

# Multiple-herbicide-resistant waterhemp control in glyphosate/glufosinate/2,4-D-resistant soybean with one- and two-pass weed control programs

## Research Article

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Waterhemp control; waterhemp biomass; waterhemp density; pre-plant herbicides; postemergence herbicides; soybean yield

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## Abstract

Waterhemp control in Ontario has increased in complexity due to the evolution of biotypes that are resistant to five herbicide modes of action (Groups 2, 5, 9, 14, and 27 as categorized by the Weed Science Society of America). Four field trials were carried out over a 2-yr period in 2021 and 2022 to assess the control of multiple-herbicide-resistant (MHR) waterhemp biotypes in glyphosate/glufosinate/2,4-D-resistant (GG2R) soybean using one- and two-pass herbicide programs. S-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, pyroxasulfone/flumioxazin, and pyroxasulfone + metribuzin applied preemergence (PRE) controlled MHR waterhemp similarly by 46%, 63%, 60%, and 69%, respectively, at 8 wk after postemergence (POST) application (WAA-B). A one-pass application of 2,4-D choline/glyphosate DMA POST provided greater control of MHR waterhemp than glufosinate. Two-pass herbicide programs of a PRE herbicide followed by (fb) a POST-applied herbicide resulted in greater MHR waterhemp control compared to a single PRE or POST herbicide application. PRE herbicides fb glufosinate or 2,4-D choline/glyphosate DMA POST controlled MHR waterhemp by 74% to 91% and by 84% to 96%, respectively, at 8 WAA-B. Two-pass herbicide applications of an effective PRE residual herbicide fb 2,4-D choline/glyphosate DMA POST in GG2R soybean can effectively manage waterhemp that is resistant to herbicides in Groups 2, 5, 9, 14, and 27.

## Introduction

Waterhemp is a competitive, dioecious, annual weed. Waterhemp reproduces by seed and can produce more than 1 million seeds on one plant in a noncompetitive environment (Costea et al. 2005; Nordby et al. 2007). The seeds produced are small and emerge from shallow depths. As a result, waterhemp is well-adapted to reduced tillage systems (Nordby et al. 2007). Growth is rapid in nitrogen-rich soils when temperatures are warm, when moderate to high soil moisture is available, and when light intensity is high (Costea et al. 2005). Waterhemp is a fast-growing weed species that can quickly acquire resources and out-compete crops and other weeds (Horak and Loughin 2000). Small flowers on the female plant are primarily wind-pollinated (Costea et al. 2005). The male plant produces pollen grains, commonly fertilizing female plants within 50 m; however, viable pollen can travel up to 800 m (Liu et al. 2012). The migration of waterhemp pollen is a crucial aspect in the spread of herbicide-resistant (HR) genes (Liu et al. 2012). Gene amplification of the glyphosate target site, 5-enolpyruvylshikimate-3-phosphate synthase, is heritable in waterhemp and can be transferred by pollen-mediated gene flow (Sarangi et al. 2017). This weed species possesses numerous advantageous traits that contribute to rapid herbicide resistance evolution including dioecious reproduction, high fecundity, and prolonged emergence (Schryver et al. 2017b).

Uncontrolled waterhemp competition has been reported to decrease the yield of soybean by 73% in Ontario (Vyn et al. 2007). Greater yield losses are observed when waterhemp emerges with the crop and is allowed to compete throughout the summer (Steckel and Sprague 2004). In Ontario, waterhemp emergence peaks in mid-June, although plants can emerge from May to October (Schryver et al. 2017b; Vyn et al. 2007). This prolonged emergence pattern contributes to the complexity of waterhemp control. New plants can emerge after residual preemergence (PRE) herbicides have dissipated (Hager et al. 2002; Steckel and Sprague 2004). Plants that emerge after application of a postemergence (POST) herbicide may not be controlled depending on the residual activity of the POST herbicide (Hager et al. 2002; Steckel and Sprague 2004).

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Late-season-emerging waterhemp plants are less competitive with reduced seed production; however, these plants can still contribute viable seeds to the soil seedbank (Nordby et al. 2007; Steckel and Sprague 2004).

