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Authors: Ferrier, James, Soltani, Nader, Hooker, David C., Robinson, Darren E., and Sikkema, Peter H.

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Author for correspondence:
Nader Soltani, Adjunct Professor, Department of Plant Agriculture, University of Guelph Ridgetown Campus, 120 Main Street East, Ridgetown, ON, Canada N0P 2C0.
Email: soltanin@uoguelph.ca

The interaction of pyroxasulfone and flumioxazin applied preemergence for the control of multiple-herbicide-resistant waterhemp (*Amaranthus tuberculatus*) in soybean

James Ferrier[1], Nader Soltani[2], David C. Hooker[3], Darren E. Robinson[4] and Peter H. Sikkema[5]

[1]Graduate Student, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; [2]Adjunct Professor; Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; [3]Associate Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; [4]Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada and [5]Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada

Abstract

Six field experiments were conducted to investigate any interaction between pyroxasulfone and flumioxazin on soybean tolerance and control of multiple-herbicide-resistant (MHR) waterhemp in soybean during 2016 and 2017 in Ontario, Canada. There was a synergistic increase in soybean injury with the co-application of pyroxasulfone and flumioxazin at all rates evaluated at 2 wk after emergence (WAE), the two highest rates evaluated (134/106 and 268/211 g ai ha^{-1}) at 4 WAE, and the highest rate (268/211 g ai ha^{-1}) evaluated at 8 WAE. Soybean injury with all pyroxasulfone and flumioxazin treatments was transient and had no adverse effect on soybean grain yield. Pyroxasulfone applied preemergence at 45, 89, 134, and 268 g ai ha^{-1} controlled MHR waterhemp up to 72%, 89%, 92%, and 95%, respectively. Flumioxazin applied preemergence at 35, 70, 106, and 211 g ai ha^{-1} controlled MHR waterhemp up to 78%, 90%, 93%, and 96%, respectively. Pyroxasulfone/flumioxazin applied preemergence at 45/35, 89/70, 134/106, and 268/211 g ai ha^{-1} controlled MHR waterhemp up to 92%, 96%, 98%, and 100%, respectively. There were no significant antagonistic or synergistic interactions for the control of MHR waterhemp with pyroxasulfone/flumioxazin at rates evaluated except at 268/211 g ai ha^{-1}, which provided a synergistic increase in MHR waterhemp control at 4 WAE. The MHR waterhemp biomass and density reductions followed a trend similar trend to visible control. Pyroxasulfone/flumioxazin at 268/211 g ai ha^{-1} caused a synergistic response in biomass reduction (9% difference). Based on these results, there is an additive increase in MHR waterhemp control and potential for a synergistic increase in soybean injury with the co-application of pyroxasulfone plus flumioxazin.

Introduction

Waterhemp can be found in most of the continental United States and the Canadian provinces of Ontario, Quebec, Manitoba, and British Columbia (Costea et al. 2005). First identified as a problematic weed in cultivated fields in Ontario in the early 2000s, waterhemp has rapidly expanded its range across southern Ontario (Benoit 2019; Costea and Tardif 2003; Schryver et al. 2017).

Waterhemp grows quickly (up to 2.5 cm per day) and produces 5 million seeds per plant under ideal growing conditions (Hartzler et al. 2004; Horak and Loughin 2000). Waterhemp grows faster than many other *Amaranthus* species and is one of the tallest, with a mature height of up to 3 m (Cole and Holch 1941; Costea et al. 2005; Horak and Laughin 2000). Waterhemp's biparental reproduction, immense fecundity, and aggressive growth allow it to thrive (Nordby et al. 2007; Waselkov and Olsen 2014. Waterhemp's prolific nature and extended germination window make it competitive and difficult to control, especially in soybean (Sellers et al. 2003). Previous studies have documented yield losses of up to 73% in soybean and 74% in corn (*Zea mays* L.) due to interference from waterhemp (Soltani et al. 2009; Steckel et al. 2010; Vyn et al. 2007). Schryver et al. (2017) reported up to 98% reduction in soybean grain yield when waterhemp density was greater than 1,200 plants m^{-2}.

