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Control of multiple-herbicide-resistant waterhemp with acetochlor-based herbicide mixtures in soybean

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Research Article

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Nomenclature:
Acetochlor; dicamba; diflufenican; flumioxazin; metribuzin; sulfentrazone; waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer.; soybean, *Glycine max* (L.) Merr.

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Abstract

Waterhemp has evolved resistance to Group 2, 5, 9, 14, and 27 herbicides in Ontario, Canada, making control of this challenging weed even more difficult. Acetochlor is a Group 15, chloroacetanilide herbicide that has activity on many small-seeded annual grasses and some small-seeded annual broadleaf weeds, including waterhemp. The objective of this study was to ascertain if acetochlor mixtures with broadleaf herbicides (dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin), applied preemergence (PRE), increase multiple-herbicide-resistant (MHR) waterhemp control in soybean. Five trials were conducted over 2 yr (2021 and 2022). The acetochlor mixtures caused ≤7% soybean injury, except acetochlor + flumioxazin, which caused 19% soybean injury. Acetochlor applied PRE controlled MHR waterhemp 89% at 4 wk after application (WAA). Dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin controlled MHR waterhemp 59%, 67%, 58%, 64%, and 86%, respectively, at 4 WAA. Acetochlor applied in a mixture with dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin provided good to excellent control of MHR waterhemp; control ranged from 91% to 98% but was similar to acetochlor applied alone. Acetochlor alone reduced MHR waterhemp density and biomass 98% and 93%; acetochlor + flumioxazin reduced waterhemp density and biomass by an additional 2% and 7%, respectively. This research concludes that acetochlor applied in a mixture with flumioxazin was the most efficacious mixture evaluated for MHR waterhemp control.

Introduction

Over the last three decades, waterhemp has become an increasingly problematic weed for growers in North America. First identified on the banks of the Mississippi River in the United States, waterhemp has since moved into Canada, threatening soybean yield and net returns for producers (Costea et al. 2005; Sauer 1957; Steckel 2007). Waterhemp is a genetically diverse weed that can grow in a range of soil types and under a range of moisture conditions (Costea et al. 2005). Its success has been attributed to its rapid growth rate, dioecious nature, season-long emergence, high fecundity, and evolution of resistance to multiple modes of action (Horak and Loughin 2000; Nordby et al. 2007; Steckel et al. 2003).

Waterhemp has evolved resistance to synthetic auxins and to the acetolactate synthase–, photosystem II–, 5-enolpyruvylshikimate-3-phosphate synthase–, protoporphyrinogen oxidase–, very-long-chain fatty-acid elongases–, and 4-hydroxyphenylpyruvate dioxygenase–inhibiting herbicides, representing Weed Science Society of America Groups 2, 4, 5, 9, 14, 15, and 27, respectively (Heap 2023). The first case of multiple-herbicide-resistant (MHR) waterhemp was documented in 1996 in Illinois, where resistance to Groups 2 and 5 was confirmed (Heap 2023). In the United States, a Missouri waterhemp population has resistance to six herbicide modes of action: Groups 2, 4, 5, 9, 14, and 27 (Shergill et al. 2018). In Ontario, Canada, four-way resistance was first recorded in 2017 to Groups 2, 5, 9, and 14 (Benoit et al. 2019). In 2022, resistance to the Group 27 herbicides and five-way-resistant waterhemp populations were confirmed (Heap 2023). The dioecious nature of waterhemp, which contributes to wide genetic diversity, is partly responsible for the rapid evolution of herbicide resistance in waterhemp. Separate male and female plants must outcross to produce viable offspring; this results in greater genetic diversity than characterizes monoecious, self-pollinated weed species (Bell and Tranel 2010).

High seed production, seed dormancy, and seed viability also contribute to the success of waterhemp. In a noncompetitive environment, waterhemp can produce up to 4.8 million seeds

Table 1. Year, location, and soil characteristics for five field trials conducted in southwestern Ontario, Canada, in 2021 and 2022.[a,b]

Year	Location	Soil texture	Sand	Silt	Clay	OM	pH	CEC
					%			
2021	Cottam	Sandy loam	62	23	15	2.3	5.9	7.7
2021	Newbury	Loamy sand	79	14	6	2.8	6.5	7.9
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	65	24	11	2.1	7.1	14.9

