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# Effect of Glyphosate on *Cercospora beticola* on Glyphosate-Resistant Sugar Beet

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DOI: 10.5274/JSBR.50.1.1

## ABSTRACT

In Minnesota and North Dakota, *Cercospora* leaf spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is the most damaging foliar disease of sugar beet (*Beta vulgaris* L.). Fungicide applications are necessary under moderate to severe disease pressure to control CLS to obtain economically viable yields of recoverable sucrose. Field trials were conducted near Foxhome, Minnesota in 2005 and 2006 to determine whether the broad-spectrum herbicide glyphosate applied post inoculation to glyphosate-resistant sugar beet controls *C. beticola* and improves crop yield and quality compared to using fungicides. The research site was artificially inoculated with *C. beticola* and multiple applications of glyphosate were applied alongside the fungicides tetraconazole, pyraclostrobin, and triphenyltin hydroxide used in rotation. CLS disease severity was high in both years as measured by the area under the disease progress curve (AUDPC). Glyphosate applied post inoculation at the same time as parallel applications of fungicides were performed did not provide effective control of CLS and resulted in similar AUDPC, yield and quality as the non-treated check. Fungicides provided effective control of *C. beticola* with lower AUDPC and resulted in significantly higher yields and recoverable sucrose than the non-treated check.

**Additional Key Words:** *Beta vulgaris*, fungicide, *Cercospora* leaf spot, disease control.

The number of crops modified through genetic engineering to be resistant to the popular broad-spectrum herbicide glyphosate is growing and includes maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum*), alfalfa (*Medicago sativa* L.) and wheat (*Triticum aestivum* L.) (Dill, 2005; James, 2011). However, commercialization of glyphosate-resistant wheat has been put on hold pending acceptance of transgenic wheat in the world market (Zhou et al., 2003). The newest commercialized glyphosate-resistant crop is sugar beet, which was approved for planting in the United States (US) in 2005. Growers rapidly adopted glyphosate-resistant sugar beet (Khan, 2010), and this technology is currently used on over 95% of US sugar beet acreage (Barnett et al., 2011; Nick Ahrends, American Crystal Sugar Company, MN; Mark Bredehoeft, Southern Minnesota Beet Sugar Cooperative, MN; Mike Metzger, Minn-Dak Farmers' Cooperative, ND; personal communication). Glyphosate inhibits 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), which is an important enzyme in the shikimate pathway (Jaworski, 1972; Steinruken and Amrhein, 1980). This pathway is important for the synthesis of several amino acids such as phenylalanine, tyrosine, and tryptophan which are found in microorganisms and plants (Hawkins et al., 1993; Roberts et al., 1998; Schmid and Amrhein, 1995). Glyphosate-resistant crops allow the use of glyphosate to control weeds present in these crops.

Some reports indicate that glyphosate has fungicidal activities on saprophytic and pathogenic fungi (Black et al., 1996; Chakravarty and Chatarpaul, 1990). Glyphosate provided control or suppressed several rust diseases in glyphosate-resistant wheat, soybean, and alfalfa, (Anderson and Kolmer, 2005; Feng et al., 2005; Feng et al., 2008; Samac and Foster-Hartnett, 2012). It will be useful to know whether glyphosate provides protection to glyphosate-resistant sugar beet from important fungal pathogens.

Minnesota and North Dakota together produce about 60% of the US sugar beet crop. Cercospora leaf spot (CLS), caused by *Cercospora beticola* Sacc., is the most damaging and economically important foliar disease of sugar beet (*Beta vulgaris* L.) in these states (Khan, 2009). Under high disease pressure, leaf spots coalesce and kill entire leaves causing defoliation of the plants which leads to reduced tonnage, low sucrose concentration and increased impurities resulting in additional processing costs (Lamey et al., 1996; Khan et al., 2007; Shane and Teng, 1992; Smith and Ruppel, 1973). Under high disease pressure, losses as high as 30% in recoverable sucrose occur (Khan, 2009; Lamey et al., 1996; Shane and Teng, 1992). Sugar beet roots are stored in piles following harvest and are processed from October through May in North Dakota and Minnesota. Roots from severely diseased plants do not store as well in storage piles as roots from healthy plants, resulting in additional economic losses (Smith and Ruppel, 1973). Over the years, varieties have been developed with higher resistance to *C. beticola* (Niehaus, 2012). However, most of the commercial sugar beet varieties require timely fungicide applications to prevent economic losses