Until recently, waterhemp biotypes found in agricultural fields in Ontario have been confirmed to be mostly resistant to Group 2 (acetolactate synthase inhibitors) and/or Group 5 (photosystem II inhibitors) herbicides. Resistance to Group 9 (5-enolpyruvyl shikimate-3-phosphate synthase-inhibitors) and Group 14 [protoporphyrinogen oxidase (PPO)-inhibitors] has evolved in the last 5 yr, resulting in some waterhemp biotypes with multiple-herbicide resistance to all four of the aforementioned herbicide groups (Benoit et al. 2019; Heap 2021). The spread of multiple-herbicide-resistant (MHR) waterhemp has made control strategies challenging, as herbicide options are limited, especially in soybean. Early-season control of MHR waterhemp with soil-applied herbicides is critical to avoid soybean yield losses and limit reproduction and spread (Schryver et al. 2017; Vyn et al. 2007). Soil-applied herbicides such as pyroxasulfone and flumioxazin, applied preplant or preemergence alone or in combination, can provide control of MHR waterhemp during the critical weed-free period in soybean (Schryver et al. 2017).

Pyroxasulfone, a Group 15 (isoxazoline) herbicide, inhibits very long-chain fatty acid elongases in susceptible plants (Anonymous 2019b). Pyroxasulfone can control waterhemp and other broadleaf weeds and grasses in soybean (Mueller and Steckel 2011; Stephenson et al. 2017). Flumioxazin is a Group 14 (*N*-phenylphthalimide) herbicide that inhibits the PPO enzyme in susceptible plants (Hartzler et al. 2004; Price et al. 2004). Flumioxazin can control waterhemp and other broadleaf weeds in soybean (Niekamp 1998; Nordby et al. 2007; Taylor-Lovell et al. 2002). In recent years, PPO-resistant biotypes of waterhemp have emerged across North America (Heap 2021). These biotypes fail to be controlled by the postemergence-applied PPO herbicides yet are still effectively controlled by preemergence-applied flumioxazin. However, length of residual control is sometimes reduced in the resistant biotypes (Dayan et al. 2014; Harder et al. 2012; Wuerffel et al. 2015).

Pyroxasulfone and flumioxazin are currently labeled for use in soybean at 125 to 247 g ai ha^{-1} and 71 to 107 g ai ha^{-1} in Canada, respectively, with rates dependent upon soil texture and organic matter content (Anonymous 2019b, 2019c). Earlier studies have shown effective control of waterhemp and other *Amaranthus* species with a preemergence co-application of pyroxasulfone and flumioxazin (Nakatani et al. 2016; Strom et al. 2019). The co-application of pyroxasulfone and flumioxazin combines two effective modes of action and can further improve the efficacy and the consistency of MHR waterhemp control in soybean. The premix formulation of pyroxasulfone/flumioxazin is currently labeled for use in soybean at 160 to 240 g ai ha^{-1} in Canada, the rate used is dependent upon soil texture and desired duration of residual control (Anonymous 2019a). The rates of active ingredients in the pre-mix product are less than the registered individual product rates. This rate discrepancy could be due to additive or synergistic weed control or increased risk of crop injury with the co-application of pyroxasulfone and flumioxazin, as the increased risk of injury is observed with mixtures of flumioxazin and *S*-metolachlor, another Group 15 herbicide (Mahoney et al. 2014; Salomao et al. 2021). Flumioxazin is precluded from mixtures with very long-chain fatty acid elongases-inhibitor herbicides other than pyroxasulfone on the commercial label, because of the likelihood of unacceptable crop injury and yield loss (Anonymous 2019c).

To our knowledge, no published study has quantified the antagonistic, additive, or synergistic interactions of pyroxasulfone and flumioxazin mixtures on soybean injury and control of MHR waterhemp. Information on the interaction of these two herbicides is critical for scientists, growers, and agronomists in developing herbicide programs for MHR waterhemp control in soybean. Understanding the interactive effects of these mixtures will also help manage potential risk or capture increased efficacy.

The objective of this research was to determine the soybean tolerance and efficacy of pyroxasulfone and flumioxazin and to quantify their interaction when applied preemergence at various rates for the control of MHR waterhemp in soybean.

