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Short Title: Diflufenican-waterhemp control

Biologically effective dose of diflufenican applied preemergence for the control of multiple herbicide-resistant waterhemp in corn

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Abstract

Waterhemp is a dioecious species which contributes to a wide genetic diversity that has enabled it to evolve resistance to several commonly used Herbicide Groups (G) in North America. Five field trials were established in Ontario to ascertain the biologically effective doses (BED) of diflufenican, a new Group 12 herbicide applied preemergence (PRE) for control of multiple herbicide-resistant (MHR) waterhemp in corn. Based on regression analysis, the predicted diflufenican doses to elicit 50, 80, and 95% MHR waterhemp control were 99, 225, and 417 g ai ha^{-1} at 2 WAA; 73, 169, and 314 g ai ha^{-1} at 4 WAA, and 76, 215, and “–“ (the effective dose was beyond the set of doses in this study) g ai ha^{-1} at 8 WAA, respectively. The diflufenican doses that caused a 50, 80, and 95% decrease in MHR waterhemp density were 42, 123, and ”–“ g ai ha^{-1}, and MHR waterhemp biomass were 72, 167, and 310 g ai ha^{-1}, respectively at 8 WAA. Diflufenican PRE at 150 g ai ha^{-1} controlled MHR waterhemp 64, 79, and 73%; isoxaflutole + atrazine PRE at 105 + 1060 g ai ha^{-1} controlled MHR waterhemp 98, 98, and 97%; and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine PRE at 1259/140/35/588 g ai ha^{-1} controlled MHR waterhemp 100, 100, and 99% at 2, 4, and 8 WAA, respectively. Diflufenican PRE reduced MHR waterhemp density and biomass by 83%; in contrast, isoxaflutole + atrazine and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine reduced MHR waterhemp density and biomass by 99%. All treatments evaluated caused either no or minimal corn injury and provided yield comparable to weed-free control. Results indicate that diflufenican applied PRE alone does not provide superior MHR waterhemp control than the commonly used herbicides isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine; however, there is potential for the utilization of diflufenican as a part of an Integrated Weed Management (IWM) strategy for the control of MHR waterhemp control in corn.

Nomenclature: Diflufenican; isoxaflutole + atrazine; *S*-metolachlor/mesotrione/bicyclopyrone/atrazine; Palmer amaranth, *Amaranthus palmeri* S.Watson; waterhemp, *Amaranthus tuberculatus* (Moq.) J.D. Sauer.; corn, *Zea mays* L.

Keywords: Preemergence herbicides, corn injury, corn yield, waterhemp control, waterhemp biomass, waterhemp density

Introduction

Corn production is an important agriculture sector in Canada and contributes substantially to the economy of Canada. Canada is the 11th top corn producer globally, producing nearly 1.5 billion kilograms of grain corn annually (Statista 2024). Nearly 65% of Canadian grain corn is produced in Ontario (OMAFRA Crop Statistics 2023). In 2022, Ontario corn growers seeded approximately 1 million ha and processed approximately 9.4 billion kilograms of grain corn with farm cash receipts of nearly $2 billion (OMAFRA 2024). In 2022, the amount of corn exported to other markets (mainly Ireland, Spain, and other European countries) amounted to nearly 1 billion kilograms, valued at $375 million (Ontario Grain Farmers 2023). The continuous increase in corn consumption globally necessitates improving corn productivity so that supply meets demand. One of the most impeding factors in corn productivity is yield loss due to weed interference, especially recently confirmed multiple herbicide-resistant (MHR) weed biotypes such as waterhemp.

Waterhemp is a dioecious weed with a wide genetic diversity which has enabled it to evolve resistance to several herbicide Groups (2, 4, 5, 9, 14, 15, and 27) (Bell and Tranel 2010; Cordes et al. 2004; Heap 2024). A recent WSSA survey has placed waterhemp among the most problematic weed species in the USA (Van Wychen 2016). Waterhemp biotypes in Ontario have evolved resistance to Groups 2, 5, 9, 14, and/or 27 (Benoit et al. 2019a; Heap 2024; Symington et al. 2022). MHR waterhemp has been found in 17 Ontario counties spanning over 800 km across the southern portion of the province (Soltani et al. 2022). A recent metadata analysis has estimated that MHR waterhemp exists in one percent of field crop hectares in Ontario. If left uncontrolled can cause an average of 19% reduction in corn yield with a farm cash receipts value of $3.1 million annually (Soltani et al. 2022). Steckel and Sprague (2004) observed as much as 74% yield loss in corn from waterhemp interference. There has been no new herbicide mode of action commercialized in Canada for use in corn in more than two decades. Corn producers need new herbicide modes of action to control yield-robbing weed species such as MHR waterhemp in their cropping systems.

