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# Biologically Effective Dose of Diflufenican for the Control of Multiple Herbicide-Resistant Waterhemp in Soybean

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

[1] Department of Plant Agriculture, University of Guelph Ridgetown Campus, Ridgetown, ON, Canada

Correspondence: Nader Soltani, University of Guelph Ridgetown Campus, 120 Main St. East, Ridgetown, ON, N0P 2C0, Canada. E-mail: soltanin@uoguelph.ca

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## Abstract

Waterhemp biotypes have evolved resistance to Weed Science Society of America (WSSA) Herbicide Groups 2, 5, 9, 14, and 27 in Ontario Canada, are present in 15 counties, spanning a distance of 800 km across southern Ontario, and cause an average soybean yield loss of 42%. Five field experiments were established in growers' fields in southwestern Ontario to determine the biologically effective doses of diflufenican (Group 12) applied preemergence (PRE) to control multiple herbicide-resistant waterhemp in soybean. The calculated diflufenican doses to elicit 50, 80, and 95% control of MHR waterhemp were 71, 164, and 304 g ai ha$^{-1}$ at 2 weeks after herbicide application (WAA); 50, 115, and 214 g ai ha$^{-1}$ at 4 WAA; and 69, 158, and 294 g ai ha$^{-1}$ at 8 WAA, respectively. The calculated diflufenican doses that caused a 50, 80, and 95% reduction in MHR waterhemp density were 28, 70, and Non-est. g ai ha$^{-1}$ and the doses that caused a 50, 80, and 95% reduction in MHR waterhemp biomass were 44, 109, and Non-est. g ai ha$^{-1}$, respectively. The calculated diflufenican doses that resulted in 50, 80, and 95% of the soybean yield of the industry standard herbicide (flumioxazin/pyroxasulfone) were 3, 12, and 57 g ai ha$^{-1}$, respectively. Diflufenican (180 g ai ha$^{-1}$) PRE controlled MHR waterhemp 89, 92, and 85%; metribuzin (300 g ai ha$^{-1}$) PRE controlled MHR waterhemp 94, 84, and 69%; and flumioxazin/pyroxasulfone (105/134 g ai ha$^{-1}$) PRE controlled MHR waterhemp 100, 99, and 98% at 2, 4, and 8 WAA, respectively. Diflufenican, metribuzin, and flumioxazin/pyroxasulfone applied PRE reduced MHR waterhemp density 96, 84, and 100% and biomass 93, 67, and 99%, respectively at 8 WAA. Diflufenican, metribuzin, and flumioxazin/pyroxasulfone applied PRE caused 9, 0, and 4% visible soybean injury, respectively but the injury was transient and caused no adverse effect on seed moisture content or seed yield of soybean. This study concludes that diflufenican and metribuzin applied PRE provide comparable MHR waterhemp control; however, control was lower than flumioxazin/pyroxasulfone.

**Keywords:** diflufenican, flumioxazin/pyroxasulfone, metribuzin, waterhemp, *Amaranthus tuberculatus* (Moq.) J.D. Sauer, soybean, *Glycine max* (L.) Merr.

## 1. Introduction

Soybean is the third most widely grown field crop and contributes nearly $3 billion annually to the economy of Canada (Soy Canada, 2023). Ontario producers grow most of the soybean produced in Canada. In Ontario, soybean was seeded on approximately 1.2 million hectares; total production was nearly 4 million tonnes with a farmgate value of around $2 billion in 2020 (OMAFRA, 2024). Controlling weeds, especially the recently confirmed glyphosate-resistant (GR) and multiple herbicide-resistant (MHR) waterhemp is critical for profitable soybean production.

Waterhemp is a dioecious species with a wide genetic diversity which has enabled it to evolve resistance to Weed Science Society of America (WSSA) Herbicide Groups 2, 4, 5, 9, 14, 15, and 27 (Bell & Tranel, 2010; Heap, 2024). Waterhemp biotypes in Ontario have evolved resistance to Groups 2, 5, 9, 14, and/or 27 (Benoit et al., 2019; Heap, 2024; Symington et al., 2022). MHR waterhemp has been confirmed in 15 Ontario counties and spans a distance of over 800 km (Soltani et al., 2016, 2022). A recent study has estimated that uncontrolled MHR waterhemp is present in 1% of field crop hectares in Ontario and causes an average 42% yield soybean loss resulting in a monetary loss of $7.1 million in the province annually (Soltani et al., 2022). Crop producers in Ontario need new herbicide modes of action that have an adequate margin of crop safety in soybean and provide control of MHR waterhemp.

