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Research Article

Control of multiple-herbicide-resistant waterhemp (*Amaranthus tuberculatus*) with acetochlor-based herbicide mixtures in corn

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Abstract

Waterhemp is a summer annual, broadleaf weed with high fecundity, short seed longevity in the soil, and wide genetic diversity. Populations have evolved resistance to five herbicide modes of action (Groups 2, 5, 9, 14, and 27), which are present across southern Ontario; this has increased the challenge of controlling this competitive weed species in corn, the most important grain crop produced worldwide and the highest-value agronomic crop in Ontario. Acetochlor is a Group 15 soil-applied residual herbicide that has activity on many grass and broadleaf weeds but has yet to be registered in Canada. The objective of this study was to ascertain whether mixtures of acetochlor with flumetsulam, dicamba, atrazine, isoxaflutole/diflufenican, or mesotrione + atrazine applied preemergence would increase the control of multiple-herbicide-resistant (MHR) waterhemp in corn. Five field trials were conducted between 2022 and 2023. No corn injury was observed. Acetochlor applied alone controlled MHR waterhemp 97% 12 wk after application (WAA). All herbicide mixtures controlled MHR waterhemp similarly at ≥98% 12 WAA; there were no differences among herbicide mixtures. Flumetsulam, dicamba, and atrazine provided lower MHR waterhemp control than all other herbicide treatments and did not reduce density or biomass. Acetochlor reduced waterhemp density 98%, while the acetochlor mixtures reduced density similarly at 99% to 100%. This study concludes that the acetochlor mixtures evaluated provide excellent waterhemp control; however, control was not greater than acetochlor alone. Herbicide mixtures should be used as a best management practice to mitigate the evolution of herbicide resistance.

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Acetochlor; flumetsulam; dicamba; atrazine; isoxaflutole/diflufenican; mesotrione; waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; corn, *Zea mays* L.

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Introduction

Corn is a very important crop for the Canadian economy and for Ontario specifically. In 2021, nearly 13 million megagrams of corn were produced in Canada, 62% was produced in Ontario (StatsCan 2015; USDA 2022). Corn is the highest-value crop grown in the province of Ontario, valued at Can$1.8 billion in 2021 (OMAFRA 2021). The majority of the remainder of Canadian corn is produced in Ontario's neighboring provinces to the east and west, Quebec and Manitoba, respectively (StatsCan 2015). The average corn yield in Canada is slightly less than U.S. yields at 9.1 Mg ha^{-1} (USDA 2022); corn is very usceptible to yield loss from weeds.

Since 2002, Ontario growers have been dealing with herbicide-resistant waterhemp, which is a summer annual, broadleaf weed (Costea et al. 2005; Heap 2022; Nordby et al. 2007) and a member of the *Amaranthus* genus. Waterhemp is difficult to distinguish from other species in the same genus. Similar to Palmer amaranth (*Amaranthus palmeri* S. Watson), waterhemp is a dioecious species; male and female reproductive organs are found on separate plants that cross-pollinate, and the female plant produces small, reddish to black seeds (Costea et al. 2005; Sarangi et al. 2017). Copious amounts of tiny, round seeds are produced from all *Amaranthus* species, including waterhemp; in one study, a single redroot pigweed (*Amaranthus retroflexus* L.) plant produced 291,000 seeds, while waterhemp produced 289,000 seeds (Sellers et al. 2003). Hartzler et al. (2004) reported that a single waterhemp plant produced 4.8 million seeds, demonstrating the species' high fecundity.

Growers in Ontario and the United States are plagued by multiple-herbicide-resistant (MHR) waterhemp populations. The first record of herbicide-resistant waterhemp in Ontario dates back to 2002, when resistance to Weed Science Society of America (WSSA) Group 2 acetolactate synthase inhibitors and WSSA Group 5 photosystem II inhibitors was confirmed (Heap 2022). Since then, five-way resistant waterhemp populations have been confirmed in seven Ontario counties; another 11 counties have two-, three-, or four-way resistance

Table 1. Year, location, and soil characteristics from three field trials (2022) and two field trials (2023) conducted in southwestern Ontario, Canada.[a,b]

Year	Location	Soil texture	Sand	Silt	Clay	OM	pH	CEC
			%					
2022	Cottam	Sandy loam	55	27	17	2.2	5.7	9.1
2022	Newbury	Loamy sand	84	11	4	2.5	6.7	11.6
2022	Walpole Island	Sandy loam	69	21	10	1.8	6.4	16.8
2023	Newbury	Loamy sand	84	11	4	2.5	6.7	11.6
2023	Walpole Island	Sandy loam	69	21	9	1.8	6.4	16.8

[a] Soil analysis was performed by A&L Canada Laboratories Inc. (London, ON, Canada) from soil cores taken from 0 to 15 cm.