Acceptable control of waterhemp is achievable in a two-pass herbicide application strategy when a soil-applied residual herbicide is followed by (fb) a POST herbicide application (Costea et al. 2005). When a two-pass program is implemented greater and more consistent waterhemp control is achieved, and crop yield is increased (Nordby et al. 2007). To ensure effective control, POST herbicides should be applied when waterhemp escapes are young (Meyer et al. 2015). In Ontario, Schryver et al. (2017b) observed >94% control of glyphosate-resistant waterhemp with a two-pass weed control consisting of a PRE fb a POST herbicide in soybean; most one-pass PRE programs did not produce satisfactory waterhemp control. The PRE-applied herbicides with activity on glyphosate-resistant waterhemp in soybean include pyroxasulfone/flumioxazin, pyroxasulfone/sulfentrazone, and S-metolachlor/metribuzin (Schryver et al. 2017b); this is supported by research conducted by Hedges et al. (2019) who found that the aforementioned herbicides controlled glyphosate-resistant waterhemp by 95%, 91%, and 87%, respectively. Studies conducted in Ontario found that a PRE herbicide fb a POST application of glufosinate or 2,4-D choline/glyphosate DMA in herbicide-resistant soybean controlled glyphosate-resistant waterhemp by  $\geq 96\%$  and 98%, respectively (Schryver et al. 2017c). Meyer et al. (2015) observed that two-pass herbicide strategies employing a total of three or more herbicide sites of action provided increased waterhemp control in soybean.

Glyphosate-resistant waterhemp was initially confirmed in 2005 in Missouri, and has now been confirmed in 19 U.S. states (Heap 2022; Legleiter and Bradley 2008). In Ontario, glyphosate-resistant waterhemp was initially reported in 2014 in a field in Lambton County (Schryver et al. 2017a). In both confirmed cases, farmers grew glyphosate-resistant soybean continuously with repeated applications of glyphosate over numerous years and limited use of other herbicide modes of action (Legleiter and Bradley 2008; Schryver et al. 2017a). The spread of glyphosate-resistant waterhemp occurs through the movement of seeds and by independent selection (Kreiner et al. 2019). Management of glyphosate-resistant waterhemp should include strategies to limit seed production, seed spread, and selection pressure for glyphosate-resistant biotypes (Kreiner et al. 2019).

Globally, waterhemp biotypes with confirmed cases of herbicide resistance to seven modes of action have been documented (Heap 2022). Populations of waterhemp with resistance to five herbicide modes of action (herbicides in Groups 2, 5, 9, 14, and 27 as categorized by the Weed Science Society of America [WSSA]) were confirmed in Ontario in 2021 (Heap 2022). Benoit et al. (2020) reported that in eastern Canada waterhemp from 92% of sites screened had single plants that exhibited resistance to at least two herbicide modes of action. This multiple-herbicide-resistant (MHR) waterhemp population limits herbicide options and intensifies selection pressure on existing effective herbicides (Benoit et al. 2020).

As new herbicide-resistant technologies come to the market, POST herbicide options have expanded in soybean and allow alternate mode of actions to be applied in-season. Growers now have access to glyphosate/glufosinate/2,4-D choline-resistant (GG2R) soybean (E3 soybean<sup>TM</sup>). Herbicide programs that use new herbicide-resistant technologies, including 2,4-D-resistant soybean, have demonstrated longer and improved control of waterhemp

(Meyer et al. 2015). In a two-pass weed control program, the application of a PRE herbicide can reduce weed size and density at the time of the POST application; the efficacy of the POST herbicide may be improved due to smaller size and less dense weed populations at application (Davis et al. 2010). The objectives of this study are two-fold: first, to assess one- and two-pass herbicide applications; and second, to compare the efficacy of glufosinate and 2,4-D choline/glyphosate DMA applied POST in a two-pass weed control strategy to control MHR waterhemp in GG2R soybean.

