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Herbicide programs for control of waterhemp (*Amaranthus tuberculatus*) resistant to three distinct herbicide sites of action in corn

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Research Article

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Abstract

Control of waterhemp is becoming more difficult in Ontario because biotypes have evolved resistance to four herbicide sites of action (SOA), including groups 2, 5, 9, and 14. The objective of this study was to compare PRE, POST, and PRE followed by (fb) POST herbicide programs for their effect on control, density, and biomass of multiple-herbicide–resistant (MHR) waterhemp as well as corn injury and grain yield. Two separate field studies, each consisting of five field trials, were conducted over a 2-yr period (2018 and 2019) in fields where corn was grown in Ontario, Canada. The first experiment evaluated MHR waterhemp control with an inhibitor of 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) applied PRE, PRE fb glufosinate applied POST, and glufosinate applied POST. The second experiment evaluated MHR waterhemp control with a non-HPPD inhibitor applied PRE, then PRE fb a POST application of atrazine + mesotrione, and then atrazine + mesotrione applied POST. Atrazine + isoxaflutole caused 3% to 5% corn injury at environment 1 (E1); no corn injury was observed with PRE and POST herbicide programs at environments E2, E3, E4, and E5. In general, atrazine/bicyclopyrone/mesotrione/*S*-metolachlor and dimethenamid-P/saflufenacil applied PRE controlled MHR waterhemp ≥95% 12 wk after POST application (WAA). A POST application of glufosinate following atrazine + tolpyralate PRE, and a POST application of atrazine + mesotrione following atrazine/dicamba or atrazine/*S*-metolachlor PRE, improved control at 4, 8, and 12 WAA in most environments. In general, PRE fb POST applications resulted in better control of MHR waterhemp throughout the growing season than single PRE and POST applications ($P < 0.05$). We conclude that herbicide programs based on multiple effective SOAs may offer effective control of MHR waterhemp where field corn is grown. It is advisable that when choosing an herbicide application program that excellent control of MHR waterhemp should be the goal given its high fecundity and competitive ability.

Introduction

Waterhemp is a competitive, summer-annual broadleaf weed that interferes with corn production. Waterhemp is difficult to manage due to its extended period of emergence, rapid growth rate, high fecundity, and dioecious reproductive system, which enables it to thrive in diverse agricultural cropping systems (Hartzler et al. 1999; Sauer 1955). A broad range of environmental conditions are conducive for waterhemp germination, which enables it to emerge from spring to late autumn (Costea et al. 2005; Hartzler et al. 1999). In Ontario, Canada, waterhemp begins to emerge in May and continues to emerge through late October (Schryver et al. 2017a). Compared to annual weeds such as giant ragweed (*Ambrosia trifida* L.) and common lambsquarters (*Chenopodium album* L.) that emerge early in the season, waterhemp emerges 5 to 25 d later, which allows it to escape weed control tactics such as preplant tillage and early nonresidual herbicide applications (Hartzler et al. 1999; Nordby and Hartzler 2004).

Waterhemp is a dioecious weed species; male and female reproductive organs occur on separate plants and viable offspring are produced following fertilization via cross-pollination (Costea et al. 2005). Obligatory out-crossing increases the rapid recombination and spread of genes between individual plants and populations thereby increasing the rate of herbicide resistance evolution (Bell and Tranel 2010; Liu et al. 2012). Following pollination, mature seed can be produced in as little as 10 to 14 d; seed typically germinates within the first 5 yr in the soil profile (Bell and Tranel 2010; Burnside et al. 1996; Hartzler et al. 1999). In contrast, when seed return is prevented, the soil seedbank can be depleted by more than 99% in 4 yr; however, a few escapes negate soil seed bank depletion efforts (Costea et al. 2005; Steckel et al. 2007).

Waterhemp is native to the Great Plains region of the United States and is now present in 18 states in the United States and 3 Canadian provinces (Heap 2020; Sauer 1957). Globally, waterhemp has evolved resistance to six herbicide sites of action (SOAs) including acetolactate synthase inhibitors (Group 2), synthetic auxins (Group 4), photosystem II inhibitors (Group 5), 5-enolpyruvyl shikimate-3-phosphate synthase inhibitors (Group 9), protoporphyrinogen oxidase inhibitors (Group 14), very-long-chain fatty-acid (Group 15), and 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) inhibitors (Group 27) (Bell et al. 2013; Heap 2020; McMullan and Green 2011; Sarangi et al. 2019). In 2018, the first waterhemp biotype resistant to six herbicide SOAs (Groups 2, 4, 5, 9, 14, and 27) was confirmed in Missouri, with 16% of individual plants possessing genes for six-way resistance (Shergill et al. 2018). In Ontario, waterhemp was first found in a commercial field in Lambton County in 2002 and has since been identified in 10 other counties as of 2019 (Costea et al. 2005; CW unpublished data). Waterhemp populations with multiple resistance to four herbicide SOAs (Groups 2, 5, 9, and 14) have been confirmed in Ontario with 35% of populations containing individuals that possess genes for four-way resistance (Benoit et al. 2019a; CW unpublished data). The ability of waterhemp to adapt to variable environments, including the ability to survive and produce seeds under water stress conditions (Sarangi et al. 2016), its genetic diversity, prolific seed production, and propensity to evolve herbicide resistance necessitates the need for season-long control to eliminate return of the weed seed to the soil, reduce the evolution of herbicide resistance, and prevent its movement to additional fields.