[b] Abbreviations: CEC, cation exchange capacity; OM, organic matter.

(Symington et al. 2022). Five-way resistant waterhemp populations are resistant to Groups 2 and 5, 5-enolpyruvateshikimate-3-phosohate synthase inhibitors (WSSA Group 9), protoporphyrinogen oxidase inhibitors (WSSA Group 14), and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (WSSA Group 27) (Symington et al. 2022). Waterhemp is a widespread problem in the United States; it has been confirmed in all but nine states (GROW, n.d.; USDA 2014). In the United States, waterhemp has evolved resistance to Groups 2, 4, 5, 9, 14, 15, and 27 herbicides (Heap 2022; Shergill et al. 2018; Strom et al. 2020). Multiple resistance drastically reduces the number of herbicides that can be used effectively to control waterhemp in corn; this is very problematic due to potential corn yield loss from waterhemp interference. High waterhemp densities cause greater crop yield losses; yet, even low waterhemp densities can reduce corn yield (Cordes et al. 2004). Corn yield losses were <10% when waterhemp was present at <82 plants m^{-2}; in contrast, yield losses as high as 74% have been reported in corn (Cordes et al. 2004; Steckel and Sprague 2004).

Corn yield can be greatly impacted by weed interference. A meta-analysis conducted by Soltani et al. (2016) concluded that there would be an average corn yield loss of 50% in North America if producers did not implement weed management tactics. Ontario growers are encouraged to keep their corn fields free of weeds from corn emergence to vegetative stage 6 (V6) to minimize yield losses from weed interference (OMAFRA 2009). This timing correlates with much of the research conducted on the critical period for weed control in corn, which varies from emergence to V14 (Hall et al. 1992; Page et al. 2012) and depends on factors including relative time of weed and crop emergence, weed species composition, weed density, soil characteristics, tillage practices, nutrient availability, environmental conditions, and planting date (Hall et al. 1992; Knezevic et al. 2002; Van Acker et al. 1993). With effective weed management programs, corn yield losses due to weed interference can be minimized (Soltani et al. 2022).

Use of effective waterhemp herbicides, such as the WSSA Group 15 herbicides, can result in reduced weed interference, higher corn yields, and fewer weed seeds returned to the soil weed seedbank (Gianessi and Reigner 2007; Gressel and Segel 1990). Acetochlor is a chloroacetanilide herbicide that can be applied preplant, preplant incorporated, preemergence (PRE), or early postemergence (ePOST) relative to the corn crop to control nonemerged small-seeded annual grass and some small-seeded annual broadleaf weeds (Anonymous 2020a; Anonymous 2020b; Shaner 2014). Approved for use in the United States in 1994 (de Guzman et al. 2005), it is now widely used for weed management in corn, cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.] (Armel et al. 2003; Cahoon et al. 2015; Jhala et al. 2015). Acetochlor inhibits very-long-chain fatty-acid elongases and is absorbed by the roots and shoots of emerging weed seedlings (Shaner 2014). Research has concluded that there is a sufficient margin of crop safety for the use of acetochlor in corn. Janak and Grichar (2016) found that even when acetochlor was applied at the 2X rate, corn injury did not exceed 3%. Additionally, acetochlor is an effective waterhemp herbicide. Jhala et al. (2015) reported that acetochlor (1,680 g ai ha^{-1}) applied PRE controlled MHR waterhemp 80% 60 d after planting. Though acetochlor can be applied ePOST relative to the crop, it has little activity on emerged weeds, which need to be controlled with another weed management tactic (Armel et al. 2003). The tolerance of corn to acetochlor postemergence (POST) allows for later applications that provide residual control of waterhemp, which can emerge throughout the growing season.

To the best of our knowledge, no research has been conducted on the efficacy of acetochlor herbicide mixtures PRE for MHR waterhemp control in corn in Ontario. The objective of this study was to evaluate MHR waterhemp control with acetochlor-based herbicide mixtures applied PRE in corn.

Materials and Methods

Experimental Methods

Three field trials were conducted in 2022 near Cottam, ON (42.149°N, 82.684°W), Newbury, ON (42.728°N, 81.823°W), and on Walpole Island, ON (42.562°N, 82.502°W), and two field trials were conducted in 2023 near Newbury, ON (42.690°N, 81.823°W), and on Walpole Island, ON (42.563°N, 82.504°W). At each site there were naturally occurring populations of waterhemp that were five-way resistant to the WSSA Groups 2, 5, 9, 14, and 27 herbicides (Symington et al. 2022). Soil characteristics for each site are presented in Table 1.