## Methods and Materials

A study consisting of four field trials was conducted during the 2021 and 2022 growing seasons on commercial farms with confirmed MHR waterhemp populations. MHR waterhemp at each site possess five-way herbicide resistance to herbicides in WSSA Groups 2, 5, 9, 14, and 27 (Symington et al. 2022). Trials were conducted near Cottam (42.15°N, 82.68°W), Newbury (42.70°N, 81.82°W), and Newbury Station (42.72°N, 81.82°W), Ontario. Site locations, soil characteristics, soybean planting, emergence, and harvest dates, and PRE and POST herbicide application information are presented in Table 1. The trials were conducted using a randomized complete block design with four replications. GG2R soybean [Brevant seeds cultivar 'B061FE' (Corteva Agriscience, Calgary, AB)] was planted to a depth of 3.75 cm at a rate of approximately 420,000 seeds ha<sup>-1</sup>. Plots were 2.25 m wide (three soybean rows spaced 75 cm apart) by 8 m in length. The treatments consisted of S-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, pyroxasulfone/flumioxazin, and pyroxasulfone + metribuzin, applied PRE; glufosinate and 2,4-D choline/glyphosate DMA applied POST; and two-pass programs of a PRE herbicide fb a POST herbicide. The PRE herbicide application timing is referred to as Application A and the POST herbicide application timing is referred to as Application B. Information on the PRE and POST herbicides included in this study is presented in Table 2. The herbicides were applied using a backpack sprayer pressurized with CO<sub>2</sub>. The spray boom was equipped with four ULD 11002 (Pentair, New Brighton, MN) spray nozzles spaced 50 cm apart producing a spray width of 2 m. The backpack sprayer was adjusted to deliver a 200 L ha<sup>-1</sup> spray volume at 240 kPa. The PRE herbicide treatments were applied after planting and prior to soybean emergence. The POST herbicide treatments were applied as soon as MHR waterhemp escapes reached an average of 10 cm in height in any PRE herbicide treatment; one POST application was made. In an effort to reduce the time-of-day at application effect on glufosinate efficacy, the POST herbicide applications were applied after 0900 and before 1100 hours (Martinson et al. 2005; Montgomery et al. 2017; Takano and Dayan 2020).

Soybean injury was visually assessed 2 wk after crop emergence (WAE), 4 wk after the PRE herbicide application (WAA-A), and 1 and 4 wk after the POST herbicide application (WAA-B). Assessments were performed on a 0% to 100% scale, where 0% represents no injury and 100% represents soybean death. MHR waterhemp control was visually assessed 2 WAA-A, just prior to the POST application (4 WAA-A), and 4 and 8 WAA-B. Waterhemp density and dry weight (biomass) were determined 4 WAA-B. Two 0.25-m<sup>2</sup> quadrats were randomly placed within each plot where MHR waterhemp plants were counted, clipped at the soil surface, and placed in labeled paper bags. The bags were then placed in a kiln and dried at 60 C until the biomass reached constant moisture (approximately 2wk), after which the dry weight was recorded. At harvest maturity, the center two rows from each

**Table 1.** Year, location, soil characteristics, soybean, and herbicide application information for four field trials.<sup>a</sup>

Year	Location	Soil characteristics			Soybean			Herbicide application			
					Planting date	Emergence date	Harvest date	PRE	POST		
		Texture	OM	pH				Application date	Application date	Waterhemp height <sup>b</sup>	Waterhemp density
			%							cm	plants m <sup>-2</sup>
2021	Cottam	Sandy loam	2.3	5.9	May 18	May 24	September 20	May 20	June 16	10	1060
	Newbury	Loamy sand	2.8	6.5	May 11	May 20	November 23	May 14	June 10	9	839
2022	Cottam	Sandy loam	2.2	5.7	May 17	May 27	September 22	May 18	June 29	11	549
	Newbury Station	Loamy sand	2.5	6.7	May 12	May 21	September 22	May 13	June 23	10	1645

<sup>a</sup>Abbreviations: OM, organic matter; PRE, preemergence; POST, postemergence.

<sup>b</sup>Waterhemp height at POST application average the nontreated control.