Weed management programs that provide season-long control are crucial to reduce interference, yield loss, and weed seed return to the soil (Horak and Loughin 2000; Schryver et al. 2017b). In the absence of weed control measures, waterhemp interference can reduce corn yield up to 74% (Steckel and Sprague 2004). Corn is sensitive to early-season weed interference; the absence of weed interference during the critical weed-free period from the 3-leaf to the 14-leaf stage is required to prevent yield loss (Hall et al. 1992). Current methods for managing multiple-herbicide–resistant (MHR) waterhemp include the use of either PRE or POST herbicides, mixing multiple effective SOAs, rotating herbicides, rotating crops, and the strategic use of tillage (Benoit et al. 2019b; Schultz et al. 2015). The HPPD inhibitors exhibit a high degree of activity on waterhemp in corn, but often provide less than 100% control. Despite the evolution of resistance, atrazine in herbicide premixes or tank mixtures is still widely used to manage weeds in corn fields due to its synergism with HPPD inhibitors and for control of other susceptible weed species (Khort and Sprague 2017b; USDA-NASS 2019; Woodyard et al. 2009). Glufosinate is a nonsystemic herbicide that controls many common annual grass and broadleaf weeds (Anonymous 2019). When used in the early POST period, glufosinate can be mixed with residual herbicides (Bradley et al. 2000; Tharp and Kells 2002). Glufosinate can also be used to manage glyphosate-resistant weeds and has foliar activity on waterhemp (Bradley et al. 2000; Jhala et al. 2017). A glufosinate application late in the season can improve season-long waterhemp control (Schultz 2015).

Although there are reports on the use of PRE followed by (fb) POST programs for broad spectrum weed control in corn, further studies are needed to develop PRE fb POST programs that specifically target MHR waterhemp given its prolificity and propensity to develop herbicide resistance. We hypothesized that herbicide programs consisting of HPPD inhibitors applied PRE fb glufosinate applied POST and PRE herbicides fb HPPD inhibitors applied POST would provide greater control of MHR waterhemp than PRE-only or POST-only herbicide programs. The objectives of these studies were to identify effective herbicide programs for control of MHR waterhemp in fields where corn is grown while upholding proper resistance management practices across various Ontario environments.

Materials and Methods

Two separate field studies were conducted over a 2-yr period (2018 and 2019). Each study consisted of five field trials conducted on Walpole Island, ON (42.561492°N, 82.501487°W) and near Cottam, ON (42.149076°N, 82.683687°W). The first experiment investigated the efficacy of HPPD inhibitors applied PRE, applied PRE fb glufosinate applied POST, and glufosinate applied POST. The herbicide mixtures containing HPPD inhibitors included atrazine + isoxaflutole, atrazine/bicyclopyrone/mesotrione/*S*-metolachlor, and atrazine + tolpyralate, and were selected to represent the isoxazole, triketone, and benzoyl pyrazole herbicide families, respectively. Atrazine and HPPD inhibitors were tank-mixed at rates that were consistent with the manufacturers' labels. The second experiment evaluated residual herbicides that did not contain HPPD inhibitors applied PRE, applied PRE fb atrazine + mesotrione applied POST, and atrazine + mesotrione applied POST. The three herbicide mixtures included atrazine/*S*-metolachlor, dimethenamid-P/saflufenacil, and atrazine/dicamba. Treatments in both experiments were selected based on previous studies that evaluated waterhemp control in Ontario (Benoit et al. 2019b; Schryver et al. 2017b; Vyn et al. 2006).

Soil characteristics and herbicide application information are listed in Tables 1 and 2; the previous crop at all environments was either corn or soybean. The Cottam environment was not tilled in the autumn; it was cultivated twice in the spring following fertilizer application to prepare the seedbed for planting. The Walpole environments were disked in the autumn and cultivated twice in the spring following fertilizer application. Glyphosate- and glufosinate-resistant DKC45-65RIB (Monsanto, St. Louis, MO) corn was planted approximately 4 cm deep at 83,000 seeds ha^{-1} in late May to late June (Table 1). Each plot measured 2.25 m wide (3 corn rows spaced 0.75 m apart) and 8 m long with a 2-m alley between blocks. Waterhemp populations at all environments were resistant to Group 2, 5, 9, and 14 herbicides (CW unpublished data). Potassium salt of glyphosate was applied early POST at 450 g ae ha^{-1} to the entire experimental area to control glyphosate-susceptible waterhemp biotypes and all other weed species.

Each trial was designed as a randomized complete block with four replications. Each replicate included a nontreated control and a weed-free control. The weed-free control was maintained with atrazine/bicyclopyrone/mesotrione/*S*-metolachlor (respectively, 588/35/140/1,259 g ai ha^{-1}) applied PRE fb atrazine/dicamba (respectively, 996/504 g ai ha^{-1}) applied POST and subsequent hand weeding as required. The PRE, PRE fb POST, and POST applications were applied using a CO_2-pressurized backpack sprayer

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Table 1. Soil characteristics of fields in which multiple herbicide–resistant waterhemp control efforts were evaluated.

Environment	Year	Location	Classification	Sand %	Silt %	Clay %	pH	OM[a] %
E1	2018	Cottam	Sandy loam	66	24	10	6.4	2.2
E2	2018	Walpole Island	Loamy sand	78	14	8	8.3	2.3
E3	2018	Walpole Island	Loamy sand	76	18	6	8.0	2.4
E4	2019	Cottam	Sandy loam	70	21	9	6.0	2.6
E5	2019	Walpole Island	Sandy loam	70	21	9	7.6	2.3

[a]Abbreviation: OM, organic matter.