[a]Abbreviations: CEC, cation exchange capacity; OM, organic matter.
[b]Soil analysis performed by A&L Canada Laboratories Inc. (Ontario, Canada) from soil cores taken from 0 to 15 cm.

per plant, though this number is reduced dramatically by inter- and intraspecies competition, environmental stresses, and delayed emergence (Costea et al. 2005; Hartzler et al. 2004; Steckel et al. 2003). The viability of waterhemp seed is high, and the seed can remain viable in the soil for up to 17 yr; however, most studies conclude that the seed remains viable for less than 10 yr (Burnside et al. 1996; Korres et al. 2018). Regardless, this results in an exponentiation of the problem in the following years. In addition, waterhemp emergence has been documented to occur from May until October in the United States and Canada, allowing late-emerging waterhemp to escape control by short-residual herbicides, produce seed, and return seed to the seedbank (Costea et al. 2005; Hartzler et al. 1999; Schryver et al. 2017b; Vyn et al. 2007). Waterhemp has a rapid growth rate of 0.30 g g^{-1} d^{-1} (Horak and Loughin 2000) and grows up to 3 m in height, allowing it to shade out lower-stature crops like soybean (Nordby et al. 2007). Waterhemp interference caused soybean yield losses of up to 73% and 55% in Ontario and the United States, respectively (Bensch et al. 2003; Steckel and Sprague 2004; Vyn et al. 2007).

Acetochlor is a Group 15 chloroacetanilide herbicide that inhibits very-long-chain fatty-acid elongases (Braswell et al. 2016). Since its registration in 1994, acetochlor has been widely used in USA corn, soybean, and cotton production; however, it is not registered for use in Canada (Armel et al. 2003; Cahoon et al. 2015). Just over 3.5 million kg of acetochlor was applied to USA soybean in 2020 (NASS 2021). Acetochlor is absorbed primarily through the elongating coleoptile and epicotyl/hypocotyl of developing grass and broadleaf species, respectively, preventing affected seedlings from emerging (Jhala et al. 2015). In the USA, encapsulated acetochlor is registered for application in soybean preplant (PP), preemergence (PRE), and postemergence (POST) (Anonymous 2020). Jhala et al. (2015) demonstrated that soybean has excellent tolerance to encapsulated acetochlor; the injury was <10% even with three sequential applications applied PRE, early postemergence (EPOST), and late postemergence (LPOST). Acetochlor primarily provides control of small-seeded monocot weeds, though it also has activity on some small-seeded dicot weeds, including waterhemp, pigweeds (*Amaranthus* spp.), nightshades (*Solanum* spp.), lambsquarters (*Chenopodium album* L.), and purslane (*Portulaca oleracea* L.) (Anonymous 2020; Jhala et al. 2015; Jursik et al. 2013). Two sequential applications of acetochlor applied PRE followed by EPOST or PRE followed by LPOST were required for full-season control of waterhemp (Jhala et al. 2015). Weed control research has been completed in the USA on acetochlor mixtures with fomesafen, flumioxazin, mesotrione, or pendimethalin (Armel et al. 2003; Cahoon et al. 2015; Grichar et al. 2015); however, five-way MHR waterhemp was not evaluated in those studies.

The level of MHR waterhemp control provided by acetochlor applied alone is often inadequate (Jhala et al. 2015; Strom et al. 2019). In addition, the newly documented Group 15–resistant waterhemp further enhances the need for mixtures to reduce selection pressure from a single herbicide mode of action. The mechanism of Group 15 resistance in waterhemp is enhanced metabolism (Strom et al. 2020). Coapplication of acetochlor with broadleaf herbicides could improve MHR waterhemp control, while reducing the selection intensity for the evolution of herbicide resistance.

Waterhemp now spans a distance of more than 800 km across southern Ontario, extending from the Michigan border in the west to the Quebec border in the east. It has also evolved resistance to five herbicide modes of action in Ontario: Groups 2, 5, 9, 14, and 27. It is imperative that more complexity be incorporated into Ontario crop/weed management programs to reduce the evolution of herbicide-resistant weeds, protect our valuable herbicide resources, and maximize net returns for growers. As MHR waterhemp continues to spread geographically through Ontario and resistance to other modes of action builds, acetochlor-based mixtures could be one tool in a diversified crop/weed management program. To the best of our knowledge, no research has been conducted on acetochlor mixtures for MHR waterhemp control in Ontario. The objective of this research was to evaluate acetochlor mixtures applied PRE for MHR waterhemp control in Ontario.