**Table 2.** Herbicides used in trials for two-pass MHR waterhemp control in soybean resistant to glyphosate/glufosinate/2,4-D.

Herbicide <sup>a</sup>	Trade name	Rate	Manufacturer	Manufacturer address
Glufosinate ammonium	Liberty <sup>®</sup> 200 SN	500	BASF Canada Inc.	100 Milverton Drive, Mississauga, ON, Canada, L5R 4H1; <a href="https://www.basf.com/ca/en.html">https://www.basf.com/ca/en.html</a>
2,4-D choline/glyphosate DMA	Enlist Duo <sup>™</sup>	1,720	Corteva Agriscience	215 2nd St. SW, Calgary, AB, Canada, T2P 1M4; <a href="https://www.corteva.ca/">https://www.corteva.ca/</a>
S-metolachlor/metribuzin	Boundary <sup>®</sup> LQD	1,443	Syngenta Canada Inc.	140 Research Lane, Guelph, ON, Canada, N1G 4Z3; <a href="https://www.syngenta.ca/">https://www.syngenta.ca/</a>
Pyoxasulfone/sulfentrazone	Authority <sup>®</sup> Supreme	250	FMC Corporation	Suite 204, 6755 Mississauga Rd, Mississauga, ON, Canada, L5N 7Y2; <a href="https://ag.fmc.com/ca/en">https://ag.fmc.com/ca/en</a>
Pyoxasulfone/flumioxazin	Fierce <sup>®</sup>	160	Valent Canada Inc.	107 Woodlawn Rd W, Guelph, ON, Canada, N1H 1B4; <a href="https://www.valent.ca/">https://www.valent.ca/</a>
Pyoxasulfone	Zidua <sup>®</sup> SC	120	BASF Canada Inc.	100 Milverton Drive, Mississauga, ON, Canada, L5R 4H1; <a href="https://www.basf.com/ca/en.html">https://www.basf.com/ca/en.html</a>
Metribuzin	Sencor <sup>®</sup> 480	400	Bayer Crop Science Inc.	160 Quarry Park Boulevard SE, Calgary, AB, Canada, T2C 3G3; <a href="https://www.cropsience.bayer.ca/en/">https://www.cropsience.bayer.ca/en/</a>

<sup>a</sup>The recommended adjuvant was applied with each herbicide used: Glufosinate ammonium included ammonium sulfate (Alpine Plant Foods, 30 Neville St, New Hamburg, ON, Canada, N3A 4G7) at 6.5 L ha<sup>-1</sup>.

plot were harvested with a small-plot combine, and soybean seed weight and moisture content were recorded. Prior to statistical analysis, soybean yield was adjusted to 13.0% moisture.

### Statistical Analysis

Statistical analysis was performed using the GLIMMIX procedure in SAS software (version 9.4; SAS, Cary, NC). The variance consisted of the fixed effect of herbicide treatment and the random effects of environment, and replication within environment. Environment incorporates differences in year and location of the trials. There was no significant treatment by environment interactions; data were pooled across all environments. Assumptions of normality were tested using the Shapiro-Wilk statistic and plotted residuals. Assumptions included that the errors are random, homogenous, independent of effects, have a mean of zero, and are normally distributed. Control data were arcsine square-root transformed to meet these assumptions. The density and dry biomass data were analyzed using a lognormal distribution. All transformed data were subject to back-transformations for the presentation of results. Contrasts were used to compare PRE vs. POST, PRE vs. PRE fb POST, POST vs. PRE fb POST,

and to compare the two POST herbicides in a two-pass system. The Tukey-Kramer test was used with a significance of  $P = 0.05$ .

## Results and Discussion

### Soybean Injury

All herbicides evaluated caused minimal soybean injury (<10%); data are not presented.

### MHR Waterhemp Control

The MHR waterhemp control among the PRE herbicides evaluated was not significantly different at 2 WAA-A; the PRE herbicides controlled MHR waterhemp by 94% to 98% (Table 3). At 4 WAA-A, pyoxasulfone/sulfentrazone and pyoxasulfone + metribuzin controlled MHR waterhemp greater than S-metolachlor/metribuzin; pyoxasulfone/flumioxazin provided intermediate control, which was similar to that of all PRE herbicides.