Table 2. Herbicide treatments, application timing, rates, and products used to evaluate control of multiple herbicide–resistant waterhempwith herbicides containing HPPD inhibitors applied PRE, herbicides containing HPPD–inhibitors applied PRE fb glufosinate applied POST, and glufosinate applied POST.

Herbicide treatment[a]	Herbicide trade name	Timing	Rate g ai ha^{-1}	Manufacturer[b]
Atrazine + isoxaflutole	Aatrex® + Converge Flexx®	PRE	800 + 79	Syngenta/ Bayer CropScience
Atrazine/bicyclopyrone/mesotrione/S-metolachlor	Acuron®	PRE	588/35/140/1,259	Syngenta
Atrazine + tolpyralate	Aatrex® + Shieldex™ 400SC	PRE	560 + 30	Syngenta/ISK Biosciences
Glufosinate	Liberty® 200SL	POST	500	BASF
Atrazine + isoxaflutole fb	Converge Flexx® fb	PRE	800 + 79	Syngenta/ Bayer CropScience
Glufosinate	Liberty® 200 SL	POST	500	BASF
Atrazine/bicyclopyrone/mesotrione/S-metolachlor fb	Acuron® fb	PRE	588/35/140/1,259	Syngenta
Glufosinate	Liberty® 200SL	POST	500	BASF
Atrazine + tolpyralate fb	Aatrex® + Shieldex™ 400SC fb	PRE	560 + 30	Syngenta/ISK Biosciences
Glufosinate	Liberty® 200SL	POST	500	BASF

[a]Abbreviation: fb, followed by.
[b]Bayer CropScience Inc. 160 Quarry Park Blvd S. E., Calgary, AB; BASF Canada Inc. 100 Milverton Drive, Mississauga, ON; ISK Biosciences Corporation. 740 Auburn Road, Concord, OH; Syngenta Canada Inc. 140 Research Lane, Research Park, Guelph, ON.

equipped with a hand-held boom fitted with four 120-02 ultra-low drift nozzles (Hypro/Pentair, New Brighton, MN). The CO_2 backpack sprayer was calibrated to deliver a water volume of 200 L ha^{-1} at 240 kPa. PRE herbicides were applied 1 to 3 d after planting and POST herbicides were applied when MHR waterhemp escapes were 10 cm tall or when the corn crop reached the V6 growth stage, whichever occurred first.

Crop injury was assessed visually at 2 and 4 wk after emergence (WAE) on a scale of 0 to 100; 0 representing no visible damage and 100 representing complete corn death. Weed control, a visual estimate of the decrease in MHR waterhemp biomass relative to the nontreated control, was evaluated at 4, 8, and 12 wk after the POST application (WAA). Weed density and biomass were determined 4 WAA using two 0.25-m^2 quadrats. The quadrats were randomly placed in each plot; MHR waterhemp within each quadrat was counted, cut at the soil surface, placed in a paper bag, and kiln-dried over a 2-wk period to a constant moisture. Samples were then weighed using an analytical balance and biomass, presented as grams per square meter (g m^{-2}), was recorded. Grain corn yield in kg per hectare (kg ha^{-1}) and harvest moisture (%) were obtained by harvesting two rows from each plot using a small plot combine. Grain yields were adjusted to 15.5% moisture content prior to statistical analysis.

The two studies were analyzed separately. Data were subjected to ANOVA and analyzed using the PROC GLIMMIX procedure in SAS v9.4 (SAS, Raleigh, NC). For both studies, an initial mixed model analysis was conducted to evaluate site-by-treatment interactions. Replication and environment were random effects and herbicide treatment was considered the fixed effect. In both studies, treatment-by-environment interactions were significant ($P < 0.05$) for all parameters, with no difference between environments E1 and E4, and E2, E3, and E5; therefore, environments were combined into two groups: E1 and E4; and E2, E3, and E5. A second mixed model analysis was conducted to evaluate herbicide treatment effects. Environments were analyzed as groups; replication was the random effect and herbicide treatment was the fixed effect. Analysis of MHR waterhemp control and corn yield did not require transformation. Density and biomass data for MHR waterhemp were analyzed on the natural log scale and means were back-transformed using the omega method (M Edwards, Ontario Agricultural College Statistician, University of Guelph, personal communication). Normality was tested using the Shapiro-Wilk statistic conducted using the PROC UNIVARIATE procedure in SAS. Normality assumptions were confirmed by plotting residuals against the predicted estimates, treatments, environments, and replications. Nonorthogonal contrasts were used to compare PRE, POST, and PRE fb POST herbicide treatments. Treatment means were separated using Tukey-Kramer grouping for least square means. Statistical comparisons were based on $P < 0.05$.

