

Control of Glyphosate-Resistant Common Waterhemp (*Amaranthus tuberculatus* var. *rudis*) in Soybean in Ontario

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Glyphosate-resistant (GR) common waterhemp is the fifth GR weed species confirmed in Canada, and the fourth in Ontario. As of 2017, GR common waterhemp has been confirmed in Lambton, Essex, and Chatham-Kent counties in Ontario. Greenhouse and field dose–response experiments revealed that GR common waterhemp in Ontario had a resistance level of 4.5 and 28, respectively, when compared with known glyphosate-susceptible populations. At 12 wk after application, pyroxa-sulfone/flumioxazin (240 g ai ha⁻¹), pyroxasulfone/sulfentrazone (300 g ai ha⁻¹), and S-metolachlor/ metribuzin (1,943 g ai ha⁻¹) controlled GR common waterhemp 97%, 92%, and 87%, respectively. Pyroxasulfone/sulfentrazone or S-metolachlor/metribuzin applied PRE followed by acifluorfen (600 g ai ha⁻¹) or fomesafen (240 g ai ha⁻¹) applied POST controlled GR common waterhemp 98% and performed better than PRE or POST alone. This research is the first to determine the resistance factor of GR common waterhemp in Ontario and identifies control strategies in soybean to mitigate the impact of common waterhemp interference in soybean crop production.

Nomenclature: Acifluorfen; flumioxazin; fomesafen; glyphosate; *S*-metolachlor; metribuzin; pyroxasulfone; sulfentrazone; common waterhemp, *Amaranthus tuberculatus* var. *rudis*; soybean, *Glycine max* (L.) Merr.

Key words: Biologically effective rate, distribution, dose response, glyphosate susceptible, management, multiple-resistant.

Common waterhemp is a small-seeded, summer annual, dioecious, broadleaf weed in the Amaranthus genus and is relatively new to Ontario. Common waterhemp is thought to have been introduced into Ontario via a demonstration combine from Illinois in the early 2000s (Costea et al. 2005). Research conducted on glyphosate-susceptible (GS) populations found that common waterhemp interference in soybean can reduce yield up to 73% (Vyn et al. 2007). Waterhemp has become a more problematic weed in Ontario following the confirmation of its resistance to glyphosate in 2014 (Heap 2017; Schryver et al. 2017). The mechanism of glyphosate resistance in the first glyphosate-resistant (GR) common waterhemp population in Ontario is due at least in part to gene amplification, with 6 to 13 copies of the EPSPS gene (PJT, unpublished data). GR common waterhemp has now been

confirmed in 40 fields across 3 Ontario counties, including Lambton, Chatham-Kent, and Essex (Schryver et al. 2017). In addition, there is resistance to herbicide Groups 2, 5, and 9 in the same field (Schryver et al. 2017). This new multiply resistant common waterhemp population presents a significant weed management challenge for Ontario soybean growers.

Waterhemp can be distinguished from other pigweed species by its narrow, lance-shaped leaves, hairless leaves and stems, and potential for growth of up to 3 m in height (Costea et al. 2005; Ontario Ministry of Agriculture and Food [OMAF] 2004). This dioecious species has high genetic diversity due to exchange of genetic traits between separate male and female plants (Costea el al. 2005). This genetic diversity results in high levels of variability in plant morphology, including prostrate to erect growth

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habit (Costea et al. 2005) and green to red/purple coloration (OMAF 2004). Although closely related to other pigweed species commonly found in Ontario, common waterhemp can be distinguished in the vegetative stage by its entirely hairless stems and leaves and lance-shaped leaves and in the reproductive stage by separate male and female plants.

Because common waterhemp is dioecious, it has the ability to acquire new traits that may confer herbicide resistance more rapidly than in monoecious species (Costea et al. 2005; Wu and Owen 2014). Waterhemp has high fecundity, with one female plant producing up to 4.8 million seeds in a noncompetitive environment (Hartzler et al. 2004). The seed can remain viable in the soil for up to 14 yr (Burnside et al. 1996). Waterhemp has an extended emergence pattern in Ontario, beginning in early spring and continuing through September (Vyn et al. 2007). Due to a number of biologically advantageous traits, including dioecious reproduction, prolific seed production, and a prolonged emergence pattern, the evolution of herbicide resistance in common waterhemp can occur rapidly.

Glyphosate is a systemic, broad-spectrum, herbicide with a unique mode of action and is widely used globally. Glyphosate is absorbed through the cuticle into the living portion of the plant and moves via the phloem with sugars to the meristematic regions, where it disrupts the shikimate biosynthetic pathway (Duke and Powles 2008; Franz et al. 1997). Glyphosate binds to enolpyruvylshikimate-3-phosphate synthase (EPSPS), displacing phosphoenol pyruvate. This replacement halts the production of 5-enolpyruvylshikamate-3-phosphate, a key precursor in the production of three aromatic amino acids: tyrosine, phenylalanine, and tryptophan (Sikorski and Gruys 1997). Due to its unique mode of action and high efficacy, glyphosate is used extensively around the world.

