

## Influence of Glyphosate on Rhizoctonia Crown and Root Rot (*Rhizoctonia solani*) in Glyphosate-Resistant Sugarbeet

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Previous greenhouse studies with a noncommercial glyphosate-resistant sugarbeet variety indicated that susceptibility to *Rhizoctonia* crown and root rot could increase after glyphosate was applied. Greenhouse and field experiments were conducted in 2008 and 2009 to determine if glyphosate influenced disease severity in potential commercially available varieties of glyphosate-resistant sugarbeet. In the first greenhouse experiment in 2008, Hilleshög 9027RR, the most tolerant variety to *Rhizoctonia* crown and root rot, exhibited an increase in disease severity when glyphosate was applied. There were no significant differences between herbicide treatments in Hilleshög 9028RR, and glyphosate decreased disease severity in Hilleshög 9032RR when compared with the no-herbicide treatment. Experiments conducted to determine if glyphosate influenced *Rhizoctonia solani* growth *in vitro* indicated that glyphosate did not increase the radial growth of *R. solani*, except at  $10\times$  ( $190\ \mu\text{g ae ml}^{-1}$ ) the normal rate of glyphosate plus ammonium sulfate (AMS). Field and additional greenhouse experiments were conducted using four commercial varieties. Differences in disease severity were observed when comparing varieties, but glyphosate did not significantly influence the severity of *Rhizoctonia* crown and root rot when compared with the no-herbicide control. Choosing a glyphosate-resistant sugarbeet variety with the best demonstrated tolerance to *Rhizoctonia* crown and root rot is an important factor in reducing disease severity and maintaining sugarbeet yield.

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

**Key words:** Glyphosate-resistant crops, standard-split, disease severity, Roundup Ready®.

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, with full commercial introduction in 2008. Since commercialization, glyphosate-resistant sugarbeet have quickly been adopted, with almost 98% of Michigan's sugarbeet area planted to glyphosate-resistant varieties in 2009 (C. G. Guza, 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 POST herbicides did not effectively control weeds with more than two leaves, so many herbicide applications were necessary and seldom resulted in 100% control (Dale and Renner 2005; Dale et al. 2006). With the introduction of glyphosate-resistant sugarbeet, growers could 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 was less expensive and fewer applications were needed to control weeds with greater

economic returns (Dexter and Luecke 1999; Guza et al. 2002; Kemp et al. 2009; Kniss et al. 2004).

Concerns have been raised about potential increases in disease pressure after glyphosate is applied, due to physiological effects of the herbicide on plants (Larson et al. 2006; Michigan Sugar Company, personal communication). 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 growth, plant defense against pathogens, and plant tolerance under stress (Pline-Srnic 2005). If 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 (Green 2009). However, this enzyme on its own in the presence of glyphosate may not be as efficient as native EPSPS and this may result in the reduced production of secondary compounds that help protect the plant from pathogens. Larson et al. (2006) reported a transient accumulation of shikimic acid when a noncommercial glyphosate-resistant sugarbeet variety was exposed to glyphosate, indicating a potential for reduced movement of compounds through the shikimic acid pathway.

Previous studies in glyphosate-resistant crops, including glyphosate-resistant sugarbeet, demonstrated an increased susceptibility to some soil-borne pathogens after glyphosate was applied (Larson et al. 2006; Sanogo et al. 2000, 2001). In greenhouse and field experiments, glyphosate-resistant soybean were more susceptible to sudden death syndrome, caused by the pathogen *Fusarium virguliforme* Akoi, O'Donnell, Homma & Lattanzi, formerly known as *Fusarium solani* (Mart.) Sacc. f. sp. *glycine*, after glyphosate was applied (Sanogo et al. 2000, 2001). In addition, greenhouse studies determined that noncommercial varieties of glyphosate-resistant sugarbeet were more susceptible to isolates of both *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht. f.

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sp. *betae* Snyder & Hans after glyphosate was applied (Larson et al. 2006).

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 other glyphosate-resistant soybean varieties, it was determined that glyphosate had no effect on soybean yield or disease severity of sudden death syndrome (Njiti et al. 2003). In glyphosate-resistant cotton, greenhouse experiments showed that glyphosate had no effect on damping off or soreshin (caused by the pathogen *R. solani*) (Pankey et al. 2005). Furthermore, in the field, glyphosate actually reduced *R. solani*-induced disease severity in cotton.

