 1996 changed the way many growers approach weed management. Growers widely adopted glyphosate-resistant crops because glyphosate made weed control easier and more effective with fewer applications, reduced the need for tillage, did not restrict crop rotations, and increased profitability (Green 2009). Currently, there are six commercialized glyphosate-resistant crops: soybean [*Glycine max* (L.) Merr], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L.), alfalfa (*Medicago sativa* L.) and, most recently in 2008, sugarbeet (*Beta vulgaris* L.). Glyphosate-resistant sugarbeet varieties were quickly adopted by growers in Michigan. Approximately 98%

of Michigan's sugarbeet hectares were planted with a glyphosate-resistant variety in 2009 (C. Guza, Agronomist, Michigan Sugar Company, Bay City, MI, personal communication).

The use of glyphosate in glyphosate-resistant sugarbeet provides growers the opportunity to achieve excellent control of many weed species that can affect sugarbeet yield and quality (Kniss et al. 2004). Conventional postemergence (POST) herbicides do not effectively control weeds with more than two leaves, so many herbicide applications are necessary and seldom result in 100% control (Dale et al. 2006; Dale and Renner 2005). Additionally, the time between herbicide applications in glyphosate-resistant sugarbeet may be longer when compared with conventional sugarbeet herbicide programs, because weed height at the time of application is generally not as limiting with glyphosate. Kemp et al. (2009) determined that fewer herbicide applications were required to improve weed control and yields in glyphosate-resistant sugarbeet. Growers can also adjust production practices, such as narrowing row width, to obtain higher yields and therefore greater economic returns despite the additional seed costs associated with using glyphosate-resistant sugarbeet varieties (Armstrong 2009). Glyphosate is less expensive when compared with conventional sugarbeet weed control programs and the potential for greater economic return is possible with fewer herbicide applications, improved weed control, and increased yields (Kniss et al. 2004).

However, one potential issue with glyphosate-resistant sugarbeet is the possible increase in diseases caused by soil-borne pathogens. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, an important component in the shikimate acid pathway. This pathway produces aromatic amino acids and

secondary compounds important for plant growth and protection (Amrhein et al. 1980; Bentley 1990; Dill 2005; Siehl 1997). While glyphosate-resistant crops have a form of the EPSPS enzyme that is not affected by glyphosate, this enzyme may not be as efficient as native EPSPS when exposed to glyphosate and therefore may result in reduced production of secondary compounds (Pline-Srnic 2005).

Studies in glyphosate-resistant crops, including glyphosate-resistant sugarbeet, have indicated a potential for increased susceptibility to some soil-borne pathogens after glyphosate was applied (Larson et al. 2006; Sanogo et al. 2000; Sanogo et al. 2001). Greenhouse and field studies with glyphosate-resistant soybean showed that these plants were more susceptible to sudden death syndrome, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*, after glyphosate was applied (Sanogo et al. 2000; Sanogo et al. 2001). Larson et al. (2006) determined that experimental varieties of glyphosate-resistant sugarbeet were more susceptible to certain isolates of both *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht. f. sp. *betae* Snyd. & Hans. after exposure to glyphosate.

In contrast, other studies demonstrated that glyphosate applications had no effect, or reduced the severity of diseases caused by soil-borne pathogens (Njiti et al. 2003; Pankey et al. 2005). In glyphosate-resistant soybean, Njiti et al. (2003) determined glyphosate had no effect on soybean yield or disease severity of sudden death syndrome. These results conflicted with greenhouse and field results reported by Sanogo et al. (2000) and (2001). There were differences between these studies concerning variety selection and varietal response to the disease. In addition, there were differences in environmental factors such as planting date, genotype, and other soil factors. This may explain why glyphosate has no effect on *F. solani* disease severity in certain varieties, but

increases disease severity in others. In glyphosate-resistant cotton, greenhouse experiments conducted by Pankey et al. (2005) showed that glyphosate had no effect on damping off or soreshin (caused by the pathogen *R. solani*). Furthermore, in the field, glyphosate actually reduced Rhizoctonia induced disease severity.

Rhizoctonia crown and root rot, caused by the soil-borne pathogen *Rhizoctonia solani*, is a problematic disease in many crops in Michigan, including sugarbeet (Kirk et al. 2008; Windels et al. 2009). Rhizoctonia crown and root rot reduces economic returns in sugarbeet by as much as 24% in the United States and up to 50% yield loss may result, depending on disease severity (Franc et al. 2001; Windels et al. 2009). The first symptoms of Rhizoctonia crown and root rot are foliar, consisting of a permanent wilting of leaves and dark lesions at the base of the petiole or on the crown of the beet. Sugarbeet leaves become dry and collapse, but remain attached to the crown and form a dry, dark rosette. Root symptoms include dark lesions that begin anywhere on the root, but may grow together and cover the entire root surface as the disease progresses. Root tissue is typically firm underneath these lesions. However, root tissue will begin to soften underneath these lesions and cracks may also develop in advanced stages of the disease.

