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When using glyphosate plus dicamba, 2,4-D, halauxifen or pyraflufen/2,4-D for glyphosate-resistant horseweed (*Erigeron canadensis*) control in soybean, which third mix partner is better, saflufenacil or metribuzin?

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Abstract

Glyphosate-resistant (GR) horseweed interference in soybean can reduce soybean yield up to 93%. Glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl or pyraflufen-ethyl/2,4-D applied preplant (PP) provide variable GR horseweed control in soybean. The objective of this study was to determine if the addition of saflufenacil or metribuzin to glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D will improve the level and consistency of GR horseweed control. Four trials were conducted over the 2020 and 2021 field seasons in fields with GR horseweed populations. Glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D controlled GR horseweed 96%, 77%, 71%, and 52%, respectively, at 8 wk after application (WAA). When saflufenacil or metribuzin was added to glyphosate plus dicamba or 2,4-D ester, GR horseweed control was not improved at 8 WAA. When saflufenacil or metribuzin was added to glyphosate plus halauxifen-methyl, GR horseweed control improved by 27% and 25%, respectively, at 8 WAA. When saflufenacil or metribuzin was added to glyphosate plus pyraflufen-ethyl/2,4-D, GR horseweed control was improved by 47% and 37%, respectively, at 8 WAA. The consistency of GR horseweed control was improved when saflufenacil or metribuzin was added to glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D compared to each herbicide applied alone. Synergism was observed when metribuzin was added to glyphosate plus halauxifen-methyl and when saflufenacil or metribuzin was added to glyphosate plus pyraflufen-ethyl/2,4-D at 8 WAA. Though GR horseweed control was improved with the addition of saflufenacil or metribuzin to glyphosate plus halauxifen-methyl or pyraflufen-ethyl/2,4-D, all treatments including saflufenacil resulted in the highest level and most consistent control.

Introduction

Horseweed is a broadleaf weed from the Asteraceae family (Weaver 2001). Horseweed has been found in the southern parts of all Canadian provinces excluding Newfoundland (Weaver 2001). It is now considered a cosmopolitan weed that is found most commonly in the north temperate geographical zone (Holm et al. 1997). The first glyphosate-resistant (GR) horseweed biotype was discovered in a soybean field in the US state of Delaware in 2000 (VanGessel 2001). In Canada, the first report of GR horseweed was in Essex County, ON, in 2010 (Byker et al. 2013d). As of 2015, 30 Ontario counties have been confirmed with GR horseweed (Budd et al. 2017).

Horseweed is a facultative winter annual with nondormant seeds that can emerge in the fall or spring (Weaver 2001). Fall-emerged horseweed first establishes a basal rosette, and the stem elongates in the spring (Loux et al. 2006; Weaver 2001). One study observed that an individual GR horseweed plant produced nearly 200,000 seeds in a noncompetitive environment (Bhowmik and Bekech 1993). More recently, Davis et al. (2009) observed a multiple-resistant horseweed plant that produced 1 million seeds in a no-till field, following a 2,4-D application. The seeds are about 1 to 2 mm long with a pappus approximately 3 to 5 mm long (Frankton and Mulligan 1987). The pappus resembles a parachute, allowing the seed to undergo wind dispersal (Frankton and Mulligan 1987). Seeds have been collected hundreds of kilometers from the mother plant (Shields et al. 2006); however, most seeds fall within 100 m. Horseweed has a low outcrossing rate of 4% despite producing approximately 95,000 pollen grains per day

(Smisek 1995). This substantial release of pollen could also contribute to the spread of resistant traits (Ye et al. 2016).