especially under heavy disease pressure (Miller et al., 1994).

The objective of this study was to determine whether glyphosate applied at times when fungicides are typically used will control *C. beticola* on glyphosate-resistant sugar beet under field conditions.

## MATERIALS AND METHODS

Field trials were conducted near Foxhome, MN during the 2005 and 2006 growing seasons. Field plots comprised six 9 m rows spaced 56 cm apart. Plots were seeded with a glyphosate-resistant sugar beet cultivar (proprietary material) on 28 and 25 April in 2005 and 2006, respectively. Terbufos (Counter 15 G; Amvac Chemical Corp., Hannibal, MO) was applied in-furrow at 13.5 kg ha<sup>-1</sup> at planting to manage sugar beet root maggot (*Tetanops myopaeformis* von Röder; Diptera: Ulidiidae). Plots were thinned manually at the 6-leaf stage to 104,000 plants ha<sup>-1</sup>. In all plots, weeds were controlled with glyphosate (Roundup WeatherMax; 48.8% a.i at 1.6 liter ha<sup>-1</sup>; Monsanto, St. Louis, MO) mixed with ammonium sulfate surfactant (AmStik 6.6 kg per 379 liter water) and applied on 1 and 17 June in 2005, and 1 and 19 June in 2006. Plots were artificially inoculated with *C. beticola* infected sugar beet leaves mixed with talc (2:1 by weight) and applied foliarly at 5.6 kg ha<sup>-1</sup> (Khan et al., 2007) on 20 June and 21 June in 2005 and 2006, respectively. Treatments consisted of multiple applications of glyphosate, and fungicides used in rotation and commenced when CLS symptoms were first observed about 35 to 36 days after inoculation. Glyphosate was applied at 1.6 litre ha<sup>-1</sup> on the same dates as the fungicide applications. In 2005, the fungicides tetraconazole (Eminent 125 SL; 1 litre ha<sup>-1</sup>; Sipcam Advan, Research Triangle Park, NC), followed by pyraclostrobin (Headline 2.09 EC; 0.8 litre ha<sup>-1</sup>, BASF, Raleigh, NC), and finally triphenyltin hydroxide (Super Tin 80 WP; 350 g ha<sup>-1</sup>; United Phosphorus, King of Prussia, PA) were used in the rotation and applied on 25 July, and on 8 and 22 August, respectively. In 2006, the same treatments as in 2005 were applied on 27 July, 10 and 24 August, respectively. In 2006, ammonium sulfate (AmStik 6.6 kg per 379 liter water) was mixed with the glyphosate treatment. Treatments were applied to the middle four rows of each plot using a four nozzle hand held sprayer operating at 139 kPa using 8002 nozzles to deliver 95 liters of spray solution ha<sup>-1</sup>. In both years, a non-treated check was included where no treatment was applied post inoculation. CLS severity was rated during the season until just prior to harvest using the 1 to 9 Kleinwanzlebener Saatucht (KWS) scale (Anonymous, 1970), where 1 = no disease and a rating of 9 = plants assessed had only new leaf growth, all earlier leaves being dead. CLS severity rating was used to calculate the area under the disease progress curve (AUDPC) (Campbell and Madden, 2006). Plots were mechanically defoliated and the middle two rows of each plot were harvested using a mechanical harvester on 3 October in both years and were weighed for root yield. Twelve to 15 random roots from each plot, not including