Results and Discussion

MHR Waterhemp Control with Herbicides Containing HPPD Inhibitors Applied PRE, Applied PRE fb Glufosinate Applied POST, and Glufosinate Alone Applied POST

Control of MHR waterhemp was comparatively lower at E1 and E4 due to greater plant density, biomass, and more competitive nature of *Amaranthus tuberculatus* var. *rudis* compared with *Amaranthus tuberculatus* var. *tuberculatus* at E2, E3, and E5 (Costea et al. 2005; Kreiner et al. 2018; Steckel and Sprague 2004; Table 4). Density of MHR waterhemp varied between environments and was 704 plants m^{-2} at E1 and E4 compared with 61 plants m^{-2} at E2, E3, and E5 at 4 WAA (data not shown). Plant density and biomass of MHR waterhemp reflect control results, which ranged from 57% to 99% with all PRE, PRE fb POST, and POST treatments (Table 4). Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor resulted in better control of MHR waterhemp, and greater reductions in density and biomass than atrazine + isoxaflutole, atrazine + tolpyralate, and glufosinate 4 WAA at E1 and E4 (Tables 4 and 5). In contrast, all PRE herbicides controlled MHR waterhemp by

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Table 3. Herbicide treatments, application timing, rates and products used to evaluate control of multiple herbicide–resistant waterhemp with herbicides that do not contain HPPD inhibitors applied PRE, herbicides that do not contain HPPD-inhibitors applied PRE fb atrazine + mesotrione POST, and atrazine + mesotrione POST.

Herbicide treatment[a]	Herbicide trade name	Timing	Rate g ai ha^{-1}	Manufacturer[b]
Atrazine/S-metolachlor	Primextra® II Magnum®	PRE	960/1200	Syngenta
Dimethenamid-P/saflufenacil	Integrity®	PRE	660/75	BASF
Atrazine/dicamba	Marksman®	PRE	996/504	BASF
Atrazine + mesotrione	Aatrex® + Callisto® 480SC	POST	280 + 100	Syngenta
Atrazine/S-metolachlor fb	Primextra® II Magnum® fb	PRE	960/1200	Syngenta
Atrazine + mesotrione	Aatrex® + Callisto 480SC	POST	280 + 100	Syngenta
Dimethenamid-P/saflufenacil fb	Integrity®	PRE	660/75	BASF
Atrazine + mesotrione	Aatrex® + Callisto® 480SC	POST	280 + 100	Syngenta
Atrazine/dicamba fb	Marksman® fb	PRE	996/504	BASF
Atrazine + mesotrione	Aatrex® + Callisto® 480SC	POST	280 + 100	Syngenta

[a]Herbicide treatments with atrazine + mesotrione included Agral® 90 (0.2% vol/vol; Syngenta Canada Inc).
[b]BASF Canada Inc., 100 Milverton Drive, Mississauga, ON; Syngenta Canada Inc. 140 Research Lane, Research Park, Guelph, ON.

95% to 99% at 4, 8, and 12 WAA, and reduced density and biomass by 68% to 100% at E2, E3, and E5. These findings are consistent with those of Benoit et al. (2019b) who reported 94%, 84%, and 93% MHR waterhemp control with PRE applications of atrazine/bicyclopyrone/mesotrione/*S*-metolachlor, atrazine + isoxaflutole, and atrazine + tolpyralate, respectively, 4 WAA. Another study reported 97% control of a triazine-resistant waterhemp population 10 WAA with atrazine + isoxaflutole applied PRE (Vyn et al. 2006). Additionally, Sarangi and Jhala (2017) reported ≥95% control of glyphosate-resistant waterhemp with atrazine/bicyclopyrone/mesotrione/*S*-metolachlor applied PRE. At E1 and E4, atrazine + tolpyralate provided the least control of MHR waterhemp when applied PRE at 4, 8, and 12 WAA. Glufosinate application resulted in 65% to 100% MHR waterhemp control at 4, 8, and 12 WAA, and plant density and biomass were reduced by 69% to 96% across all environments. These results are consistent with those of Bradley et al. (2000) who reported 73% to 100% control of waterhemp 5 WAA with glufosinate (400 g ha^{-1}) applied early POST and those of Jhala et al. (2017) who reported 76% control 14 d after late POST application. Greater MHR waterhemp control with glufosinate at E2, E3, and E5 can be attributed to MHR waterhemp biotype, lower density, and possibly the result of greater competition between MHR waterhemp and corn due to natural thinning of waterhemp populations that occurs as the season progresses (Benoit et al. 2019c; Heneghan and Johnson 2017). Weather conditions and time of day both influence glufosinate efficacy; control can be variable from year to year and environment to environment (Peterson and Hurle 2000). Poor control of MHR waterhemp with glufosinate relative to other herbicides evaluated in this study can also be attributed to the lack of residual activity with glufosinate and continual waterhemp emergence throughout the growing season (Anonymous 2019; Costea et al. 2005).

All PRE fb POST herbicide applications provided >89% control of MHR waterhemp at 4, 8, and 12 WAA. Similarly, Schryver et al. (2017b) reported application of PRE herbicides fb glufosinate controlled glyphosate-resistant waterhemp by >96% in soybean at 2, 4, 8, and 12 WAA. Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor provided 98% to 99% control of MHR waterhemp across all environments at 4, 8, and 12 WAA; the contrast analysis suggested that control was not improved when its application was followed by that of glufosinate. This is consistent with a report by Sarangi and Jhala (2017) who noted superior control of waterhemp with atrazine/bicyclopyrone/mesotrione/*S*-metolachlor applied PRE, which resulted in 98% and 91% control 14 and 63 d after treatment, respectively. Based on nonorthogonal contrasts, when glufosinate was applied POST following application of atrazine + isoxaflutole or atrazine + tolpyralate, MHR waterhemp control increased by 17% to 37% at 4, 8, and 12 WAA at E1 and E4. Contrast analysis suggested PRE fb POST applications resulted in better control of MHR waterhemp than PRE-only and POST-only applications 4, 8, and 12 WAA ($P < 0.05$), which is consistent with results reported by Jhala et al. (2017) and Schryver et al. (2017b). This is due to MHR waterhemp that escape PRE-only applications and the lack of residual soil activity associated with glufosinate (Anonymous 2019; Soltani et al. 2012). Based on nonorthogonal contrasts, PRE-only applications resulted in greater reductions in MHR waterhemp density than POST-only applications, and PRE fb POST applications reduced density and biomass more than PRE- and POST-only herbicide applications ($P < 0.05$). These results are consistent with those reported by Benoit et al. (2019b) and Schryver et al. (2017b) who noted greater MHR waterhemp density and biomass reductions with PRE and PRE fb POST herbicide applications, respectively.