If no control strategies are implemented, GR horseweed interference can decrease cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean yield up to 46%, 69%, and 93%, respectively (Byker et al. 2013b; Ford et al. 2014; Steckel and Gwathmey 2009). In conventional tillage systems, small plants can be controlled using aggressive fall and/or spring tillage (Kapusta 1979). In no-tillage crop production systems, the use of herbicides is imperative to manage GR horseweed (Bruce and Kells 1990). Generally, only preplant (PP) and preemergence (PRE) herbicides are effective for providing control of GR horseweed in soybean, because postemergence herbicides provide limited control (Byker et al. 2013c). Using PP or PRE herbicide mixes that consist of multiple effective modes of action can broaden the range of weeds controlled and slow the onset of herbicide resistance (Green and Owen 2011; Loux et al. 2006).

Variable GR horseweed control has been reported with glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D applied PP in soybean. Byker et al. (2013b) observed that glyphosate (900 g ae ha^{-1}) plus dicamba (300 g ae ha^{-1}) and glyphosate (900 g ae ha^{-1}) plus 2,4-D ester (560 g ae ha^{-1}) applied PP to soybean controlled GR horseweed 68% to 100% and 73% to 95%, respectively, at 8 WAA. Soltani et al. (2020a) and Zimmer et al. (2018) reported 71% and 87% GR horseweed control, respectively, with glyphosate (900 g ae ha^{-1}) plus halauxifen-methyl (5 g ai ha^{-1}) applied PP to soybean. Soltani et al. (2020a) reported that glyphosate (900 g ae ha^{-1}) plus pyraflufen-ethyl/2,4-D (532 g ai ha^{-1}) applied PP controlled GR horseweed 60% at 8 WAA. The aforementioned studies confirm variable GR horseweed control when glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D is applied PP to soybean.

The addition of an effective third herbicide mix partner such as saflufenacil or metribuzin may improve the level and consistency of GR horseweed control (Mellendorf et al. 2013). In addition, three-way mixes that include different effective modes of action can be useful to delay the evolution of herbicide resistance (Monks et al. 1993; Scott et al. 1998). Glyphosate (900 g ae ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) plus dicamba (600 g ae ha^{-1}) or 2,4-D ester (500 g ae ha^{-1}) controlled GR horseweed 98% and 95%, respectively, at 8 WAA (Budd et al. 2016). Similarly, Zimmer et al. (2018) observed that glyphosate (1,120 g ae ha^{-1}) plus 2,4-D (560 g ae ha^{-1}) plus either metribuzin (210 g ai ha^{-1}) or saflufenacil (37 g ai ha^{-1}) controlled GR horseweed 82% and 97%, respectively, at 5 WAA. Glyphosate (900 g ae ha^{-1}) plus metribuzin (400 g ai ha^{-1}) plus pyraflufen-ethyl/2,4-D (532 g ai ha^{-1}), applied PP, controlled GR horseweed 96% at 8 WAA (Soltani et al. 2020a). GR horseweed control with three-way mixes including saflufenacil or metribuzin appear to improve GR horseweed control, but further investigation is needed to see which provide better and more consistent GR horseweed control.

Variable GR horseweed control has been reported with glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D in soybean. With the aforementioned two-way mixes, it is not known whether the addition of saflufenacil or metribuzin would be the better third mix partner. The objective of this research was to ascertain if saflufenacil or metribuzin is a better mix partner with glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl or pyraflufen-ethyl/2,4-D, applied PP, for GR horseweed control in soybean. Our hypothesis is that the addition of saflufenacil or metribuzin to glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D, applied PP, will provide better and more consistent GR horseweed control in soybean.