Waterhemp control data 4 and 8 WAA-B, waterhemp density, waterhemp dry biomass, and seed yield of soybean data are presented in Table 4. The PRE application of S-metolachlor/metribuzin, pyoxasulfone/sulfentrazone, pyoxasulfone/flumioxazin, and

**Table 3.** Multiple herbicide-resistant waterhemp control with PRE herbicides evaluated prior to a POST herbicide application from four field trials.<sup>a,b</sup>

Herbicide treatment	Application timing	Rate g ai ha <sup>-1</sup>	Visible control	
			2 WAA-A	4 WAA-A
S-metolachlor/metribuzin	PRE	1,443	94 a	85 b
Pyroxasulfone/sulfentrazone	PRE	250	95 a	92 a
Pyroxasulfone/flumioxazin	PRE	160	94 a	90 ab
Pyroxasulfone + metribuzin	PRE	120 + 400	98 a	93 a

<sup>a</sup>Abbreviations: PRE, preemergence; POST, postemergence; WAA-A, weeks after application A (preemergence herbicide treatment).

<sup>b</sup>Means in the same column followed by the same letter are not statistically different.

**Table 4.** Means and nonorthogonal contrasts for multiple-herbicide resistant waterhemp control, density, dry biomass, and soybean yield.<sup>a,b,c</sup>

Treatment	Rate g ai ha <sup>-1</sup>	Application timing	4 WAA-B		Density plants m <sup>-2</sup>	Dry biomass g m <sup>-2</sup>	Soybean yield kg × 1,000 ha <sup>-1</sup>
			%				
Nontreated control			0	0	1,306 g	196.7 f	1.46 b
Glufosinate	500	POST	40 e	26 e	480 fg	65.5 ef	2.23 a
2,4-D choline/glyphosate DMA	1,750	POST	79 abcd	75 abc	145 ef	15.7 abcde	2.39 a
S-metolachlor/metribuzin	1,443	PRE	63 d	46 de	49 de	77.6 ef	2.28 a
fb glufosinate	500	POST	81 abcd	74 abc	38 bcde	5.9 abcd	2.47 a
fb 2,4-D choline/glyphosate DMA	1,750	POST	90 abc	84 abc	40 abcd	1.4 ab	2.57 a
Pyroxasulfone/sulfentrazone	250	PRE	71 cd	63 bcd	29 cde	37.7 cde	2.39 a
fb glufosinate	500	POST	91 abc	90 ab	13 abcd	2.6 abc	2.73 a
fb 2,4-D choline/glyphosate DMA	1,750	POST	97 a	96 a	5 abc	0.9 a	2.59 a
Pyroxasulfone/flumioxazin	160	PRE	72 bcd	60 cd	30 bcde	75.2 de	2.28 a
fb glufosinate	500	POST	92 abc	87 abc	6 abc	1.8 ab	2.65 a
fb 2,4-D choline/glyphosate DMA	1,750	POST	97 a	94 a	3 a	0.3 a	2.55 a
Pyroxasulfone + metribuzin	120 + 400	PRE	75 bcd	69 abcd	19 abcd	26.1 bcde	2.51 a
fb glufosinate	500	POST	94 ab	91 a	8 abc	1.7 abc	2.69 a
fb 2,4-D choline/glyphosate DMA	1,750	POST	97 a	96 a	10 ab	0.8 a	2.68 a
Contrasts							
PRE vs. POST			70 vs. 59*	60 vs. 51	37 vs. 399*	78.7 vs. 58.0	2.36 vs. 2.31
PRE vs. PRE fb POST			70 vs. 92*	60 vs. 89*	37 vs. 21*	78.7 vs. 2.1*	2.36 vs. 2.62*
POST vs. PRE fb POST			59 vs. 92*	51 vs. 89*	399 vs. 21*	58.0 vs. 2.1*	2.31 vs. 2.62*
PRE fb glufosinate vs. PRE fb 2,4-D choline/glyphosate DMA			89 vs. 95*	86 vs. 92	20 vs. 22	3.5 vs. 0.9	2.63 vs. 2.59

<sup>a</sup>Abbreviations: fb, followed by; NS, nonsignificant; POST, postemergence; PRE, preemergence; WAA-B, weeks after application B (POST herbicide treatment).