The widespread and repeated use of glyphosate has resulted in the evolution of GR weed populations (Powles 2008; Shaner 2009). The increase in glyphosate use is correlated with the introduction of GR crops in Ontario in 1997. In soybean, Young (2006) reported that before the introduction of GR soybean, less than 3 million kg year⁻¹ of glyphosate was used annually; this had increased to 30 million kg year⁻¹ by 2002 in the United States. The first confirmed GR weed was rigid ryegrass (*Lolium rigidum* Gaudin) in Australia in 1996; now there are 37 GR weed species (Heap 2017). The overreliance on glyphosate has led to the rapid evolution of GR weeds.

Although new to Ontario, GR common waterhemp was first reported in Missouri in 2005, and has since been reported in 18 U.S. states as of 2016 (Heap 2017; Legleiter and Bradley 2008). There are three known mechanisms that confer resistance to glyphosate in GR common waterhemp. The first is gene amplification, first found in closely related Palmer amaranth (Amaranthus palmeri S. Wats.) (Gaines et al. 2010). A recent multistate study found that EPSPS gene copy number was correlated with the level of glyphosate resistance, which is consistent with previous findings of a positive relationship between gene copy number and resistance factor (Chatham et al. 2015a; Shaner et al. 2012; Tranel et al. 2010). This resistance mechanism was found in 91% of 80 common waterhemp populations examined (Chatham et al. 2015b). A second mechanism of glyphosate resistance in common waterhemp is due to an altered target site with a serine to proline substitution at position 106 of the EPSPS enzyme (Pro-106-Ser) (Bell et al. 2013). The third mechanism of glyphosate resistance, found in a common waterhemp population in Mississippi, is reduced translocation in conjunction with an altered target site, which conferred a 5-fold level of resistance (Nandula et al. 2013). There was a 10% reduction in glyphosate translocated out of the treated leaf in GR compared with GS populations (Nandula et al. 2013).

A wide range in the resistance factor to glyphosate exists for common waterhemp. In 2008, a common waterhemp population in Texas, exposed to three glyphosate applications per year in a continuous cotton production field, was found to have a resistance factor of 4 to 60 (Light et al. 2011). Similarly, in 2012, following 8 yr of GR corn and soybean production, a common waterhemp population in Nebraska was found to have a resistance factor of 3 to 39 (Sarangi et al. 2015a). This vast range in the resistance factor to glyphosate in common waterhemp demonstrates the variability within this species.

Heap (2017) now reports resistance in common waterhemp to six herbicide groups: 2, 4, 5, 9, 14, and 27, making it increasingly difficult to manage, as fewer herbicide options remain to control this weed. Multiple resistance is becoming increasingly common, with a recent discovery of a common waterhemp population in Illinois with resistance to five modes of action, including Groups 2, 4, 5, 14, and 27 (Heap 2017). Research on control of common waterhemp in soybean in Canada is limited to GS populations. Vyn et al. (2007) reported that acifluorfen or fomesafen (Group 14 herbicides) applied POST provided greater than 80% control.

A number of studies found that the use of a PRE herbicide is an important component of a successful control strategy for GR common waterhemp in soybean. Although the Group 15 herbicides (pyroxasulfone, dimethenamid-P, and S-metolachlor) provide excellent control in some studies, control has been variable. Legleiter et al. (2009) found that S-metolachlor/metribuzin applied PRE compared with glyphosate applied POST for the control of GR common waterhemp resulted in greater net returns. In that research, densities of GR common waterhemp were reduced from up to 70 plants m⁻² with glyphosate applied POST to less than 5 plants m^{-2} when a PRE herbicide was applied. Similarly, research conducted in Ontario reported that S-metolachlor/metribuzin applied PRE controlled GS common waterhemp by 94% (Vyn et al. 2007). Sarangi et al. (2015b) reported higher soybean yields with a full rate of a Group 15 applied PRE compared with a sequential application, with yields of 2,100 and 1,800 kg ha^{-1} , respectively. In contrast, Behnken et al. (2015) reported that layered applications of a PRE herbicide controlled GR common waterhemp 90% to 95% compared with 62% to 81% with a single PRE application, resulting in an increase in soybean yield of 0.40-0.94 T ha⁻¹. Finally, acetochlor, an additional Group 15 herbicide, provided GR common waterhemp control equivalent to the weed-free control, but this herbicide is not available in Ontario (Jhala et al. 2015).

The objectives of this research were: 1) to determine the glyphosate resistance factor of GR common waterhemp populations in Ontario in controlled and field environments, 2) to ascertain the efficacy of soil-applied residual herbicides for the control of GR common waterhemp, and 3) to evaluate two-pass weed control programs for the control of GR common waterhemp in soybean. This is the first research on the control of GR common waterhemp in soybean in Canada.

Materials and Methods

Biologically Effective Rate (BER). These experiments were conducted in both the greenhouse and the field.