Materials and Methods

Experimental Methods

Two field trials were conducted in 2021 and three in 2022 for a total of 5 site-years. In 2021, trial locations were near Cottam, ON (42.149°N, 82.684°W) and Newbury, ON (42.691°N, 81.823°W); in 2022, field locations were near Cottam, near Newbury (42.728°N, 81.823°W), and on Walpole Island, ON (42.562°N, 82.502°W) (Table 1). All sites were infested with naturally occurring populations of waterhemp resistant to Groups 2, 5, 9, 14, and 27. Soil characteristics for each site are presented in Table 1.

The previous crop at each site was corn. Sites were vertical tilled in the fall followed by one pass with a tandem disk and field cultivator in the spring. Glyphosate/dicamba-resistant (Roundup Ready Xtend®) soybean cultivars DKB10-20® and DKB0817® (Bayer Crop Science, Calgary, AB, Canada) were planted in 2021 and 2022, respectively, at a rate of approximately 400,000 seeds ha^{-1} to a depth of 3.75 cm in rows spaced 75 cm apart. Plots measured 8 m long × 2.25 m wide (three soybean rows). The field trials were established as a randomized complete block design (RCBD) with four replicates. The trial included 14 herbicide treatments plus a nontreated control and a weed-free control. Acetochlor (1,700 g ai ha^{-1}) and the broadleaf herbicides of dicamba (600 g ai ha^{-1}), metribuzin (413 g ai ha^{-1}), diflufenican (90 g ai ha^{-1}), sulfentrazone (140 g ai ha^{-1}), and flumioxazin (107 g ai ha^{-1}) were

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Table 2. Herbicide active ingredient, rate, trade name, and manufacturer of products used to investigate acetochlor-based mixtures in soybean for multiple-herbicide-resistant waterhemp control for five trials completed in southwestern Ontario, Canada, in 2021 and 2022

Herbicide treatment	Rate	Trade name	Manufacturer
	g ai ha^{-1}		
Acetochlor	1,700	Warrant®	Bayer Crop Science (Calgary, AB, Canada)
Dicamba	600	XtendiMax®	Bayer Crop Science
Metribuzin	413	Sencor® 75DF	Bayer Crop Science
Diflufenican	90	Brodal®	Bayer Crop Science
Sulfentrazone	140	Authority®	FMC Canada (Mississauga, ON, Canada)
Flumioxazin	107	Valtera™ EZ	Valent Canada (Guelph, ON, Canada)
S-metolachlor/metribuzin	1,943	Boundary® LQD	Syngenta Canada (Guelph, ON, Canada)
Pyroxasulfone/sulfentrazone	300	Authority® Supreme	FMC Canada
Pyroxasulfone/flumioxazin	240	Fierce® EZ	Valent Canada

Table 3. Year, location, cultivar, soybean planting, herbicide application, soybean emergence, and soybean harvest dates for five field trials conducted in southwestern Ontario, Canada, in 2021 and 2022

Year	Location	Soybean cultivar	Planting date	Application date	Emergence date	Harvest date
2021	Cottam	DKB10-20	18 May	21 May	24 May	20 Sep
2021	Newbury	DKB10-20	11 May	14 May	20 May	23 Nov
2022	Cottam	DKB0817 A5	17 May	18 May	27 May	22 Sep
2022	Newbury	DKB0817 A5	12 May	13 May	21 May	22 Sep
2022	Walpole Island	DKB0817 A5	21 Jun	23 Jun	26 Jun	6 Oct

each applied on its own. Each broadleaf herbicide was also evaluated in combination with acetochlor. The commercial standards of *S*-metolachlor/metribuzin (1,943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}), and pyroxasulfone/flumioxazin (240 g ai ha^{-1}) were used as comparisons. Herbicide active ingredient, rate, trade name, and manufacturer are presented in Table 2. Pyroxasulfone/flumioxazin (Fierce® EZ, Valent Canada, Guelph, ON, Canada) (160 g ai ha^{-1}) applied PRE followed by glyphosate/dicamba (Roundup Xtend®, Bayer Crop Science) (1,800 g ae ha^{-1}) applied POST was used to prevent weeds from being present in the weed-free control; hand weeding was completed when required. Herbicide treatments were applied PRE with a CO_2-pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 240 kPa. Four nozzles (ULD120-02, Hypro, Pentair, London, UK) spaced 50 cm apart on a 1.5-m boom were used for treatment application, producing a spray width of 2 m. Year; location; soybean cultivar; planting, emergence, and harvest dates; and herbicide application dates are presented in Table 3.

