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

Nader Soltani[1] , Christian Willemse[2] and Peter H. Sikkema[1]

[1]Adjunct Professor, University of Guelph, Ridgetown, ON, Canada and [2]Former Graduate Student, University of Guelph, Ridgetown, ON, Canada

Abstract

Waterhemp is a dioecious species with wide genetic diversity which has enabled it to evolve resistance to several commonly used herbicide groups in North America. Five field trials were established in Ontario to ascertain the biologically effective doses of diflufenican, a new Group 12 herbicide applied preemergence 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}, respectively, at 2 wk after application (WAA); 73, 169, and 314 g ai ha^{-1}, respectively, at 4 WAA; and 76, 215, and — (meaning the effective dose was beyond the set of doses in this study) g ai ha^{-1}, respectively, at 8 WAA. The predicted diflufenican doses that would cause a 50%, 80%, and 95% decreases 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 applied preemergence at 150 g ai ha^{-1} controlled MHR waterhemp by 64%, 79%, and 73% at 2, 4, and 8 WAA, respectively. Isoxaflutole + atrazine applied preemergence at 105 + 1,060 g ai ha^{-1} controlled MHR waterhemp by 98%, 98%, and 97% at 2, 4, and 8 WAA, respectively; and *S*-metolachlor/mesotrione/bicyclopyrone/ atrazine applied preemergence at 1,259/140/35/588 g ai ha^{-1} controlled MHR waterhemp by 100%, 100%, and 99% at 2, 4, and 8 WAA, respectively. Diflufenican applied preemergence 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 resulted in corn yield that was similar with the weed-free control. Results indicate that diflufenican applied alone preemergence does not provide superior MHR waterhemp control over the commonly used herbicides isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/ atrazine; however, there is potential for using diflufenican as part of an integrated weed management strategy for the control of MHR waterhemp control in corn.

Introduction

Corn is an important agricultural product in Canada and contributes substantially to the nation's economy. Canada ranks 11th in global corn production, with nearly 1.5 billion kg of grain corn produced annually (Statista 2024). Nearly 65% of Canadian grain corn is produced in Ontario (OMAFRA 2023). In 2022, Ontario corn growers seeded approximately 1 million ha and produced approximately 9.4 billion kg of grain corn with farm cash receipts of nearly Can$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 kg, valued at Can $375 million (McCulloch 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 wide genetic diversity that has enabled it to evolve resistance to several herbicide groups (groups 2, 4, 5, 9, 14, 15, and 27 as categorized by the Weed Science Society of America [WSSA]) (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 United States (Van Wychen 2016). Waterhemp biotypes in Ontario have evolved resistance to herbicides in WSSA 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 more than 800 km across the southern portion of the province (Soltani et al. 2022). A recent metadata analysis has estimated that MHR waterhemp exists in 1% of field crop hectares in Ontario. If left uncontrolled MHR waterhemp caused an average of 19% reduction in corn yield with a farm cash receipts value of Can$3.1 million annually (Soltani et al. 2022). Steckel and Sprague (2004) observed as much as 74% corn yield loss from waterhemp interference. No new herbicide mode

of action has been commercialized in Canada for use on corn in more than two decades. Corn producers need new herbicide modes of action to control yield-robbing weed species such as MHR waterhemp.

Diflufenican ($C_{19}H_{11}F_5N_2O_2$) 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 2021). Diflufenican was just registered for use in February 2024 by Canada's Pest Management Regulatory Agency and is pending approval from the U.S. Environmental Protection Agency for use on soybean and corn (Effertz 2021). Diflufenican, combined with other herbicides, can contribute to the control of two important weed species in North America: MHR waterhemp and MHR Palmer amaranth (Effertz 2021). No other herbicide from WSSA Group 12 has been marketed for weed management in corn and soybean in North America (Effertz 2021). Diflufenican can be applied preemergence 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 additional herbicide-resistant weed biotypes. The biologically effective dose 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 used on corn for the control of MHR waterhemp, including isoxaflutole + atrazine and S-metolachlor/mesotrione/bicyclopyrone/atrazine.