Rhizoctonia solani has many host crops in addition to sugarbeet, which makes it difficult to control with crop rotation alone (Rush and Winter 1990; Schuster and Harris 1960). Soybean, dry bean (*Phaseolus vulgaris* L.), corn, and many weed species are alternate hosts for Rhizoctonia, further increasing the buildup of disease inoculum (Windels et al. 2009). The availability of sugarbeet varieties tolerant to Rhizoctonia crown and root rot provides an additional option to manage this disease, and varieties with varying levels of tolerance are readily available to Michigan sugarbeet growers.

Although these varieties do not completely prevent infection, they certainly limit fungal colonization and disease severity (Ruppel 1973).

Additional methods for controlling Rhizoctonia crown and root rot in sugarbeet include applications of strobilurin fungicides, such as azoxystrobin. Single fungicide treatments are typically applied either in-furrow at planting or postemergence (POST) to sugarbeet at the 4- to 8-leaf stage (Karaolglanidis and Karadimos 2006; Whitney and Duffus 1986). In-furrow applications of azoxystrobin can reduce infection early in the season, but may not prevent later infections (Karaoglanidis and Karadimos 2006; Kiewnick et al. 2001; Jacobsen et. al. 1999; Windels and Brantner 2000). If glyphosate-resistant sugarbeet are more susceptible to plant pathogens after glyphosate is applied, then fungicide applications may be important in controlling sugarbeet diseases such as Rhizoctonia crown and root rot. Therefore, the objectives of this research were to: 1) investigate potential interactions between glyphosate and fungicide applications of azoxystrobin on management of Rhizoctonia crown and root rot in four glyphosate-resistant sugarbeet varieties, and 2) determine the effectiveness of in-furrow and foliar applications of azoxystrobin when *Rhizoctonia solani* is present.

Materials and Methods

A field experiment was conducted in 2008 and 2009 in the Saginaw Valley region of Michigan. The 2008 experiment was located in St. Charles, Michigan on a Misteguay silty clay (fine, mixed, semiactive, calcareous, mesic Aeric Endoaquepts) soil with a pH of 7.8 and 3.0 % organic matter. The 2009 experiment was located in Frankenmuth, Michigan and the soil type was a Tappan-Londo complex (fine-loamy, mixed, active,

calcareous, mesic Typic Endoaquolls) with a pH of 7.7 and 2.4 % organic matter. Experiments followed dry bean in both 2008 and 2009. Fields were fall-chisel plowed followed by spring field cultivation twice prior to planting. Fertilizer applications were standard for sugarbeet production in Michigan. The glyphosate-resistant sugarbeet varieties Crystal RR827[1], Hilleshög 9027RR[2], Hilleshög 9028RR, and Hilleshög 9029RR were planted 2.5-cm deep in 76-cm rows at a population of 122,000 seeds/ha on April 25, 2008 and April 16, 2009. Plots were six rows wide by 9.1 m in length. Each variety was planted, one per row, in rows two through five. Rows one and six served as border rows. Commercial sugarbeet varieties selected for this experiment were approved by Michigan Sugar Company and were thought to have varying degrees of Rhizoctonia crown and root rot tolerance.

The experimental design was a split-strip-plot with four replications. The main plot was herbicide treatment, the sub-plot was fungicide treatment, and the strip-plot was variety. Herbicide treatments consisted of a glyphosate program, a standard-split program (used in conventional sugarbeet), and a hand-weeded control (no herbicide). The glyphosate program consisted of glyphosate[3] at 0.84 kg ae/ha plus ammonium sulfate at 2% v/v, applied three times at 2- to 4-leaf, 4- to 6-leaf, and 6- to 8-leaf sugarbeet. The standard-split program consisted of a combination of desmedipham at 180 g/ha plus phenmedipham[4] at 180 g ai/ha, triflusulfuron[5] at 9 g ai/ha, clopyralid[6] at 104 g ai/ha, and non-ionic surfactant at 0.25% v/v, applied twice when sugarbeet was at the cotyledon to 2-leaf and 2- to 4-leaf stages. The rates of desmedipham plus phenmedipham were each increased to 270 g ai/ha for the second application. All plots

were maintained weed-free by hand-weeding throughout the growing season. Plots were rated for herbicide injury 7 days after the last herbicide application timing. Fungicide treatments consisted of azoxystrobin[7] applied in-furrow at planting at 140 mg ai/m of row, foliar applications of azoxystrobin at 0.82 kg ai/ha to 4- to 6-leaf sugarbeet, and a no-fungicide control. Foliar applications of azoxystrobin were tank-mixed and applied with glyphosate for the glyphosate program. POST herbicide and fungicide treatments were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 178 L/ha at 207 kPa through 10003 AirMix[8] nozzles. Nozzles were spaced 51 cm apart and were positioned approximately 56 cm above the sugarbeet canopy.