Minimal corn injury due to herbicide application was observed in this study. Atrazine + isoxaflutole resulted in 3% to 5% corn injury at E1 and did not affect corn grain yield (data not shown). Corn injury was not observed from PRE, PRE fb POST, or POST herbicide applications at all other environments. Despite the high density and biomass of MHR waterhemp and the more competitive nature of *A. tuberculatus* var. *rudis*, there were no differences in corn grain yield among herbicide treatments or between PRE, POST, or PRE fb POST applications (Table 5). At the V5 to V6 corn growth stage, MHR waterhemp in the nontreated control ranged from 3 to 10 cm in height (data not shown). Steckel and Sprague (2004) reported corn yield reductions when waterhemp emerged before the V6 corn growth stage and suggested that corn yield is closely associated with the length of time waterhemp interferes. Soil cultivation prior to planting may have placed MHR waterhemp at a competitive disadvantage with the corn crop resulting in no yield penalty (Steckel et al. 2007). In contrast, Aulakh and Jhala (2015) and Schryver et al. (2017b) reported greater soybean yield with PRE fb POST applications than PRE- or POST-only applications.

MHR Waterhemp Control with Residual Herbicides Containing Non-HPPD Inhibitors Applied PRE, Applied PRE fb Atrazine + Mesotrione Applied POST, and Atrazine + Mesotrione Applied POST

Control of MHR waterhemp was comparatively lower at E1 and E4 due to greater plant density, biomass, and more

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Table 4. Means and nonorthogonal contrasts of efforts to control multiple herbicide–resistant waterhemp (4, 8, and 12 wk after POST application) of herbicides containing HPPD inhibitors applied PRE, herbicides containing HPPD-inhibitors applied PRE fb glufosinate applied POST, and glufosinate applied POST.[a,b]

			Control %					
			E1, E4			E2, E3, E5		
Treatment	Rate g a.i. ha^{-1}	Application giming	4 WAA	8 WAA	12 WAA	4 WAA	8 WAA	12 WAA
Atrazine + isoxaflutole	800 + 79	PRE	70 b	77 ab	78 abc	97 a	97 ab	97 ab
Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor	588/35/140/1,259	PRE	94 a	93 a	95 ab	99 a	99 a	99 a
Atrazine + tolpyralate	560 + 30	PRE	57 b	62 b	58 c	94 a	96 ab	95 ab
Glufosinate	500	POST	64 b	77 ab	76 bc	86 b	92 b	91 b
Atrazine + isoxaflutole fb Glufosinate	800 + 79 fb 500	PRE fb POST	92 a	96 a	93 ab	98 a	99 a	98 ab
Atrazine/bicyclopyrone/ mesotrione/*S*-metolachlor fb Glufosinate	588/35/140/1,259 fb 500	PRE fb POST	97 a	98 a	98 a	99 a	99 a	99 ab
Atrazine + tolpyralate fb Glufosinate	560 + 30 fb 500	PRE fb POST	89 a	93 a	94 ab	99 a	99 a	99 ab
Contrasts								
Atrazine + isoxaflutole vs. atrazine + isoxaflutole fb glufosinate			70 vs. 92*	77 vs. 96*	78 vs. 93*	97 vs. 98	97 vs. 99	97 vs. 98
Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor vs. atrazine/bicyclopyrone/mesotrione/ *S*-metolachlor fb glufosinate			94 vs. 97	93 vs. 98	96 vs. 99	99 vs. 99	99 vs. 99	99 vs. 99
Atrazine + tolpyralate vs. atrazine + tolpyralate fb glufosinate			57 vs. 89*	62 vs. 93*	58 vs. 95*	94 vs. 99*	96 vs. 99	95 vs. 99
PRE vs. PRE fb POST			*	*	*	NS	NS	NS
POST vs. PRE fb POST			*	*	*	*	*	*
PRE vs. POST			NS	NS	NS	*	*	*

[a]Abbreviations: E, environment; fb, followed by; NS, nonsignificant.
[b]Means followed by the same letter within a column are not significantly different using Tukey's LSD ($P > 0.05$). *Indicates nonorthogonal contrasts that are significantly different ($P < 0.05$).