roots at the end of plots were analyzed for yield parameters including net root yield, sugar concentration, and sugar loss to molasses at the American Crystal Sugar Company Quality Tare laboratory, Moorhead, MN. Recoverable sucrose was determined from net yield and sucrose concentration after discounting losses from sugar to molasses and other impurities. The experimental design was a randomized complete block with four replicates per treatment. Analysis of variance was performed on the data using PROC GLM in SAS (version 9.1; SAS Institute, Cary, NC). Test for homogeneity of variances was performed by calculating the  $F$  ratio between error mean variance for the repeated trial (Gomez and Gomez, 1984). Treatment means were compared using Fisher's Least Significant Difference (LSD) Test at  $P = 0.05$ .

## RESULTS AND DISCUSSION

The test for homogeneity on the variances of the trials was not significant at  $P = 0.05$  therefore the trials were combined. In both years CLS symptoms were observed first in late July and disease developed rapidly in mid- to late-August. Disease severity was high in the non-treated check and had an AUDPC of 689 just prior to harvest (Table 1). All plants received treatments at the same time. Plants treated with glyphosate did not control *C. beticola* and resulted in similar leaf spot ratings and AUDPC as the non-treated check. In contrast, plants treated with the fungicides in rotation, as expected, showed effective control of *C. beticola* with significantly lower AUDPC (200) compared to glyphosate applied at the time of fungicide applications and the non-treated check. Fungicide use resulted in significantly higher tonnage and sugar concentration, significantly lowered sugar loss to molasses, and in significantly higher recoverable sucrose compared to glyphosate applied post inoculation (Table 1).

Based on two years field testing with combined analysis, glyphosate applied post inoculation at the same time as fungicides, starting after CLS symptoms were first observed, did not appear to display any fungicidal activity against *C. beticola* and did not control CLS in the glyphosate-resistant sugar beet nor did it significantly increase any of the yield parameters evaluated relative to the untreated check. These observations are contrary to earlier research results with soybean where application of glyphosate provided preventative and curative control against stripe rust (*Puccinia striiformis* f. sp. *tritici* (Erikss) CO Johnston) and leaf rust (*Puccinia triticina* Erikss) in glyphosate-resistant wheat, and suppressed Asian soybean rust (*Phakospora pachyrhizi* Syd & P Syd) in glyphosate-resistant soybean (Anderson and Kolmer, 2005, Feng et al., 2005; Feng et al., 2008). These results are also contrary to greenhouse research which showed that glyphosate at the recommended field application rate provided effective control against alfalfa rust (*Uromyces striatus* Schrot.) (Samak and Foster-Hartnett, 2012).

**Table 1.** Effect of glyphosate and fungicide treatments on Cercospora leaf spot severity measured as area under the disease progress curve, and yield and quality of sugar beet averaged over 2005 and 2006 near Foxhome, MN.

| Treatments <sup>†</sup> | AUDPC <sup>‡</sup>  | Net Yield<br>(t ha <sup>-1</sup> ) | SC <sup>§</sup><br>(%) | SLM <sup>¶</sup><br>(%) | RS <sup>††</sup><br>(kg ha <sup>-1</sup> ) |
|-------------------------|---------------------|------------------------------------|------------------------|-------------------------|--|
| Non-treated check       | 689 b <sup>**</sup> | 46 b                               | 13.5 b                 | 1.8 a                   | 4894 b                                     |
| Glyphosate              | 692 b               | 48 b                               | 13.3 b                 | 1.8 a                   | 4975 b                                     |
| Fungicides              | 200 a               | 61 a                               | 14.8 a                 | 1.5 b                   | 7378 a                                     |

<sup>†</sup> Treatments were non-treated control (no treatment after inoculation); glyphosate (three applications at 14 d intervals; and fungicides (tetraconazole, Eminent 125 S at 1 litre ha<sup>-1</sup> followed by pyraclostrobin, Headline 2.09 EC at 0.8 litre ha<sup>-1</sup> and triphenyltin hydroxide, Super Tin 80WP at 350 g ha<sup>-1</sup>) at 14 d interval.