Materials and Methods

The study consisted of six field experiments; three were conducted in 2016, and three in 2017, in commercial soybean fields located in southwestern Ontario with waterhemp previously confirmed resistant to Group 2 (imazethapyr), Group 5 (atrazine), and Group 9 (glyphosate) (Heap 2021; Schryver et al. 2017). The waterhemp biotypes present in the experimental fields survived application of 75 g ai ha^{-1} imazethapyr, and of 1,000 g ai ha^{-1} atrazine, and have a resistance factor of 5 to 28 for glyphosate (Schryver et al. 2017). One experiment each year was completed near Cottam, ON, Canada (42.149076º N, 82.683687º W) and two experiments (at separate sites) in each year were completed on Walpole Island, ON, Canada (42.561492º N, 82.501487º W and 42.554334° N, 82.515518° W).

The experimental design was a randomized complete block with four replications. Plots were 2.25 m wide and 8 m long, containing three soybean rows with 0.75 m inter-row spacing. Prior to planting, the plot area was tilled twice with a cultivator and harrow. Glyphosate/dicamba-resistant soybean cultivars DKB 30-61 (2016) and DKB 10-01 (2017) were seeded at a depth of 4 cm and at a rate of approximately 400,000 seeds ha^{-1} on planting dates listed in Table 1. Treatments included a nontreated weedy control, a weed-free control, pyroxasulfone (K-I Chemical USA Inc, Durham, NC) applied preemergence at 45, 89, 134, and 268 g ai ha^{-1}, flumioxazin applied preemergence at 35, 70, 106, and 211 g ai ha^{-1}, and a preformulated combination of pyroxasulfone/flumioxazin (Valent Canada, Guelph, ON) applied preemergence at $45 + 35$, $89 + 70$, $134 + 106$, and $268 + 211$ g ai ha^{-1}. Herbicide rates were chosen based on titration of the individual active ingredients in the preformulated pyroxasulfone/flumioxazin combination. Herbicides were applied 1–5 d after seeding with a compressed CO_2 backpack sprayer calibrated to apply 200 L ha^{-1} of spray solution through a 1.5-m hand-held boom equipped with four ULD120-02 nozzles (Pentair, 375 5th Ave NW, New Brighton, MN) producing a spray width of 2.0 m.

Soybean injury was estimated visually at 2, 4, and 8 wk after soybean emergence (WAE), and MHR waterhemp control was estimated visually compared to the nontreated control at 2, 4, 8, and 12 wk after herbicide application (WAA). Plots were evaluated on a 0–100 scale where 0 = no visible soybean injury/no waterhemp control and 100 = complete soybean necrosis/total waterhemp control. The waterhemp density (plants m^{-2}) and dry biomass (g m^{-2}) was determined from two randomly placed 0.25-m^{-2} frames within each plot 8 WAA. MHR waterhemp plants within each quadrat were counted, then cut with hand clippers as close to the soil surface as practical, bagged in paper, dried until no moisture was left in the sample, and weights recorded. Soybean grain yield was harvested and weighed by a self-propelled research combine. The final grain yield was standardized to 13% moisture prior to statistical analysis.

Statistical Analysis

The GLIMMIX procedure in SAS v. 9.4 (SAS Institute, Cary, NC) was used to analyze the data variance for this study. Site-by-treatment interactions was evaluated with a mixed-model analysis where treatment was the fixed effect. It was determined that site, site-by-treatment, and replication within the site were random effects. Because the site-by-treatment interactions were considered nonsignificant ($P > 0.05$), data from all sites were combined for analysis.

Means were generated for soybean injury at 2, 4, and 8 WAE; MHR waterhemp control at 2, 4, 8, and 12 WAA; MHR waterhemp

Table 1. Location, year, soil characteristics, soybean planting and emergence dates, and herbicide application date for the interaction of pyroxasulfone and flumioxazin for multiple herbicide-resistant waterhemp control for six field experiments conducted in southwestern Ontario, Canada, during 2016 and 2017.

		Soil parameters					
Location	Year	Soil type[a]	OM[a,b]	pH[a]	Planting date	Herbicide application Date	Crop emergence date
			%				
Walpole I	2016	Sandy Loam	6.4	7.6	May 30	June 2	June 7
Cottam	2016	Sandy Loam	2.9	6.5	May 23	May 24	May 30
Walpole II	2016	Sandy Loam	4.3	7.8	May 30	June 2	June 7
Walpole I	2017	Sandy Loam	2.1	8.0	June 8	June 9	June 14
Cottam	2017	Sandy Loam	2.2	6.4	May 19	May 23	May 29
Walpole II	2017	Loamy Sand	2.3	8.3	June 2	June 7	June 9

[a]Based on soil test results from the top 15 cm of the soil profile.
[b]Abbreviation: OM, organic matter.