Diflufenican (C19H11F5N2O2) is a WSSA Group 12 selective contact and residual herbicide from the phenyl ether chemical family. In Europe, diflufenican has been commercialized for weed management in cereals and lentils for several years (Effertz 202). In Canada and the USA, diflufenican was just registered (Feb. 2024) by the PMRA (Pest Management Regulatory Agency) and is pending approval from the EPA (Environmental Protection Agency) for utilization in soybean and corn (Effertz 2021). Diflufenican, combined with other herbicides, can contribute to controlling two important weed species in North America: MHR waterhemp and MHR Palmer amaranth (Effertz 2021). No other herbicide from WSSA Group 12 has ever been marketed for weed management in corn and soybean in North America (Effertz 2021). Diflufenican can be applied preemergence (PRE) to control MHR waterhemp (Effertz 2021; Haynes and Kirkwood 1992; Tejada 2009). It is primarily absorbed by the shoots of seedlings and has limited translocation within plants (Ashton et al. 1994; Conte et al. 1998; Haynes and Kirkwood 1992). Diflufenican disrupts the biosynthesis of carotenoids, a crucial pigment for photosynthesis, and the protection of plants from harmful high-energy light (Miras-Moreno et al. 2019). In the absence of carotenoids, susceptible plants cannot shield their cells from harmful high-light energy, leading to growth cessation and total necrosis of plants within days (Haynes and Kirkwood 1992). Diflufenican has low water solubility and low volatility, low toxicity to honeybees and mammals if ingested, does not persist in the soil, and has a relatively favorable environmental profile (Ashton et al. 1994; Bending et al. 2006).

Waterhemp has not evolved resistance to herbicides from Group 12; therefore, diflufenican offers a new mode of action for the control of MHR waterhemp in corn and can be an ideal herbicide partner with other available herbicides to diversify modes of action and minimize selection pressure for the evolution of herbicide-resistant weed biotypes. The biologically effective dose (BED) of diflufenican for MHR waterhemp control in corn has not been assessed under Ontario environmental conditions. Additionally, there has been little research to compare the efficacy of diflufenican compared to herbicides currently utilized in corn for the control of MHR waterhemp including isoxaflutole + atrazine and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine.

This research was conducted to determine the BED of diflufenican for PRE control of MHR waterhemp in corn and to compare the control of MHR waterhemp with diflufenican to isoxaflutole + atrazine, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine.

Materials and Methods

Five field trials were carried out in 2017 and 2018 in growers' fields with naturally occurring MHR waterhemp in southwestern Ontario, Canada. In 2017, two trials were conducted on Walpole Island, ON, and one near Cottam, ON, and in 2018, one trial was conducted on Walpole Island, ON, and one near Cottam, ON.

Field trials were set up as a randomized complete block design with 4 replicates. Experiment treatments included a weed-free control, diflufenican applied PRE at 60, 90, 120, 150, 180, and 210 g ai ha^{-1}, isoxaflutole + atrazine applied PRE at 105 + 1060 g ai ha^{-1}, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE at 1259/140/35/588 g ai ha^{-1}. Plots were 8 m long and 3.0 m wide and consisted of four rows (0.75 m apart) of glyphosate/glufosinate-resistant corn (DKC45-65RIB®/DKC42-60RIB®) seeded at the rate of ~80,000 seeds ha^{-1}.

Treatments were applied PRE with a CO_2-pressurized backpack sprayer adjusted to deliver 200 L ha^{-1} at 240 kPa. The spray boom was 1.5 m long and had four nozzles (ULD120-02, Hypro, Pentair, New Brighton, Minnesota, USA) spaced 50 cm apart, producing a spray width of 2.0 m.