<sup>‡</sup> AUDPC - area under the disease progress curve.

<sup>§</sup> SC – sugar concentration

<sup>¶</sup> SLM – sugar loss to molasses

<sup>††</sup> RS – recoverable sucrose

<sup>\*\*</sup> Means within a column followed by the same letter do not significantly differ ( $P = 0.05$ , Fisher's protected least significant difference)

There are several potential reasons for lack of control of CLS with glyphosate on glyphosate-resistant sugar beet in our study. Glyphosate is a water soluble molecule, which needs a surfactant to break the cuticular layer of the leaves for effective uptake and translocation (Feng et al., 1998; Feng et al., 2003). In 2005, adjuvant was not added to the glyphosate because the WeatherMax formulation already has a full adjuvant load (Zollinger, 2012). Additional surfactant was added to glyphosate in 2006 but still did not provide control of *C. beticola* compared to the fungicides treatment.

In this research, glyphosate was applied twice for weed control to all treatments before inoculation, and CLS symptoms were observed in all plots around late July, about four weeks after the last glyphosate application used for weed control. Early glyphosate application for weed control did not appear to have an impact on delaying CLS symptoms because symptoms typically are observed after row closure in late July in artificially inoculated trials (Khan et al., 2009). *C. beticola* is a polycyclic pathogen which produces several generations within a growing season. Under favorable environmental conditions, the fungus can complete one sporulation cycle in 12 days (Weiland and Koch, 2004). This means that the three applications of glyphosate timed as fungi-

cide treatments would have been present at the different stages of the sporulation cycle but did not impact disease severity at any time as measured by the AUDPC. The total amount of glyphosate that can be used on sugar beet from emergence through harvest is 7.6 L ha<sup>-1</sup>. In this trial, a total of 8 L ha<sup>-1</sup> of glyphosate was used in two applications for weed control and three for CLS control, which already is above the labeled rate. The inability of glyphosate to control *C. beticola*, relative to fungicides, does not make it a candidate to be used for controlling CLS.

Reports have indicated variable responses of fungal pathogens to glyphosate. Sumac and Foster-Hartnett (2012) showed that glyphosate provided effective control against *Uromyces striatus* Schrot., limited protection to *Colletotrichum trifoli* Bain & Essary, and no control of *Phoma medicaginis* Malbr. & Roum. An obvious explanation for the non-control of CLS may be the insensitivity of *C. beticola* to glyphosate.

There are reports that indicate glyphosate use has resulted in unintended nutrient deficiency and increasing disease pressures, especially soil-borne diseases of some crops (Altman and Rovira, 1989; Johal and Huber, 2009; Larson et al., 2006). In this study, no nutrient deficiency symptoms nor symptoms of common diseases of sugar beet, such as *Rhizoctonia* root rot caused by *Rhizoctonia solani*, and *Aphanomyces* damping off and root rot caused by *Aphanomyces cochlioides*, were observed.

The use of glyphosate for weed control before inoculation did not appear to affect the efficacy of subsequent fungicides used for controlling *C. beticola* in glyphosate-resistant sugar beet. Similar results were obtained by Barnett et al. (2011), who found that glyphosate did not affect the efficacy of azoxystrobin fungicide in controlling *Rhizoctonia solani* in glyphosate-resistant sugar beet.

This research suggests that glyphosate used at the time required for fungicide applications does not provide control against *C. beticola* in glyphosate-resistant sugar beet. Growers should continue to use fungicides judiciously as one of their tools for managing CLS in glyphosate-resistant sugar beet.

### ACKNOWLEDGEMENTS

This project was supported in part by a grant from the Sugar Beet Research and Education Board of Minnesota and North Dakota.

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