*Rhizoctonia solani* Kühn 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 can result in up to 50% yield loss and it is estimated that this disease affects the economic returns of 24% of the sugarbeet hectares grown in the United States (Windels et al. 2009). The previously mentioned potential for increased Rhizoctonia disease severity when glyphosate is applied to glyphosate-resistant sugarbeet has sugarbeet growers concerned. 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 *R. solani* *in vitro*.

## Materials and Methods

**Greenhouse Experiment 1: Response of Three Sugarbeet Varieties.** Glyphosate-resistant sugarbeet varieties Hilleshög 9027RR, Hilleshög 9028RR, and Hilleshög 9032RR (Syngenta Seeds Inc., 1020 Sugarmill Rd., Longmont, CO 80501) were planted 2.5 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  $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  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 (3 L). At 14 d after planting, sugarbeet were fertilized weekly with 50 ml of a solution containing  $6.61 \text{ g L}^{-1}$  of 20–20–20.

The experiment was arranged in a three-factor completely randomized design with five replications, and repeated in time. Factors included *R. solani* inoculation (inoculated or non-inoculated), sugarbeet variety (Hilleshög 9027RR, Hilleshög 9028RR, or Hilleshög 9032RR), and herbicide treatment. Herbicide treatments consisted of two glyphosate (Roundup WeatherMAX, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167) rates (0.84 and  $1.68 \text{ kg ae ha}^{-1}$ ) plus ammonium sulfate at  $3.62 \text{ kg ha}^{-1}$ , a standard conventional sugarbeet herbicide mixture (phenmedipham plus desmedipham [Betamix, Bayer CropScience AG, Alfred-Nobel-Str. 50, D-40789 Monheim am Rhein, Germany], each at  $270 \text{ g ai ha}^{-1}$ ; triflurosulfuron [UpBeet, E.I. du Pont de Nemours and Co., Crop Protection, 1007 Market St., Wilmington, DE 19898] at  $9 \text{ g ai ha}^{-1}$ , and clopyralid [Stinger, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis,

IN 46268] at  $104 \text{ g ai ha}^{-1}$ ), and a no-herbicide control. Herbicide applications were made when sugarbeet were at the six- to eight-leaf growth stage using a single-tip track-sprayer with a Teejet 8001E (Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) even flat-fan nozzle. The sprayer was calibrated to deliver  $187 \text{ L ha}^{-1}$  at a pressure of 234 kPa at a speed of  $1.6 \text{ km h}^{-1}$ .

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 IIIB 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 (*R. 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 noninoculated control pots. After inoculation, pots were watered lightly over the crown area to ensure sufficient moisture for infection and good contact between the inoculum and soil.

Sugarbeet were harvested approximately 21 d after treatment (DAT). The noninoculated, no-herbicide treated sugarbeet plants were at the 10- to 12-leaf stage at this time. Sugarbeet were harvested by removing the whole plant from the pot and washing taproots to remove any excess soil. Each plant (leaves and taproots) was blotted dry with paper toweling to remove excess water. Each sugarbeet root was rated for disease severity using the 0 to 7 Rhizoctonia crown and root rot rating scale (0 = no visible signs of disease, 1 = inactive lesions on less than 5% of the root surface, 2 = less than 5% of the root with 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). Fresh weights of the sugarbeet leaves and taproots 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 (PDA; Becton & Dickinson Co., 7 Loveton Circle, Sparks, MD 21152) to confirm the presence of *R. solani*. The remaining samples were air dried for 1 wk at 28 C and dry weights were recorded. Fresh weight results followed similar trends as dry weight results; therefore only plant dry weight data are presented.

***Rhizoctonia solani* Growth In Vitro.** A laboratory experiment measured the fungal growth of *R. 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 by 15 mm) were filled with 25 ml of herbicide-amended water agar (1.5% w/v) (Sigma Chemical Co., 6050 Spruce St., St. Louis, MO 63103). Herbicide rates were calculated based on the area of the plate ( $56.5 \text{ cm}^2$ ). All herbicide and additive aqueous stock solutions were filter-sterilized ( $0.2 \mu\text{m}$ ) before being added to autoclaved PDA. Herbicide treatments included the following: glyphosate