All plots were inoculated with *R. solani* AG-2-2-IIIB when sugarbeet was at the 6- to 8-leaf stage. Subgroup AG-2-2-IIIB is the most common and virulent *R. solani* subgroup found in Michigan (Kirk et al. 2008). *R. solani* inoculum was produced in bulk on a barley medium. Pans of barley, saturated with water, were autoclaved and 9 plugs (7 mm) of *R. solani* grown on potato dextrose agar were placed into the pans. The pans were sealed with Parafilm and incubated at 25 C $\pm$ 2 for 3 wk. Once the barley was colonized, it was air dried and ground into a fine flour. Inoculum was applied directly over each sugarbeet row at 2 g/m using a modified drop spreader.[9] Rate was confirmed by determining the amount of leftover inoculum and calculating the kg applied per m of row. All plots were cultivated following inoculation to put soil and inoculum in the crown for increased disease severity (Ruppel et al. 1979).

Sugarbeet stand counts were recorded for each variety at 4 wk after planting and at harvest. Approximately 8 wk after inoculation, sugarbeet were lifted from the soil

using a modified lift harvester.[10] Each sugarbeet root was rated for disease severity using the 0 to 7 Rhizoctonia crown and root rot rating scale as follows: 0 – no visible signs of disease; 1 = inactive lesions; 2 = less than 5 % active lesions; 3 = 6 to 25 % of the root rotted; 4 = 26 to 50% of the root rotted; 5 = 51 to 75 % of the root rotted; 6 = greater than 75 % of the root rotted, but still some living tissue; 7 = roots completely rotted and dead (Ruppel et al. 1979). Stand counts were used to determine how many sugarbeet were missing from each plot due to advanced disease severity. Values were adjusted by assigning each of the missing sugarbeet a disease severity rating of 7. An average disease index was determined for each variety in each plot. The disease index was calculated as a weighted average based on the number of sugarbeet in each of the eight disease classes (Ruppel et al. 1979). Healthy sugarbeet were determined by calculating the percent of sugarbeet that had a disease severity rating of 0 or 1. Harvestable sugarbeet were determined by calculating the percent of sugarbeet with a disease severity rating of 3 or less.

Precipitation data was recorded by weather stations operated by the Michigan Automated Weather Network[11] (Table 9). Weather stations were located within 3 km of the experimental locations.

Data were analyzed using the PROC MIXED procedure in SAS 9.1.[12] An analysis of variance was performed and treatment means for disease index, percent of healthy sugarbeet, and percent of harvestable sugarbeet were compared using Fisher's Protected LSD at the $p \leq 0.05$ significance level. Interactions between main effects were analyzed using the SLICE option in the LSMEANS statement. Data were combined

across year, variety, herbicide treatment, or fungicide treatment when interactions were not significant.

Results and Discussion

Herbicide Injury. The glyphosate-resistant sugarbeet varieties did not show visible signs of damage from glyphosate treatments. However, applications of the standard-split herbicide program uniformly caused 13% injury for each of the four glyphosate-resistant sugarbeet varieties evaluated (data not shown). Injury symptoms consisted of yellowing and stunting when compared with the non-treated control, which are consistent with what others have observed with this combination (Wilson 1994, 1995). Approximately 2 wks after this evaluation sugarbeet recovered from this damage. In-furrow or foliar applications of azoxystrobin neither significantly increased nor decreased herbicide injury. An increase in herbicide injury was a potential concern with the glyphosate and azoxystrobin tank-mixture, since previous research has indicated an increase in sugarbeet injury from tank-mixtures of azoxystrobin and other sugarbeet herbicides (Sprague et al. 2005).

Effect of Variety, Herbicide, and Fungicide on Rhizoctonia Crown and Root Rot. *Rhizoctonia solani* subgroup AG-2-2-IIIB inoculation was highly effective. The combination of cultivation and precipitation (Table 9) following Rhizoctonia inoculation resulted in an average disease index of 5.9 in the non-fungicide controls which provided a good basis for treatment separation. Natural *R. solani* infestations were low each year

based on disease index (2 or less) evaluations taken in adjacent non-inoculated sugarbeet plots.