Table 5. Means and nonorthogonol contrasts of multiple herbicide–resistant waterhemp density and biomass (4 wk after POST application) and corn grain yield resulting from herbicides containing HPPD inhibitors applied PRE, herbicides containing HPPD-inhibitors applied PRE fb glufosinate applied POST, and glufosinate applied POST. were applied PRE, PRE fb glufosinate applied POST, and glufosinate applied POST.[a,b]

			Density plants m^{-2}		Biomass g m^{-2}		Yield kg ha^{-1}	
Treatment	Rate g ai ha^{-1}	Application timing	E1, E4	E2, E3, E5	E1, E4	E2, E3, E5	E1, E4	E2, E3, E5
Nontreated control	–	–	–	–	–	–	9,900 a	10,800 a
Weed-free control	–	–	–	–	–	–	11,400 a	11,600 a
Atrazine + isoxaflutole	800 + 79	PRE	43 b	1 a	32.4 bc	0.1 a	11,200 a	10,700 a
Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor	588/35/140/1,259	PRE	4 a	0 a	2.1 a	0 a	11,900 a	11,600 a
Atrazine + tolpyralate	560 + 30	PRE	100 b	4 ab	48.9 c	0.4 a	10,700 a	11,400 a
Glufosinate	500	POST	215 b	19 b	20.1 bc	2 b	10,500 a	11,100 a
Atrazine + isoxaflutole fb glufosinate	800 + 79 fb 500	PRE fb POST	3 a	0 a	1 a	0.2 a	11,500 a	11,200 a
Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor fb glufosinate	588/35/140/1,259 fb 500	PRE fb POST	3 a	0 a	0.9 a	0 a	11,700 a	11,300 a
Atrazine + tolpyralate fb glufosinate	560 + 30 fb 500	PRE fb POST	42 ab	0 a	3.4 ab	0 a	11,300 a	10,800 a
Contrasts								
Atrazine + isoxaflutole vs. atrazine + isoxaflutole fb glufosinate			43 vs. 3*	1 vs. 0	32.4 vs. 1*	0.1 vs. 0.2	11,200 vs. 11,500	10,700 vs. 11,200
Atrazine/bicyclopyrone/mesotrione/*S*-metolachlor vs. atrazine/ bicyclopyrone/mesotrione/*S*-metolachlor fb glufosinate			4 vs. 3	0 vs. 0	2.1 vs. 0.9	0 vs. 0	11,900 vs. 11,700	11,600 vs. 11,300
Atrazine + tolpyralate vs. atrazine + tolpyralate fb glufosinate			100 vs. 42	4 vs. 0*	48.9 vs. 3.4*	0.4 vs. 0	10,700 vs. 11,300	11,400 vs. 10,800
PRE vs. PRE fb POST			*	*	*	NS	NS	NS
POST vs. PRE fb POST			*	*	*	*	NS	NS
PRE vs. POST			*	*	NS	*	NS	NS

[a]Abbreviations: E, environment; fb, followed by; NS, nonsignificant.
[b]Means followed by the same letter within a column are not significantly different using Tukey's LSD ($P > 0.05$). *Indicates nonorthogonal contrasts that are significantly different ($P < 0.05$).

competitive nature of *A, tuberculatus* var. *rudis* compared with *A. tuberculatus* var. *tuberculatus* at E2, E3, and E5 (Costea et al. 2005; Kreiner et al. 2018; Steckel and Sprague 2004; Table 7). MHR waterhemp density varied among environments and was 1,172 plants m^{-2} at E1 and E4 compared with 85 plants m^{-2} at E2, E3, and E5 at 4 WAA (data not shown). MHR waterhemp density and biomass reflect control results, which ranged from 72% to 99% with PRE, PRE fb POST, and POST treatments (Table 6). At E1 and E4, atrazine/*S*-metolachlor and atrazine/dicamba provided the least control (72% to 83%) of MHR waterhemp at 4, 8, and 12 WAA. Atrazine/dicamba provided the least control (ranging from 87% to 90%) at E2, E3, and E5 at 4, 8, and 12 WAA. These results are consistent with those reported by Benoit et al. (2019b), Steckel et al. (2002), and Vyn et al. (2006) who found 91%, 98%, and 97% to 100% control of MHR waterhemp 4 WAA with dimethenamid-P/saflufenacil, atrazine/dicamba, and atrazine/*S*-metolachlor, respectively. In contrast to the PRE herbicides evaluated in this study, Khort and Sprague (2017a) reported 81% and 92% control of MHR Palmer amaranth at 10 WAA when dimethenamid-P + saflufenacil ($660 + 75$ g ai ha^{-1}) and atrazine + *S*-metolachlor ($1{,}100 + 1{,}400$ g ai ha^{-1}), respectively, were used. Use of atrazine + mesotrione provided 80% to 99% control of MHR waterhemp at 4, 8, and 12 WAA and reduced plant density and biomass 88% to 100% (Tables 6 and 7). These results are consistent with a report by Woodyard et al. (2009) that atrazine + mesotrione ($560 + 105$ g ai ha^{-1}) provided up to 99% control of waterhemp 10 d after application. More recent studies conducted in Ontario have reported atrazine + mesotrione controlled MHR waterhemp 90% to 91% at 4 WAA (Benoit et al. 2019b, 2019c).