For the BER greenhouse study, seeds from two populations of common waterhemp representing a GS population (collected near Petrolia, ON) and a GR population (collected near Walpole, ON) were stratified by refrigeration at 4 C for 8 wk. The GS population was from a known GS site (Vyn et al. 2007). The GR seed was collected in 2014 from a grower's field with the first confirmed case of GR common waterhemp in Ontario (Schryver et al. 2017). Waterhemp seeds were germinated in trays filled with soilless mixture (Pro-Mix[®] PGX, Premier Tech Horticulture, Québec, Canada). When the seedlings were at the cotyledon to first true-leaf stage, they were transplanted into individual pots (10-cm diameter) containing the same soilless mixture. When the common waterhemp seedlings were 10 cm in height, glyphosate was applied at 14, 28, 56, 113, 225, 450, 900, 1,800, or 3,600 g ae ha⁻¹ for the GS population, and 56, 113, 225, 450, 900, 1,800, 3,600, 7,200, or 14,400 g ae ha⁻¹ for the GR population. Glyphosate was applied in a spray chamber with a flat-fan nozzle calibrated to apply $200 \text{ L} \text{ ha}^{-1}$ at 280 kPa while moving at 2.15 km h⁻¹ Plants were then arranged in a randomized complete block design with 10 replicates. An untreated control was included in each replicate. Common waterhemp control was estimated visually at 1, 2, 3, and 4 wk after application (WAA) on a scale of 0% to 100%. A rating of 0% represented a healthy plant, and 100% represented complete mortality. Dry weight was determined at 4 WAA by cutting any remaining plant material at the soil line, placing it in a paper bag, and drying it in a kiln at 60 C to a constant moisture. The experiment was repeated three times.

For the BER field study, eight field experiments (four with GS and four with GR common waterhemp) were conducted over a 2-yr period (2015 and 2016) in commercial soybean fields with known GS or GR common waterhemp. For the GR common waterhemp studies, DeKalb[®] '30-61RY' soybean was seeded in rows spaced 75-cm apart at approximately 400,000 seeds ha⁻¹ at a depth of 3.75 cm. Field location and year, soil characteristics, seeding date, herbicide application date, and common waterhemp size and density at application are presented in Table 1. The GS population research was set up in established soybean fields. The experiments followed a randomized completed block design with four replications. Each replicate included a weedy and a weedfree control. The weed-free plots were maintained

I able 1. Field site and herbicide ap glyphosate-susceptible (GS) and -resist:	plication infor ant (GR) comi	mation f non wate	or biologically el rhemp in 2015 a	ffective rate a and 2016 in (nd one Dntario.	- and ª	two-pass coi	mmon wat	ernemp co	ontrol studies	conducted on
									Herbici	ide application ^t	
				Soil chara	cteristics			PRE		POST	
Experiment	Location	Ye	ar Nearest City	Texture	ОМ	Ηd	Seeding date	App date	App date	Height (cm)	Density (m ⁻²)
Biologically effective rate	Walpole -1	GR 20	15 Wallaceburg	Sandy loam	5.5	7.7	May 28		July 2	10	382
	Walpole - 2	GR 20	15 Wallaceburg	Sandy loam	5.5	7.7	May 28		July 8	14	484
	Walpole	GR 20	16 Wallaceburg	Sandy loam	6.4	7.6	May 30		July 6	18	114
	Nelson	GR 20	l6 Cottam	Sandy loam	2.9	6.5	May 23		June 21	14	989
	Trimble -1	GS 20	l6 Cottam	Loam	5.3	7.1	May 22		June 21	7	162
	Trimble -2	GS 20	l6 Cottam	Loam	5.3	7.1	May 22		June 21	6	48
	Merrit - 1	GS 20	16 Cottam	Loam	3.8	7.2	May 27		June 29	10	69
	Merrit - 2	GS 20	l6 Cottam	Loam	3.8	7.2	May 27		July 5	13	7
GR common waterhemp control studies	Walpole1	20	15 Wallaceburg	Sandy loam	5.5	7.7	May 28	May 29	July 28	25	103
4	Walpole2	20	15 Wallaceburg	Sandy loam	5.5	7.7	May 28	June 2	August 5	48	182
	Walpole1	20	16 Wallaceburg	Sandy loam	6.4	7.6	May 30	June 1	July 6	25	285
	Nelson	20	l6 Cottam	Sandy loam	2.9	6.5	May 23	May 24	June 29	45	390
^a Abbreviations: App, application; O ^b Size and density assessments were o	M, percent org conducted in t	ganic mat he untrea	ter. ted check plots.								