Interactions between the years were not significant, therefore all data are presented as a combination of the 2008 and 2009 experiments. The three-way interaction of variety x herbicide x fungicide was not significant for any of the parameters evaluated (Table 10). All two-way interactions were not significant for any of the parameters measured, except for the variety x fungicide interaction for the percentage of harvestable sugarbeet. Therefore, data are discussed as the main effects of variety, herbicide, and fungicide, except for the variety x fungicide interaction for the percentage of harvestable sugarbeet.

Variety. There was a difference in how the four glyphosate-resistant sugarbeet varieties responded to inoculation of *R. solani.* Averaged across herbicide and fungicide treatments, Hilleshög 9027RR and Hilleshög 9029RR were the most tolerant varieties to *R. solani* subgroup AG-2-2-IIIB with disease index evaluations of 3.6 and 3.7, respectively (Table 11). Crystal RR827 was the most susceptible glyphosate-resistant variety with a disease index of 4.7 and Hilleshög 9028RR showed moderate tolerance with a disease index of 4.0. The percentage of healthy sugarbeet, based on disease severity ratings of 1 or less, followed a similar trend. The percentage of healthy sugarbeet was less than 25% for all four glyphosate-resistant sugarbeet varieties. However, the percentage of healthy sugarbeet for the most susceptible variety, Crystal RR827 (14%), was considerably lower than the more Rhizoctonia tolerant varieties, Hilleshög 9027RR and Hilleshög 9029RR (Table 11). As observed with other studies,

varieties have varying levels of Rhizoctonia crown and root rot susceptibility and tolerance (Ruppel 1973). Although Hilleshög 9027RR and Hilleshög 9029RR do not completely prevent *R. solani* infection, they exhibited more tolerance and are more effective at managing Rhizoctonia crown and root rot when compared with Crystal RR827.

Herbicide. One of the objectives was to determine if there were interactions between glyphosate and fungicide applications on Rhizoctonia crown and root rot. There were no significant interactions with herbicide and the main effect of herbicide was not significant (Table 10). This indicated that glyphosate had no influence on the disease index, the percentage of harvestable sugarbeet, or percentage of healthy sugarbeet when compared with the standard-split or no herbicide treatments. This is in contrast to what Larson et al. (2006) observed in greenhouse experiments with non-commercial glyphosate-resistant sugarbeet varieties. Their results indicated that a glyphosate-resistant sugarbeet variety with tolerance to Rhizoctonia crown and root rot demonstrated increased susceptibility to the disease after glyphosate was applied. The increased disease severity did not appear to be a fungal response because there was not a significant difference in the growth rate of *Rhizoctonia solani* or in the production of sclerotia after exposure to glyphosate. They concluded that differences in disease severity were explained by a particular cultivar or isolate pathogen response. Only one of the glyphosate-resistant varieties demonstrated a significant increase in disease severity with AG-2-2-IIIB (not AG-2-2-IV) after glyphosate application. In addition, other studies suggest the timing of glyphosate application in relation to disease infection is important. In our field experiment,

sugarbeet were inoculated days after the last herbicide application. However, if sugarbeet were inoculated prior to herbicide applications, it may have influenced disease severity differently than what was observed in our study. Experiments with glyphosate-resistant wheat (*Triticum aestivum* L.) have indicated that glyphosate actually decreased disease severity of leaf rust (caused by the pathogen *Puccinia triticina*) and stem rust fungus (cause by the pathogen *Puccinia graminis* f. sp. *tritici* Eriks) when exposed to glyphosate 21 d to 35 d after inoculation (Anderson and Kolmer 2005). This may explain why differences between herbicide treatments were not observed in our field experiment, while greenhouse studies by Larson et al. (2006) indicated that glyphosate applications increased disease severity.

Herbicides may synergize or antagonize fungicide activity against different diseases in different crops. Kataria and Gisi (1990) found that when used alone in wheat, the herbicides DNOC, dicamba, ioxynil, and bromoxynil had a low to moderate effect on reducing the disease severity of *Rhizoctonia cerealis* Van der Hoeven and *Pseudocercosporella herpotrichoides* (Fron) Deighton. However, herbicide combinations with the fungicide cyproconazole were synergistic and effective in reducing the disease severity. Hill and Stratton (1991) concluded from *in vitro* tests, that the herbicide metribuzin, when used in combination with the fungicide chlorothalonil, was antagonistic and reduced efficacy on *Alternaria solani* (Ell. And Martin) Sor. Unlike these examples, the herbicide treatments in our field trial did not synergize or antagonize Rhizoctonia crown and root rot management with azoxystrobin.