Table 2. Observed and Colby's (1967) expected soybean injury and grain yield after the application of pyroxasulfone and flumioxazin, applied preemergence, alone and in combination, from six field experiments conducted in southwestern Ontario, Canada during 2016 and 2017.[a]

		Soybean injury									
		2 WAE[b]			4 WAE			8 WAE			
	Rate	Obs	Exp	P value[c]	Obs	Exp	P value[c]	Obs	Exp	P value[c]	Grain yield
Herbicide common name	g ai ha^{-1}	%									kg ha^{-1}
Nontreated control	–	0			0			0 b			921 c
Weed-free control	–	0			0			0 b			1,931 a
Pyroxasulfone	45	0 d			0 b			0 b			1,187 bc
	89	0 d			0 b			0 b			1,311 abc
	134	0 d			1 b			0 b			1,593 ab
	268	1 d			1 b			0 b			1,568 abc
Flumioxazin	35	1 d			0 b			0 b			1,205 bc
	70	2 cd			0 b			0 b			1,311 abc
	106	4 bcd			1 b			0 b			1,348 abc
	211	9 b			2 b			0 b			1,543 abc
Pyroxasulfone/flumioxazin	45 + 35	1 d	1 b	^	0 b	0 b	NS	0 b	0	–	1,461 abc
	89 + 70	4 bcd	2 b	^	1 b	0 b	NS	0 b	0	–	1,681 ab
	134 + 106	8 bc	4 b	^	4 ab	2 ab	^	0 b	0	NS	1,666 ab
	268 + 211	17 a	9 a	^	8 a	3 a	^	2 a	0	^	1,774 ab

[a]Means followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, α = 0.05.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value; WAE, weeks after crop emergence.
[c]A caret symbol ^ indicates that the observed value was significantly greater than the expected value; NS indicates that the observed value was not significantly different from the expected value; a dash – indicates that the difference could not be calculated. Expected values were calculated using Colby's equation [$E = (X + Y) - (XY)/100$]; P = 0.05.

density and dry biomass; and relative soybean seed yield. A Tukey-Kramer test was used to compare means ($P < 0.05$). Expected values for the soybean injury, MHR waterhemp control, biomass, and density were calculated with the following equations used for the analysis.

Colby's (1967) equation was applied to control and injury observations:

$$\mathrm{Exp} = (X + Y) - (XY)/100 \qquad [1]$$

where Exp = expected value, X = observed pyroxasulfone value and Y = observed flumioxazin value.

Modified Colby's equation (applied to density and biomass observations):

$$\mathrm{Exp} = XY/C \qquad [2]$$

where X = measured parameter value for pyroxasulfone, Y = measured parameter value for flumioxazin, and C = measured parameter value of the nontreated plot.

Colby's equation was selected because the different modes of action of flumioxazin and pyroxasulfone are best in fitting an independent-action model (Abendroth et al. 2011).

Expected values generated from the equations were compared to the observed means with a Student's T-test. Where the expected and observed values did not differ, the interaction effect between the two herbicides was deemed additive. Where the difference between the observed and expected values was statistically significant, the interaction was determined to be antagonistic if lower, or synergistic if higher. Where computation of the difference between expected and observed values was not possible, a dash was inserted into the tables.

Results and Discussion

Soybean Injury

Pyroxasulfone applied preemergence at 45, 89, 134, and 268 g ai ha^{-1} caused minimal soybean injury (<1%) at 2, 4, and 8 WAE (Table 2). The yield reduction with pyroxasulfone applied preemergence at 45 g ai ha^{-1} was due to MHR waterhemp interference and not crop injury. Flumioxazin applied preemergence at 35, 70, 106, and 211 g ai ha^{-1} caused up to 9% injury at 2 WAE and 2% injury at 4 WAE; soybean showed no injury at 8 WAE (Table 2). Pyroxasulfone/flumioxazin applied preemergence at 45 + 35, 89 + 70, 134 + 106, and 268 + 211 g ai ha^{-1} caused 1%, 4%, 8%

and 17% injury at 2 WAE; 0%, 1%, 4%, and 8% injury at 4 WAE; and 0%, 0%, 0%, and 2% injury at 8 WAE, respectively (Table 2). There was a synergistic increase in soybean injury with pyroxasulfone/flumioxazin at all rates evaluated at 2 WAE, the two highest rates evaluated (134 + 106 and 268 + 211 g ai ha^{-1}) at 4 WAE, and the highest rate (268 + 211 g ai ha^{-1}) evaluated at 8 WAE (Table 2). Soybean injury from pyroxasulfone/flumioxazin was transient with ≤2% injury at 8 WAE.