All PRE fb POST applications provided ≥ 96% control of MHR waterhemp at 4, 8, and 12 WAA and reduced plant density and biomass by 99% to 100%. Based on nonorthogonal contrasts, MHR waterhemp control at E1 and E4 was improved 16% to 18% at 4, 8, and 12 WAA when use of atrazine/*S*-metolachlor was followed by atrazine + mesotrione. When atrazine/dicamba use was followed by a POST application of atrazine + mesotrione, control of MHR across all environments increased by 9% to 26% at 4, 8, and 12 WAA. Khort and Sprague (2017a) reported excellent control (95%) of MHR Palmer amaranth 2 WAA with atrazine + *S*-metolachlor ($1{,}820 + 1{,}410$ g ai ha^{-1}) PRE fb atrazine + mesotrione ($670 + 100$ g ai ha^{-1}) POST. Contrast analysis suggested that PRE fb POST herbicide applications resulted in better control of MHR waterhemp than both PRE- and POST-only applications ($P < 0.05$); however, PRE-only and POST-only applications provided similar control of MHR waterhemp. Comparably greater reductions in plant density and biomass were the result of PRE fb POST applications at E1 and E4 compared with those at E2, E3, and E5. After all PRE applications, the addition of a POST application of atrazine + mesotrione reduced density and biomass 99% to 100%. These results are consistent with reports in previous studies of reductions in plant density and biomass of up to 100% when PRE and PRE fb POST applications were used (Benoit et al. 2019b; Khort and Sprague 2017a). Greater control of MHR waterhemp with PRE fb POST herbicide applications can again be attributed to a POST application providing control of later-emerging seedlings that escape PRE applications, especially at higher plant densities (Costea et al. 2005; Soltani et al. 2012).

Corn injury was not observed with the herbicide treatments evaluated in this study (data not shown). Despite the higher density and biomass of MHR waterhemp at E1 and E4, corn grain yield was similar among all herbicide treatments and between PRE, PRE fb POST, and POST herbicide applications in all environments (Table 7). At the V5 to V6 corn growth stage, MHR waterhemp was 3 to 10 cm in height (data not shown). As mentioned earlier, spring tillage before seeding could have delayed waterhemp emergence and placed the weed at a competitive disadvantage in respect to the corn crop (Steckel et al. 2007). The ability of waterhemp to interfere with corn growth and development depends on plant density and relative time of crop and weed emergence. Cordes et al. (2004) reported no corn yield loss when waterhemp was controlled before reaching 15 cm in height. These results are inconsistent with those reported by Aulakh and Jhala (2015) and Schryver et al. (2017b) that greater soybean yield occurred with PRE fb POST applications than with PRE-only or POST-only applications.

In conclusion, atrazine/bicyclopyrone/mesotrione/*S*-metolachlor and dimethenamid-P/saflufenacil applied PRE to corn resulted in 88% to 99% control of MHR waterhemp across all environments at 4, 8, and 12 WAA. At most environments, a POST application of glufosinate following a PRE application of atrazine + tolpyralate and a POST application of atrazine + mesotrione following atrazine/dicamba or atrazine/*S*-metolachlor PRE, improved MHR waterhemp control at 4, 8, and 12 WAA. In general, both studies found that PRE fb POST applications resulted in better control of MHR waterhemp and greater reductions in density and biomass than PRE-only and POST-only herbicide applications 4, 8, and 12 WAA, whereas results with PRE-only and POST-only herbicide applications were similar. This study did not observe decreased control of MHR waterhemp with PRE herbicides over the course of the growing season as was reported by Hedges et al. (2018); however, in the event of early-season weed escapes with PRE herbicides, glufosinate or atrazine + mesotrione applied POST, will provide season-long MHR waterhemp control with the PRE fb POST herbicide applications evaluated in this study. Previous research has demonstrated that use of PRE herbicides is the foundation for full-season control of MHR waterhemp and that weed escapes can be managed with POST applications later in the growing season (Benoit et al. 2019b; Jhala et al. 2017; Khort and Sprague 2017a; Soltani et al. 2012). The results of this study complement those reported by Hedges et al. (2018), Jhala et al. (2017) and Schryver et al. (2017b) that superior control of MHR waterhemp in soybean was achieved using PRE fb POST herbicides. Given the ability of MHR waterhemp to emerge continually during the growing season and evolve resistance to multiple SOAs, preventing its survival and reproduction through the use of PRE fb POST herbicide applications will help mitigate the evolution of resistance to other currently used SOAs, which warrants the use of PRE fb POST herbicides, given that in both studies, PRE, PRE fb POST, and POST applications resulted in similar corn grain yields. The application of herbicide tank mixes that included multiple, effective SOAs is an effective resistance management strategy for controlling genetically diverse weeds such as MHR waterhemp that evolve herbicide-resistance rapidly. The herbicide programs presented in this study should be used in combination with other modern agronomic practices such as rotating crops, cover crops, and strategic tillage to ensure longer use of currently available herbicides while reducing the evolution, spread, and interference of MHR waterhemp in Ontario corn production.

Table 6. Means and nonorthogonal contrasts of efforts to control multiple herbicide–resistant waterhemp(4, 8 and 12 wk after POST application) of residual herbicides containing non-HPPD inhibitors applied PRE, residual herbicide containing non-HPPD inhibitors applied PRE fb atrazine + mesotrione applied POST, and atrazine + mesotrione applied POST.[a,b]