Diflufenican is a new herbicide from Group 12 (phenyl ether herbicide family) that is currently under review for weed management in corn and soybean in North America (Bayer, 2021). Diflufenican has been sold for weed management in cereals and lentils in Europe for many years (Bayer, 2021). A Group 12 herbicide has never been sold in Ontario or other regions of North America (Bayer, 2021). Diflufenican can be applied preemergence (PRE) to control troublesome broadleaf weeds including MHR waterhemp (Bayer, 2021; Haynes & Kirkwood, 1992; Tejada, 2009). Diflufenican absorption is primarily through the shoots of seedlings and has limited translocation within plants (Ashton et al., 1994; Haynes & Kirkwood, 1992). Diflufenican inhibits the enzyme phytoene desaturase which is required for carotenoid synthesis within plants (Miras-Moreno et al., 2019). Sensitive plants cease growth and die within days (Haynes & Kirkwood, 1992). Diflufenican has a favorable toxicological and ecological profile and degrades rapidly in tolerant crops and soil (Ashton et al., 1994; Bending et al., 2006; Conte et al., 1998).

There is limited published research on the biologically effective doses (BED) of diflufenican for MHR waterhemp control in soybean under Ontario environmental conditions. Furthermore, diflufenican has not been evaluated for soybean tolerance and weed control efficacy compared to other available, commonly utilized herbicides for the control of problematic weeds including MHR waterhemp in soybean production in Ontario. If soybean tolerance is acceptable and MHR waterhemp control is adequate, diflufenican would provide Ontario producers with a new herbicide mode of action for control of this problematic weed.

The objectives of this research were to determine the biologically effective dose of diflufenican applied PRE for the control of MHR waterhemp in soybean and evaluate soybean tolerance and MHR waterhemp control with diflufenican, metribuzin, and flumioxazin/pyroxasulfone.

## 2. Materials and Methods

### *2.1 Experimental Methods*

A total of 5 field experiments were conducted over two years (2017, 2018) in growers' fields in southwestern Ontario, Canada. In 2017, two field experiments were conducted on Walpole Island, ON (49.7898979°N, -85.9662831°W) and (45.573299°N, -76.772118°W) and one near Cottam, ON (42.149046°N, -82.683986°W). In 2018, one experiment was conducted on Walpole Island, ON (49.7898979°N, -85.9662831°W) and the second was near Cottam, ON (42.149046°N, -82.683986°W). The experimental sites had naturally occurring waterhemp that was resistant to Groups 2, 5, 9, 14, and 27 herbicides.

Field experiments were set up as a randomized complete block design (RCBD) with four replications. Experiment treatments consisted of a weedy control, weed-free control, diflufenican applied at 60, 90, 120, 150, 180, and 210 g ai ha$^{-1}$, metribuzin at 300 g ai ha$^{-1}$, and pyroxasulfone/flumioxazin premixed at 105/134 g ai ha$^{-1}$. Each plot was 8 m long and 3.0 m wide and consisted of 4 rows of glyphosate/dicamba-resistant (Roundup Ready Xtend®) soybean seeded in rows spaced 0.75 m apart at approximately 400,000 seeds ha$^{-1}$.

Herbicide treatments were applied PRE (after planting and before soybean emergence) with a $CO_2$-pressurized backpack sprayer calibrated 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, 375 5th Avenue NW, New Brighton, Minnesota, USA, 55112) spaced 50 cm apart that produced a spray width of 2 m.

Soybean injury was visually evaluated at 3 weeks after herbicide application (WAA) and MHR waterhemp control was evaluated at 2, 4, and 8 WAA on a scale of 0 to 100% (0 = no injury/control and 100 = soybean/waterhemp death). Waterhemp density and aboveground biomass were determined at 8 WAA by counting and cutting the waterhemp plants at the soil surface within two 0.25 m$^2$ randomly placed quadrats in each plot. The clipped waterhemp plants from each plot were placed in paper bags and dried at 60ºC for at least 48 hours and then the waterhemp dry biomass was weighed and recorded. Soybean from the middle two rows of each plot was harvested at maturity with a small-plot combine; soybean seed weight and moisture content were recorded. Soybean yield was adjusted to 13.5% moisture content before statistical analysis.