Soybean injury in this study is similar to Mahoney et al. (2014), McNaughton et al. (2014), and Steppig et al. (2018), who found that soybean recovered quickly following preemergence treatments of flumioxazin and pyroxasulfone alone or in combination. However, other researchers have reported potential interactions between pyroxasulfone and flumioxazin that may result in a significant risk of injury in soybean (Hartzler 2017).

Soybean Yield

MHR waterhemp interference reduced soybean yield by 53% (Table 2). Reduced interference from the waterhemp population resulted in soybean yield that was similar to the weed-free control in all treatments except for the lowest examined dose of pyroxasulfone and flumioxazin.

MHR Waterhemp Control

MHR waterhemp control with pyroxasulfone, flumioxazin, and pyroxasulfone/flumioxazin increased with rate and decreased over time (Table 3). There were no significant antagonistic or synergistic interactions with pyroxasulfone and flumioxazin at rates evaluated except with pyroxasulfone/flumioxazin applied preemergence at 268 + 211 g ai ha^{-1}, which provided a synergistic increase in MHR waterhemp control at 4 WAE (Table 3). Control results are similar to Schryver et al. (2017), who found 96%, 97%, 97%, and 97% control at 2, 4, 8, and 12 WAA, respectively, with pyroxasulfone/flumioxazin applied preemergence at a rate similar to 134 + 106 g ai ha^{-1} in soybean, but the study did not examine interaction effects. Meyer et al. (2016) reported 98% waterhemp control with pyroxasulfone/flumioxazin applied preemergence 3 WAA. Pyroxasulfone and flumioxazin interactions for control of other weed species were mostly additive in research conducted by Presoto (2020) and mostly synergistic in a study conducted by Sievernich et al. (2011). Interaction effects of the co-application of pyroxasulfone and flumioxazin appear to be specific to weed species and herbicide rate.

Aboveground MHR Waterhemp Biomass and Density

Pyroxasulfone applied preemergence at 45 and 89 g ai ha^{-1} did not reduce MHR waterhemp biomass but did reduce MHR waterhemp biomass 79% and 94% when applied at 134 and 268 g ai ha^{-1}, respectively (Table 4). Pyroxasulfone applied preemergence at 45, 89, 134, and 268 g ai ha^{-1} reduced MHR waterhemp density 76%, 72%, 88%, and 92%, respectively (Table 4).

Flumioxazin applied preemergence at 35, 70, and 106 g ai ha^{-1} had no effect on MHR waterhemp biomass but reduced MHR waterhemp biomass 79% when applied at 211 g ai ha^{-1} (Table 4). Flumioxazin applied preemergence at 35, 70, 106, and 211 g ai ha^{-1} reduced MHR waterhemp density 85%, 90%, 94%, and 99%, respectively (Table 4).

Pyroxasulfone/flumioxazin applied preemergence at 45 + 35 g ai ha^{-1}did not reduce MHR waterhemp biomass, but reduced

Table 3. Observed and Colby's (1967) expected control of waterhemp at 2, 4, 8, and 12 wk after application (WAA) of pyroxasulfone and flumioxazin, applied preemergence, alone and in combination, from six field experiments conducted in southwestern Ontario, Canada during 2016 and 2017.[a]