<sup>b</sup>Means followed by the same lowercase letter within the same column are not statistically different according to the Tukey-Kramer test ( $P < 0.05$ ).

<sup>c</sup>An asterisk (\*) indicates  $P < 0.05$ .

pyroxasulfone + metribuzin controlled MHR waterhemp by 63%, 71%, 72%, and 75%, respectively at 4 WAA-B; control decreased to 46%, 63%, 60%, and 69%, respectively, at 8 WAA-B. 2,4-D choline/glyphosate DMA applied POST provided greater control of MHR waterhemp by 39 and 49 percentage points compared to glufosinate at 4 and 8 WAA-B, respectively. In greenhouse studies, Chahal et al. (2015) observed 95% control of glyphosate-resistant waterhemp plants that were  $\leq 10$  cm tall with 2,4-D choline/glyphosate DMA (1,640 g ae ha<sup>-1</sup>). Following a PRE application of S-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, pyroxasulfone/flumioxazin, or pyroxasulfone + metribuzin, a POST application of glufosinate or 2,4-D choline/glyphosate DMA improved MHR waterhemp control by 18% to 27%, 20% to 26%, 20% to 25%, and 19% to 22%, respectively, 4 WAA-B. Based on nonorthogonal contrasts a single PRE herbicide application provided 11% greater MHR waterhemp control than a single POST application, a two-pass herbicide program provided greater MHR waterhemp control compared to a single-pass of a PRE (22%) or POST (33%) herbicide program, and in a two-pass program, a POST 2,4-D choline/glyphosate DMA application provided greater control of MHR waterhemp than glufosinate (6%) at 4 WAA-B. Results are

similar to research conducted by Schryver et al. (2017c) who found enhanced MHR waterhemp control when applying 2,4-D choline/glyphosate DMA POST compared to glufosinate POST. Glufosinate or 2,4-D choline/glyphosate DMA applied POST following S-metolachlor/metribuzin applied PRE improved control by 28 and 38 percentage points, respectively, 8 WAA-A. Following pyroxasulfone/sulfentrazone or pyroxasulfone/flumioxazin applied PRE, 2,4-D choline/glyphosate DMA applied POST improved MHR waterhemp by 33 and 34 percentage points, respectively, 8 WAA-B. Based on nonorthogonal contrasts a two-pass herbicide strategy of a PRE fb a POST application resulted in increased MHR waterhemp control than a single PRE (29%) or POST (38%) application; however, no significant differences were observed between a single PRE compared to POST program, and when applied following a PRE-applied herbicide.

#### MHR Waterhemp Density and Dry Biomass

All herbicide treatments decreased MHR waterhemp density (89% to 100%) as a percentage of the nontreated control, except for glufosinate applied POST at 4 WAA-B. The PRE-applied herbicides

lowered MHR waterhemp density by 96% to 99%. All two-pass herbicide programs reduced MHR density by  $\geq 97\%$ . Based on nonorthogonal contrasts herbicide programs consisting of a PRE fb a POST treatment caused a greater decrease in MHR waterhemp density than one-pass PRE or POST herbicides, and a PRE herbicide application caused a greater decrease in MHR waterhemp density than a single POST application. When applied following a PRE herbicide there was no difference in MHR waterhemp control between the POST application of 2,4-D choline/glyphosate DMA and glufosinate.