Fungicide. The main effect of fungicide was significant for Rhizoctonia disease indices and the percentage of healthy sugarbeet (Table 10). Combined across all varieties and herbicide treatments, foliar application of azoxystrobin to 4- to 6- leaf sugarbeet provided the greatest suppression of Rhizoctonia crown and root rot (Table 12). Foliar applications of azoxystrobin resulted in a disease index rating of 2.0 and 42% of the sugarbeet were considered healthy (disease severity rating of one or less). This was in contrast to the no fungicide treatment where the disease index rating was 4.0 and only 1% of the sugarbeet were considered healthy. In-furrow applications of azoxystrobin also provided some protection against Rhizoctonia crown and root rot. However, in-furrow applications were not as effective as foliar applied azoxystrobin (Table 12). Others have reported that in-furrow applications of azoxystrobin were just as effective as foliar applications to 4- to 6-leaf sugarbeet in reducing Rhizoctonia crown and root rot (Kirk et al. 2008). Differences in the results of our experiment may be related to the timing of *R. solani* inoculation, which occurred when sugarbeet was at the 6- to 8-leaf stage. In-furrow azoxystrobin applications may be more effective against earlier infections of *R. solani* and may not last long enough to prevent later infections. In addition, method of fungicide application may also have influenced fungicide efficacy. In-furrow applications were banded onto the rows; therefore soil in between rows would not have been treated. Foliar applications were broadcast applied to the sugarbeet in the rows, as well as soil in between the rows, and this may have been more effective in reducing disease severity of Rhizoctonia crown and root rot. Several studies indicate that environmental factors influence fungicide efficacy, therefore variations in temperature and moisture may explain differences between in-furrow and foliar applications.

Previous studies indicated that fungicide treatments applied between 18 and 21 C are optimal for disease management, therfore later fungicide application timings are more effective with cool, spring temperatures (Jacobsen et al. 2004; Poindexter 2010). In addition, Stump et al. (2004) determined that fungicide treatments applied at the time of inoculation resulted in the lowest disease severity and that treatments (in-furrow) applied at planting were too early for optimal control of Rhizoctonia crown and root rot in sugarbeet.

Harvestable Sugarbeet. There was a fungicide by variety interaction for the percentage of harvestable sugarbeet. Sugarbeet that were considered harvestable have a disease severity rating of 3 or less, which means that less than 25% of the sugarbeet is rotted and there are no deep penetrating cracks. Regardless of variety, fewer than 20% of sugarbeet were harvestable when a fungicide was not applied (Table 13). In-furrow and foliar applications of azoxystrobin increased the number of harvestable sugarbeet for all varieties, excluding the in-furrow azoxystrobin treatment on the most susceptible variety, Crystal RR827. A foliar application of azoxystrobin was the only treatment that improved the percentage of harvestable sugarbeet for this variety (73%). In contrast, Hilleshög 9027RR, Hilleshög 9028RR, and Hilleshög 9029RR benefited from both in-furrow and foliar applications of azoxystrobin for the percentage of harvestable sugarbeet (Table 13), although the foliar azoxystrobin application resulted in the greatest percentage of harvestable sugarbeet (88% or greater).

In summary, the four glyphosate-resistant sugarbeet varieties that we investigated had a range of responses to *R. solani*. Hilleshög 9027RR and Hilleshög 9029RR were

most tolerant, Hilleshög 9028RR was moderately tolerant, and Crystal RR827 was the most susceptible variety to Rhizoctonia crown and root rot. Herbicide treatment, whether it was the glyphosate program or the standard conventional herbicide program, did not affect Rhizoctonia crown and root rot development or management in the field. This is in contrast to a greenhouse study by Larson et al. (2006) where applications of glyphosate increased the disease severity of Rhizoctonia crown and root rot in a Rhizoctonia-tolerant glyphosate-resistant sugarbeet variety. Across the four glyphosate-resistant sugarbeet varieties, a foliar application of azoxystrobin provided the most protection against Rhizoctonia crown and root rot. However, both foliar and in-furrow applications of azoxystrobin reduced the disease index and resulted in more healthy and harvestable sugarbeet than treatments lacking a fungicide application. The exception was Crystal RR827, the most susceptible variety to *R. solani*, where harvestable sugarbeet did not differ between the in-furrow fungicide treatment and no fungicide application. From this field research, there is no evidence that Michigan sugarbeet growers should be concerned about the potential for an increase in Rhizoctonia crown and root rot in glyphosate-resistant sugarbeet when glyphosate is applied. Choosing varieties that exhibit some tolerance to Rhizoctonia crown and root rot and applying a fungicide like azoxystrobin will be the key factors to help growers manage this disease.