### *2.2 Statistical Analysis*

#### 2.2.1 Non-Linear Regression Analysis

The NLIN procedure in SAS Studio v9.4 (SAS Institute, Cary, NC) was used to regress waterhemp control, density, aboveground biomass, and soybean yield data against the dose of diflufenican (0 to 210 g ha$^{-1}$). An exponential to maximum model (Equation 1) was used to regress waterhemp control at 2, 4, and 8 WAA against the dose of diflufenican. Waterhemp density and biomass were regressed against diflufenican dose using an inverse exponential model (Equation 2) and soybean yield, expressed as a percentage of the industry standard herbicide (flumioxazin/pyroxasulfone), was regressed using Cousens' (1985) rectangular hyperbola (Equation 3) and back transformed to kg $ha^{-1}$ for presentation.

Exponential to Maximum:

$$y = a - be^{(-c \cdot dose)} \tag{1}$$

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

Inverse Exponential:

$$y = a + be^{(-c \cdot dose)} \tag{2}$$

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

Rectangular Hyperbola:

$$y = (b \cdot dose)/[1 + (b \cdot dose/a)] \tag{3}$$

where, $y$ = response parameter, $a$ = upper asymptote, and $b$ = initial slope.

Parameters created from each regression analysis were used to calculate the expected dose ($ED_n$) of diflufenican for 50, 80, and 95% waterhemp control, a 50, 80, and 95% reduction in waterhemp plant density and aboveground biomass, and to achieve 50, 80, and 95% yield of the industry standard herbicide.

Model Goodness of Fit:

Model efficiency (ME; Equation 4) and root mean square error (RMSE; Equation 5) were calculated to determine the goodness of fit for each regression model as suggested by Soltani et al. (2020).

$$\text{ME} = 1 - \left[\frac{\sum_{i=1}^{n} (Oi - Pi)^2}{\sum_{i=1}^{n} (Oi - i)^2}\right] \tag{4}$$

$$\text{RMSE} = \sqrt{\frac{\text{RSS}}{(n - p - 1)}} \tag{5}$$

where, $Oi$ is the observed, $Pi$ is the calculated, $Oi$ 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 indicate better goodness of fit.

### 2.2.2 Least-Square Means Comparisons

Data were analyzed in SAS Studio v9.4, OnDemand for Academics (SAS Institute, Cary, NC) using generalized linear mixed modeling via the GLIMMIX procedure. Variance was divided into the random effects of environment, block nested within environment, and the environment-by-treatment interaction and the fixed effect herbicide treatment. Parameters were analyzed using distributions that best met the assumptions of ANOVA. Pearson chi-square/df values were assessed to prevent model overdispersion and assumptions of normality and homogeneity of variance were confirmed using the Shapiro-Wilk test and plots of studentized residuals. The untreated control was assigned a value of 0 for crop injury and waterhemp control and was excluded from the analysis of these parameters due to zero variance. Treatment means were compared to the untreated control based on the P-value associated with the t-test conducted on least-square means. Waterhemp control 2, 4, and 8 WAA was arcsine-square root transformed prior to analyses with 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 a lognormal distribution with identity link. Soybean injury 3 WAA, moisture, and yield were analyzed using a normal distribution with identity link. For presentation, data analyzed using the lognormal distribution were back-transformed using the omega method. Means were separated using Tukey's Least Significant Difference (LSD) and grouped accordingly. An alpha level of 0.05 was used for the analyses.

## 3. Results and Discussion

The experimental sites had good waterhemp pressure averaging 490 $m^{-2}$ at 8 WAA (Tables 1 and 2).

### *3.1 Biologically Effective Dose of Diflufenican Applied Preemergence for the Control of MHR Waterhemp*

Based on the regression analysis, the calculated diflufenican doses to elicit 50, 80, and 95% control of MHR waterhemp at 2 WAA were 71, 164, and 304 g $ha^{-1}$; the calculated diflufenican doses to elicit 50, 80, and 95% control of MHR waterhemp at 4 WAA were 50, 115, and 214 g $ha^{-1}$, respectively; and the calculated diflufenican doses to elicit 50, 80, and 95% control of MHR waterhemp at 8 WAA were 69, 158, and 294 g $ha^{-1}$, respectively (Table 1). In another study to obtain 50, 80, and 90% MHR waterhemp control the calculated flumioxazin doses were 28, 80, and 147 g $ha^{-1}$ and the calculated pyroxasulfone doses were 40, 110, and 247 g $ha^{-1}$, respectively (Ferrier et al., 2022).