Materials and Methods

Experimental Methods

Field research trials were completed over a 2-yr period at four different site locations in southwestern Ontario, Canada. Two trials were conducted in 2020, and two were conducted in 2021. The site, year, the nearest town to the site location, location coordinates, soil information, weather at the treatment application, treatment spray date, and soybean seeding and emergence are presented in Table 1. The horseweed resistance profile for each location was confirmed in greenhouse screenings. Horseweed seed was collected randomly from multiple plants at each location. Transplanting flats (25 cm × 25 cm × 5 cm) were filled with potting mix (Berger Growing Media with sphagnum peat moss, perlite, wetting agent, dolomitic and calcitic limestone) and were watered until the soil was completely saturated. Horseweed seeds (approximately 300) were sprinkled onto the soil surface. Approximately 1 mm of the potting mix was used to cover the seed on the soil surface. The trays remained in the greenhouse (16-h photoperiod with 26 C day and 17 C night temperatures) and were watered daily with approximately 20 ml of water. Once the seedlings had at least four leaves, 100 horseweed plants from each population were transplanted into individual circular pots measuring 10 cm diam. Once the horseweed reached 10 cm diam, 40 horseweed plants from each population were sprayed with glyphosate (900 g ae ha^{-1}), and another 40 were sprayed with cloransulam-methyl (17.5 g ai ha^{-1}). The horseweed was sprayed in a spray chamber equipped with flat-fan nozzles calibrated to deliver 205 L ha^{-1} at 2.6 km h^{-1} and 280 kPa. Two untreated checks for every 10 horseweed plants were used as comparisons to conduct the visible-control ratings. Visible-control ratings were completed 1, 3, and 5 WAA with a 0% to 100% scale; 0% represented no control, 100% represented complete necrosis (Canadian Weed Science Society 2018). The values in Table 2 represent the percent of horseweed resistant to glyphosate and cloransulam-methyl at each location at 5 WAA. Seed was not collected at the Bothwell site, and therefore resistance screening was not conducted for this location.

The study was a 3-by-5 factorial structured as a randomized complete block design with four replications. Factor One was the Group 4 herbicides control, dicamba, 2,4-D ester, halauxifen-methyl, and pyraflufen-ethyl/2,4-D, and Factor Two was control, saflufenacil, and metribuzin. Glyphosate (900 g ae ha^{-1}) was included in each herbicide treatment to remove the confounding effects of glyphosate-susceptible horseweed and other weed species. There were a total of 14 treatments plus one weedy control. The plot width was 2.25 m, encompassing three soybean rows with 0.75-m spacings. The plot length was 8 m, and a 2-m alleyway separated each replicate. A CO_2-pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 240 kPa was used to make the PP herbicide applications when horseweed approached 10 cm in height or diameter. The handheld boom measured 1.5 m in length equipped with 4 ULD 120-02 nozzles spaced 50 cm apart. The spray width was 2 m. Roundup Ready 2 Xtend® soybean cultivar (DKB12-16) was seeded to a 3.8-cm depth at about 416,000 seeds ha^{-1} following the PP herbicide applications. Glyphosate (450 g ae ha^{-1}) was applied postemergence to the entire experimental area to control glyphosate-susceptible horseweed

Table 1. The site, year, the nearest town to the site location, location coordinates, soil characteristics, weather at the treatment application, treatment spray date, and soybean seeding and emergence dates for four field trials conducted in southwestern Ontario, Canada in 2020 and 2021.

				Soil characteristics						Weather at treatment application			Agronomic information		
Site	Year	Nearest town	Location coordinates	Texture	Sand	Silt	Clay	OM[a]	pH	Air temperature	Relative humidity	Wind speed	Treatment spray date	Soybean seeding date	Soybean emergence date
						%				C	%	km h^{-1}			
S1	2020	Ridgetown	42.46° N, 81.85° W	Sandy loam	75	17	7	1.9	7.1	25	60	7.5–8.8	May28	June 5	June 11
S2	2020	Zone Centre	42.64° N, 81.94° W	Loamy sand	85	9	5	2.9	6.5	25	26	3.6–4.8	June 17	June 24	July 3
S3	2021	Kintyre	42.56° N, 81.77° W	Sandy loam	53	29	18	4.4	6.9	21	50	0.5–1.2	May 19	May 20	May 25
S4	2021	Bothwell	42.62° N, 81.91° W	Loamy Sand	85	11	4	3.3	6.8	20	62	1.5–5.6	May 27	June 12	June 18

[a]Abbreviations: OM, organic matter.

Table 2. The site, year, the nearest town to the site location, glyphosate-resistant (GR) horseweed size and density at the time of the treatment application, and resistance profile for four field trials in southwestern Ontario, Canada in 2020 and 2021.