This research was conducted to determine the biologically effective dose of diflufenican applied preemergence for 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 four replicates. Experiment treatments included a weedy control, weed-free control, diflufenican applied preemergence at 60, 90, 120, 150, 180, and 210 g ai ha^{-1}; isoxaflutole + atrazine applied preemergence at 105 + 1,060 g ai ha^{-1}, and S-metolachlor/

mesotrione/bicyclopyrone/atrazine applied preemergence at 1,259/140/35/588 g ai ha^{-1}. Plots were 8 m long and 3 m wide and consisted of four rows (0.75 m apart) of glyphosate/glufosinate-resistant corn (DKC45-65RIB®/DKC42-60RIB®; Bayer Cropscience, Mississauga, ON) seeded at a rate of approximately 80,000 seeds ha^{-1}.

Treatments were applied preemergence 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; Pentair Hypro, New Brighton, MN) spaced 50 cm apart, producing a spray width of 2 m.

Corn injury evaluations were completed at 1, 2, 4, and 8 wk after emergence, and MHR waterhemp control evaluations were completed at 2, 4, and 8 wk after application (WAA) on a scale of 0% (no corn injury/waterhemp control) to 100% (corn/ waterhemp death). The MHR waterhemp density and above-ground 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 harvest maturity, the two 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 with SAS software (SAS Institute Inc., Cary, NC). 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:

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

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

Inverse exponential:

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

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

Parameters generated from 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 — when 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):

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

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

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. Model efficiency ranges from negative infinity ($-\infty$) to 1; values closer to 1 signify better goodness of fit.

Least-Square Means Comparisons

Data were analyzed with SAS software 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 log-normal 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 log-normal 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.

Biologically Effective Doses of Diflufenican Applied Preemergence 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 — (the 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 biologically effective dose of diflufenican for MHR waterhemp control in corn. Studies conducted by Sarangi and Jhala (2017) determined that the calculated doses of *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence to elicit 50% and 90% control of glyphosate-resistant waterhemp in corn were 94 and 586 g ha^{-1} at 2 WAA; 149 and 1,173 g ha^{-1} at 5 WAA, and 251 and 2,796 g ha^{-1} at 9 WAA, respectively. The same study determined that the calculated doses of *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence to elicit 50% and 90% reduction in density of glyphosate-resistant waterhemp were 274 and 2,824 g ai ha^{-1} and glyphosate-resistant waterhemp biomass were 229 and 2,389 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 + 1,060 g ai ha^{-1}), and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine (1,259/140/35/588 g ai ha^{-1}) applied preemergence controlled MHR waterhemp by 64%, 98%, and 100%, respectively, at 2 WAA; by 79%, 98%, and 100%, respectively, at 4 WAA; and by 73%, 97%, and 99%, respectively, at 8 WAA (Table 2). Studies on the efficacy of diflufenican for the control of MHR waterhemp in corn are scant. Studies conducted by Benoit et al. (2019b) with single-active-ingredient herbicides demonstrated only 73% to 83% control of MHR waterhemp with *S*-metolachlor, 71% to 79% control with dimethenamid-P, 74% to 81% control with pyrox-asulfone, 44% to 55% control with pethoxamid, 65% to 73% control with atrazine, and 42% to 54% control with dicamba. However, Willemse et al. (2021) observed that multiple-active-ingredient herbicide mixtures such as isoxaflutole + atrazine applied preemergence controlled MHR waterhemp by 70% to 97%, 77% to 97%, and 78% to 97% at 4, 8, and 12 WAA, respectively. In the same study, *S*-metolachlor/mesotrione/bicyclopyrone/atrazine controlled MHR waterhemp by 93% to 99% at various evaluation timings (Willemse et al. 2021). Sarangi and Jhala (2017) observed >95% MHR waterhemp control with *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence to corn. Additionally, Legleiter and Bradley (2009) observed 98% control of glyphosate-resistant waterhemp with atrazine + mesotrione + *S*-metolachlor applied to corn 12 wk after emergence.