Corn injury evaluations were completed at 1, 2, 4, and 8 weeks after emergence (WAE), and MHR waterhemp control evaluations were completed at 2, 4, and 8 weeks after application (WAA) on a scale of 0 (no corn injury/waterhemp control) to 100% (corn/waterhemp death). The MHR waterhemp density and aboveground biomass were determined at 8 WAA by clipping all waterhemp plants within two 0.25 m^2 randomly placed quadrats in each plot. Aboveground dry biomass was then determined by oven-drying clipped waterhemp plants at 65 C to constant moisture. At corn maturity, the 2 middle rows of each plot were harvested with a small-plot research combine; corn grain moisture content and mass were recorded. Corn yield was adjusted to 15.5% moisture.

Non-Linear Regression Analysis

Waterhemp control, density, and biomass data were regressed against the dose of diflufenican using the NLIN procedure in SAS (SAS 2013). An exponential to maximum model (Equation 1) was used to model waterhemp control at 2, 4, and 8 WAA against the dose of diflufenican from 0 to 210 g ha^{-1}. Similarly, waterhemp density and biomass were regressed against diflufenican dose using an inverse exponential model (Equation 2).

Exponential to Maximum:

$$y = a - be^{(-c*dose)} \quad [1]$$

where y = response parameter, a = upper asymptote, b = magnitude, and c = slope.

Inverse Exponential:

$$y = a + be^{(-c*dose)} \quad [2]$$

where y = response parameter, a = lower asymptote, b= change in Y from intercept to a, and c = slope.

Parameters generated form each regression analysis were used to calculate the expected dose (ED$_n$) of diflufenican for 50, 80, and 95% waterhemp control, and a 50, 80, and 95% reduction in waterhemp plant density and biomass. Diflufenican dose was reported as "–" where it could not be calculated by the model.

Model Goodness of Fit

Model efficiency (ME; Equation 3) and root mean square error (RMSE; Equation 4) were calculated to determine goodness of fit for each regression model as suggested by Soltani et al. (2020).

$$ME = 1 - \left[\frac{\sum_{i=1}^{n}(Oi-Pi)^2}{\sum_{i=1}^{n}(Oi-i)^2}\right] \quad [3]$$

$$RMSE = \sqrt{\frac{RSS}{(n-p-1)}} \quad [4]$$

where Oi is the observed, Pi is the predicted, Oi is the mean observed value, RSS is the residual sum of squares, n is the number of data points, and p is the number of parameters. ME ranges from $-\infty$ to 1; values closer to 1 signify better goodness of fit.

Least-Square Means Comparisons

Data were analyzed in SAS (SAS 2013) using the GLIMMIX procedure. Variances were partitioned into the fixed effect of herbicide treatment and the random effects of environment (location-year combinations), block nested within environment, and the environment-by-treatment interaction. Waterhemp control at 2, 4, and 8 WAA were arcsine square-root transformed prior to analysis using a normal distribution with identity link; non-transformed means were presented based on the interpretation of transformed data. Waterhemp density and biomass were analyzed using the lognormal distribution with identity link. The Pearson chi-square/degrees of freedom ratio and Shapiro-wilk statistic were used to determine model fitness for each parameter and eliminate potential overdispersion. Studentized residual plots and normal probability plots were used to confirm the homogeneity of variance and the assumptions of normality, respectively. Means were separated using Tukey's least significant difference at an alpha level of 0.05. Data analyzed using a lognormal distribution were back-transformed using the omega method.

Results and Discussion

Corn injury was minimal and environment-specific; therefore, regression equations were not generated for injury data (Tables 1 and 2).

Biologically Effective Doses of Diflufenican PRE for MHR Waterhemp Control

The predicted diflufenican doses to elicit 50, 80, and 95% control of MHR waterhemp were 99, 225, and 417 g ai ha^{-1} at 2 WAA; 73, 169, and 314 g ai ha^{-1} at 4 WAA; and 76, 215, and "–" (effective dose was beyond the set of doses in this study) g ai ha^{-1} at 8 WAA, respectively (Table 1). The predicted diflufenican doses that caused a 50, 80, and 95% decrease in MHR waterhemp density were 42, 123, and "–" g ai ha$^{-1,}$ and the doses that caused a 50, 80, and 95% decrease in MHR waterhemp biomass were 72, 167, and 310 g ai ha^{-1}, respectively (Table 1). No other studies have been published on the BED of diflufenican for managing MHR waterhemp in corn. Studies conducted by Sarangi and Jhala (2017) determined that the calculated doses of *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE to elicit 50 and 90% control of glyphosate-resistant (GR) waterhemp in corn were 94 and 586 g ha^{-1} at 2 WAA; 149 and 1173 g ha^{-1} at 5 WAA, and 251 and 2796 g ha^{-1} at 9 WAA, respectively. The same study determined that the calculated doses of *S*-metolachlor/mesotrione/bicyclopyrone/atrazine PRE to elicit 50 and 90% reduction in density of GR waterhemp were 274 and 2824 g ai ha^{-1} and GR waterhemp biomass were 229 and 2389 g ai ha^{-1}, respectively at 9 WAA.