Visible soybean injury assessments were completed at 2 and 4 wk after emergence (WAE) on a 0 to 100 scale, where 0 represents no soybean injury and 100 designates complete plant death. Visible MHR waterhemp control as an approximation of the biomass reduction compared to the nontreated control was assessed at 4, 8, and 12 wk after application (WAA) on a 0 to 100 scale, where 0 indicates no control and 100 indicates complete waterhemp control. At 8 WAA, density was recorded by counting and hand harvesting waterhemp plants from two 0.25-m^2 quadrants from each plot. The waterhemp plants were clipped at the soil surface, placed into paper bags, kiln dried for 2 wk, and weighed using an analytical balance to determine dry shoot biomass. Two soybean rows were harvested with a small-plot research combine at harvest maturity; percent seed moisture content and weight were recorded. Soybean seed yield was adjusted to 13.5% moisture prior to statistical analysis.

Statistical Analysis

Statistical analysis was performed as an RCBD in SAS 9.4 (SAS Institute, Cary, NC, USA) using the PROC GLIMMIX procedure. The fixed effect was herbicide treatment, and the random effects were environment (site-year), replicate within environment, and treatment by environment. All environments were combined and analyzed together. The PROC UNIVARIATE procedure was used to ensure that variances were normal and homogenous. The Shapiro–Wilk test statistic, linear studentized residuals, and a test for overdispersion were analyzed to ensure that the assumptions of normality (residuals are random, independent, and normally distributed; have a mean of 0; and are homogenous) were met. The nontreated control and weed-free control were omitted from the data set for analysis of waterhemp control and soybean injury; the weed-free control was not included for analysis of waterhemp density and biomass. An arcsine square root distribution was used for soybean injury, and lognormal distribution was used for weed density and biomass. Visible control and yield residuals fit a normal distribution. Injury, density, and biomass data were back-transformed from their appropriate distributions for presentation of results.

Colby's equation (Equation 1) was used to determine the expected level of soybean injury and MHR waterhemp control by replicate for the treatments involving a mixture with acetochlor from the observed injury and control 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

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

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

where

A = value of first herbicide in mixture applied alone

B = value of second herbicide in mixture applied alone

W = value of nontreated control

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Table 4. Multiple-herbicide-resistant waterhemp control at 4, 8, and 12 WAA and density and biomass at 8 WAA after PRE application of soybean herbicides and acetochlor-based mixtures for five field trials conducted in southwestern Ontario, Canada, in 2021 and 2022.[a,b,c]

Treatment	Rate	Visible waterhemp control			Density, 8 WAA	Biomass, 8 WAA
		4 WAA	8 WAA	12 WAA		
	g ai ha^{-1}	——— % ———			plants m^{-2}	g m^{-2}
Weed-free control		100	100	100	0	0
Nontreated control		0	0	0	882 h	127.2 e
Acetochlor	1,700	89 ab	88 a	82 ab	17 b–d	8.8 b
Dicamba	600	59 d	42 c	37 d	445 gh	54.9 de
Metribuzin	413	67 b–d	60 bc	53 cd	147 e–g	41.8 cd
Diflufenican	90	58 d	44 c	38 d	378 f–h	67.6 de
Sulfentrazone	140	64 cd	61 bc	55 cd	56 d–f	38.2 cd
Flumioxazin	107	86 a–c	86 a	81 ab	19 b–d	12.2 b
Acetochlor + dicamba	1,700 + 600	94 (95) a	93 (93) a	89 (87) ab	16 (9)* a–c	2.6 (3.8) ab
Acetochlor + metribuzin	1,700 + 413	92 (96) a	93 (95) a	90 (92) ab	11 (3)** a–c	4.6 (2.9) ab
Acetochlor + diflufenican	1,700 + 90	93 (95) a	89 (93) a	89 (89) ab	13 (7)* a–c	2.0 (4.7) ab
Acetochlor + sulfentrazone	1,700 + 140	91 (96) ab	91 (95) a	92 (92) a	11 (1)** a–c	6.1 (2.6)* ab
Acetochlor + flumioxazin	1,700 + 107	98 (98) a	99 (98) a	97 (97) a	3 (0)* a	0.2 (0.8) a
S-metolachlor/metribuzin	1,943	78 a–d	76 ab	66 bc	41 c–e	18.5 bc
Pyroxasulfone/sulfentrazone	300	81 a–d	84 a	77 a–c	11 a–c	16.0 b
Pyroxasulfone/flumioxazin	240	90 ab	95 a	91 a	4 ab	4.1 ab

[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.
*, $P < 0.05$; **, $P < 0.01$, between observed and expected values based on a two-tailed *t*-test.