weed-free primarily with S-metolachlor/metribuzin (1,943 g ai ha⁻¹) applied PRE, but in one instance, due to crop emergence, glyphosate $(900 \text{ g ae ha}^{-1})$ plus imazethapyr (75 ai ha⁻¹) was applied POST. Following the initial herbicide application, subsequent hand hoeing was done as required. Plots were 2.25-m wide (three soybean rows spaced 75-cm apart) by 8-m long, and glyphosate was applied at 14, 28, 56, 113, 225, 450, 900, 1,800, 2,700, or 5,400 g ae ha⁻¹ for the GS populations and 113, 225, 450, 900, 1,800, 2,700, 5,400, 10,800, 21,600, or 43,200 g ae ha⁻ for the GR populations. Glyphosate was applied when the majority of common waterhemp reached 10 cm in height using a CO₂-pressurized backpack sprayer and 1.5-m handheld boom with four ULD 120-02 nozzles (Hypro, New Brighton, MN) spaced 50-cm apart and calibrated to deliver 200 L ha⁻¹ at 280 kPa. Common waterhemp control was estimated visually at 1, 2, 4, 8, and 12 WAA on a scale of 0% to 100%. Zero percent indicated no common waterhemp control, and 100% was complete common waterhemp death. Density and dry weight were determined at 4 WAA. Waterhemp plants were counted in two 0.5-m² quadrats, cut at the soil surface, placed in paper bags, and dried in a kiln at 60 C to a constant moisture, and the dry weight was recorded. Soybean yield from the weedy and weedfree controls was determined at maturity by hand cutting the soybean from two 1-m sections of a row taken from the center row of each plot and threshed in a stationary thresher. Soybean yield was adjusted to 13% moisture.

GR Common Waterhemp Control in PRE and PRE followed by POST Programs. Eight field experiments were conducted over a 2-yr period (2015 and 2016) to evaluate 14 herbicides applied PRE for the control of GR common waterhemp and to evaluate a PRE followed by POST program.

Four field experiments were completed to address each of the above objectives, with two studies at Walpole Island in 2015 (separated in time) and one at Walpole Island and one near Cottam, ON, in 2016. These experiments included a PRE experiment and a PRE followed by POST experiment conducted in four different environments.

Glyphosate $(1,800 \text{ g ae ha}^{-1})$ was applied before seedbed preparation to remove the confounding effect of other weed species. Field location and year, soil characteristics, seeding date, herbicide application date, and common waterhemp size and density at application are presented in Table 1. DeKalb[®] 30-61RY soybean was seeded in rows spaced 75-cm apart at approximately 400,000 seeds ha^{-1} at a depth of 3.75 cm. All field experiments were established as a randomized complete block design with four replicates. A weedy and weed-free control was included in each replicate. The weed-free check was maintained weed-free with S-metolachor/metribuzin (1,943 g ai ha⁻¹) applied PRE followed by hand hoeing as required. The plots were 2.25 m in width (three soybean rows spaced 75-cm apart) and 8 m in length. Herbicides were applied with a CO_2 -pressurized backpack sprayer and a 1.5-m handheld boom with four ULD 120-02 nozzles (Hypro) spaced 50-cm apart and calibrated to deliver 200 L ha⁻¹ at 280 kPa. PRE herbicides were applied within 5 d after seeding (Table 1) and POST applications were applied when common waterhemp escapes reached approximately 10 cm in height in the PRE treatments.

The PRE herbicides were applied at the highest labeled rate in the province of Ontario. Herbicides used in the PRE followed by POST weed control study included pyroxasulfone/sulfentrazone (300 g ai ha⁻¹) or *S*-metolachlor/metribuzin (1,943 g ai ha⁻¹) applied PRE and glyphosate (900 g ae ha⁻¹), glyphosate (900 g ae ha⁻¹) plus acifluorfen (600 g ai⁻¹ ha), or glyphosate (900 g ae ha⁻¹) plus fomesafen (240 g ai ha⁻¹) + Turbocharge[®] 0.5% v/v applied POST. All herbicides were evaluated alone and in a combination of a PRE followed by a POST herbicide.

Waterhemp control was assessed visually at 1, 2, 4, 8, and 12 WAA after the POST application on a scale of 0% to 100%, with a rating of 0% indicating no control and 100% indicating complete common waterhemp control. Waterhemp density and dry weight were determined 4 WAA from a 0.5 m^{-2} subsample of each plot. Waterhemp was counted, cut at the soil surface, placed in paper bags, and dried in a kiln at 60 C, and the dry weight was recorded. Soybean seed yield and moisture was recorded from each plot at crop maturity, and yield was adjusted to 13% moisture.

Statistical Analysis. For BER studies (greenhouse and field), statistical analysis was conducted in SAS v. 9.4 PROC NLIN (SAS Institute 2012). For the greenhouse study, regression analysis was completed using an ascending dose–response curve (Equation 1)

for all control assessments, density, and dry weight; this model provided the best fit (Bowley 2008):

$$Y = C + \frac{D - C}{1 + \exp[b(\log(X) - \log(I_{50}))]}$$
[1]

where variable Y is the percent control of common waterhemp, C is the lower asymptote, D is the upper asymptote, b is the slope of the line (negative for all control ratings, positive for density and dry weight) and I₅₀ is the rate to achieve 50% response between the upper and lower asymptotes (Bowley 2008). For the field study, the common waterhemp density and percent dry weight predicted values were modeled using an inverse exponential equation (Equation 2):

$$Y = a + b * \exp(-c * \operatorname{rate})$$
 [2]

where the variable Y is the percent control of common waterhemp, a represents the lower asymptote, b the reduction in Y from the initial value to a, and c denotes the slope from the initial point to a.