		Control											
		2 WAA			4 WAA			8 WAA			12 WAA		
Herbicide common name	Rate	Obs[b]	Exp	P value[c]	Obs[b]	Exp	P value[c]	Obs[b]	Exp	P value[c]	Obs[b]	Exp	P value[c]
	g ai ha^{-1}	%	%		%	%		%	%		%	%	
Pyroxasulfone	45	72 c			56 d			52 f			48 f		
	89	89 abc			75 bcd			67 def			66 de		
	134	92 ab			86 ab			80			78 bcd		
	268	95 ab			93 ab			89 abc			90 abc		
Flumioxazin	35	78 bc			63 cd			54 ef			49 ef		
	70	90 abc			75 bcd			70 de			67 d		
	106	93 ab			86 ab			76 cd			73 cd		
	211	96 ab			93 ab			89 abc			88 abc		
Pyroxasulfone/flumioxazin	45 + 35	92 ab	88 a	NS	81 abc	77 b	NS	75 cd	75 c	NS	75 cd	71 c	NS
	89 + 70	96 ab	94 a	NS	92 ab	88 ab	NS	89 abc	86 b	NS	88 abc	85 b	NS
	134 + 106	98 a	97 a	NS	98 a	95 a	NS	95 ab	93 ab	NS	95 abc	92 ab	NS
	268 + 211	100 a	99 a	NS	100 a	99 a	^	99 a	98 a	NS	99 a	98 a	NS

[a]Means followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, α = 0.05.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]A caret symbol ^ indicates that the observed value was significantly greater than the expected value, P = 0.05; NS indicates that the observed value was not significantly different from the expected value. Expected values were calculated using Colby's equation [$E = X + Y) - (XY)/100$].

Table 4. Observed and Colby's (1967) expected waterhemp biomass and density at 8 wk after application of pyroxasulfone and flumioxazin, applied preemergence, alone and in combination, from six field experiments conducted in southwestern Ontario, Canada during 2016 and 2017.[a]

		Biomass			Density		
Common name	Rate	Obs	Exp	P value[c]	Obs	Exp	P value[c]
	g ai ha^{-1}	g DM m^{-2}	g DM m^{-2}		Plants m^{-2}	Plants m^{-2}	
Nontreated control		205 a			299 a		
Pyroxasulfone	45	151 a			71 b		
	89	124 abc			83 bcd		
	134	43 bcd			35 cdef		
	268	13 cd			23 cdefg		
Flumioxazin	35	165 a			46 b		
	70	177 ab			30 bcd		
	106	111 abc			19 bcde		
	211	43 bcd			4 efg		
Pyroxasulfone/flumioxazin	45 + 35	137 abc	82 a	NS	37 bc	20 a	NS
	89 + 70	51 bcd	55 ab	NS	5 defg	13 ab	NS
	134 + 106	9 cd	39 ab	NS	1 fg	5 bc	NS
	268 + 211	0 d	18 ab	*	0 g	1 c	NS

[a]Means followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, α = 0.05.
[b]Abbreviations: DM, dry matter; Exp, expected value; NS, not significant; Obs, observed value.
[c]An asterisk * indicates that the observed value was significantly different from the expected value, P = 0.05; NS indicates that the observed value was not significantly different from the expected value. Expected values were calculated using a modified version of Colby's equation (Exp = XY/C), where Exp is the expected parameter estimate, X and Y are the measured parameter values of pyroxasulfone and flumioxazin, respectively, and C is the measured parameter value of the nontreated control plot.

MHR waterhemp biomass 75%, 96%, and 100% when applied at 89 + 70, 134 + 106, and 268 + 211 g ai ha^{-1}, respectively (Table 4). There were no significant antagonistic or synergistic interactions with pyroxasulfone/flumioxazin at rates evaluated except at 268 + 211 g ai ha^{-1}, which caused a synergistic decrease in MHR biomass of 9% (Table 4). Pyroxasulfone/flumioxazin applied preemergence at 45 + 35, 89 + 70, 134 + 106, and 268 + 211 g ai ha^{-1} reduced MHR waterhemp density 88%, 98%, 100%, and 100%, respectively (Table 4); all interactions for MHR waterhemp density were additive (Table 4). These results are similar to other studies that reported large reductions in waterhemp biomass and density with pyroxasulfone and flumioxazin applied alone or in combination at comparable rates (Hedges et al. 2018; Perkins et al. 2020; Schryver et al. 2017).