Control of MHR Waterhemp with Diflufenican compared to Isoxaflutole + Atrazine and S-metolachlor/Mesotrione/Bicyclopyrone/Atrazine

Diflufenican (150 g ai ha^{-1}), isoxaflutole + atrazine (105 + 1060 g ai ha^{-1}), and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/588 g ai ha^{-1}) applied PRE controlled MHR waterhemp 64, 98, and 100% at 2 WAA; 79, 98, and 100% at 4 WAA, and 73, 97, and 99% at 8 WAA, respectively (Table 2). There has been little research published on the efficacy of diflufenican for the control of MHR waterhemp in corn. Studies conducted by Benoit et al. (2019b) with single active ingredient herbicides, *S*-metolachlor, dimethenamid-P, pyroxasulfone, pethoxamid, atrazine, and dicamba showed only 73-83, 71-79, 74-81, 44-55, 65-73, and 42-54% MHR waterhemp control. However, Willemse et al. (2021) observed that multiple active ingredient herbicide mixtures such as isoxaflutole + atrazine PRE controlled MHR waterhemp 70-97, 77-97, and 78-97% at 4, 8, and 12 WAA, respectively. In the same study, *S*-metolachlor/mesotrione/bicyclopyrone/atrazine controlled MHR waterhemp 93-99% at various timings (Willemse et al. 2021). Sarangi and Jhala (2017) observed >95% MHR waterhemp control with *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn. Additionally, Legleiter and Bradley (2009) observed 98% GR waterhemp control with atrazine + mesotrione + *S*-metolachlor applied PRE at 12 WAA in corn.

Diflufenican (150 g ai ha^{-1}), isoxaflutole + atrazine (105 + 1060 g ai ha^{-1}), and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/588 g ai ha^{-1}) applied PRE reduced MHR waterhemp density 83, 99, and 99%, respectively (Table 2). In other studies, Willemse et al. (2021) observed 94 and 99% reductions in density of MHR waterhemp with isoxaflutole + atrazine and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn, respectively which is comparable to this study. Similarly, Benoit et al. (2019b) documented 94 and 98% reductions in density of MHR waterhemp with isoxaflutole + atrazine and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn, respectively. Vyn et al. (2006) reported that the density of a triazine-resistant waterhemp population was reduced 97% at 10 WAA with isoxaflutole + atrazine applied PRE in corn.

Diflufenican (150 g ai ha^{-1}), isoxaflutole + atrazine (105 + 1060 g ai ha^{-1}), and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/588 g ai ha^{-1}) applied PRE reduced MHR waterhemp biomass 83, 99, and 99%, respectively (Table 2). Similarly, in other studies, Willemse et al. (2021) and Benoit et al. (2019b) observed up to 98% reductions in aboveground dry biomass of MHR waterhemp with isoxaflutole + atrazine and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn.

Diflufenican applied PRE at 150 g ha^{-1}, isoxaflutole + atrazine applied PRE at 105 + 1060 g ai ha^{-1}, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE at 1259/140/35/588 g ai ha^{-1} caused no crop injury in corn at 2, 4, and 8 WAA (data not shown). Additionally, all herbicide treatments evaluated provided comparable corn yield (Table 2). These results are similar to those found by Willemse et al. (2021) which documented no or minimal corn injury with isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn. Benoit et al. (2019b) also found transient visible corn injury with isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE in corn. Similarly, Brown et al., (2016) found no corn injury with isoxaflutole + atrazine applied preplant (PP). Jha (2021), Lawson (2017), and Richburg et al. (2019) also observed no visible corn injury or yield loss in corn with *S*-metolachlor/atrazine/mesotrione/bicyclopyrone applied PP.