Sources of Materials

[1] BetaSeed, Inc., 1788 Marschall Road, Shakopee, MN 55379.

[2] Syngenta Seeds Inc., 1020 Sugarmill Rd., Longmont, CO 80501.

[3] Roundup WeatherMAX, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[4] Betamix, Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein, Germany.

[5] UpBeet, E.I. du Pont de Nemours and Co., Crop Protection, 1007 Market St., Wilmington, DE 19898.

[6] Stinger, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268.

[7] Quadris, Syngenta International AG, P.O. Box CH – 4002, Basel, Switzerland

[8] AirMix 11003, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

[9] Gandy Company, 528 Gandrud Road, Owatonna, MN 55060.

[10] Tractor Supply Company, 200 Powell Place, Brentwood, TN 37027.

[11] Michigan Automated Weather Network, Web site: http://www.agweather.geo.msu.edu/

[12] The SAS System for Windows, Version 9.1, SAS Institute, Inc., 100 SAS Campus Dr., Cary NC 27513.

Table 9. Monthly precipitation[a] and the 30-year average for experiments located in the Saginaw Valley region of Michigan in 2008 and 2009.

	Precipitation (mm)		
	2008	2009	30 yr.
April	51	119	72
May	29	31	71
June	99	122	83
July	100	69	70
August	53	88	96
Total	332	429	392

[a] Precipitation data was collected from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/).

Table 10. P-values for main effects and interactions of herbicide and fungicide treatments on *Rhizoctonia solani*[a] AG-2-2-IIIB disease index and healthy and harvestable sugarbeet of four glyphosate-resistant sugarbeet varieties. Data are combined across years.

	Disease index	Harvestable sugarbeet	Healthy sugarbeet
		p-value	
Variety	<0.0001	<0.0001	0.0248
Herbicide	0.6361	0.5194	0.9533
Fungicide	0.0003	0.0006	<0.0001
Variety x herbicide	0.9514	0.9729	0.9326
Variety x fungicide	0.4919	0.0045	0.4484
Herbicide x fungicide	0.7364	0.5717	0.5662
Variety x herbicide x fungicide	0.9999	0.9971	0.9966

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

Table 11. Disease index ratings and percent healthy sugarbeet of four glyphosate-resistant sugarbeet varieties inoculated with *Rhizoctonia solani.*[a] Data are combined across herbicide treatments, fungicide treatments, and years.

Variety	Disease index[b]	Healthy sugarbeet[c]
	0 to 7 scale	%
Hilleshog 9027RR	3.6a[d]	20a
Hilleshog 9028RR	4.0b	19ab
Hilleshog 9029RR	3.7a	22a
Crystal RR827	4.7c	14b

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Disease is rated based on a 0 to 7 scale (0 = no disease and 7 = completely rotted) and the disease index is calculated by determining a weighted average based on the number of sugarbeet in each of the eight disease classes.

[c] Healthy sugarbeet is determined by calculating the percent of sugarbeet that have a disease severity rating of 0 or 1.

[d] Means followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 12. Disease index ratings and percent healthy sugarbeet for fungicide treatments applied to glyphosate-resistant sugarbeet inoculated with *Rhizoctonia solani.*[a] Data are combined across varieties, herbicide treatments, and years.

Fungicide	Rate	Disease index[b]	Healthy sugarbeet[c]
		0 to7 scale	%
Foliar azoxystrobin	0.8 kg/ha	2.0a[d]	42a
In-furrow azoxystrobin	140 g/m row	4.0b	13b
No fungicide	—	5.9c	1c

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Disease is rated based on a 0 to 7 scale (0 = no disease and 7 = completely rotted) and the disease index is calculated by determining a weighted average based on the number of sugarbeet in each of the eight disease classes.

[c] Healthy sugarbeet is determined by calculating the percent of sugarbeet that have a disease severity rating of 0 or 1.

[d] Means followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 13. Percent harvestable[a] sugarbeet for fungicide treatment applied to four glyphosate-resistant sugarbeet varieties inoculated with *Rhizoctonia solani.*[b] Data are combined across herbicide treatments and years.