This research concludes that there is the potential for a synergistic increase in soybean injury with the co-application of pyroxasulfone and flumioxazin. Although pyroxasulfone/flumioxazin caused up to 17% soybean injury, no decrease in soybean yield was detected in this study, which demonstrates that soybean injury was transient. In addition, this study found that the co-application of pyroxasulfone and flumioxazin results in an additive increase in MHR waterhemp control and an additive decrease in biomass and density. The results from this study can help farmers better manage MHR waterhemp in soybean. This study provided much-needed insight into the interaction of pyroxasulfone and flumioxazin. Pyroxasulfone/flumioxazin demonstrated overlapping and complementary visible control of MHR waterhemp. The long-term residual activity at commercially registered rates of pyroxasulfone/flumioxazin (89 + 70 and 134 + 106 g ai ha^{-1}) (Anonymous 2019a) can help manage MHR waterhemp with its extended emergence pattern, allowing soybean growers to optimize yield and economic returns.

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References

Abendroth J, Blankenship E, Martin A, Roeth Fred. (2011). Joint action analysis utilizing concentration addition and independent action models. Weed Technol 25:436–446

Anonymous (2019a) Specimen label, Fierce™ herbicide English label – 2019-2679. Guelph, ON: Valent Canada Inc

Anonymous (2019b) Specimen label, Pyroxasulfone 85 WG English label – 2019-6220. Durham, NC: K-I Chemical USA Inc

Anonymous (2019c) Specimen label, Valtera™ herbicide English label – 2019-3771. Guelph, ON: Valent Canada Inc

Benoit L (2019) The distribution and control of herbicide-resistant waterhemp (*Amaranthus tuberculatus*) in Ontario. MS Thesis. Department of Plant Agriculture, University of Guelph, Guelph, ON. 191 p

Benoit L, Hedges B, Schryver MG, Soltani N, Hooker DC, Robinson DE, Laforest M, Soufiane B, Tranel PJ, Giacomini D, Sikkema PH (2019) The first record of protoporphyrinogen oxidase and four-way herbicide resistance in eastern Canada. Can J Plant Sci 100:327–331

Colby SR (1967) Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20–22

Cole HE, Holch AE (1941) The root habits of certain weeds of southern Nebraska. Ecology 22:141–147

Costea M, Tardif FJ (2003) Conspectus and notes on the genus *Amaranthus* in Canada. Rhodora 105:260–281

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

Dayan FE, Owens DK, Tranel PJ, Preston C, Duke SO (2014) Evolution of resistance to phytoene desaturase and proto- porphyrinogen oxidase inhibitors––state of knowledge. Pest Manag Sci 70:1358–1366

Harder DB, Nelson KA, Smeda RJ (2012) Management options and factors affecting control of a common waterhemp (*Amaranthus rudis*) biotype resistant to protoporphyrinogen oxidase-inhibiting herbicides. Int J Agron 2012:1

Hartzler B (2017) Evaluating herbicide injury on soybean. Integrated Crop Management News. https://crops.extension.iastate.edu/cropnews/2017/05/evaluating-herbicide-injury-soybean. Accessed: October 7, 2021

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

Heap I (2021) The international herbicide-resistant weed database. www.weedscience.com. Accessed: September 21, 2021

Hedges BK, Soltani N, Hooker DC, Robinson DE, Sikkema PH (2018) Control of glyphosate-resistant waterhemp with preemergence herbicides in glyphosate- and dicamba-resistant soybean. Can J Plant Sci 99:34–39

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. Weed Sci 48:347–355

Mahoney K, Tardif F, Robinson D, Nurse R, Sikkema P (2014) Tolerance of soybean (*Glycine max* L.) to protoporphyrinogen oxidase inhibitors and very long chain fatty acid synthesis inhibitors applied preemergence. Am J Plant Sci 5:1117–1124

McNaughton K, Shropshire C, Robinson D, Sikkema P (2014) Soybean (*Glycine max*) tolerance to timing applications of pyroxasulfone, flumioxazin, and flumioxazin. Weed Technol 28:494–500

Meyer C, Norsworthy J, Young B, Steckel L, Bradley K, Johnson W, Butts T (2016) Early-season Palmer amaranth and waterhemp control from preemergence programs utilizing 4-hydroxyphenylpyruvate dioxygenase–inhibiting and auxinic herbicides in soybean. Weed Technol 30:67–75

Mueller TC, Steckel LE (2011) Efficacy and dissipation of pyroxasulfone and three chloroacetamides in Tennessee field soil. Weed Sci 59:574–579

Nakatani M, Yamaji Y, Honda H, Uchida Y (2016) Development of the novel pre-emergence herbicide pyroxasulfone. J Pest Sci 41:107–112