alone at 0, 9.5, 19, 38, or 190  $\mu\text{g ae ml}^{-1}$  (0, 0.5, 1, 2, and 10 $\times$  the recommended use rate); glyphosate at the same rates plus ammonium sulfate at 0, 41, 82, 164, or 818  $\mu\text{g ml}^{-1}$ ; ammonium sulfate alone at 82  $\mu\text{g ml}^{-1}$ ; and the standard conventional sugarbeet herbicide mixture of phenmedipham plus desmedipham; triflusaluron; and clopyralid at 6, 6, 0.2, and 2.4  $\mu\text{g ai ml}^{-1}$ , respectively. Mycelial plugs (7 mm diam) of *R. solani* AG-2-2 IIIB were removed from 3-wk-old stock cultures on PDA and transferred to the center of each plate. Plates were sealed with Parafilm and incubated in the dark at 27  $\pm$  2 C. Radial growth was measured daily for 5 d until mycelia reached the edge of the plate on control plates. The experiment was arranged in a completely randomized design with five replications, and 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, MI, 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, MI, 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 (BetaSeed, Inc., 1788 Marschall Road, Shakopee, MN 55379) were planted 2.5 cm deep in rows spaced 76 cm apart at a population of 122,000 seeds  $\text{ha}^{-1}$  on April 25, 2008, and April 16, 2009. Hilleshög 9032RR was not used in these experiments because 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 variety per row, in rows two through five. Rows one and six served as border rows. Sugarbeet varieties selected for this experiment were approved for commercial planting in the Michigan sugarbeet growing region and were thought to have varying degrees of Rhizoctonia crown and root rot tolerance.

The experimental design was a split-split-plot with all treatments replicated four times. Herbicide treatment was the main-plot factor, *R. solani* inoculation was the split-plot factor, and variety was the split-split-plot factor. When sugarbeet were at the six- to eight-leaf stage, plots were inoculated with *R. solani* AG-2-2-IIIB. This age was chosen because beets have a developmental change from a seedling to a mature plant around the four- to six-leaf stage, which is associated with a number of effects, including disease response (Trebbi and McGrath 2008). *Rhizoctonia* inoculum was grown on barley (*Hordeum vulgare* L. subsp. *vulgare*) medium according to procedures outlined by Ruppel et al. (1979). Briefly, pans of barley were saturated with water and autoclaved and nine (7-mm) plugs of *R. solani* grown on PDA were placed into the pans. Parafilm-sealed pans were incubated at 25  $\pm$  2 C for 3 wk. Once the barley was colonized, it was air-dried and ground into fine flour. Inoculum was applied bidirectionally over each sugarbeet row at 2 g  $\text{m}^{-1}$  of row using a modified drop spreader (Gandy Company, 528 Gandrud Road, Owatonna, MN 55060). This

level of inoculum has been reported to give a moderate level of root rot (Ruppel and Hecker 1988). The inoculum rate was confirmed by weighing the amount of leftover inoculum and calculating the number of grams of inoculum applied per meter of row. Plots that were not inoculated served as a control. All plots were cultivated following inoculation to place soil and inoculum in the sugarbeet crown to increase disease infection (Franc et al. 2001; 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  $\text{ha}^{-1}$  plus ammonium sulfate at 3.62 kg  $\text{ha}^{-1}$ , applied three times at two- to four-leaf, four- to six-leaf, and six- to eight-leaf sugarbeet. The standard-split program consisted of a combination of desmedipham at 180 g ai  $\text{ha}^{-1}$  plus phenmedipham at 180 g ai  $\text{ha}^{-1}$ , triflusaluron at 9 g ai  $\text{ha}^{-1}$ , clopyralid at 104 g ai  $\text{ha}^{-1}$ , and nonionic surfactant at 0.25% v v $^{-1}$ , applied twice at the cotyledon to two-leaf and two- to four-leaf stages of sugarbeet. The desmedipham plus phenmedipham rates were increased to 270 g ai  $\text{ha}^{-1}$  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  $\text{ha}^{-1}$  at 207 kPa through 11003 AirMix nozzles (Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434) spaced 51 cm apart at approximately 56 cm above the canopy. Plots were evaluated for herbicide injury 14 d after the last herbicide application timing on a scale from 0 (no injury) to 100 (plant death).