The previous crop at each site was soybean. Seedbed preparation consisted of vertical tillage in the fall followed by cultivation in the spring. Corn hybrids were seeded at a rate of ~83,000 seeds ha^{-1} to a depth of 4.0 to 5.0 cm in rows spaced 75 cm apart. Plot measurements were 2.25 m wide (three corn rows) × 8 m long. Glyphosate (Roundup WeatherMAX®, Bayer Crop Science, Calgary, AB, Canada) (450 g ai ha^{-1}) was applied POST to the entire experimental area to control glyphosate-susceptible waterhemp and all other weed species. The trials were established as a randomized complete block design (RCBD) with four blocks. Each trial included 15 herbicide treatments plus a nontreated (weedy) and a weed-free control. Herbicide active ingredient, rate, trade name, and manufacturer are presented in Table 2. The weed-free control was maintained weed-free with *S*-metolachlor/atrazine/mesotrione/bicyclopyrone (Acuron®, Syngenta Canada, Guelph, ON, Canada) (2,026 g ai ha^{-1}) applied PRE, followed by glufosinate (Liberty® 200 SN, BASF, Mississauga, ON, Canada) (500 g ai ha^{-1}) applied POST; hand weeding was completed when

Table 2. Herbicide active ingredient, rate, trade name, and manufacturer of products used to investigate acetochlor-based herbicide mixtures in corn for multiple-herbicide-resistant waterhemp control from three field trials (2022) and two field trials (2023) conducted in southwestern Ontario, Canada

Herbicide	Rate	Trade name	Manufacturer
	g ai/ae ha^{-1}		
Acetochlor	2,950	Harness®	Bayer Crop Science (Calgary, AB, Canada)
Flumetsulam	50	Broadstrike™ RC	Corteva Agriscience (Calgary, AB, Canada)
Dicamba	600	XtendiMax®	Bayer Crop Science
Atrazine	1,490 or 800[a]	AAtrex®	Syngenta Canada (Guelph, ON, Canada)
Isoxaflutole/diflufenican	191	Brodal®	Bayer Crop Science
Mesotrione	140	Callisto®	Syngenta Canada
Isoxaflutole/thiencarbazone-methyl	74/30	Corvus®	Bayer Crop Science
Dimethenamid-p/saflufenacil	660/75	Integrity®	BASF (Mississauga, ON, Canada)
S-metolachlor/atrazine/mesotrione/bicyclopyrone	1,259/588/140/35	Acuron®	Syngenta Canada

[a] Applied at 1,490 g ai ha^{-1} for all treatments besides the coapplication of isoxaflutole/thiencarbazone-methyl + atrazine and isoxaflutole/diflufenican + atrazine.

Table 3. Year, location, corn hybrid, corn planting, herbicide application, corn emergence, and corn harvest dates from five field trials (2022) and two field trials (2023) conducted in southwestern Ontario, Canada

Year	Location	Corn hybrid	Planting date	Application date	Emergence date	Harvest date
2022	Cottam	DKC46-82RIB	17 May	18 May	25 May	20 Oct
2022	Newbury	DKC46-82RIB	12 May	13 May	20 May	24 Oct
2022	Walpole Island	DKC46-82RIB	21 Jun	23 Jun	26 Jun	9 Nov
2023	Newbury	P0075YHR	26 May	29 May	2 Jun	—
2023	Walpole Island	Pride 7197G8	27 May	15 Jun	5 Jun	—

required. Herbicide treatments were applied PRE with a CO_2-pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 240 kPa. A spray width of 2 m was produced from a 1.5-m boom equipped with four ultra-low drift nozzles (ULD 120–02, Hypro, Pentair, London, UK) spaced 50 cm apart. Owing to miscommunication with the grower for Walpole Island 2023, the PRE application was made after corn emergence; therefore glufosinate (500 g ai ha^{-1}) was applied to control all emerged waterhemp. Corn hybrid, corn planting, herbicide application, corn emergence, and corn harvest dates are presented in Table 3.

