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SHORT COMMUNICATION

Influence of glyphosate/dicamba application rate and timing on the control of glyphosate-resistant waterhemp in glyphosate/dicamba-resistant soybean

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Abstract: The objective of this study was to determine the effect of premixed glyphosate/dicamba application timing and rate on GR waterhemp control. Glyphosate/dicamba applied to 5, 15 and 25 cm tall plants, controlled GR waterhemp 81, 73 and 61% at 2 WAA and 61, 68 and 72% at 8 WAA, respectively.

Key words: Application rate, application timing, dicamba, glyphosate resistance, height, soybean, yield

Introduction

Herbicides are important for weed control in crop production systems. They are a common choice due to ease, cost, and efficacy. Glyphosate is a non-selective, systemic herbicide that can be used as a pre-plant (PP) burndown for weed control; however, it has no residual activity. The

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Abbreviations: GR, glyphosate-resistant; POST, postemergence; PP, pre-plant; PRE, preemergence; WAA, weeks after application; WAE, weeks after emergence.

introduction of glyphosate-resistant (GR) crops resulted in multiple in-season applications of glyphosate and led to glyphosate becoming the most widely used herbicide globally. The repeated and continuous use of glyphosate for weed control has increased selection pressure for GR weeds (Cobb and Reade 2010). Currently, there are 41 known GR weed species globally. Glyphosate-resistant waterhemp [(*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif)] was first found in the United States of America in Texas and Kansas in 2006 and Canada in 2014 (Costea and Tardiff 2003). Glyphosate-resistant waterhemp has been confirmed in three counties in Ontario as of 2017 (Schryver 2017). Waterhemp can be distinguished from other *Amaranthus* species by lanceolate leaves that are smooth and glossy, and a hairless stem (Pratt and Clark 2001). Waterhemp has an extended emergence pattern from May until the end of October in Ontario (Vyn et al. 2007; Schryver et al. 2017). Waterhemp has male and female reproductive structures on separate plants (dioecious), therefore, both are needed for populations to establish in a new area (Costea and Tardiff 2003). Upon maturity, waterhemp seeds have primary dormancy; there is greater emergence after cold stratification (Leon and Owen 2003). Waterhemp densities of up to 989 plants m⁻² have been documented in Ontario fields (Schryver et al. 2017), which have reduced soybean yield up to 73% (Vyn et al. 2007).

Important components of weed control are weed height and herbicide application rate. Previous research found that at 8 weeks after application (WAA), glyphosate (840 g ae ha⁻¹) controlled glyphosate-susceptible waterhemp 58% when applied to plants 10 cm in height; however, increased to 90% when glyphosate rate was increased to 1120 g ae ha⁻¹ and application was delayed to when waterhemp was 15 cm in height (Krausz and Young 2003). Krausz et al. (1996) observed an increase in glyphosate-susceptible waterhemp control from 88 to 100% with

glyphosate as the rate was increased from 560 to 2800 g ae ha⁻¹, respectively. Klingman et al. (1992) reported an increase in control of waterhemp from 97 to 100% when the rate of imazethapyr was increased from 53 to 140 g ai ha⁻¹, respectively. Hager et al (2003) reported a 7 to 9% decrease in control when the height of waterhemp at the time of acifluorfen (70 to 260 g ai ha⁻¹) application was 10 cm compared to 5 cm. Mayo et al. (1995) observed a decrease in waterhemp control with acifluorfen (420 g ai ha⁻¹) when applied at 30 cm compared to application at 4-12 cm in height; in contrast there was no decrease in control when lactofen (220 g ai ha⁻¹), chlorimuron (9 g ai ha⁻¹), thifensulfuron (4 g ai ha⁻¹), imazethapyr (70 g ai ha⁻¹) or imazaquin (70 g ai ha⁻¹) was applied to 30 cm compared to 4-12 cm waterhemp. Robinson et al. (2012) reported an increase in waterhemp control when 2,4-D was applied at 20 cm compared to 30 cm in height and an increase in control as 2,4-D rate was increased from 280 to 1120 g ae ha⁻¹. There was no increase in waterhemp control when 280 to 1120 g ae ha⁻¹ of 2,4-D were added to glyphosate (840 g ae ha⁻¹) (Robinson et al. 2012). In summary, the efficacy of a number of herbicides for the control of waterhemp is influenced by application rate and the height of waterhemp at the time of application.

Glyphosate/dicamba-resistant soybean is a herbicide-resistant technology that was introduced in North America in 2017. Dicamba-resistance in glyphosate/dicamba-resistant soybean is conferred by the insertion of a gene that codes for dicamba monooxygenase (DMO) from *Pseudomonas maltophilia* (Behrens et al. 2007). This gene enhances the metabolism of dicamba through the removal of a methyl group, converting it to inactive 3,6-dichlorosalicylic acid (Behrens et al. 2007). Dicamba is a Group 4, synthetic auxin (growth regulator), benzoic acid herbicide (Cobb and Reade 2010). Dicamba provides approximately 2 weeks residual weed control, however the actual length of residual activity is influenced by soil type, soil organic

matter content, rainfall, and weed species sensitivity (Cobb and Reade 2010). New low-volatile formulations of dicamba (Roundup Xtend, Monsanto, Winnipeg, MB) were developed in parallel with glyphosate/dicamba resistant soybeans to reduce off-site injury to sensitive plants.