S-metolachlor/metribuzin applied PRE and glufosinate applied POST did not decrease MHR waterhemp dry biomass compared to that of the nontreated control. Pyroxasulfone/sulfentrazone, pyroxasulfone/flumioxazin, and pyroxasulfone + metribuzin applied PRE reduced MHR waterhemp biomass similarly at 62% to 87%. The two-pass weed control programs reduced MHR waterhemp biomass 97% to 100%. A POST application of glufosinate or 2,4-D choline/glyphosate DMA following a PRE application of S-metolachlor/metribuzin or pyroxasulfone/flumioxazin decreased dry biomass compared to a single PRE application of the aforementioned PRE herbicides. 2,4-D choline/glyphosate DMA applied POST following pyroxasulfone/sulfentrazone or pyroxasulfone + metribuzin applied PRE decreased dry biomass compared to a single PRE application. Based on nonorthogonal contrasts, a two-pass system of a PRE herbicide fb glufosinate or 2,4-D choline/glyphosate DMA applied POST reduced MHR waterhemp dry biomass compared to a single-pass herbicide application.

### Soybean Yield

Soybean seed yield decreased 47% due to MHR waterhemp presence (highest yielding treatment compared to nontreated control). Based on nonorthogonal contrasts reduced waterhemp interference with two-pass weed control strategies resulted in greater soybean yield than a single PRE or POST application. This is consistent with research carried out by Schryver et al. (2017b) that showed increased soybean seed yield with PRE fb POST herbicide treatments evaluated for the control of glyphosate-resistant waterhemp.

In summary, two-pass weed control programs of a PRE fb POST herbicide provided improved MHR waterhemp control, reduced waterhemp density and dry biomass, and greater soybean seed yield. Results are similar to those reported by Craigmyle et al. (2013) who observed improved waterhemp control with PRE fb POST strategies that incorporate glufosinate and 2,4-D applied POST. 2,4-D choline/glyphosate DMA applied POST provided superior MHR waterhemp control than the POST application of glufosinate. Reduced control with glufosinate may be due to the herbicide's limited translocation in the weed compared to 2,4-D, which has symplastic translocation (Sterling and Hall 1997; Takano et al. 2020). Herbicide programs that use a PRE residual herbicide and a POST treatment of 2,4-D choline/glyphosate DMA provide GG2R soybean growers with solutions for managing MHR waterhemp populations. Season-long control of MHR waterhemp reduces the number of seeds in the soil seedbank and limits the spread of MHR waterhemp. By using a diverse selection of integrated weed management (IWM) strategies growers can reduce the herbicide resistance selection pressure placed on waterhemp populations. IWM strategies include a reduction in crop row width, cover crops, alternating herbicide modes of action, and crop rotation. Herbicide-resistant crop technologies can be an integral

part of an IWM program; however, they should be used judiciously to reduce resistance selection and maintain their effectiveness.