Visible corn injury assessments were completed at 2 and 4 wk after emergence (WAE) on a percentage scale; 0 represented no corn injury, and 100 designated complete corn death. Visible MHR waterhemp control as an estimation of the biomass reduction relative to the nontreated control was assessed at 4, 8, and 12 wk after application (WAA) on a percentage scale; 0 indicated no control, and 100 indicated complete waterhemp control. At 8 WAA, waterhemp density was determined by counting and hand harvesting plants from two arbitrarily placed 0.25-m^2 quadrats within each plot. Waterhemp plants were clipped at the soil surface, placed into paper bags, and kiln-dried to consistent moisture. Samples were weighed using an analytical balance, and the dry shoot biomass was recorded. In 2022, at harvest maturity, two corn rows were combined with a small plot combine; seed moisture content (%) and weight were recorded. Corn was not combined in 2023. Corn grain yield was adjusted to 15.5% moisture prior to statistical analysis.

Statistical Analysis

Statistical analysis was performed as a RCBD using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC, USA). Herbicide treatment was the fixed effect; random effects included the environment (site by year), the replicate within environment, and the treatment by environment. All environments were pooled together for analysis. Variances were verified to be normal and homogenous with the use of the PROC UNIVARIATE procedure. The Shapiro–Wilk test statistic and linear studentized residuals were analyzed to ensure the assumptions of normality that residuals are random, are independent, are normally distributed, have a mean of zero, and are homogenous were met. The nontreated control and weed-free control were omitted from the data set for analysis of waterhemp control and corn injury; the weed-free control was not included for analysis of waterhemp density and biomass. Corn injury and visible waterhemp control utilized an arcsine square root transformation and normal distribution, whereas density and biomass fit a lognormal distribution. Corn yield used a normal distribution. All data that were transformed or analyzed with non-Gaussian distributions were back-transformed for presentation of results.

To determine the expected level of corn injury, and the expected level of MHR waterhemp control, Colby's equation (Equation 1) was used. Expected values were computed by replicate for the treatments involving a mixture with acetochlor from the observed corn injury and waterhemp values for each herbicide applied alone:

$$\text{Expected} = (A + B) - [(A * B)/100] \qquad [1]$$

where

A = value of first herbicide in herbicide mixture applied alone

B = value of second herbicide in herbicide mixture applied alone

Colby's equation was modified (Equation 2) to calculate the expected values for waterhemp density and biomass by replicate. This was completed for the mixtures containing acetochlor by using the observed density and biomass values for herbicides applied alone and the density and biomass from the nontreated control:

Table 4. Multiple-herbicide-resistant waterhemp control at 4, 8, and 12 wk after acetochlor-based herbicide mixtures applied preemergence from five field trials conducted in 2022 and 2023 in southwestern Ontario, Canada.[a,b,c]

		Visible waterhemp control		
Treatment	Rate	4 WAA	8 WAA	12 WAA
	g ai/ae ha^{-1}	%		
Weed-free control		100	100	100
Nontreated control		0	0	0
Acetochlor	2,950	99 a	98 a	97 a
Flumetsulam	50	79 b	60 b	53 b
Dicamba	600	79 b	57 b	55 b
Atrazine	1,490	81 b	66 b	64 b
Isoxaflutole/diflufenican	191	99 a	97 a	97 a
Mesotrione + atrazine	140 + 1,490	99 a	95 a	94 a
Acetochlor + flumetsulam	2,950 + 50	100 (100) a	99 (97) a	98 (96) a
Acetochlor + dicamba	2,950 + 600	100 (100) a	99 (98) a	99 (97) a
Acetochlor + atrazine	2,950 + 1,490	100 (99) a	99 (97) a	99 (96) a
Acetochlor + isoxaflutole/diflufenican	2,950 + 191	100 (100) a	99 (99) a	99 (99) a
Acetochlor + mesotrione + atrazine	2,950 + 140 + 1,490	100 (100) a	99 (99) a	99 (98) a
Isoxaflutole/thiencarbazone-methyl + atrazine	104 + 800	99 a	96 a	95 a
Isoxaflutole/diflufenican + atrazine	191 + 800	100 a	99 a	99 a
Dimethenamid-p/saflufenacil	735	99 a	97 a	95 a
S-metolachlor/atrazine/mesotrione/bicyclopyrone	2,026	99 a	98 a	97 a

[a] Abbreviation: WAA, weeks after application.

[b] Means followed by the same letter within a column are not significantly different according to Tukey–Kramer at $P < 0.05$.

[c] Values in parentheses represent expected values from Colby's equation.

$$\text{Expected} = (A * B)/W \qquad [2]$$

where

A = value of first herbicide in mixture applied alone
B = value of second herbicide in mixture applied alone
W = value of nontreated control

After expected values were calculated, a two-tailed *t*-test was run in SAS to compare the expected values to the observed values for the acetochlor-based mixtures. A significance level of $\alpha = 0.05$ was used to determine the nature of the relationship. The relationship was antagonistic when the observed value was less than the expected value, additive when the two values were similar, and synergistic if the observed value was greater than the expected value.