The objective of this study was to determine the impact of glyphosate/dicamba rates and application timings on the control of glyphosate-resistant waterhemp. The results will be used to develop guidelines for optimal glyphosate/dicamba rates and application timings for the control of GR waterhemp in glyphosate/dicamba resistant soybean.

Materials and Methods

Six experiments were completed over a two-year period (2016, 2017) in Ontario, Canada. Two experiments were located near Walpole Island (42.592650, -82.476869) and one near Cottam Ontario (42.128549, -82.744135) in each year. A 3 x 4 factorial was arranged in a randomized complete block design (RBCD) with four replications. Factor one was application timings (when the height of waterhemp was approximately 5, 15 or 25 cm); factor two was three labelled rates of glyphosate/dicamba premixed at 900 (600/300), 1350 (900/450) or 1800 (1200/600) g ae ha⁻¹. A 2 to 1 premix formulation of glyphosate and dicamba was used in these experiments. The formulation contained 240 g ae L⁻¹ of glyphosate and 120 g ae L⁻¹ of dicamba. The seedbed was prepared with one pass of a tandem disc followed by a second pass with a field cultivator. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO) were planted in 2016 and 2017, respectively, at a rate of 400 000 seeds ha⁻¹ to a depth of 4 cm. Each plot was 2.25 m wide (3 soybean rows spaced 75 cm apart) and 8 m in length. Herbicides were applied with a CO₂ pressurized backpack sprayer at a rate of 200 l ha⁻¹. Percent visible soybean injury was assessed at 2 and 4 WAA, where 0% was no injury compared to the weedy control

and 100% was plant death. Waterhemp control was estimated visually at 2, 4, and 8 weeks after application (WAA), where 0% was no decrease in the apparent waterhemp biomass compared to the weedy control and 100% was complete control. Waterhemp density and biomass were measured at 6 WAA. Waterhemp within two 0.25 m² quadrants were counted, cut at the soil surface, and then placed in a paper bag. The bag was placed in a kiln set to 60°C for two weeks, after which the samples were weighed. Soybean was harvested at maturity in 2016 by harvesting a 1m subsample from 2 rows and threshing in a stationary Almaco threshing machine (Almaco, Nevada, IA). In 2017, two rows of soybean were harvested with a small plot Almaco combine. Soybean weight and moisture content were recorded; seed weights were adjusted to 14.5% moisture content before analysis.

Statistical analysis was completed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The fixed effect was the herbicide rate-timing combinations, and the random effects were environment and block. In all analyses, residuals were analyzed using the UNIVARIATE procedure to test for normality, homogeneity and independence of each other. Weedy controls were not included in the analysis for waterhemp control since they were artificially set to 0% control. Soybean yield and waterhemp control data at 2, 4, and 8 WAA were fit to a normal distribution using the identity link. Waterhemp density and biomass data were analyzed using a lognormal distribution with the identity link and the means backtransformed for presentation purposes. Waterhemp control and soybean yield data did not need transformation. Treatment means were separated by Fisher's Protected LSD and Tukey-Kramer adjustment with alpha set at $P=0.05$.

Results and discussion

Soybean Injury

There was no soybean visible injury detected in any treatment (data not presented). There was no interaction between glyphosate/dicamba application timing and rate for waterhemp control, density and biomass or soybean yield; therefore, the simple effects will be presented (Table 1).

Application Timing

At 2, 4 and 8 WAA, there was an effect of application timing on GR waterhemp control. At 2 WAA, there was a 20% reduction in waterhemp control as application timing was delayed from 5 to 15 to 25 cm tall waterhemp (Table 1). The reduced control with the late application timing (25 cm) is attributed to slower herbicide activity on weeds with greater biomass at the time of application. At 4 WAA, glyphosate/dicamba provided significant reduction (5-7%) in 5 cm tall waterhemp control compared to 15 or 25 cm application timing. At 8 WAA, glyphosate/dicamba applied to waterhemp at 25 cm provided 72% control compared to 61% at the 5 cm application height. The improved control can be attributed to the extended emergence pattern of waterhemp in Ontario. When glyphosate/dicamba application was delayed until waterhemp was 25 cm tall, a greater proportion of the weeds had emerged at the time of application. There was no impact of waterhemp height at the time of glyphosate/dicamba application on waterhemp density or biomass and soybean yield.