Sugarbeet stand counts were recorded for the entire length of the plot for each variety 4 wk after emergence and at harvest. Approximately 8 wk after inoculation, when the majority of the *R. solani*-inoculated most-susceptible sugarbeet variety was dead, individual sugarbeet plants were lifted from the soil using a modified lift harvester (Tractor Supply Company, 200 Powell Place, Brentwood, TN 37027). Each sugarbeet root for the entire length of row was evaluated for Rhizoctonia crown and root rot disease severity using the 0 to 7 scale described previously (Ruppel et al. 1979). Differences between the original stand counts and the final 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 split-split-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) according to the following equation:

$$\text{disease index} = \frac{\sum (\text{disease class} \times \text{number of roots within that class})}{\text{total initial number of plants within plot}}$$

The percentage of healthy sugarbeet was determined by calculating the percentage of sugarbeet that had a disease severity rating of 0 or 1. Harvestable sugarbeet were

Table 1. Monthly precipitation<sup>a</sup> and the 30-yr average for experiments located in the Saginaw Valley region of Michigan in 2008 and 2009.

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

<sup>a</sup> Precipitation data were collected from the Michigan Automated Weather Network (<http://www.agweather.geo.msu.edu/mawn/>).

determined by calculating the percentage of sugarbeet with a disease severity rating 3 or less (Panella et al. 2008).

Precipitation data were recorded by weather stations operated by the Michigan Automated Weather Network (Michigan Automated Weather Network, Web site: <http://www.agweather.geo.msu.edu/>) (Table 1), which were located within 3 km of the experimental locations.

### Greenhouse Experiment 2: Response of Four Sugarbeet Varieties.

This greenhouse experiment evaluated the four commercial sugarbeet varieties that were used in the 2008 and 2009 field experiments: Hilleshög 9027RR, Hilleshög 9028RR, Hilleshög 9029RR, and Crystal RR827. Two of these varieties, Hilleshög 9027RR and Hilleshög 9028RR, also were evaluated in greenhouse experiment 1. Methods for this experiment were similar to experiment 1, with certain exceptions. These exceptions include that sugarbeet were planted in a professional potting mix (Baccto Professional Potting Mix, Michigan Peat Company, P.O. Box 980129, Houston, TX 77098), instead of pasteurized sandy loam soil. In addition to weekly fertilization of 20–20–20, at the four-leaf stage sugarbeet were fertilized once with 1 g cm<sup>-2</sup> of a micronutrient mixture (MicroMax, Grace-Sierra, 1001 Yosemite Dr., Milpitas, CA 95035). Micronutrients were added because variety Hilleshög 9027RR had shown boron deficiency symptoms when plants were maintained in the greenhouse for longer than 2 mo (L. Hanson, unpublished data). Similar procedures were used for *Rhizoctonia* inoculation, except the inoculum was grown on a barley medium as described for the field experiment. Pots were inoculated by spreading 0.5 ml of the finely ground barley inoculum around the sugarbeet crown within 24 h after the herbicide treatments. The noninoculated 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.** Greenhouse and field data were analyzed using the PROC MIXED procedure in SAS version 9.1 (SAS Institute, Inc., 100 SAS Campus Dr., Cary NC 27513). An ANOVA was performed to test for significant interactions and main effects. All effects except replication were considered fixed. Data were combined over experiments, years, or both, 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

Table 2. Response of three glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*<sup>a</sup> AG-2-2 IIIB in the presence and absence of herbicides.

Herbicide treatment	Sugarbeet variety		
	H 9027RR	H 9028RR	H 9032RR
	— <i>Rhizoctonia</i> crown and root rot severity <sup>b</sup> —		
No herbicide	2.8 ab <sup>d</sup>	4.8 cde	4.9 de
Standard conventional program <sup>c</sup>	4.0 abcd	4.7 cde	2.5 a
Glyphosate (0.84 kg ae ha <sup>-1</sup> )	4.7 cde	4.4 cde	3.0 abc
Glyphosate (1.68 kg ae ha <sup>-1</sup> )	5.9 e	4.7 cde	4.0 abcd

<sup>a</sup> *Rhizoctonia solani* inoculum was prepared with a millet medium.

<sup>b</sup> Sugarbeet roots were rated for disease severity on a 0 to 7 scale (0 = no disease and 7 = completely rotted, as described in the text).

<sup>c</sup> The standard conventional herbicide program included phenmedipham at 270 g ai ha<sup>-1</sup> plus desmedipham at 270 g ai ha<sup>-1</sup>, triflusalufuron at 9 g ai ha<sup>-1</sup>, and clopyralid at 104 g ai ha<sup>-1</sup>.