Results and Discussion

Rainfall at all sites varied from 0.2 to 30.7 mm 7 d after treatment application. Control in low-rainfall environments, though, showed the same trends as control in high-rainfall-environments.

Corn Injury

The herbicide treatments evaluated caused <1% corn injury at 2 and 4 WAE (data not presented). These results are similar to a study conducted by Janak and Grichar (2016), who reported that acetochlor (5,165 g ai ha^{-1}) caused <3% corn injury.

Multiple-Herbicide-Resistant Waterhemp Visible Control

Acetochlor (2,950 g ai ha^{-1}) controlled MHR waterhemp 99% at 4 WAA (Table 4). Strom et al. (2019) reported only 75% control of waterhemp at 4 WAA with acetochlor applied at 2,700 g ai ha^{-1}. In the study by Strom et al., the encapsulated formulation of acetochlor was used; in contrast, the emulsifiable concentrate was used in the current study. Hausman et al. (2013) found that the emulsifiable concentrate formulation of acetochlor at 1,680 and 3,360 g ai ha^{-1} provided 85% and 94% control of waterhemp, respectively, at 4 WAA. Flumetsulam (50 g ai ha^{-1}), dicamba (600 g ai ha^{-1}), and atrazine (1,490 g ai ha^{-1}) controlled waterhemp 79%, 79%, and 81%, respectively. This low level of control with flumetsulam and atrazine was expected because there were Group 2– and Group 5–resistant biotypes at all trial locations. Meyer et al. (2016) reported that dicamba PRE provided poor waterhemp control and suggested that this was due to rainfall, which reduced the length of residual waterhemp control with dicamba. Isoxaflutole/diflufenican (191 g ai ha^{-1}), mesotrione + atrazine (140 + 1,490 g ai ha^{-1}), all acetochlor mixtures, isoxaflutole/thiencarbazone-methyl + atrazine (104 + 800 g ai ha^{-1}), isoxaflutole/diflufenican + atrazine (191 + 800 g ai ha^{-1}), dimethenamid-p/saflufenacil (735 g ai ha^{-1}), and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone (2,026 g ai ha^{-1}) controlled waterhemp 99% to 100%; control was similar to acetochlor applied alone but greater than control with flumetsulam, dicamba, or atrazine applied alone. All acetochlor mixture interactions were additive. Willemse et al. (2021b) reported 99% control of waterhemp 4 WAA with mesotrione + atrazine and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone PRE, which are similar to the control in the current study.

Acetochlor controlled MHR waterhemp 98%, which was similar to all herbicide treatments except flumetsulam, dicamba, and atrazine, which provided between 57% and 66% control 8 WAA (Table 4). At 60 d after treatment, or 8.5 wk, Hausman et al. (2013) reported that acetochlor (3,360 g ai ha^{-1}) controlled waterhemp 87%, which is slightly lower than the findings from this study. All acetochlor-based mixtures were additive and controlled waterhemp 99%, which was similar to acetochlor, isoxaflutole/diflufenican, mesotrione + atrazine, isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine, dimethenamid-p/saflufenacil, and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone. Armel et al. (2003) reported that acetochlor + mesotrione (1,800 + 160 g ai ha^{-1}) PRE controlled smooth pigweed (*Amaranthus hybridus* L.), a relative of waterhemp, 95% to 99% at 8 WAA, which is similar to the control with mesotrione + atrazine or acetochlor + mesotrione + atrazine in

Table 5. Multiple-herbicide-resistant waterhemp density and biomass at 8 wk after acetochlor-based herbicide mixtures applied preemergence and corn yield from five field trials conducted in 2022 and 2023 in southwestern Ontario, Canada.[a,b,c]