			GR horseweed		Resistance profile	
Site	Year	Nearest town	Size	Density	Glyphosate	Cloransulam-methyl
			cm	plants m^{-2}	%	
S1	2020	Ridgetown	6	384	100	99
S2	2020	Zone Centre	10	57	79	100
S3	2021	Kintyre	8	147	98	85
S4	2021	Bothwell	9	46	–	–

Table 3. Herbicides and surfactants used in four field trials examining the control of glyphosate-resistant horseweed using herbicide mixes in southwestern Ontario, Canada in 2020 and 2021.

Common name	Trade name	Manufacturer
Herbicides		
Metribuzin	Sencor 480SC	Bayer CropScience Inc., Calgary, AB
Saflufenacil[a]	Eragon LQ	BASF Canada Inc., Mississauga, ON
Pyraflufen-ethyl/2,4-D	Blackhawk	Nufarm Canada, Calgary, AB
Halauxifen-methyl[b]	Elevore	Dow AgroSciences Canada Inc. Calgary, AB
2,4-D ester	Ester 700	Nufarm Canada
Dicamba	Xtendimax	Bayer CropScience Inc.
Surfactants		
Nonionic surfactant	Merge	BASF Canada Inc.
Methylated seed oil	MSO concentrate	Dow AgroSciences Canada Inc.

[a]The surfactant Merge (1 L ha^{-1}) was included in treatments with saflufenacil.
[b]The surfactant MSO (1% v/v) was included in all treatments with halauxifen-methyl.

plus other weed species. The postemergence application was applied when soybean was at the V1 to V4 growth stages at all locations. Horseweed size and density at the time of the PP treatment application are included in Table 2. The herbicides used in this study are included in Tables 3 and 4.

A visual rating (%) was used to assess horseweed control 2, 4, and 8 WAA using a rating scale of 0 to 100; 0 indicated no control, and 100 indicated plant death (Canadian Weed Science Society 2018). Horseweed density was collected 8 WAA. Quadrats measuring 0.25 m^2 were placed randomly in the plot–one quadrat in the front and one in the back. Plants were counted in each quadrat to determine density. Plant biomass was assessed 8 WAA by cutting all plants within both quadrats close to the soil surface, putting all samples in bags, and drying the samples in a kiln at 60 C for approximately 2 wk or until the samples reached stable moisture and the biomass was recorded. Soybean injury (%) was visually evaluated 2 and 4 wk after soybean emergence using a rating scale of 0 to 100; 0 indicated no soybean injury and 100 indicated soybean death (Canadian Weed Science Society 2018). A small-plot harvester combined two soybean rows per plot and recorded soybean moisture and weight. Before analysis, soybean grain yield was corrected to 13.5% moisture content.

Statistical Analysis

PROC GLIMMIX in SAS version 9.4 (Statistical Analysis Systems 2020) was used for the analyses. The study was a mixed model with

Table 4. Herbicide treatments and rates used in the present study for glyphosate-resistant horseweed control in southwestern Ontario, Canada in 2020 and 2021.

Treatment[a]	Rate
	g ai/ae ha^{-1}
Saflufenacil[b]	25
Metribuzin	400
Dicamba	600
Dicamba + saflufenacil	600 + 25
Dicamba + metribuzin	600 + 400
2,4-D ester	528
2,4-D ester + saflufenacil	528 + 25
2,4-D ester + metribuzin	528 + 400
Halauxifen-methyl[c]	5
Halauxifen-methyl + saflufenacil	5 + 25
Halauxifen-methyl + metribuzin	5 + 400
Pyraflufen-ethyl/2,4-D	532
Pyraflufen-ethyl/2,4-D + saflufenacil	532 + 25
Pyraflufen-ethyl/2,4-D + metribuzin	532 + 400

[a]Glyphosate (900 g ae ha^{-1}) was included in all herbicide treatments.
[b]The surfactant Merge (1 L ha^{-1}) was included in all treatments with saflufenacil.
[c]The surfactant MSO (1 % v/v) was included in all treatments with halauxifen-methyl.