Table 1. Regression parameters and the calculated doses of diflufenican applied preemergence required to obtain 50, 80, and 95% control of multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) 2, 4, and 8 weeks after application (WAA), a 50, 80, and 95% reduction in density and biomass at 8 WAA, and 50, 80, and 95% soybean yield of the industry standard herbicide (flumioxazin/pyroxasulfone) from five field experiments conducted in Ontario, Canada in 2017 and 2018

| Variable | Regression parameters (±SE) | | | Calculated diflufenican dose | | |
|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | $ED_{50}$ | $ED_{80}$ | $ED_{95}$ |
| Control | | | | -------------- g ai $ha^{-1}$ ------------- | | |
| 2 WAA[a] | 100 (0) | 100.5 (2.42) | 0.01 (0) | 71 | 164 | 304 |
| 4 WAA[a] | 100 (0) | 100.2 (3.11) | 0.01 (0) | 50 | 115 | 214 |
| 8 WAA[a] | 100 (0) | 100.6 (3.68) | 0.01 (0) | 69 | 158 | 294 |
| Density[b] | 30.15 (64.32) | 528.3 (102) | 0.03 (0.02) | 28 | 70 | Non-est. |
| Biomass[b] | 9.21 (20.14) | 146.7 (24.4) | 0.02 (0.01) | 44 | 109 | Non-est. |
| Yield[c] | 99.81 (14.1) | 33.3 (152.6) | - | 3 | 12 | 57 |

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

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

[c] Regression parameters: $y = (b \cdot \text{dose})/[1 + (b \cdot \text{dose}/a)]$; Where *a* is the upper asymptote, and *b* is the initial slope.

[d] Abbreviations: $ED_n$, effective dose to elicit response level n; Non-est., non-estimable; WAA, weeks after application.

Based on the regression analysis, the calculated diflufenican doses to elicit 50, 80, and 95% reduction in MHR waterhemp density 8 WAA were 28, 70, and Non-est. g $ha^{-1}$, respectively (Table 1). In another study, the calculated doses to obtain 50, 80, and 90% reduction in MHR waterhemp density at 8 WAA were 50, 73, and 97 g $ha^{-1}$ with flumioxazin and 117, Non-est., and Non-est. g $ha^{-1}$ with pyroxasulfone, respectively (Ferrier et al. 2022).

Based on the regression analysis, the calculated diflufenican doses to elicit 50, 80, and 95% reduction in MHR waterhemp biomass 8 WAA were 44, 109, and Non-est. g $ha^{-1}$, respectively (Table 1). In another study, the calculated doses to obtain 50, 80, and 90% reduction in MHR waterhemp biomass at 8 WAA were 141, 210, and 301 g $ha^{-1}$ with flumioxazin and 204, 382, and Non-est. g $ha^{-1}$ with pyroxasulfone, respectively (Ferrier et al. 2022).

Based on the regression analysis, lower doses of diflufenican PRE were needed to result in 50, 80, and 95% of the soybean yield of the industry standard herbicide. The calculated diflufenican doses to elicit 50, 80, and 95% of the soybean yield of the industry standard herbicide (flumioxazin/pyroxasulfone) were 3, 12, and 57 g $ha^{-1}$, respectively (Table 1). In another study, the calculated doses to elicit 50, 80, and 95% yield of the weed-free control were 3, 14, and 65 g $ha^{-1}$ with flumioxazin and 6, 24, and 112 g $ha^{-1}$ with pyroxasulfone, respectively (Ferrier et al., 2022).

### *3.2 Control of MHR Waterhemp With Diflufenican Compared to Metribuzin and Flumioxazin/Pyroxasulfone*

Diflufenican (180 g $ha^{-1}$) was compared with the commonly used herbicides metribuzin (300 g $ha^{-1}$) and pyroxasulfone/flumioxazin (105/134 g $ha^{-1}$) applied PRE for the control of MHR waterhemp. Diflufenican controlled MHR waterhemp 89, 92, and 85%; metribuzin controlled MHR waterhemp 94, 84, and 69%; and pyroxasulfone/flumioxazin controlled MHR waterhemp 100, 99, and 98% at 2, 4, and 8 WAA, respectively (Table 2). In other studies, Symington et al. (2024) reported that MHR waterhemp was controlled 53 to 67% with metribuzin (413 g $ha^{-1}$), 38 to 58% with diflufenican (90 g $ha^{-1}$), and 90 to 95% with pyroxasulfone/flumioxazin (240 g $ha^{-1}$) applied PRE in soybean. Schryver et al. (2017a) reported 92% control of GR waterhemp with pyroxasulfone/flumioxazin (240 g $ha^{-1}$) PRE which is comparable to this study. In another study, Schryver et al. (2017b) reported that GR waterhemp control was 76% with metribuzin (1,120 g $ha^{-1}$), and 77% with flumioxazin

(108 g ha$^{-1}$) applied PRE in soybean. Hedges et al. (2018a) documented 86 to 99% GR waterhemp control with *S*-metolachlor/metribuzin (1943 g ha$^{-1}$) PRE and 96 to 99% MHR waterhemp control with pyroxasulfone/flumioxazin (240 g ha$^{-1}$) PRE in soybean. Similarly, Duenk et al. (2023) reported 94% control of MHR waterhemp with pyroxasulfone/flumioxazin (160 g ha$^{-1}$) PRE in soybean. Westerveld et al. (2021) documented 93 to 99% control of MHR waterhemp with pyroxasulfone/flumioxazin (240 g ha$^{-1}$) PRE in soybean.

