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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 NOP 2CO. Email: soltanin@uoguelph.ca

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

James Ferrier¹, Nader Soltani², David C. Hooker³, Darren E. Robinson⁴ and Peter H. Sikkema⁵

¹Graduate Student, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; ²Adjunct Professor; Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; ³Associate Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; ⁴Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada and ⁵Professor, 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 at 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 (42.149076° N, 82.683687° W) and two experiments (at separate sites) in each year were completed on Walpole Island, ON, Canada (42.561492° N, 82.501487° 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 at 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 CO₂ 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

		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	

 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.

^aBased on soil test results from the top 15 cm of the soil profile.

^bAbbreviation: 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									
	Rate	2 WAE ^b			4 WAE			8 WAE			
		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	۸	0 b	0 b	NS	0 b	0	-	1,461 abc
	89 + 70	4 bcd	2 b	۸	1 b	0 b	NS	0 b	0	-	1,681 ab
	134 + 106	8 bc	4 b	۸	4 ab	2 ab	۸	0 b	0	NS	1,666 ab
	268 + 211	17 a	9 a	۸	8 a	3 a	۸	2 a	0	^	1,774 ab

^aMeans followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, $\alpha = 0.05$.

^bAbbreviations: Exp, expected value; NS, not significant; Obs, observed value; WAE, weeks after crop emergence.

^cA 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:

$$Exp = (X + Y) - (XY)/100$$
 [1]

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

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

$$Exp = XY/C$$
[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 $(134 + 106 \text{ and } 268 + 211 \text{ g ai } ha^{-1})$ at 4 WAE, and the highest rate $(268 + 211 \text{ g ai } ha^{-1})$ evaluated at 8 WAE (Table 2). Soybean injury from pyroxasulfone/flumioxazin was transient with $\leq 2\%$ 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 + 35g ai ha⁻¹did 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

Weans followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, $\alpha = 0.05$ Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.

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 values were calculated using Colby's equation [E = X + Y) - (XY)/100]

NS NS NS

71 c 85 b 92 ab 98 a

NS NS NS

75 c 86 b 93 ab 98 a

> NS NS > NS

77 b 88 ab 95 a 99 a

NS NS NS

ס ס ס ס

88 94 97

 $\begin{array}{r} 45 + 35 \\ 89 + 70 \\ 134 + 106 \end{array}$

Pyroxasulfone/flumioxazin

268 + 21

	Rate	Biomass				Density			
Common name		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	0	205 a	0		299 a				
Pyroxasulfone	45	151 a			71 b				
2	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		

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

^aMeans followed by the same letter within column do not significantly differ from each other according to Tukey-Kramer's multiple range test, $\alpha = 0.05$.

^bAbbreviations: DM, dry matter; Exp, expected value; NS, not significant; Obs, observed value.

^cAn 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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