A two-tailed *t*-test in SAS was then used to compare the expected values to the observed values for the acetochlor mixtures. Significance values of $P < 0.05$ were used to determine the relationship; where applicable, $P < 0.01$ levels are also shown. If the observed value was greater than the expected value, the relationship was characterized as synergistic; when the observed value was similar to the expected value, the relationship was termed additive, and when the observed value was less than the expected value, the relationship was deemed antagonistic.

Results and Discussion

In 2021 at the Cottam site, there was 31.3 and 49.4 mm of rain in the 7 and 14 d that followed herbicide application, respectively. In 2021 at the Newbury site, there was 0 and 31.6 mm of rain in the 7 and 14 d that followed herbicide application, respectively. In 2022 at the Cottam site, 15.7 and 27.7 mm of rain fell within 7 and 14 d after application, respectively. At the Newbury site in 2022, 30.7 and 45.7 mm of rain fell within 7 and 14 d after application, respectively. Weather data for the Walpole Island site in 2022 were retrieved from the neighboring town of Wallaceburg because there were problems with the on-site weather station. At Walpole Island in 2022, 0.2 and 7.3 mm of rainfall fell within 7 and 14 d after application, respectively. Data were pooled across site-years, as there was no interaction.

Multiple-Herbicide-Resistant Waterhemp Visible Control

Acetochlor controlled MHR waterhemp 89%, 88%, and 82% at 4, 8, and 12 WAA, respectively (Table 4). Hay et al. (2018) reported 68% waterhemp control at 4 WAA with encapsulated acetochlor (1,220 g ai ha^{-1}). Flumioxazin provided 86%, 86%, and 81% waterhemp control at 4, 8, and 12 WAA, respectively. At 4 WAA, dicamba, metribuzin, diflufenican, and sulfentrazone controlled MHR waterhemp similarly at 58% to 67%; control at 8 WAA ranged from 42% to 61% and from 37% to 55% at 12 WAA. Schryver et al. (2017b) reported 48% waterhemp control with metribuzin (420 g ai ha^{-1}) applied PRE at 4 WAA, which is similar to the findings of the current study. At 4, 8, and 12 WAA, the mixtures of acetochlor + dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin controlled MHR similarly at 91% to 98%, 89% to 99%, and 89% to 97%, respectively; all interactions were additive. The industry standards *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin controlled MHR waterhemp 66% to 78%, 77% to 84%, and 90% to 95%, respectively. All the acetochlor mixtures and industry standards provided similar MHR waterhemp control, with the exception that acetochlor + sulfentrazone, acetochlor + flumioxazin, and pyroxasulfone/flumioxazin provided greater MHR waterhemp control than *S*-metolachlor/metribuzin at 12 WAA. Similarly, Han et al. (2002) reported good control of several annual weeds with acetochlor + flumioxazin; lambsquarters, redroot pigweed (*Amaranthus retroflexus* L.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], and black nightshade (*Solanum nigrum* L.) were controlled 89% with acetochlor + flumioxazin (800 + 50 g ai ha^{-1}) at 2 WAE.

Acetochlor controlled MHR waterhemp 88% at 8 WAA, which was greater than dicamba, metribuzin, diflufenican, and sulfentrazone but similar to flumioxazin (Table 4). Similarly, Jhala et al. (2015) reported 80% waterhemp control 8 wk after planting with acetochlor applied PRE at either 1,680 or 3,370 g ai ha^{-1}; in the current study, 88% control was achieved with a rate of 1,700 g ai ha^{-1}. In another study, acetochlor (1,260 g ai ha^{-1}) provided 84% control of Palmer amaranth (*Amaranthus palmeri* S. Watson), a close relative of waterhemp, in 2- to 3-leaf cotton 3 WAA (Cahoon et al. 2015). Similar to 4 WAA, at 8 WAA, there were no differences in MHR waterhemp control among acetochlor mixtures and industry standards. Hedges et al. (2018a) reported 87%, 91%, and 95% waterhemp control with *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin, respectively, at 8 WAA.