	Fungicide		
Variety	Foliar azoxystrobin	In-furrow azoxystrobin	No fungicide
	%		
Hilleshog 9027RR	95a[c]	62bcd	15f
Hilleshog 9028RR	88abc	46e	9f
Hilleshog 9029RR	92ab	57cd	12f
Crystal RR827	73de	25fg	2g

[a] Harvestable sugarbeet is determined by calculating the percent of sugarbeet that have a disease severity rating of 3 or less.

[b] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[c] Means followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

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Appendix A: Additional Parameters for Greenhouse *Experiment 1*

Fresh root weight and dry weight were also determined for sugarbeet in greenhouse *Experiment 1*. Fresh root weights were determined by dividing the fresh root weight of the Rhizoctonia-inoculated plants by the fresh root weight of non-inoculated plants for each treatment. Dry weights were determined by dividing the dry weight of the Rhizoctonia-inoculated plants by the dry weight of non-inoculated plants for each treatment. Fresh root weight and dry weight are presented as a percent of the non-inoculated. Data were combined across experiments when interactions were not significant.

Fresh root weight and dry weight followed a similar trend to disease severity and fresh plant weight. Inoculation with *R. solani* AG-2-2-IIIB was significant and the average disease severity for plants that were inoculated was 4.2. Non-inoculated plants were removed for further analysis. In Hilleshög 9027RR, glyphosate at 1.68 kg ae/ha reduced fresh root weight when compared with the no-herbicide control (Table 14). However glyphosate did not affect fresh root weight in Hilleshög 9028RR. In Hilleshög 9032RR glyphosate at 0.84 kg ae/ha and 1.68 kg ae/ha did not reduce fresh root weight when compared with the no-herbicide control, but did significantly reduce fresh root weight when compared with the standard conventional program. In Hilleshög 9027RR, glyphosate at 1.68 kg ae/ha also reduced dry weight when compared with the no-herbicide control (Table 15). Glyphosate did not affect dry weight in Hilleshög 9028RR, However, in Hilleshög 9032RR glyphosate applied at 0.84 kg ae/ha increased dry weight when compared with the no-herbicide control.

Table 14. Fresh root weights of three glyphosate-resistant sugarbeet varieties exposed to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in the presence and absence of herbicides (*Experiment 1*).

Herbicide treatment	H 9027RR	H 9028RR	H 9032RR
	% of non-inoculated[c]		
No herbicide	78ab[d]	44c	34c
Standard conventional program[b]	57bc	32c	97a
Glyphosate (0.84 kg ae/ha)	52bc	56bc	52bc
Glyphosate (1.68 kg ae/ha)	33c	51bc	56bc

[a] *Rhizoctonia solani* inoculum was prepared with a millet medium.

[b] The standard conventional herbicide program included phenmedipham at 270 g ai/ha plus desmedipham at 270 g ai/ha, triflusulfuron at 9 g ai/ha, and clopyralid at 104 g ai/ha.

[c] Fresh root weights were determined by dividing the fresh root weight of the Rhizoctonia-inoculated plants by the fresh root weight of non-inoculated plants for each treatment.

[d] Means followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 15. Dry weights of three glyphosate-resistant sugarbeet varieties exposed to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in the presence and absence of herbicides (*Experiment 1*).

Herbicide treatment	H 9027RR	H 9028RR	H 9032RR
	% of non-inoculated[c]		
No herbicide	85ab[d]	59bcd	48d
Standard conventional program[b]	75abcd	52cd	91a
Glyphosate (0.84 kg ae/ha)	59bcd	70abcd	77abc
Glyphosate (1.68 kg ae/ha)	52cd	64abcd	65abcd

[a] *Rhizoctonia solani* inoculum was prepared with a millet medium.

[b] The standard conventional herbicide program included phenmedipham at 270 g ai/ha plus desmedipham at 270 g ai/ha, triflusulfuron at 9 g ai/ha, and clopyralid at 104 g ai/ha.

[c] Dry weights were determined by dividing the dry weight of the Rhizoctonia-inoculated plants by the dry weight of non-inoculated plants for each treatment.

[d] Means followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Appendix B: Additional Parameters for Greenhouse *Experiment 2*

Fresh root weight and dry weight were also determined for sugarbeet in greenhouse *Experiment 2*. Fresh root weights were determined by dividing the fresh root weight of the Rhizoctonia-inoculated plants by the fresh root weight of non-inoculated plants for each treatment. Dry weights were determined by dividing the dry weight of the Rhizoctonia-inoculated plants by the dry weight of non-inoculated plants for each treatment. Fresh root weight and dry weight are presented as a percent of the non-inoculated. Data were combined across experiments and herbicide treatments when interactions were not significant.