<sup>d</sup> Means followed by the same letter are not different according to Fisher's Protected LSD at  $P < 0.05$ .

the laboratory experiment, radial fungal growth was regressed over time for each treatment (cm d<sup>-1</sup>) using TableCurve 2D 5.01 software package (TableCurve 2D 5.01, Systat Software Inc., 501 Canal Blvd., Richmond, CA 94804-2028). Regression analysis of the different glyphosate rates indicated a poor fit with SAS. Therefore, paired *t* tests were then used to determine differences in radial growth between the control and standard-split treatment and individual glyphosate treatments.

## Results and Discussion

**Greenhouse Experiment 1: Response of Three Sugarbeet Varieties.** Greenhouse experiment 1 was conducted twice in early 2008, prior to the full commercial release of glyphosate-resistant sugarbeet. Experimental replication-by-treatment interactions were not significant; therefore the data were combined for analysis. *Rhizoctonia* crown and root rot disease severity was significant ( $P < 0.0001$ ) for sugarbeet inoculated with *R. solani* AG-2-2-IIIB compared with noninoculated sugarbeet (treated with sterile-autoclaved millet seed). The average disease severity for *R. solani*-inoculated sugarbeet was 4.2 (Table 2). *Rhizoctonia* crown and root rot was not present on any of the noninoculated sugarbeet and *R. solani* was isolated only from inoculated sugarbeet. The noninoculated treatments were dropped from further analysis. However, dry weights from each replication of the noninoculated plants were used to standardize sugarbeet dry weights for comparisons among the three varieties. Dry weight data are presented as a percent of the noninoculated 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 in plant dry weight with the different herbicide treatment-variety combinations. The most *Rhizoctonia*-tolerant variety, Hilleshög 9027RR, had 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 ae ha<sup>-1</sup> to Hilleshög 9027RR increased the disease severity rating from 2.8 to 4.7 and 5.9, respectively (Table 2). Increased disease severity also was reflected with reduced plant dry weight

Table 3. Dry weights of three glyphosate-resistant sugarbeet varieties exposed to *Rhizoctonia solani*<sup>a</sup> AG-2-2 IIIB in the presence and absence of herbicides.

Herbicide treatment	Sugarbeet variety		
	H 9027RR	H 9028RR	H 9032RR
	—% of noninoculated <sup>c</sup> —		
No herbicide	85 ab <sup>d</sup>	59 de	48 e
Standard conventional program <sup>b</sup>	75 abcd	53 de	91 a
Glyphosate (0.84 kg ae ha <sup>-1</sup> )	59 de	70 bcd	77 abc
Glyphosate (1.68 kg ae ha <sup>-1</sup> )	52 e	64 cde	65 cde

<sup>a</sup> *Rhizoctonia solani* inoculum was prepared with a millet medium.

<sup>b</sup> The standard conventional herbicide program included phenmedipham at 270 g ai ha<sup>-1</sup> plus desmedipham at 270 g ai ha<sup>-1</sup>, triflusaluron at 9 g ai ha<sup>-1</sup>, and clopyralid at 104 g ai ha<sup>-1</sup>.

<sup>c</sup> Dry weights were determined by dividing the dry weight of the *Rhizoctonia*-inoculated plants by the dry weight of noninoculated plants for each treatment.

<sup>d</sup> Means followed by the same letter are not different according to Fisher's Protected LSD at P < 0.05.

(Table 3). There was a 26 and 33% reduction in plant dry weight when glyphosate was applied at 0.84 and 1.68 kg ae ha<sup>-1</sup>, respectively, as compared with the no-herbicide control (Table 3). This response was similar to results observed in previous greenhouse work, where an increase in *Rhizoctonia* crown and root rot disease severity occurred when glyphosate was applied to a *Rhizoctonia*-tolerant glyphosate-resistant sugarbeet variety (Larson et al. 2006).

Although the glyphosate-resistant sugarbeet varieties Hilleshög 9028RR and Hilleshög 9032RR had similar disease severity ratings as the no-herbicide control, they responded differently to the herbicide treatments. None of the herbicide treatments significantly changed the disease severity rating or plant dry 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 ae ha<sup>-1</sup> when compared with the no-herbicide control (Table 2). Sugarbeet dry weight also was higher for these treatments (Table 3). This indicates that varieties can respond differently to herbicide-by-disease interactions. For example, previous greenhouse and field studies demonstrated that glyphosate applications influenced sudden death syndrome disease severity in some varieties of glyphosate-resistant soybean, but this response was variety-dependent (Sanogo et al. 2000, 2001).