Application Rate

At 2, 4 and 8 WAA there was an increase in waterhemp control as the rate of glyphosate/dicamba was increased from 900 to 1350 to 1800 g ae ha⁻¹ (Table 1). Waterhemp

density and biomass decreased with an increase in the rate of glyphosate/dicamba. The weedy control had the highest waterhemp density and biomass, and glyphosate/dicamba applied at 1800 g ae ha⁻¹ had the lowest waterhemp density and biomass. Glyphosate/dicamba applied at 900, 1350 and 1800 g ae ha⁻¹ reduced waterhemp density by 65, 78 and 86% and biomass by 67, 81 and 90%, respectively, compared to the weedy control. The application of glyphosate/dicamba at 900 g ae ha⁻¹ increased soybean yield compared to the weedy check, but there was no difference in soybean yield among the three rates of glyphosate/dicamba.

Based on these results, the application of glyphosate/dicamba did not cause any injury in glyphosate/dicamba soybean in these trials. This was not unexpected since glyphosate/dicamba soybean contains transgenes that confer resistance to both glyphosate and dicamba and acceptable tolerance has been observed up to 2.8 kg ha⁻¹ (Behrens et al. 2007).

In this study, control of GR waterhemp was affected by the herbicide rate of glyphosate/dicamba at 2, 4 and 8 WAA. At 4 WAA, glyphosate/dicamba applied at 900, 1350 and 1800 g ae ha⁻¹ controlled GR waterhemp up to 66, 78 and 87%, respectively. Only glyphosate/dicamba (1800 g ae ha⁻¹) provided >80% control of GR waterhemp 2 and 4 WAA. At 8 WAA, there was improved control of GR waterhemp with the higher rates of glyphosate/dicamba. Hager et al. (2003) observed a 64 to 85% increase in waterhemp control when the rate of acifluorfen was increased from 70 to 280 g ai ha⁻¹, 83 to 95% increase when lactofen rate was increased from 55 to 218 g ai ha⁻¹, and 68 to 90% control of waterhemp when fomesafen rate increased from 87 to 350 g ai ha⁻¹.

There was an effect of glyphosate application timing in this study. At 2 WAA, the control of GR waterhemp decreased as the size of the waterhemp at the time of application increased. The results from this study are consistent with Spaunhorst and Bradley (2013) who found that

glyphosate + dicamba (860 + 560 g ae ha⁻¹) applied to GR waterhemp that was at 7.5, 15 and 30 cm in height provided 62, 40 and 30% control, respectively. However, this response reversed by 8 WAA, where improved control was observed with the late application timing. At 4 WAA, 82% control GR waterhemp was observed at the 5 cm application timing. At 8 WAA, control of 5 cm waterhemp decreased to 61%, which can be attributed to the extended emergence pattern of waterhemp in Ontario and the relatively short residual activity provided by dicamba (Vyn et al. 2007; Schryver et al. 2017). Season-long control of weeds is important to increase ease of harvest and reduce weed seed return to the soil.

Sequential applications of glyphosate/dicamba may be needed for full season control of GR waterhemp. However, this strategy is not preferred due to the increased selection pressure for glyphosate and dicamba-resistant waterhemp. Research on the control of GR waterhemp in narrow row soybean should be conducted to determine if earlier canopy closure will result in improved control. For full season control of GR waterhemp, a two-pass waterhemp control program of an effective soil applied herbicide followed by an effective postemergence may be required.

This study concludes that there was no interaction between application timing and herbicide rate. An increase in herbicide rate increased control of GR waterhemp, and application timings when waterhemp was smaller in size were more efficacious than applications to taller waterhemp (15 or 25 cm tall) early in the growing season but not at the end of the growing season.

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Table 1. Interactions between application timing, rate, and timing x rate. Means for GR Waterhemp control, density, biomass and soybean yield for application timings and herbicide rates at six site-years locations in 2016 and 2017 in Ontario.^a

Main effects	Visible waterhemp control (%)			Density (plants m ⁻²)	Biomass (g m ⁻²)	Yield (t ha ⁻¹)
	2 WAA	4 WAA	8 WAA			
Application timing (cm)	*	*	*	NS	NS	NS
5	81a	82a	61b	61	74	1.4
15	73b	77b	68ab	65	71	1.4
25	61c	72b	72a	71	73	1.3
Glyphosate/dicamba premix rate ^c (g ae ha ⁻¹)	*	*	*	*	*	*
0	0	0	0	202a	255a	1.0b
900 (600/300)	61c	66c	58c	71b	85b	1.5a
1350 (900/450)	72b	78b	68b	45c	49c	1.5a
1800 (1200/600)	82a	87a	77a	28d	26d	1.6a
Timing x rate	NS	NS	NS	NS	NS	NS

Note: WAA, weeks after application; ns, not significant at a level of P=0.05.

^aMeans followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (P=0.05); *significant difference between application timings, herbicide rates or timings x rates.

^bVisible control estimates based on comparisons made to weedy and weed-free control.

^c Two to one premix formulation of glyphosate and dicamba. The formulation contained 240 g ae L⁻¹ of glyphosate and 120 g ae L⁻¹ of dicamba.