Fresh root weight and dry weight followed a similar trend to disease severity and fresh plant weight. Adequate moisture in the greenhouse following Rhizoctonia inoculation resulted in an average disease severity rating of 5.9. Non-inoculated plants were removed for further analysis. In this greenhouse experiment, herbicide treatment did not influence fresh root weight or dry weight. Therefore, data are combined across herbicide treatment. Hilleshög 9028RR had the highest fresh root weight and dry weight when compared with Hilleshög 9027RR and Crystal RR827 (Table 16). Hilleshög 9029RR also had a higher fresh root weight and dry weight than Crystal RR827, but was not significantly different from Hilleshög 9027RR for these parameters.

Table 16. Response of four glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IIIB in greenhouse *Experiment 2.* Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

Variety	Fresh root weight[b]	Dry weight[c]
	—% of non-inoculated—	—% of non-inoculated—
Hilleshog 9027RR	23bc[d]	41bc
Hilleshog 9028RR	39a	55a
Hilleshog 9029RR	30ab	45ab
Crystal RR827	11c	33c

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Fresh root weight is determined by weighing the root and dividing that weight by the weight of the same un-inoculated treatment.

[c] Dry weight is determined by weighing the whole plant and dividing that weight by the dry weight of the same un-inoculated treatment

[d] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Appendix C: Response of glyphosate-resistant sugarbeet to *R. solani* AG-2-2-IV

An additional greenhouse experiment was conducted to determine the response of five glyphosate-resistant sugarbeet varieties to *R. solani* AG-2-2-IV. Similar methods for previous greenhouse studies were also used in this experiment. Factors included *R. solani* inoculation (inoculated or $_{\text{non-inoculated}}$), sugarbeet variety (Hilleshög 9027RR, Hilleshög 9028RR, Hilleshög 9029RR, Hilleshög 9032RR, and Crystal RR827), and herbicide treatment. Herbicide treatments consisted of two rates of glyphosate (0.84 and 1.68 kg ae/ha) plus ammonium sulfate at 2% v/v, a standard conventional sugarbeet herbicide mixture (phenmedipham at 270 g/ha plus desmedipham at 270 g/ha, triflusulfuron at 9 g/ha, and clopyralid at 104 g/ha), and a no-herbicide control. Disease severity (0 to 7 scale), fresh plant weight, fresh root weight, and dry weight were the parameters tested. Data were combined across experiments and herbicide treatments when interactions were not significant.

Adequate moisture in the greenhouse following Rhizoctonia inoculation resulted in an average disease severity rating of 2.1. Non-inoculated plants were removed for further analysis. In this greenhouse experiment, herbicide treatment did not influence fresh root weight or dry weight. Therefore, data are combined across herbicide treatment. Hilleshög 9032RR had the lowest disease severity when compared with all other varieties (Table 17). Crystal RR827 was the most susceptible to Rhizoctonia crown and root rot and the highest disease severity when compared with the four Hilleshög varieties. However, there were no significant differences for fresh plant weight. For fresh root weight and dry weight, Crystal RR827 had the lowest weights when compared with the Hilleshög 9027RR, Hilleshög 9028RR, and Hilleshög 9032RR varieties (Table 18).

Table 17. Response of five glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IV in the greenhouse. Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

Variety	Disease severity[b]	Fresh weight[c]
	—disease severity (0-7 scale)—	—% of non-inoculated—
Hilleshog 9027RR	2.1b[d]	47a
Hilleshog 9028RR	2.3b	48a
Hilleshog 9029RR	2.3b	48a
Hilleshog 9032RR	1.7a	47a
Crystal RR827	2.1c	46a

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Sugarbeet roots were rated for disease severity on a 0 to 7 scale (0 = no disease and 7 = completely rotted).

[c] Fresh whole weight is determined by weighing the whole plant and dividing that weight by the weight of the same un-inoculated treatment.

[d] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Table 18. Fresh root weight and dry weight of five glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*[a] isolate AG-2-2-IV in the greenhouse. Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

Variety	Fresh root weight[b]	Dry weight[c]
	—% of non-inoculated—	—% of non-inoculated—
Hilleshog 9027RR	97a[d]	95a
Hilleshog 9028RR	96a	93a
Hilleshog 9029RR	92bc	91ab
Hilleshog 9032RR	95ab	95a
Crystal RR827	90c	86b

[a] *Rhizoctonia solani* inoculum was prepared with a barley medium.

[b] Fresh root weight is determined by weighing the root and dividing that weight by the weight of the same un-inoculated treatment.

[c] Dry weight is determined by weighing the whole plant and dividing that weight by the dry weight of the same un-inoculated treatment

[d] Means within each column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.