Using the predicted values from each respective variable equation, ED_{50} , ED_{80} , and ED_{95} values (representing effective dose required to achieve 50%, 80%, and 95% control, respectively) were determined.

For the common waterhemp control study, data analysis was completed using the PROC MIXED procedure in SAS. The variance was partitioned into fixed effects of treatment and random effects of block, environment, and environment by treatment interaction. To confirm that the assumptions of variance analysis were met, including errors being independent, homogeneous, and normally distributed, residuals were plotted by predicted, treatment, and block for each variable. Normality was tested by Shapiro-Wilk test (Shapiro and Wilk 1965) in PROC UNIVARIATE in SAS. Data transformations included an arcsine square-root transformation, square-root transformation, and log transformation to meet the above assumptions.

For statistical analysis for the PRE herbicide study, transformed data underwent a Tukey's test, and the pdmix800 macro was used to separate the means (Saxton 1998). Least-squares means comparisons were performed with a type I error rate set at P = 0.05. Treatment means from transformed data were back-transformed for presentation purposes; the standard error was calculated from untransformed data.

In the PRE followed by POST weed control study, nonorthogonal contrasts using PROC MIXED COVTEST for the two PRE herbicides, the three POST herbicides, and the herbicide timings of PRE vs. POST, PRE vs. PRE followed by POST, and POST vs. PRE followed by POST were completed. Tukey's test was used with significance level of P = 0.05.

Results and Discussion

Biologically Effective Rate of Glyphosate. In greenhouse studies, based on the ED_{50} at 4 WAA, the resistance factor was 4.5 when comparing the amount of glyphosate required to achieve 50% control of common waterhemp in the GR and GS populations (Table 2). Interestingly, the resistance factor was similar for the ED_{80} , ED_{90} , and ED_{95} , reflecting a consistent response across populations over time when compared with the GS populations. Based on the ED_{80} , ED_{90} , and ED_{95} at 4 WAA, the resistance factor was 4.6, 4.7, and 4.9, respectively (Table 2). This study suggests that any of these four parameters can be used to determine the resistance factor for the tested populations.

In field study, at 1 WAA, there was little response, because glyphosate is a slow-acting herbicide, while beyond 4 WAA, new common waterhemp plants were emerging, because glyphosate does not provide residual control, which confounded the results in the field studies. Thus, the ratings completed at 2 and 4 WAA are presented and compared with the GS population (Table 3). Based on the ED_{50} at 2 and 4 WAA, the resistance factors were 14.2 and 27.9, respectively, which were much higher than in the greenhouse study. Based on the ED₅₀, the resistance factors based on common waterhemp density and dry weight were 2.6 and 8.6, respectively; while based on the ED_{80} , the resistance factors based on common waterhemp density and dry weight were 3.9 and 10.7, respectively (Table 3). In the field studies, GR common waterhemp interference reduced soybean yield 50% (unpublished data). In the study in which common waterhemp density was the highest, soybean yield was reduced 98% due to GR common waterhemp interference, with the highest yield of $3.50 \text{ T} \text{ ha}^{-1}$ found in the weed-free check and the lowest of 0.06 T ha⁻¹ found in the nontreated control (unpublished data).

Although there were similarities in common waterhemp response to glyphosate in the greenhouse and field studies, there was more variability in the field study. In the field, common waterhemp continued to emerge following the application of glyphosate, because glyphosate does not provide residual weed control. The later-emerging common waterhemp made control assessments difficult, because it was difficult to know which plants were present at the time of glyphosate application. In addition, the greenhouse experiments were conducted on a single GR population from Walpole, discovered in 2014, and did not include GR common waterhemp from near Cottam, which was discovered in 2015 (Schryver et al. 2017), after the greenhouse experiments were completed. Due to the pooling of field BER results, there was more variability in the regression analysis, as populations exhibited slightly different responses to glyphosate. The GR common waterhemp population from near Cottam was more resistant to glyphosate than the Walpole population. In general, the greenhouse experiments provided a better determination of the resistance factor, because the results are not confounded by the lateemerging common waterhemp cohort and were taken from one population, resulting in more uniform results. Despite these differences, the objective of this research was to determine the resistance factor of GR common waterhemp in Ontario. Future research could explore

		Estin	nate (± SE)		Effectiv	ve dose (ED) f	for predetermin	ned level
Variable	D	С	В	I ₅₀	50%	80%	90%	95%
Susceptible 4WAA	100.0(0)	8.5 (1.6)	2.11(0.2)	588.7 (33.6)	538.7	1,077.2	1,592.6	2,276.1
Resistant	0.9(0)	0.3(0)	2.39(1.7)	234.1 (04.8)	-	-	- 7 502 8	-
DW	0.3 (0)	0.7(0)	-3.84(0.4)	2716.2 (448.8)	2,44).0	4,9/2.9	/,)02.8	- 11,239.2

Table 2. Rate response of glyphosate on the control of resistant and susceptible common waterhemp populations 1, 2, 3, and 4 wk after application (WAA) and the dry weight at 4 WAA in greenhouse experiments conducted in 2015.^a

^a Average of three experimental runs of each population. D is the upper limit and C is the lower limit. B is the slope of the line. I₅₀ is the rate to achieve 50% response between the upper and lower asymptotes.