MHR waterhemp control with acetochlor fell slightly to 82% at 12 WAA; control with dicamba, metribuzin, diflufenican, sulfentrazone, and flumioxazin ranged from 37% to 81%. MHR waterhemp control with dicamba, metribuzin, diflufenican, and

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Table 5. Mean percent injury of soybean 2 and 4 wk after crop emergence and soybean yield for five field trials evaluating control of multiple-herbicide-resistant waterhemp with soybean herbicides and acetochlor-based mixtures applied PRE in southwestern Ontario, Canada, in 2021 and 2022.[a,b,c]

Treatment	Rate	Visible injury		Yield
		2 WAE	4 WAE	
	g ai ha^{-1}	%	%	t ha^{-1}
Weed-free control		0 a	0.0 a	2.81 ab
Nontreated control		0 a	0.0 a	1.90 c
Acetochlor	1,700	4 bc	1.0 bc	2.82 ab
Dicamba	600	0 ab	0.0 a–c	2.36 bc
Metribuzin	413	0 ab	0.1 a–c	2.48 ab
Diflufenican	90	1 a–c	0.3 bc	2.59 ab
Sulfentrazone	140	0 ab	0.1 a–c	2.49 ab
Flumioxazin	107	3 bc	1.0 bc	2.95 a
Acetochlor + dicamba	1,700 + 600	4 (4) bc	1.3 (1) c	2.88 ab
Acetochlor + metribuzin	1,700 + 413	5 (4) bc	1.0 (1) bc	2.71 ab
Acetochlor + diflufenican	1,700 + 90	7 (5)** cd	1.5 (1) c	2.74 ab
Acetochlor + sulfentrazone	1,700 + 140	5 (4) bc	0.5 (1) bc	2.83 ab
Acetochlor + flumioxazin	1,700 + 107	19 (7)** d	10.6 (2)** d	2.91 a
S-metolachlor/metribuzin	1,943	1 a–c	0.3 bc	2.54 ab
Pyroxasulfone/sulfentrazone	300	0 ab	0.0 a–c	2.85 ab
Pyroxasulfone/flumioxazin	240	3 bc	0.9 bc	2.74 ab

[a]Abbreviation: WAE, weeks after crop emergence.
[b]Means followed by the same letter within a column are not significantly different according to Tukey–Kramer grouping at $P < 0.05$.
[c]Values in parentheses represent expected values from Colby's equation.
*, $P < 0.05$; **, $P < 0.01$, between observed and expected values based on a two-tailed *t*-test.

sulfentrazone was lower than with acetochlor. Schryver et al. (2017a) reported 76%, 58%, and 77% control of glyphosate-resistant waterhemp with metribuzin (1,120 g ai ha^{-1}), sulfentrazone (210 g ai ha^{-1}), and flumioxazin (108 g ai ha^{-1}), respectively. The results of the current study found 53%, 55%, and 81% control with metribuzin, sulfentrazone, and flumioxazin, respectively, although the rates of metribuzin and sulfentrazone used in the study by Schryver et al. (2017a) were 2.7X and 1.5X greater, which partially explains the higher control in that study. Adding acetochlor to dicamba, metribuzin, diflufenican, sulfentrazone, and flumioxazin increased waterhemp control by 52%, 37%, 51%, 37%, and 16%, respectively, at 12 WAA. Control with the acetochlor-based mixtures was ≥89%; acetochlor + flumioxazin controlled waterhemp 97%, which exceeded control with *S*-metolachlor/metribuzin (66%). All relationships were additive based on the observed and expected values obtained from Colby's equation. The industry standards pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin controlled MHR waterhemp 77% and 91%, respectively, which was similar to the acetochlor mixtures evaluated. Schryver et al. (2017b) reported 75% and 92% control of glyphosate-resistant waterhemp with pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and pyroxasulfone/flumioxazin (240 g ai ha^{-1}) applied PRE, which closely parallels the results of this study.