			Control %					
			E1, E4			E2, E3, E5		
Treatment[c]	Rate g ai ha^{-1}	Application timing	4 WAA	8 WAA	12 WAA	4 WAA	8 WAA	12 WAA
Atrazine/*S*-metolachlor	960/1,200	PRE	78 bc	79 bc	83 bc	98 a	98 a	99 a
Dimethenamid-P/saflufenacil	660/75	PRE	88 abc	94 abc	95 abc	99 a	98 a	99 a
Atrazine/dicamba	996/504	PRE	72 c	77 c	80 c	87 b	90 b	87 b
Atrazine + mesotrione	280 + 100	POST	80 abc	86 abc	86 abc	99 a	99 a	99 a
Atrazine/*S*-metolachlor fb atrazine + mesotrione	960/1,200 fb 280 + 100	PRE fb POST	96 ab	97 abc	99 ab	98 a	99 a	99 a
Dimethenamid-P/saflufenacil fb atrazine + mesotrione	660/75 fb 280 + 100	PRE fb POST	99 a	99 a	99 a	99 a	99 a	99 a
Atrazine/dicamba fb atrazine + mesotrione	996/504 fb 280 + 100	PRE fb POST	98 a	98 ab	98 ab	99 a	99 a	99 a
Contrasts								
Atrazine/*S*-metolachlor vs. atrazine/*S*-metolachlor fb atrazine + mesotrione			78 vs. 96*	79 vs. 97*	83 vs. 99*	98 vs. 98	98 vs. 99	99 vs. 99
Dimethenamid-P/saflufenacil vs. dimethenamid-P/saflufenacil fb atrazine + mesotrione			88 vs. 99	94 vs. 99	95 vs. 99	99 vs. 99	98 vs. 99	99 vs. 99
Atrazine/dicamba vs. atrazine/dicamba fb atrazine + mesotrione			72 vs. 98*	77 vs. 98*	80 vs. 98*	87 vs. 99*	90 vs. 99*	87 vs. 99*
PRE vs. PRE fb POST			*	*	*	*	*	*
POST vs. PRE fb POST			*	*	*	NS	NS	NS
PRE vs. POST			NS	NS	NS	*	*	*

[a]Abbreviations: E, environment; fb, followed by; NS, nonsignificant.
[b]Means followed by the same letter within a column are not significantly different using Tukey's LSD ($P > 0.05$). *Indicates nonorthogonal contrasts that are significantly different ($P < 0.05$).
[c]Atrazine + mesotrione treatments included Agral® 90 (0.2% v/v) (Syngenta Canada Inc. 140 Research Lane, Research Park, Guelph, ON).

Table 7. Means and nonorthogoanl contrasts of efforts to control multiple herbicide-–resistant waterhemp density and biomass (4 wk after POST application) and corn grain yield at resulting from residual herbicides containing non-HPPD inhibitors applied PRE, residual herbicides containing non-HPPD inhibitors applied PRE fb atrazine + mesotrione applied POST, and atrazine + mesotrione applied POST.[ab]

			Density plants m^{-2}		Biomass g m^{-2}		Yield kg ha^{-1}	
Treatment[c]	Rate g a.i. ha^{-1}	Application timing	E1, E4	E2, E3, E5	E1, E4	E2, E3, E5	E1, E4	E2, E3, E5
Weed-free control	–	–					9,400 a	10,400 a
Untreated control	–	–					10,600 a	11,000 a
Atrazine/*S*-metolachlor	2160	PRE	37 cd	0 a	22.6 bc	0 a	11,000 a	10,500 a
Dimethenamid-P/saflufenacil	735	PRE	8 bc	0 a	7.6 bc	0 a	11,000 a	10,100 a
Atrazine/dicamba	1,500	PRE	43 cd	15 b	36 c	2.9 b	10,800 a	10,800 a
Atrazine + mesotrione	280 + 100	POST	120 d	0 a	9.8 bc	0 a	9,800 a	10,500 a
Atrazine/*S*-metolachlor fb Atrazine + mesotrione	2,160 fb 280 + 100	PRE fb POST	5 ab	0 a	0.5 ab	0 a	11,400 a	11,100 a
Dimethenamid-P/saflufenacil fb Atrazine + mesotrione	735 fb 280 + 100	PRE fb POST	0 a	0 a	0 a	0 a	11,000 a	10,300 a
Atrazine/dicamba fb Atrazine + mesotrione	1,500 fb 280 + 100	PRE fb POST	0 a	0 a	0 a	0 a	11,100 a	10,500 a
Contrasts								
Atrazine/*S*-metolachlor vs. atrazine/S-metolachlor fb atrazine + mesotrione			37 vs. 5*	0 vs. 0	22.6 vs. 0.5*	0 vs. 0	11,000 vs. 11,400*	10,500 vs. 11,100
Dimethenamid-P/saflufenacil vs. dimethenamid-P/saflufenacil fb atrazine + mesotrione			8 vs. 0*	0 vs. 0	7.6 vs. 0*	0 vs. 0	11,000 vs. 11,000	10,100 vs. 10,300
Atrazine/dicamba vs. atrazine/dicamba fb atrazine + mesotrione			43 vs. 0*	15 vs. 0*	36 vs. 0*	2.9 vs. 0*	10,800 vs. 11,100	10,800 vs. 10,500
PRE vs. PRE fb POST			*	*	*	*	NS	NS
POST vs. PRE fb POST			*	NS	*	NS	NS	NS
PRE vs. POST			*	*	NS	NS	NS	NS

[a]Abbreviations: E, environment; fb, followed by; NS, nonsignificant.
[b]Means followed by the same letter within a column are not significantly different using Tukey's LSD ($P > 0.05$). *Indicates nonorthogonal contrasts that are significantly different ($P < 0.05$).
[c]Atrazine + mesotrione treatments included Agral® 90 (0.2% vol/vol; Syngenta Canada Inc. 140 Research Lane, Research Park, Guelph, ON).

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