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Variable		Parameter est	imates (± SE)		Predicted glyphosate rate (g ai ha ⁻¹)				
Dose response	D	С	В	I ₅₀	ED ₅₀	ED ₈₀	ED ₉₀	ED ₉₅	
Susceptible									
2 WAA	100 (0)	0.0(0)	1.5 (0.1)	151.2 (6.7)	151.2	371.7	629.0	1021.3	
4 WAA	100 (0)	1.0 (1.9)	1.7 (0.1)	165.3 (9.0)	163.4	371.7	600.2	933.0	
Resistant									
2 WAA	89.9 (2.3)	0.0(0)	1.2 (0.1)	1,772.6 (140.5)	2,145.9	10353.9	_	-	
4 WAA	65.2 (4.6)	0.0 (0)	1.4 (0.4)	1,934.4 (260.7)	4,556.9	-	-	-	
Inverse exponential	е	f	g		ED ₅₀	ED ₈₀	ED ₉₀	ED ₉₅	
Variable ^b									
% DEN GS	0 (0)	344 (116.3)	0.0(0)		1,451.5	984.0	1,805.1	2,158.8	
%DW GS	0 (0)	126.8 (14.2)	0.0 (0)		226.4	449.4	618.0	786.7	
%DEN GR	34.3 ((7.0)	73.3 (9.2)	0.0 (0)		3,810.4		_	_	
%DW GR	9.3 (5.3)	101.5 (7.2)	0.0 (0)		1,956.3	4,815.6	10,627.0		

Table 3. Rate response of glyphosate on the control of resistant and susceptible common waterhemp populations at 2 and 4 wk after application (WAA) and the percent dry weight at 4 WAA in field experiments conducted in 2015 and 2016.^a

^a Average of four experimental runs for each population. Dose-response parameters (Equation 1): *B*, slope; *C*, lower asymptote; *D*, upper asymptote; I_{50} , rate required for 50% response.

Inverse exponential (Equation 2): e, lower asymptote; f, magnitude of response; g, slope of response.

^b Abbreviations: DEN, percent density of nontreated control; DW, percent dry weight of nontreated control; GS, glyphosate susceptible, GR, glyphosate resistant.

the response of specific populations in a greenhouse setting to gain insight into the differing resistance factors of certain populations and how they relate to grower management practices.

GR Common Waterhemp Control in a PRE followed by POST Programs. In the PRE herbicide control study, common waterhemp had an extended emergence pattern. In these studies, common waterhemp began emerging shortly after seedbed preparation and continued to emerge until the last assessment late in October (Figure 1), as was also observed in studies by Vyn et al. (2007) in Ontario. The most efficacious herbicides applied PRE were pyroxasulfone, flumioxazin+ imazethapyr+ metribuzin, metribuzin, S-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/ flumioxazin. Those herbicides provided between 87% and 97% control of GR common waterhemp at 8 WAA (Table 4). In general, these herbicides were the most efficacious at all assessment timings. Of the 14 herbicide treatments evaluated, only 2 provided greater than 90% full-season residual control of GR common waterhemp. The control of GR common waterhemp with most PRE herbicides did not provide acceptable season-long control. For instance, the control of GR common waterhemp with dimethenamidP/saflufenacil, saflufenacil, imazethapyr, dimethenamid-P, and S-metolachlor decreased by 23% to 36% from 2 to 12 WAA. In contrast, there was no decrease in GR common waterhemp control from week 2 to 12 with pyroxasulfone/flumioxazin. At 12 WAA, some herbicides such as imazethapyr, pendimethalin, and saflufenacil provided equivalent control to the nontreated control with 4%, 11%, and 26% GR common waterhemp control respectively. The poor control with imazethapyr can be attributed to common waterhemp at all sites having Group 2 resistance (Schryver et al. 2017; PJT, unpublished data).