Multiple-Herbicide-Resistant Waterhemp Density and Biomass

The waterhemp density in the nontreated control was 882 plants m^{-2} at 8 WAA. Acetochlor reduced the density of MHR waterhemp by 98%; Han et al. (2002) reported that acetochlor (1,500 g ai ha^{-1}) reduced the density of grass and broadleaf weeds by 72%, and Hausman et al. (2013) found that acetochlor (1,680 g ai ha^{-1}) reduced waterhemp density by 88% averaged over 2 yr. The application of dicamba or diflufenican alone did not reduce waterhemp density relative to the nontreated control. Meyer et al. (2016) reported that dicamba (560 g ae ha^{-1}) reduced waterhemp density 19%, compared to 50% in the current study. Sulfentrazone and flumioxazin reduced waterhemp density by 94% and 98%, respectively, which was greater than what was found in a study by Schryver et al. (2017a), where sulfentrazone (210 g ha^{-1}) and flumioxazin (108 g ai ha^{-1}) reduced waterhemp density 67% and 77%, respectively. The mixtures of acetochlor + dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin reduced waterhemp density by 98% to 100%. The addition of acetochlor reduced waterhemp density relative to the broadleaf herbicides applied alone; conversely, only the mixture of acetochlor + flumioxazin reduced waterhemp density over acetochlor applied alone. Based on Colby's equation, there was an antagonistic relationship when acetochlor was coapplied with dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin; however, the numeric decrease in density was 99% to 100%. *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin reduced waterhemp density by 95%, 99%, and 100%, respectively. Previous research has found that waterhemp density reductions relative to the nontreated control from the application of *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin at the same rates as evaluated in this study ranged from 84% to 98%, 87% to 99%, and 96% to 99%, respectively (Hedges et al. 2018a, 2018b; Schryver et al. 2017a, 2017b).

All treatments evaluated, except dicamba and diflufenican, reduced waterhemp shoot biomass. Acetochlor reduced shoot biomass by 93%. Shoot biomass reductions were greatest from the mixture of acetochlor + flumioxazin, which reduced biomass by >99%, though there were no differences among acetochlor mixtures. The addition of acetochlor to dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin reduced biomass by an additional 41%, 29%, 51%, 25%, and 10%, respectively, although the reduction was statistically significant only with the mixture of acetochlor + flumioxazin. All interactions were additive except the mixture of acetochlor and sulfentrazone, which was determined to be antagonistic, although shoot biomass was reduced 95% relative to the untreated check. The industry standards *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin reduced shoot biomass of waterhemp relative to the nontreated control by 85%, 87%, and 97%, respectively, which was similar to acetochlor applied alone. This aligns closely with results presented by Hedges et al. (2018a) where the same premixtures reduced waterhemp biomass 93%, 93%, and 96%, respectively.

Soybean Injury and Yield

Acetochlor applied PRE caused 4% soybean injury at 2 WAE (Table 5). Flumioxazin caused 3% soybean injury; dicamba, metribuzin, diflufenican, and sulfentrazone did not cause any soybean injury. The mixtures of acetochlor + dicamba, metribuzin, diflufenican, or sulfentrazone caused similar soybean injury of 4% to 7%; acetochlor + flumioxazin caused 19% soybean injury at 2 WAE, which was similar to acetochlor + diflufenican. Soybean injury in acetochlor mixtures included stunting and cupped and puckered leaves with shortened midribs. Han et al. (2002) did not observe any soybean injury with the mixture of acetochlor + flumioxazin (800 + 50 g ai ha^{-1}) applied PRE; however, the rates used in the current study were more than double. There was a synergistic interaction in terms of soybean injury with the mixtures of acetochlor + diflufenican and acetochlor + flumioxazin. The coapplication of acetochlor + flumioxazin caused 19% soybean injury, which was greater than the expected injury of 7% based on Colby's equation, thus demonstrating a synergistic increase in injury. In contrast to results from this study, Jhala et al. (2015) reported that acetochlor applied alone (1,680 g ai ha^{-1}) and when coapplied with flumioxazin (1,680 + 110 g ai ha^{-1}) caused <10% soybean injury. However, the soils in the current study had a much greater percentage of sand and a significantly lower proportion of clay than the soils in the study by Jhala et al. (2015), which can influence herbicide adsorption to the soil and subsequent uptake by soybean (Weber and Peter 1982). *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin caused 1%, 0%, and 3% injury, respectively.