Treatment	Rate	Density	Biomass	Yield
	g ai/ae ha^{-1}	plants m^{-2}	g m^{-2}	Mg ha^{-1}
Weed-free control		0	0	9.91 a
Nontreated control		482 d	93.1 c	8.56 a
Acetochlor	2,950	9 a–c	4.2 a	9.99 a
Flumetsulam	50	258 d	51.4 bc	8.68 a
Dicamba	600	279 d	47.4 c	8.69 a
Atrazine	1,490	382 d	41.9 bc	8.92 a
Isoxaflutole/diflufenican	191	18 a–c	5.2 a	9.44 a
Mesotrione + atrazine	140 + 1,490	48 c	8.1 ab	9.84 a
Acetochlor + flumetsulam	2,950 + 50	6 (3) a–c	2.5 (5.4) a	9.56 a
Acetochlor + dicamba	2,950 + 600	3 (2) ab	0.7 (3.9) a	8.80 a
Acetochlor + atrazine	2,950 + 1,490	6 (4) a–c	2.8 (1.8) a	9.93 a
Acetochlor + isoxaflutole/diflufenican	2,950 + 191	2 (1) a	0.1 (0.3) a	9.47 a
Acetochlor + mesotrione + atrazine	2,950 + 140 + 1,490	5 (1)* a–c	1.0 (1.8) a	9.82 a
Isoxaflutole/thiencarbazone-methyl + atrazine	104 + 800	25 bc	3.5 a	9.43 a
Isoxaflutole/diflufenican + atrazine	191 + 800	6 a–c	1.8 a	9.44 a
Dimethenamid-p/saflufenacil	735	9 a–c	9.7 a	9.46 a
S-metolachlor/atrazine/mesotrione/bicyclopyrone	2,026	7 a–c	5.1 a	9.38 a

[a] Means followed by the same letter within a column are not significantly different according to Tukey–Kramer at $P < 0.05$.

[b] Values in parentheses represent expected values from Colby's equation.

[c] An asterisk (*) denotes significance at $P < 0.05$ between observed and expected values based on a two-tailed *t*-test.

the current study. Steckel et al. (2002) published that acetochlor/atrazine provided 91% waterhemp control at 8 WAA, which is similar to the control (99%) with acetochlor + atrazine in this study. Isoxaflutole/diflufenican, mesotrione + atrazine, acetochlor, all acetochlor-based mixtures, isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine, dimethenamid-p/saflufenacil, and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone provided greater waterhemp control than dicamba, atrazine, and flumetsulam applied alone.

Dicamba, atrazine, and flumetsulam controlled waterhemp 53%, 55%, and 64%, respectively, at 12 WAA (Table 4). Acetochlor controlled MHR waterhemp 97%, and all acetochlor mixtures provided 98% to 99% control; all acetochlor mixtures were additive. Isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine (191 + 800 g ai ha^{-1}), dimethenamid-p/saflufenacil, and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone controlled waterhemp 95% to 99%.

Multiple-Herbicide-Resistant Waterhemp Density and Biomass

At 8 WAA, 482 waterhemp plants m^{-2} were in the nontreated control (Table 5). All locations contained naturally high seedbank infestation levels that varied from 54 to 6,741 plants m^{-2}. Acetochlor reduced MHR waterhemp density 98% relative to the nontreated control. Similarly, Hausman et al. (2013) reported that acetochlor (3,360 g ai ha^{-1}) reduced resistant waterhemp density 96%. Flumetsulam, dicamba, and atrazine did not reduce waterhemp density relative to the nontreated control. Similarly, Meyer et al. (2016) reported that dicamba (560 g ae ha^{-1}) reduced waterhemp density by only 19%. Isoxaflutole/diflufenican and mesotrione + atrazine reduced waterhemp density by 96% and 90%, respectively. All acetochlor mixtures reduced MHR waterhemp density 99% to 100%. The mixtures of acetochlor with flumetsulam, dicamba, atrazine, or isoxaflutole/diflufenican were additive. On the basis of Colby's equation, one waterhemp plant was expected in the mixture of acetochlor + mesotrione + atrazine; however, five plants were observed, demonstrating an antagonistic interaction. Isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine, dimethenamid-p/saflufenacil, and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone reduced waterhemp density 95% to 99%. Willemse et al. (2021b) reported that *S*-metolachlor/atrazine/mesotrione/bicyclopyrone reduced waterhemp density 100%, which is similar to the 99% reduction in the current study.

There was 93.1 g m^{-2} of waterhemp biomass in the nontreated control at 8 WAA (Table 5). Acetochlor reduced waterhemp biomass by 95%, which was similar to all other herbicide treatments evaluated, except flumetsulam, dicamba, and atrazine, which reduced waterhemp biomass by 45%, 49%, and 55%, respectively. All acetochlor mixtures reduced waterhemp biomass by 97% to 100%; all interactions were additive. Isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine, dimethenamid-p/saflufenacil, and *S*-metolachlor/atrazine/mesotrione/bicyclopyrone reduced waterhemp biomass 90% to 98%.

Corn Yield

There was no difference in corn yield in this study. Despite large densities of MHR waterhemp in the nontreated control, the various herbicide treatments evaluated were able to delay waterhemp emergence long enough that when waterhemp did emerge, the corn crop was successfully able to outcompete it. The majority of emerged waterhemp likely remained small due to a lack of light as explained by the red:far-red light ratio (Markham and Stoltenberg 2010).