In the two-pass weed control study, GR common waterhemp control was influenced by herbicide application timing and herbicide choice. Pyroxasulfone/sulfentrazone and *S*-metolachlor/metribuzin applied PRE provided equivalent control of GR common waterhemp with estimates of 79% and 67% control, respectively, at 12 WAA (Table 5). Fomesafen + glyphosate was more efficacious than acifluorfen + glyphosate for the control of GR common waterhemp, although differences were not always statistically significant. Across all control assessments, glyphosate with fomesafen provided 14% to 20% better control of GR common waterhemp than did acifluorfen. Both fomesafen and acifluorfen combined with glyphosate applied POST following a PRE



Figure 1. Weekly common waterhemp emergence counts conducted from June to late October in Ontario in 2015 and 2016 represented as average plants per m^2 .

herbicide provided better control of GR common waterhemp than glyphosate alone applied POST for all assessments, with the exception of common waterhemp density. Following the application of PRE herbicide, there was no difference in GR common waterhemp control between either fomesafen or acifluorfen when combined with glyphosate applied POST. The PRE herbicides provided better control of GR common waterhemp than the POST-only herbicides evaluated for all control estimates, with the exception of common waterhemp dry weight. At 4 and 12 WAA, herbicides applied PRE provided 45% and 53% better control of GR common waterhemp than herbicides applied POST, respectively. In general, control of GR common waterhemp was better when a POST herbicide followed a PRE herbicide in contrast to PRE only. At 4 and 12 WAA, the addition of a herbicide applied POST following a PRE herbicide improved GR common waterhemp control 17% and 21%, respectively, while the addition of a PRE

Table 4. Means for percent common waterhemp control, density, biomass, and soybean yield with PRE herbicides at Walpole and Cottam, ON, in 2015 and 2016.^a

Treatment	Rate	Control 2 WAA ^b	Control 4 WAA	Control 8 WAA	Control 12 WAA	Density 4 WAA ^c	Dry weight 4 WAA ^d	Soybean yield
	g ai ha ⁻¹			%				T ha ⁻¹
Weedy check	8	0 f	0 f	0 h	0 f	100 ab	100 ab	0.8 abc
Weed-free check		100 a	100 a	100 a	100 a	0 d	0 f	1.2 a
Dimethenamid-P	693	83 bc	85 bc	74 cde	58 bcde	32 bcd	19 def	0.7 abc
Pendimethalin	1,000	30 e	38 e	22 fg	11 ef	96 abc	79 abc	0.6 bc
Pyroxasulfone	150	94 ab	91 Ь	87 abcd	85 abc	14 d	8 def	0.7 abc
S-metolachlor	1,600	88 bc	87 bc	82 bcd	65 bcd	25 cd	17 def	0.9 abc
Flumioxazin	108	94 ab	93 ab	85 bcd	77 abcd	23 d	18 def	0.9 abc
Metribuzin	1,120	92 ab	94 ab	88 abcd	76 abcd	24 cd	10 def	0.9 abc
Saflufenacil	25	54 de	56 de	44 ef	26 def	42 abcd	39 bcd	0.7 abc
Sulfentrazone	210	71 cd	68 cd	66 de	58 bcde	33 bcd	32 cde	0.7 abc
Imazethapyr	100	31 e	41 de	12 gh	4 f	100 a	100 a	0.6 c
Dimethenamid-P/saflufenacil	245	88 bc	84 bc	73 cde	52 cde	47 abcd	32 cde	0.8 abc
Pyroxasulfone/flumioxazin	240	96 ab	97 ab	97 ab	97 ab	4 d	1 f	0.9 abc
Pyroxasulfone/sulfentrazone	300	95 ab	96 ab	95 abc	92 abc	7 d	4 ef	1.0 abc
S-metolachlor/metribuzin	1,943	96 ab	96 ab	93 abcd	87 abc	16 d	7 def	1.2 ab
Flumioxazin	93	96 ab	95 ab	87 abcd	84 abc	17 d	5 def	1.0 abc
Metribuzin	420							
Imazethapyr	75							
SE		1.94	1.87	2.09	2.26	0.11	0.04	0.05

^a Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD (P = 0.05).

^b Abbreviation: WAA, weeks after application.

^c Percentage of untreated.

^d Visual control estimates based on comparisons made to weedy and weed-free check.