***Rhizoctonia solani* Growth In Vitro.** A laboratory experiment was conducted to determine the effect of herbicides on the rate of mycelial growth of *R. solani*. The addition of ammonium sulfate to glyphosate did not affect mycelial growth rate. Therefore, data for the glyphosate treatments are combined over the glyphosate alone and the glyphosate plus ammonium sulfate treatments.

Similar to results of Larson et al. (2006) the only glyphosate treatment different from the control was the highest rate of glyphosate. Our highest rate of glyphosate (190 µg ml<sup>-1</sup>), equivalent to 10× the normal use rate of glyphosate, inhibited mycelial growth by 8% when compared with the control (P ≤ 0.0005). This rate also was different from the standard conventional herbicide mixture of phenmedipham plus desmedipham; triflusaluron; and clopyralid (P ≤ 0.0059). The lower rates of glyphosate (0.5, 1, or 2×) and the standard conventional herbicide mixture did not influence the growth rate of *R. solani* when compared with the control. There are

Table 4. 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 <sup>a</sup>	Disease index <sup>b</sup>	Harvestable <sup>c</sup>	Healthy <sup>d</sup>
Herbicide	0.8762	0.9714	0.5835
Variety	< 0.0001	< 0.0001	0.5152
Variety × herbicide	0.9904	0.9991	0.7081

<sup>a</sup> Inoculation was removed from further analysis because it was highly significant and noninoculated plants had a disease severity rating of less than 2.

<sup>b</sup> 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.

<sup>c</sup> Harvestable sugarbeet is the percentage of sugarbeet in the plot with a disease severity rating of 3 or less.

<sup>d</sup> Healthy sugarbeet is the percentage of sugarbeet in the plot with a disease severity rating of 1 or less.

three possible effects that herbicides may have on mycelial growth. First, herbicides can be a food source and increase fungal growth (Klimek et al. 2001). Secondly, herbicides can inhibit fungal growth of foliar diseases (Anderson and Kolmer 2005; Pavreena et al. 2007). For example, Feng et al. (2005) indicated that glyphosate had antifungal activity and decreased the disease severity of *Puccinia triticina* and *Puccinia striiformis* in glyphosate-resistant wheat. Finally, herbicides may have no effect on fungal growth (Roberti et al. 2006). The evidence from our work and Larson et al. (2006) indicate that the potential for glyphosate to have antifungal or enhanced growth activity is unlikely to be a factor for *R. solani* at rates used in the field.

**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. There was no year-by-treatment interactions, therefore all data are presented as a combination of the 2008 and 2009 experiments. The two-way interaction of variety by herbicide was not significant (Table 4) 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 allowed for adequate *R. solani* infection with an overall disease index of 5.9 (Table 5). 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 noninoculated 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 nontreated control and are consistent with what others have observed with this program (Wilson 1994, 1995). Approximately 2 wk after this evaluation, sugarbeet recovered from this injury.

Table 5. Response of four glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*<sup>a</sup> AG-2-2 IIIB in field experiments conducted in 2008 and 2009.<sup>b</sup>

Variety	Disease index <sup>c</sup>	Harvestable <sup>d</sup>	Healthy <sup>e</sup>
	0–7 scale	—	%
Hilleshög 9027RR	5.5 a <sup>f</sup>	15 a	2 a
Hilleshög 9028RR	5.9 b	9 b	1 a
Hilleshög 9029RR	5.7 ab	12 ab	1 a
Crystal RR827	6.6 c	2 c	0 a

<sup>a</sup> Inoculation was removed from further analysis because it was highly significant and noninoculated plants had a disease severity rating of less than 2.

<sup>b</sup> Data are combined over herbicide treatments because there was not a significant variety by herbicide interaction.

<sup>c</sup> 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.

<sup>d</sup> Harvestable sugarbeet is the percent of sugarbeet in the plot with a disease severity rating of 3 or less.

<sup>e</sup> Healthy sugarbeet is the percent of sugarbeet in the plot with a disease severity rating of 1 or less.