In summary, acetochlor mixtures with flumetsulam, dicamba, atrazine, isoxaflutole/diflufenican, and mesotrione + atrazine controlled MHR waterhemp ≥98% at 4, 8, and 12 WAA and reduced density and biomass ≥99% and ≥97%, respectively; however, these values were similar to acetochlor applied alone. At 8 WAA, flumetsulam, dicamba, and atrazine controlled waterhemp 57% to 66%, reduced density 21% to 46%, and reduced biomass 45% to 55%. At 8 WAA, isoxaflutole/thiencarbazone-methyl + atrazine, isoxaflutole/diflufenican + atrazine, dimethenamid-p/saflufenacil, and S-metolachlor/atrazine/mesotrione/bicyclopyrone controlled waterhemp 96% to 99%, reduced density 95% to

99%, and reduced biomass 90% to 98%. No corn yield differences were present at harvest. Although acetochlor-based herbicide mixtures did not improve waterhemp control and did not reduce waterhemp density and biomass relative to acetochlor, these herbicide mixtures might reduce the selection intensity for the evolution of further herbicide-resistant waterhemp biotypes in Ontario fields. Delaying herbicide resistance should be an important consideration when developing best management practices for waterhemp control programs in Ontario corn production.

Practical Implications

Waterhemp continues to develop resistance to new herbicide modes of action and has become a challenging weed to control in many parts of North America. Waterhemp populations have evolved resistance to five herbicide modes of action (Groups 2, 5, 9, 14, and 27), which are present across southern Ontario; this has increased the challenge of controlling this competitive weed species in corn, the most important grain crop produced worldwide and the highest-value agronomic crop in Ontario. Acetochlor is a Group 15 soil-applied residual herbicide that has activity on many small-seeded annual grasses and some small-seeded annual broadleaf weeds. The mixtures of acetochlor with flumetsulam, dicamba, atrazine, isoxaflutole/diflufenican, or mesotrione + atrazine applied PRE caused minimal injury or yield reduction in corn. Acetochlor applied alone provided excellent control of MHR waterhemp. Similarly, the mixtures of acetochlor with flumetsulam, dicamba, atrazine, isoxaflutole/diflufenican, or mesotrione + atrazine applied PRE provided ≥98% control of MHR waterhemp at 12 WAA. There were no differences among herbicide mixtures for waterhemp control or corn yield. This study shows that the acetochlor herbicide mixtures evaluated provide excellent waterhemp control; however, control was not greater than with acetochlor alone. Combining acetochlor with the broadleaf herbicides evaluated could reduce selection intensity for the evolution of herbicide-resistant biotypes.

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References

Anonymous (2020a) Harness® herbicide label. St. Louis, MO: Bayer Crop Science. 13 p

Anonymous (2020b) Warrant® herbicide label. St. Louis, MO: Bayer Crop Science. 13 p

Armel GR, Wilson HP, Richardson RJ, Hines TE (2003) Mesotrione, acetochlor, and atrazine for weed management in corn (*Zea mays*). Weed Technol 17:284–290

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Braswell LR, Jennings KM (2015) Weed control in cotton by combinations of microencapsulated acetochlor and various residual herbicides applied preemergence. Weed Technol 29:740–750

Cordes JC, Johnson WG, Scharf P, Smeda RJ (2004) Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed Technol 18:999–1005

Costea M, Weaver SE, Tardif FJ (2005) The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer). Can J Plant Sci 85:507–522

de Guzman NP, Hendley P, Gustafson DI, van Wesenbeeck I, Klein AJ, Fuhrman JD, Travis K, Simmons ND, Teskey WE, Durham RB (2005) The Acetochlor registration partnership state ground water monitoring program. J Environ Qual 34:1454

Gianessi LP, Reigner NP (2007) The value of herbicides in U.S. crop protection. Weed Technol 21:559–566

Gressel J, Segel LA (1990) Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. Weed Technol 4:186–198

GROW (n.d.) Waterhemp. https://growiwm.org/weed/waterhemp/. Accessed: August 15, 2022

Hall MR, Swanton CJ, Anderson GW (1992) The critical period of weed control in grain corn (*Zea mays*). Weed Sci 40:441–447

Hartzler RG, Battles BA, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci 52:242–245

Hausman NE, Tranel PJ, Riechers DE, Maxwell DJ, Gonzini LC, Hager AG (2013) Responses of an HPPD inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) population to soil-residual herbicides. Weed Technol 27: 704–711.