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Treatment	Rate	Application timing	2 WAA	4 WAA	8 WAA	12 WAA	Density	Dry weight	Yield
	g ai ha ⁻¹			———% Со	ntrol ^b ———		% of nontre	eated control	T ha ⁻¹
Nontreated control	0		0	0	0	0	100	100	1.00
Weed-free			100	100	100	100	0	0	1.64
Pyroxasulfone/sulfentrazone	300	PRE	84	85	81	79	36	51	1.62
S-metolachlor/ metribuzin	1,943	PRE	80	75	71	67	12	56	1.25
Glyphosate	900	POST	4	6	1	0	131	84	1.34
Glyphosate	900	POST	50	47	40	32	129	60	1.26
Acifluorfen	600	POST							
Glyphosate	900	POST	68	61	55	47	106	43	0.97
Fomesafen ^c	240	POST							
Pyroxasulfone/sulfentrazone	300	PRE	94	92	89	87	10	12	1.83
Glyphosate	900	POST							
Pyroxasulfone/sulfentrazone	300	PRE	99	99	98	97	2	2	1.54
Glyphosate	900	POST							
Acifluorfen	600	POST							
Pyroxasulfone/sulfentrazone	300	PRE	99	99	98	97	3	1	1.37
Glyphosate	900	POST							
Fomesafen	240	POST							
S-metolachlor/metribuzin	1,943	PRE	88	86	83	82	9	17	1.68
Glyphosate	900	POST							
S-metolachlor/metribuzin	1,943	PRE	98	98	97	96	3	3	1.31
Glyphosate	900	POST							
Acifluorfen	600	POST							
S-metolachlor/metribuzin	1,943	PRE	100	100	99	98	1	0	1.53
Glyphosate	900	POST							
Fomesafen	240	POST							
Contrasts									
Pyroxasulfone/sulfentrazone vs S-metolachlor/metribuzin	5.		84 vs. 80	85 vs. 75	81 vs. 71	79 vs. 67	36 vs. 12	51 vs. 56	1.62 vs. 1.25
Acifluorfen vs. fomesafen			50 vs. 68**	47 vs. 61**	40 vs. 55**	32 vs. 47**	129 vs. 106	60 vs. 43	1.26 vs. 0.97
PRE fb fomesafen vs. PRE fb	glyphosate		99 vs. 91*	99 vs. 89*	99 vs. 86*	98 vs. 85*	2 vs. 10	0 vs. 14*	1.45 vs. 1.75
PRE fb acifluorfen vs. PRE fb	glyphosate		99 vs. 91*	98 vs. 89*	97 vs. 86*	97 vs. 85*	2 vs. 10	2 vs. 14*	1.42 vs. 1.75
PRE fb acifluorfen vs. PRE fb	fomesafen		99 vs. 99	98 vs. 99	97 vs. 99	97 vs. 98	2 vs. 2	2 vs. 0	1.42 vs. 1.45
PRE vs. POST			82 vs. 37**	80 vs. 35**	76 vs. 27**	73 vs. 20**	23 vs. 122**	54 vs. 61	1.43 vs. 1.19
PRE vs. PRE fb POST			82 vs. 97**	80 vs. 97**	76 vs. 95**	73 vs. 94**	23 vs. 4	54 vs. 4**	1.43 vs. 1.54
POST vs. PRE fb POST			37 vs. 97**	35 vs. 97**	27 vs. 95**	20 vs. 94**	122 vs. 4**	61 vs. 4**	1.19 vs. 1.54*

Table 5. Contrasts of herbicides and herbicide timing PRE, POST, and PRE followed by POST in the control of glyphosate-resistant (GR) common waterhemp in GR soybean summarizing four experiments in 2015 and 2016 in Ontario.^a

^a Abbreviations: fb, followed by; WAA, weeks after application.
^b Visual control estimates based on comparisons made to weedy and weed-free check.
^c Herbicide always included addition of Turbocharge[®] surfactant at 0.5% v/v.

*Significance at P < 0.05. **Significance at P < 0.0001.

herbicide to a POST herbicide improved control 62% and 74%, respectively. Soybean yield was greater in PRE followed by POST when compared with POST alone with 1.54 and 1.19 T ha⁻¹, respectively (Table 5).

A limitation of the methodology used in this experiment was that the POST application timing was when GH common waterhemp escapes in the PRE herbicides reached 10 cm in height. Consequently, the GR common waterhemp was much taller where no PRE herbicide was applied, which resulted in reduced weed control when glyphosate was combined with fomesafen and acifluorfen applied POST without a PRE herbicide. Although this research was conducted with GR soybean, the findings presented could also be adopted for GR common waterhemp control in identity-preserved soybean with the removal of glyphosate from the POST tank mixes tested.

In conclusion, glyphosate is no longer an efficacious POST option for the control of common waterhemp in many fields in Ontario. The glyphosate resistance factor at 4 WAA, based on greenhouse studies, was 4.5, while it was up to 27.9 based on field studies. This study concludes that pyroxasulfone/flumioxazin, pyroxasulfone/sulfentrazone, and S-metolachlor/metribuzin are the most efficacious soil-applied herbicides for the control of GR common waterhemp in soybean in Ontario. A twopass weed control program of a PRE followed by a POST herbicide provided ≥94% GR common waterhemp control. Although use of a PRE residual herbicide provides a basis for acceptable control, weed escapes should also be controlled with a POST herbicide in a two-pass weed control program. The POST herbicides acifluorfen and fomesafen are effective for the control of GR common waterhemp, but use should be monitored due to the existence of widespread protoporphyrinogen oxidase resistance in common waterhemp in the United States and could be argued to be a short-term solution in Ontario (Heap 2017). Moving forward, there is a vital need for an increased awareness of herbicide resistance. Growers need to diversify their crop production/ weed management systems. Research such as the comprehensive study published by Meyer et al. (2015) finds that future technologies show more effective common waterhemp and Palmer amaranth control than current technologies. Specifically, synthetic auxins were found to improve GR

waterhemp and Palmer amaranth control over treatments not containing synthetic-auxin herbicides in soybean (Meyer et al. 2015). In combination with future technologies, the use of companion crops, cover crops, narrow row spacing, high seeding rates, and crops with different herbicide-resistant traits are all potential tools to reduce the evolution of herbicide resistance. The continuing evolution of singly and multiply resistant weed populations is a threat to the economic viability of field crop production in Ontario.

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