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

| Herbicide | Rate | Visible control | | | Density | Biomass |
|---|---|---|---|---|---|---|
| | | 2 WAA | 4 WAA | 8 WAA | | |
| | g ai ha$^{-1}$ | ----------------- | % | ----------------- | Plants m$^{-2}$ | g m$^{-2}$ |
| Non-treated control | - | 0 c | 0 c | 0 d | 490 c | 169 c |
| Diflufenican | 180 | 89 b | 92 ab | 85 b | 20 ab | 12 ab |
| Metribuzin | 300 | 94 ab | 84 b | 69 c | 79 b | 55 b |
| Flumioxazin/pyroxasulfone | 105/134 | 100 a | 99 a | 98 a | 0 a | 1 a |

*Note*. [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).

Diflufenican PRE reduced MHR waterhemp density similar to metribuzin and pyroxasulfone/flumioxazin (Table 2). Diflufenican, metribuzin, and flumioxazin/pyroxasulfone PRE reduced MHR waterhemp density 96, 84, and 100%, respectively (Table 2). In contrast, Symington et al. (2024) reported that MHR waterhemp density was decreased 83% with metribuzin (413 g ha$^{-1}$), 57% with diflufenican (90 g ha$^{-1}$), and 100% with pyroxasulfone/flumioxazin (240 g ha$^{-1}$) PRE in soybean.

Diflufenican PRE reduced MHR waterhemp aboveground biomass comparable to metribuzin and pyroxasulfone/flumioxazin (Table 2). Diflufenican, metribuzin, and flumioxazin/pyroxasulfone PRE reduced MHR aboveground waterhemp biomass 93, 67, and 99%, respectively (Table 2). Symington et al. (2024) reported that MHR waterhemp aboveground biomass was decreased 67% with metribuzin (413 g ha$^{-1}$), 47% with diflufenican (90 g ha$^{-1}$), and 97% with pyroxasulfone/flumioxazin (240 g ha$^{-1}$) PRE in soybean.

Diflufenican, metribuzin, and flumioxazin/pyroxasulfone PRE at 3 WAA caused 9, 0, and 4% visible soybean injury, respectively; however, the injury was transient and there was no impact on soybean seed moisture content or yield (Table 3). Similarly, Symington et al. (2024) found transient visible soybean injury and comparable soybean grain yield with metribuzin (413 g ha$^{-1}$), diflufenican (90 g ha$^{-1}$), and pyroxasulfone/flumioxazin (240 g ha$^{-1}$) PRE. In other studies, Hedges et al. (2018a, b) and Mahoney et al. (2014) reported minimal and transient injury with no yield loss with pyroxasulfone/flumioxazin PRE in soybean.

Table 3. Soybean injury 3 weeks after application (WAA), seed moisture content, and grain yield following herbicides applied preemergence from five field trials conducted in Ontario, Canada in 2017 and 2018

| Herbicide | Rate | Injury | Moisture | Yield |
|---|---|---|---|---|
| | | 3 WAA | | |
| | g ai ha$^{-1}$ | % | % | kg ha$^{-1}$ |
| Non-treated control | - | 0 a | 13.6 a | 1,730 a |
| Diflufenican | 180 | 9 b | 11.5 a | 2,220 a |
| Metribuzin | 300 | 0 a | 11.9 a | 2,020 a |
| Flumioxazin/pyroxasulfone | 105/134 | 4 ab | 11.5 a | 2,340 a |

*Note*. [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).

## 4. Conclusions

This study concludes that there is potential for the utilization of diflufenican as one component in a diversified, integrated MHR waterhemp control program in soybean. Diflufenican PRE generally provides comparable MHR waterhemp control with metribuzin, but control was less than flumioxazin/pyroxasulfone PRE. The evaluation of diflufenican with other efficacious herbicides for waterhemp control should be explored in future studies.

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Drs. Peter Sikkema and Nader Soltani were responsible for the study design and writing of this manuscript. Christian A. Willemse conducted the statistical analysis of the data collected.

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