In conclusion, diflufenican PRE at 150 g ai ha^{-1} provided lower MHR waterhemp control than the currently used multiple active ingredient herbicide mixtures, isoxaflutole + atrazine (105 + 1060 g ai ha^{-1}) and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/588 g ai ha^{-1}). There is potential for the utilization of diflufenican with its unique site of action as a part of an Integrated Weed Management (IWM) strategy for controlling MHR waterhemp in corn. Future studies are needed to evaluate PRE applications of diflufenican combined with other effective herbicides for control of MHR waterhemp and other weed species in corn.

Practical Implications

MHR Waterhemp biotypes are present in 17 counties over a distance of 800 km causing an average of 19% corn yield loss in Ontario, Canada. Herbicides with new modes of action are crucial in managing MHR waterhemp in corn. Diflufenican is a new Group 12 herbicide from the phenyl ether chemical family that has been just registered in Canada for MHR waterhemp in corn and soybean. Based on regression analysis, the doses of diflufenican to elicit 95% MHR waterhemp control in corn were 417, 314, and “–“ g ai ha^{-1} at 2, 4, and 8 WAA, respectively. Additionally, the doses of diflufenican to elicit 50, 80, and 95% decrease in MHR waterhemp density were 42, 123, and “–“ g ai ha^{-1} and MHR waterhemp biomass was 72, 167, and 310 g ai ha^{-1}. Diflufenican PRE caused no crop injury or yield reduction in corn. Based on these results, diflufenican applied PRE alone does not provide superior MHR waterhemp control than the commonly used corn herbicides isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine. However, diflufenican offers a new mode of action for the control of MHR waterhemp in corn and can be a complementary herbicide partner with other available herbicides to diversify modes of action and minimize the intensity of natural selection for the evolution of additional HR weed biotypes.

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Competing Interests

The authors declare none.

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Table 1. Regression parameters and the predicted doses of diflufenican for 50, 80, and 95 multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) control at 2, 4, and 8 weeks after application (WAA), and a 50, 80, and 95% reduction in density and biomass at 8 WAA from five field trials conducted in southwestern Ontario, Canada in 2017 and 2018.

	Regression parameters (±SE)			Predicted diflufenican dose		
Variable	*a*	*b*	*c*	ED_{50}	ED_{80}	ED_{95}
Control				------------------------ g ai ha^{-1} ------------------------		
2 WAA[a]	100 (0)	102 (3.34)	0.01 (0)	99	225	417
4 WAA[a]	100 (0)	100 (3.46)	0.01 (0)	73	169	314
8 WAA[a]	88.6 (9.08)	87.69 (9.15	0.01 (0)	76	215	-
Density[b]	58 (63.43)	401.6 (90.48)	0.02 (0.01)	42	123	-
Biomass[b]	0 (0)	175.1 (24.59)	0.01 (0)	72	167	310

[a] Regression parameters: $y = a - b(e^{-c*dose})$; Where *a* is the upper asymptote, *b* is the magnitude, and *c* is the slope.

[b] Regression parameters: $y = a + b(e^{-c*dose})$; Where *a* is the lower asymptote, *b* is the change in *y* from the intercept to *a*, and *c* is the slope.

[c] Abbreviations: ED_n, effective dose to elicit response level n; WAA, weeks after application. –, the effective dose could not be estimated by the model or was beyond the set of doses in this study.

Table 2. Multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) control 2, 4, and 8 weeks after application (WAA), density and biomass at 8 WAA, and corn yield provided by diflufenican and industry-standard herbicides applied PRE from five field trials across Ontario, Canada in 2017 and 2018.

		Visible control											
Herbicide treatment	Rate	2 WAA		4 WAA		8 WAA		Density		Biomass		Yield	
	g ai ha^{-1}	---------------- % ----------------						Plants m^{-2}		g m^{-2}		kg ha^{-1}	
Non-treated control	-	0	c	0	c	0	c	372	c	168	c	9,660	a
Diflufenican	150	64	b	79	b	73	b	63	a	28	a	9,910	a
Isoxaflutole + atrazine	105 + 1060	98	a	98	a	97	a	4	a	1	a	10,100	a
S-metolachlor/mesotrione/bicyclopyrone/atrazine	1259/140/35/588	100	a	100	a	99	a	3	a	1	a	10,400	a

[a] Abbreviations: WAA; weeks after application.

[b] Means followed by the same letter within a column are not significantly different according to Tukey's LSD (P > 0.05).