<sup>f</sup> Means within each column followed by the same letter are not different according to Fisher's Protected LSD at  $P \leq 0.05$ .

**Variety.** The main effect of variety was significant for *Rhizoctonia* disease indices and the percentage of harvestable sugarbeet (Table 4). Sugarbeet that are considered harvestable have a disease severity rating of 3 or less (Panella et al. 2008). However, there was no difference in the percentage of healthy sugarbeet for any of the varieties examined. 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 5). The disease index rating for Hilleshög 9028RR was higher than Hilleshög 9027RR, but was not 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 5). However, regardless of variety, 15% or fewer of the sugarbeet were considered harvestable. Fewer than 3% of the sugarbeet were considered healthy (Table 5).

**Herbicide.** The main effect of herbicide was not significant (Table 4). 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 greenhouse experiment 1 results and to previous greenhouse findings by other researchers (Larson et al. 2006).

Although glyphosate applications in greenhouse experiment 1 increased disease severity for Hilleshög 9027RR, 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. Both millet (Cotterill et al. 1990; Nagendran et al. 2009) and barley (Pierson and Gaskill 1961; Ruppel and Hecker 1988) have been used for producing *Rhizoctonia* inoculum. We switched from millet seed to ground barley inoculum for better quantitation of inoculum. When whole seeds are used, there is the potential to use a seed that has not been colonized by the fungus. We examined all millet seeds before inoculation and observed evidence of fungal hyphae, and we did not have any inoculated beets that showed

Table 6. P values for main effects and interactions of herbicide treatments on *Rhizoctonia solani*<sup>a</sup> AG-2-2 IIIB disease severity and plant fresh weight of four glyphosate-resistant sugarbeet varieties for greenhouse experiment 2.

Effects <sup>b</sup>	Disease severity	Dry weight
	P values	
Herbicide	0.3672	0.1136
Variety	< 0.0001	0.0015
Variety × herbicide	0.2330	0.2669

<sup>a</sup> *Rhizoctonia solani* inoculum was prepared with a barley medium.

<sup>b</sup> Inoculation was removed from further analysis since it was highly significant and noninoculated plants had a disease severity rating of less than 1.

no disease, but potential of each individual seed to serve as an inoculum source is not known. When ground barley inoculum is used, multiple grains are ground and the resultant material is mixed. This allows quantifying inoculum. Our ground barley inoculum was in the same range as that of Ruppel and Hecker (1988) of approximately 80 colony-forming units (cfu)  $g^{-1}$  of inoculum and Larson et al. (2006) of approximately 25 cfu  $0.6 ml^{-1}$  (L. Hanson, unpublished data).

Another possibility is that disease severity could have been affected by the different soil types used in each of these experiments. The presence of additional soil-borne pathogens or other soil-borne microorganisms, as well as additional environmental factors, could have resulted in differences between these experiments. In addition, it is possible that the timing of herbicide application to plants in relation to timing of infection events may influence susceptibility to pathogens. In both greenhouse experiments, sugarbeet were inoculated within 24 h of herbicide treatment. However, in the field, sugarbeet were inoculated 7 d after the last herbicide application. This should not impact the results, as Larson et al. (2006) reported no difference in response of beets treated 1 or 9 d before fungal inoculation, and even longer time periods between glyphosate application and fungal inoculation can show effects. For example, studies with glyphosate-resistant wheat (*Triticum aestivum* L.) indicated that glyphosate-associated decrease in disease severity of leaf rust (*P. triticina*) and stem rust (*Puccinia graminis* f. sp. *tritici*) occurred even when plants were exposed to glyphosate 21 to 35 d after inoculation (Anderson and Kolmer 2005).

### Greenhouse Experiment 2: Response of Four Sugarbeet Varieties.

An additional greenhouse experiment (experiment 2) was conducted using the four commercial varieties of glyphosate-resistant sugarbeet and the barley inoculum used in the field to confirm earlier field results and conflicting greenhouse results. This greenhouse experiment was repeated and experimental replication-by-treatment interactions were not significant, so data were combined for analysis. The two-way interaction of variety by herbicide was not significant in the greenhouse (Table 6) for any of the parameters evaluated. Therefore, data are discussed as the main effects of variety and herbicide for disease severity and dry plant weight.