Heap I (2022) The International Herbicide-Resistant Weed Database. http://www.weedscience.org/. Accessed: October 12, 2021

Janak TW, Grichar JW (2016) Weed control in corn (*Zea mays* L.) as influenced by preemergence herbicides. Int J Agron 2016:2607671

Jhala AJ, Malik MS, Willis JB (2015) Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean. Can J Plant Sci 95:973–981

Knezevic SZ, Evans SP, Blankenship EE, Van Acker RC, Lindquist JL (2002) Critical period for weed control: the concept and data analysis. Weed Sci 50:773–786

Markham MY, Stoltenberg DE (2010) Corn morphology, mass, and grain yield as affected by early-season red:far-red light environments. Crop Sci 50:273–280

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 (2016) Early-season Palmer amaranth and waterhemp control from preemergence programs utilizing 4-hydroxyphenylpyruvate dioxygenase-inhibiting and auxinic herbicides in soybean. Weed Technol 30:67–75

Nordby D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. Publication GWC-13. West Lafayette, IN: Purdue Extension. 12 p

[OMAFRA] Ontario Ministry of Agriculture, Food, and Rural Affairs (2009) Critical weed-free period: Ontario crop IPM. http://www.omafra.gov.on.ca/IPM/english/weeds-herbicides/critical-weed-free.html. Accessed: June 16, 2022

[OMAFRA] Ontario Ministry of Agriculture, Food, and Rural Affairs (2021) Area, yield, production and farm value of specified field crops (imperial and metric units): 2015–2021 by year. https://data.ontario.ca/dataset/ontario-field-crops-production-estimate/resource/02daebd7-a430-4220-83fa-7e7afc3d5efa. Accessed: June 23, 2022

Page ER, Cerrudo D, Westra P, Loux M, Smith K, Foresman C, Wright H, Swanton CJ (2012) Why early season weed control is important in maize. Weed Sci 60:423–430

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

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus* species in Missouri. Weed Sci 51:329–333

Shaner DL (2014) Herbicide handbook. 10th ed. Lawrence, KS: Weed Science Society of America. 513 p

Shergill LS, Barlow BR, Bish MD, Bradley KW (2018) Investigations of 2,4-D and multiple herbicide resistance in a Missouri waterhemp (*Amaranthus tuberculatus*) population. Weed Sci 66:386–394

Soltani N, Dille AJ, Burke IC, Everman WJ, VanGessel MJ, Davis VM, Sikkema PH (2016) Potential corn yield losses from weeds in North America. Weed Technol 30:979–984

Soltani N, Geddes C, Laforest M, Dille JA, Sikkema PH (2022) Economic impact of glyphosate-resistant weeds on major field crops grown in Ontario. Weed Technol 36:629–635

[StatsCan] Statistics Canada (2015) Corn: Canada's third most valuable crop. https://www150.statcan.gc.ca/n1/pub/96-325-x/2014001/article/11913-eng.htm. Accessed: April 27, 2022

Steckel LE, Sprague CL (2004) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci 52:359–364

Steckel LE, Sprague CL, Hager AG (2002) Common waterhemp (*Amaranthus rudis*) control in corn (*Zea mays*) with single preemergence and sequential applications of residual herbicides. Weed Technol 16: 755–761

Strom SA, Gonzini LC, Mitsdarfer C, Davis AS, Riechers DE, Hager AG (2019) Characterization of multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus*) populations from Illinois to VLCFA-inhibiting herbicides. Weed Sci 67:363–379.

Strom SA, Hager AG, Seiter NJ, Davis AS, Riechers DE (2020) Metabolic resistance to *S*-metolachlor in two waterhemp (*Amaranthus tuberculatus*) populations from Illinois, USA. Pest Manag Sci 76:3139–3148

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 Agric Sci 14:53–58

[USDA] U.S. Department of Agriculture (2014) *Amaranthus tuberculatus* (Moq.) Sauer. https://plants.usda.gov/home/plantProfile?symbol=AMTU. Accessed: August 16, 2022

[USDA] U.S. Department of Agriculture (2022) Corn area, yield, and production. Table 4 *in* World Agricultural Production. Circular WAP 3-24. Washington, DC: USDA. 37 p

Van Acker RC, Swanton CJ, Weise SF (1993) The critical period of weed control in soybean [*Glycine max* (L.) Merr.]. Weed Sci 41:194–200

Willemse C, Soltani N, Hooker DC, Jhala AJ, Robinson DE, Sikkema PH (2021b) Interaction of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and atrazine alternative photosystem II (PS II) inhibitors for control of multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus*) in corn. Weed Sci 69:492–450