Diflufenican, isoxaflutole + atrazine, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence at the rates mentioned above reduced MHR waterhemp density by 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, respectively, applied preemergence to corn, which is comparable to the findings in 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, respectively, applied preemergence to corn. Vyn et al. (2006) reported that the density of a triazine-resistant waterhemp population was reduced by 97% at 10 WAA with isoxaflutole + atrazine applied preemergence to corn.

Diflufenican, isoxaflutole + atrazine, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence at the rates mentioned above reduced MHR waterhemp biomass by 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 preemergence to corn.

Diflufenican, isoxaflutole + atrazine, and *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence caused no crop injury to corn at 2, 4, and 8 WAA (data not shown). Additionally, all herbicide treatments evaluated resulted in similar corn yield (Table 2). These results are similar to those reported by Willemse et al. (2021) who documented no or minimal corn injury with isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence to corn. Benoit et al. (2019b) also found transient visible corn injury with isoxaflutole + atrazine or *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied preemergence to corn. Similarly, Brown

Table 1. Regression parameters and the predicted doses of diflufenican for 50%, 80%, and 95% control of multiple herbicide–resistant waterhemp at 2, 4, and 8 wk after application; and reductions of 50%, 80%, and 95% in density and biomass at 8 wk after application from five field trials in 2017 and 2018.[a]

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

[a]Abbreviations: ED_n, effective dose to elicit response level n; WAA, weeks after application. –,.
[b]Regression parameter values in parentheses indicate ±SE.
[c]Regression parameters: $y = a - b(e^{-c \cdot dose})$, where a is the upper asymptote, b is the magnitude, and c is the slope.
[d]Regression parameters: $y = a + b(e^{-c \cdot dose})$, where a is the lower asymptote, b is the change in y from the intercept to a, and c is the slope.
[e]A dash (—) indicates 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 control 2, 4, and 8 wk after application; density and biomass at 8 wk after application; and corn yield provided by diflufenican and industry-standard herbicides applied preemergence from five field trials in 2017 and 2018.[a,b]

Herbicide treatment	Rate	Visible control			Density	Biomass	Yield
		2 WAA	4 WAA	8 WAA			
	g ai ha^{-1}		%		Plants m^{-2}	g m^{-2}	kg ha^{-1}
Nontreated 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 + 1,060	98 a	98 a	97 a	4 a	1 a	10,100 a
S-metolachlor/mesotrione/ bicyclopyrone/atrazine	1,259/140/35/588	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 test (P > 0.05).

et al., (2016) found no corn injury with isoxaflutole + atrazine applied preplant to corn. Jha (2021), Lawson (2017), and Richburg et al. (2019) also observed no visible corn injury or yield loss of corn with S-metolachlor/atrazine/mesotrione/bicyclopyrone applied preplant.

In conclusion, diflufenican applied preemergence at 150 g ai ha^{-1} provided lower MHR waterhemp control than the currently used multiple active ingredient herbicide mixtures of isoxaflutole + atrazine (105 + 1060 g ai ha^{-1}) and S-metolachlor/mesotrione/ bicyclopyrone/atrazine (1259/140/35/588 g ai ha^{-1}). Diflufenican, with its unique site of action, has potential use as part of an integrated weed management strategy for the control of MHR waterhemp in corn. Future studies are needed to evaluate preemergence 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. Herbicides with new modes of action are needed for managing MHR waterhemp in corn. Diflufenican is a new group 12 herbicide from the phenyl ether chemical family that has just been registered in Canada for control of MHR waterhemp in corn and soybean. Based on regression analysis, the predicted 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 predicted doses of diflufenican to elicit 50%, 80%, and 95% decreases 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}. Diflufenican applied preemergence caused no corn injury

or yield reduction to corn. Based on these results, diflufenican applied preemergence 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 selection intensity for the evolution of additional herbicide-resistant weed biotypes.

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