Generally, soybean injury decreased from 2 to 4 WAE (Table 5). Acetochlor alone caused 1% injury, which was greater than the weed-free and nontreated control but similar to all other treatments except acetochlor + flumioxazin. Acetochlor + flumioxazin was more injurious than any other treatment; there was 11% injury at 4 WAE. Dicamba, metribuzin, diflufenican, sulfentrazone, flumioxazin, acetochlor + metribuzin, and acetochlor + sulfentrazone caused ≤1% soybean injury. The coapplication of acetochlor + flumioxazin at 4 WAE caused a synergistic increase in soybean injury. Soybean injury was ≤1% in *S*-metolachlor/metribuzin-, pyroxasulfone/sulfentrazone-, and pyroxasulfone/flumioxazin-treated plots, slightly lower than the findings of Hedges et al. (2018a) and Mahoney et al. (2014).

MHR waterhemp interference in untreated control plots reduced soybean yield by 32% (Table 5). In previous studies conducted in Ontario, soybean yield losses as a result of waterhemp interference were 34% to 56% (Hedges et al. 2018a, 2018b; Schryver et al. 2017a, 2017b). The yields from all herbicide treatments were similar to the yield of the weed-free control. Reduced waterhemp interference with acetochlor alone increased yield by 0.92 t ha^{-1} compared to the nontreated control. Flumioxazin and acetochlor + flumioxazin resulted in the highest numeric yields, 2.95 and 2.91 t ha^{-1}, respectively, which were greater than dicamba and similar to all other treatments. Jhala et al. (2015) found that there was no difference in soybean yield between acetochlor applied alone (1,680 g ai ha^{-1}) and as a mixture with flumioxazin, consistent with the findings of this study.

In summary, the coapplication of acetochlor with dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin controlled MHR waterhemp ≥89% at 12 WAA, reduced waterhemp density ≥98%, decreased waterhemp shoot biomass ≥95%, and resulted in soybean yield that was similar to the weed-free control. Acetochlor + flumioxazin caused 11% soybean injury at 4 WAE; injury from the other herbicide mixtures was ≤2%. Waterhemp interference caused a 32% soybean yield loss. This study concludes that the coapplication of acetochlor with dicamba, metribuzin, diflufenican, sulfentrazone, or flumioxazin results in excellent season-long control of MHR waterhemp and results in soybean yield similar to the weed-free control. There is potential for soybean injury when acetochlor is coapplied with flumioxazin, but soybean yield was not affected. Acetochlor provides effective control of small-seeded annual grass and some small-seeded annual broadleaf weeds, including waterhemp; the coapplication of acetochlor with a broadleaf herbicide will increase the spectrum of weeds controlled and may provide more consistent control across a range of weed densities, soil types, and weather conditions. Herbicide mixtures are recommended to decrease the pressure applied to single modes of action to delay herbicide resistance. As waterhemp continues to evolve multiple resistances, growers must have diverse management strategies that mitigate the risk of further evolution of herbicide resistance. Other nonchemical methods of weed control, including crop rotation, planting of cover crops, and tillage, should be used in combination with herbicides to ensure the lasting efficacy of these products.

Practical Implications

Waterhemp is an ongoing challenge to control not only in Ontario but globally as well. Its ability to rapidly evolve resistance to multiple herbicide modes of action makes it difficult to control. Integrated pest management strategies are crucial to its control, and chemical methods are one aspect of that approach. The use of single herbicide modes of action is becoming less common given waterhemp's wide resistance to multiple modes of action. Instead, a mixture of multiple modes of action is preferred. Encapsulated acetochlor would be a new herbicide for Ontario growers, although it is and has widely been used in the USA for many years. Acetochlor is a Group 15 herbicide, and to date, there is no reported Group 15 waterhemp resistance in Ontario, making it an attractive option for growers who struggle with this weed. However, to help delay resistance development, this research was carried out to look at acetochlor's efficacy and compatibility with other effective waterhemp herbicides. This research showed that not only is acetochlor an effective herbicide on its own for waterhemp control but it is also efficacious when mixed with other common soybean herbicides. Additionally, this information is very important for chemical manufacturers, retailers, and agronomists alike who will be marketing these mixtures and products to growers. Understanding how much crop injury is possible from these mixtures, in addition to how they perform on waterhemp, is pivotal in making recommendations. Overall, waterhemp is a significant issue facing Ontario agriculture, and this research will provide the industry with new information that will contribute to better weed control and thus improved soybean yields, easier harvesting, and improved quality.

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