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### References

- Benoit L, Hedges B, Schryver MG, Soltani N, Hooker DC, Robinson DE, Laforest M, Soufiane B, Tranel PJ, Giacomini D, Sikkema PH (2020) The first record of protoporphyrinogen oxidase and four-way herbicide resistance in eastern Canada. *Can J Plant Sci* 100:327–331
- Chahal PS, Aulakh JS, Rosenbaum K, Jhala AJ (2015) Growth stage affects dose response of selected glyphosate-resistant weeds to premix of 2,4-D choline and glyphosate (Enlist Duo herbicide). *J Agr Sci* 7:1–10
- Costea M, Weaver SE, Tardif FJ (2005) The biology of invasive alien plants in Canada 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. *Can J Plant Sci* 85:507–522
- Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of herbicide programs on weed management in soybean with resistance to glufosinate and 2,4-D. *Weed Technol* 27:78–84
- Davis JT, Kruger GR, Young BG, Johnson WG (2010) Fall and spring preplant herbicide applications influence spring emergence of glyphosate-resistant horseweed (*Conyza canadensis*). *Weed Technol* 24:11–19
- Hager AG, Wax LM, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. *Weed Sci* 50:607–610
- Heap I (2022) The international survey of herbicide-resistant weeds. [www.weedscience.org](http://www.weedscience.org) Accessed: September 14, 2022
- Hedges BK, Soltani N, Hooker DC, Robinson DE, Sikkema PH (2019) Control of glyphosate-resistant waterhemp with preemergence herbicides in glyphosate- and dicamba-resistant soybean. *Can J Plant Sci* 99:34–39
- Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. *Weed Sci* 48:347–355
- Kreiner JM, Giacomini DA, Bemm F, Waithaka B, Regalado J, Lanz C, Hildebrandt J, Sikkema PH, Tranel PJ, Weigel D, Stinchcombe JR, Wright SI (2019) Multiple modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*. *Proc Natl Acad Sci USA* 116:21076–21084
- Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. *Weed Sci* 56:582–587
- Liu J, Davis AS, Tranel PJ (2012) Pollen biology and dispersal dynamics in waterhemp (*Amaranthus tuberculatus*). *Weed Sci* 60:416–422
- Martinson KB, Durgan BR, Gunsolus JL, Sothorn RB (2005) Time of day of application effect on glyphosate and glufosinate efficacy. *Crop Manag Res* 4:1–7
- Meyer CJ, Norsworthy JK, Young BG, Steckel LE, Bradley KW, Johnson WG, Loux MM, Davis VM, Kruger GR, Bararpour MT, Ikley JT, Spaunhorst DJ, Butts TR (2015) Herbicide program approaches for managing glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus* and *Amaranthus rudis*) in future soybean-trait technologies. *Weed Sci* 29:716–729
- Montgomery GB, Treadway JA, Reeves JL, Steckel LE (2017) Effect of time of day of application of 2,4-D, dicamba, glufosinate, paraquat, and saflufenacil on horseweed (*Conyza canadensis*) control. *Weed Technol* 31:550–556
- Nordby D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. West Lafayette, IN: Purdue Extension. <https://extension.missouri.edu/media/wysiwyg/Extensiondata/Pub/pdf/miscpubs/mx1137.pdf>. Accessed: September 19, 2022
- Sarangi D, Tyre AJ, Patterson EL, Gaines TA, Irmak S, Knezevic SZ, Lindquist JL, Jhala AJ (2017) Pollen-mediated gene flow from glyphosate-resistant common waterhemp (*Amaranthus rudis* Sauer): consequences for the dispersal of resistance genes. *Sci Rep* 7:44913
- Schryver MG, Soltani N, Hooker DC, Robinson DE, Tranel PJ, Sikkema PH (2017a) Glyphosate-resistant waterhemp (*Amaranthus tuberculatus* var. *rudis*) in Ontario, Canada. *Can J Plant Sci* 97:1057–1067

- Schryver MG, Soltani N, Hooker DC, Robinson DE, Tranel PJ, Sikkema PH (2017b) Control of glyphosate-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) in soybean in Ontario. *Weed Technol* 31:811–821
- Schryver MG, Soltani N, Hooker DC, Robinson DE, Tranel PJ, Sikkema PH (2017c) Control of glyphosate-resistant common waterhemp (*Amaranthus rudis*) in three new herbicide-resistant soybean varieties in Ontario. *Weed Technol* 31:828–837
- Steckel LE, Sprague CL (2004) Late-season common waterhemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. *Weed Technol* 18:947–952
- Sterling TM, Hall CJ (1997) Mechanism of action of natural auxins and the auxinic herbicides. Pages 111–142 in Roe RM, Burton JD, Kuhr RJ, eds. *Herbicide Activity: Toxicology, Biochemistry and Molecular Biology*. Burke VA: IOS Press
- Symington HE, Soltani N, Sikkema PH (2022) Confirmation of 4-hydroxyphenylpyruvate dioxygenase inhibitor-resistant and 5-way multiple-herbicide-resistant waterhemp in Ontario, Canada. *J Agr Sci* 14:53–58
- Takano HK, Dayan FE (2020) Glufosinate-ammonium: a review of the current state of knowledge. *Pest Manag Sci* 76:3911–3925
- Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2020) Physiological factors affecting uptake and translocation of glufosinate. *J Agr Food Chem* 68:3026–3032
- Vyn JD, Swanton CJ, Weaver SE, Sikkema PH (2007) Control of herbicide-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) with pre- and post-emergence herbicides in soybean. *Can J Plant Sci* 87:175–182