***Rhizoctonia* Inoculation.** Inoculation of *R. solani* subgroup AG-2-2 IIIB was highly effective in the greenhouse. *Rhizoctonia solani* inoculation resulted in an average disease severity rating of 5.9 (Table 7). *Rhizoctonia* crown and root rot was not present on any of the non-inoculated sugarbeet and *R. solani* was isolated only from inoculated sugarbeet.

Table 7. Response of four glyphosate-resistant sugarbeet varieties to *Rhizoctonia solani*<sup>a</sup> AG-2-2 IIIB in greenhouse experiment 2.<sup>b</sup>

Variety	Disease severity <sup>c</sup>	Dry weight <sup>d</sup>
	0–7 scale	% of noninoculated
Hilleshög 9027RR	5.9 b <sup>e</sup>	41 b
Hilleshög 9028RR	4.8 a	55 a
Hilleshög 9029RR	6.1 bc	45 b
Crystal RR827	6.7 c	33 c

<sup>a</sup> *Rhizoctonia solani* inoculum was prepared with a barley medium.

<sup>b</sup> Data are combined over herbicide treatments since there was not a significant variety by herbicide interaction.

<sup>c</sup> Sugarbeet roots were rated for disease severity on a 0 to 7 scale (0 = no disease and 7 = completely rotted).

<sup>d</sup> Dry whole weight is determined by weighing the whole plant and dividing that weight by the weight of the same noninoculated treatment.

<sup>e</sup> Means within each column followed by the same letter are not different according to Fisher's Protected LSD at  $P \leq 0.05$ .

Therefore, the noninoculated treatments were dropped from further analysis.

However, similar to greenhouse experiment 1, dry weights from each replication of the noninoculated plants were used to standardize sugarbeet dry weights for comparisons among the three varieties. Dry weight data are presented as a percentage of the noninoculated treatments.

**Variety.** The main effect of variety was significant for *Rhizoctonia* disease severity and sugarbeet dry weight (Table 6). The order of *Rhizoctonia* tolerance of the varieties was different in the greenhouse compared with the field results. In the greenhouse, Hilleshög 9028RR had the lowest disease severity rating (4.8) (Table 7). 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 different from Hilleshög 9029RR. The dry weight of *Rhizoctonia*-inoculated sugarbeet was reduced by 45% or more when compared with the noninoculated controls (Table 7). The relative dry weight of Hilleshög 9028RR was higher than dry weights of the other varieties, following similar trends as disease severity ratings.

**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 dry weight in the greenhouse (Table 6). 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 similar to what we observed in the field. However, it is contradictory to our greenhouse experiment 1 results and greenhouse findings by other researchers (Larson et al. 2006).

In greenhouse experiment 1, glyphosate applications increased disease severity for Hilleshög 9027RR and reduced disease severity of Hilleshög 9032RR. However, field and additional greenhouse experiments did not support these findings. In experiment 1, the overall *Rhizoctonia* crown and root rot 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-borne 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-borne pathogens in these soil or soil-less mix media sources could have resulted in differences between these experiments. In addition, sugarbeet in experiment 2 were fertilized with a micronutrient solution. This micronutrient solution was added to in experiment 2 because sugarbeet showed boron deficiency when maintained for over 2 mo in the greenhouse (L. Hanson, unpublished data). Hilleshög 9027RR appeared to demonstrate the most severe deficiency symptoms of the four varieties and this may be a factor in 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 deficiency symptomology compared to conventional varieties (Loecker et al. 2010). Some micronutrients can affect disease severity, and some studies have shown impacts on diseases caused by *R. solani* on some field crops (Datnoff et al. 2007). Interactions between variety response to micronutrients, pathogens, and herbicides is an area that warrants further investigation.

We also observed a difference in the ranking of *Rhizoctonia* crown and root rot tolerance among the varieties when comparing the greenhouse and field experiments. Although Hilleshög 9027RR was the most *Rhizoctonia* crown and root rot 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). 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 in the field. Growers can make several glyphosate applications to glyphosate-resistant sugarbeet varieties without increasing susceptibility to *Rhizoctonia* crown and root rot. Although in one greenhouse study glyphosate was found to either increase or decrease disease severity depending on the variety, glyphosate applications did not influence disease severity in subsequent field or greenhouse studies. Variety selection is the most important factor in reducing disease severity of *Rhizoctonia* crown and root rot in glyphosate-resistant sugarbeet. To prevent yield loss as a result of reduced stand, using a variety with excellent tolerance to *R. solani* is recommended.

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