Niekamp JW (1998) Weed management with sulfentrazone and flumioxazin in no-tillage soybean. MS Thesis, University of Missouri, Columbia, MO. 157 pp

Nordby D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. GWS13. USDA/Perdue Extension—Knowledge to Go. https://weedscience.missouri.edu/publications/gwc-13.pdf. Accessed: October 12, 2021

Perkins CM, Gage KL, Norsworthy JK, Young BG, Bradley KW, Bish MD, Hager A, Steckel LE (2020) Efficacy of residual herbicides influenced by cover-crop residue for control of *Amaranthus palmeri* and *A. tuberculatus* in soybean. Weed Technol 35:77–81

Presoto JC (2020) Interaction and efficacy of flumioxazin and pyroxasulfone and influence of the dry period after application in a sugarcane environment Master's Thesis, University of Sao Paulo. USP Agency for Academic Information Management. https://core.ac.uk/display/345070326. Accessed: October 12, 2021. 65 p

Price AJ, Pline WA, Wilcut JW, Cranmer JR, Danehower D (2004) Physiological basis for cotton tolerance to flumioxazin applied postemergence directed. Weed Sci 52:1–7

Salomao H, Trezzi M, Viecelli M, Pagnoncelli F, Patel F, Damo L, Frizzon G (2021) Weed management with pre-emergent herbicides in soybean crops. Commun Plant Sci 11:60–66

Schryver MG, Soltani N, Hooker DC, Robinson DE, Tranel PJ, Sikkema PH (2017) Control of glyphosate-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) in soybean in Ontario. Weed Technol 31:811–812

Sellers B, Smeda R, Johnson W, Kendig A, Ellersieck M (2003) Comparative growth of six *Amaranthus* species in Missouri. Weed Sci 51:329–333

Sievernich B, Simon A, Moberg WK, Evans RR, inventors; BASF SE, Ludwigshafen (DE), assignee (2011) January 20. Herbicidal compositions comprising pyroxasulfone V. US patent 2011/0015068 A1. https://patentimages.storage.googleapis.com/8f/5e/3f/8877b551a18724/US20110015068A1.pdf. Accessed: October 12, 2021

Soltani N, Vyn JD, Sikkema PH (2009) Control of common waterhemp (*Amaranthus tuberculatus* var. *rudis*) in corn and soybean with sequential herbicide applications. Can J Plant Sci 89:127–132

Steckel LE, Main CL, Mueller TC (2010) Glyphosate-resistant horseweed in the United States. Pages 185–193 *in* Nandula VK, ed. Glyphosate Resistance in Crops and Weeds: History, Development, and Management. Hoboken, NJ: John Wiley & Sons, Inc

Stephenson D, Blouin D, Griffin J, Landry R, Woolam B, Hardwick J (2017) Effect of pyroxasulfone application timing and rate on soybean. Weed Technol 31:202–206

Steppig NR, Norsworthy JK, Scott RC, Lorenz GM (2018) Insecticide seed treatments reduced crop injury from flumioxazin, chlorsulfuron, saflufenacil, pyroxasulfone, and flumioxazin + pyroxasulfone + chlorimuron in soybean. Int J Agron, doi:.org/10.1155/2018/9107549. 7 p

Strom SA, Gonzini LC, Mitsdarfer C, Davis AS, Riechers DE, Hager AG (2019) Characterization of multiple herbicide–resistant waterhemp (*Amaranthus tuberculatus*) populations from Illinois to VLCFA-inhibiting herbicides. Weed Sci 67:369–379

Taylor-Lovell S, Wax LM, Bollero G (2002) Preemergence flumioxazin and pendimethalin and postemergence herbicide systems for soybean (*Glycine max*). Weed Technol 16:502–511

Waselkov K, Olsen K (2014) Population genetics and origin of the native North American agricultural weed waterhemp (*Amaranthus tuberculatus*; Amaranthaceae). Am J Bot 101:1726–1736

Wuerffel RJ, Young JM, Matthews JL, Young BG (2015) Characterization of PPO-Inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting herbicides. Weed Sci 63, 511–521

Vyn JD, Swanton CJ, Weaver SE, Sikkema PH (2007) Control of herbicide-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) with pre- and post-emergence herbicides in soybean. Can J Plant Sci 87:175–182