a fixed effect (saflufenacil or metribuzin, Group 4 herbicide, saflufenacil or metribuzin by Group 4 herbicide) and random effects (location, block within the location, location-by-saflufenacil or metribuzin, location-by-Group 4 herbicide). Normality was confirmed after conducting the Shapiro-Wilk test and reviewing studentized residual plots. An arcsine square-root back-transformation was used for control 2, 4, and 8 WAA. A log-normal distribution was used for density and biomass. Soybean yield was analyzed using a normal distribution. The least-square means were analyzed in the analysis format then converted back to the data format using the ilink option except when log-normal was specified, which used the omega procedure to back-transform means. Tukey-Kramer's multiple-range test ($\alpha = 0.05$) was used to compare the least-square means. Letter codes were assigned in Tables 5 and 6 to indicate significant data.

A common method to investigate herbicide interactions is with Colby's equation (Colby 1967). Because glyphosate (900 g ae ha^{-1}) is not effective on GR horseweed and was only included in the tank to control glyphosate-susceptible horseweed and other weed species, only two-way interactions between the Group 4 herbicides and saflufenacil or metribuzin were evaluated. Previous literature used the two-way Colby's equation to determine the expected control of a three-way mix when one of the herbicides was not effective on the weed of interest (Meyer and Norsworthy 2019). The expected control mean was calculated using the observed control means for A (Group 4 herbicide) and B (Saflufenacil or metribuzin) in the Colby's equation.

$$\text{Expected} = (A + B) - \left(\frac{A \times B}{100}\right) \qquad (1)$$

The expected density and biomass data were calculated using an adjusted Colby's equation. The nontreated control mean was represented as W in the equation.

$$\text{Expected} = \left(\frac{A \times B}{W}\right) \qquad (2)$$

A t-test was used to compare the observed and expected values. Significance was noted when $P < 0.05$. If the control values differ from one another (i.e., if the observed value is greater than or less than the expected value), then the interaction is considered synergistic or antagonistic, respectively. If the observed value is the same as expected, the interaction is considered additive (Colby 1967). In contrast, if the density or biomass values differ from one another (i.e., if the observed value is greater than or less than the expected value), then the interaction is considered antagonistic or synergistic, respectively (Colby 1967).

The coefficient of variation (CV) was calculated to determine the consistency of GR horseweed control for each least-square mean estimate. When comparing treatments, a lower CV would indicate greater consistency in control, whereas a higher CV would indicate less consistency in control (Shechtman 2013).

Results and Discussion

Soybean Injury

There was minimal soybean injury (≤5%) at all sites across both years (data not shown).

Glyphosate-Resistant Horseweed Control, Density, and Biomass

There was a significant interaction between Factor One (control, dicamba, 2,4-D ester, halauxifen-methyl, and pyraflufen-ethyl/2,4-D) and Factor Two (control, saflufenacil, and metribuzin) for GR horseweed control 2, 4, and 8 WAA, density, and biomass, so the simple effects are presented (Table 6).

Glyphosate plus dicamba controlled GR horseweed 48%, 87%, and 96% at 2, 4, and 8 WAA, respectively, and decreased density and biomass 93% and 98%, respectively (Table 6). Byker et al. (2013b) observed that glyphosate (900 g ae ha^{-1}) plus dicamba (300 g ae ha^{-1}) provided a minimum of 68% GR horseweed control at 8 WAA. The decreases in GR horseweed density and biomass reported in this study are similar to those reported by Eubank et al. (2008) and Byker et al. (2013b), respectively. When saflufenacil was added to glyphosate plus dicamba, GR horseweed control improved by 43% at 2 WAA; control did not improve at 4 or 8 WAA, and there was no decrease in density or biomass. Similarly, Hedges et al. (2018) observed a 43% increase in GR horseweed control at 2 WAA and no reduction in density or biomass when saflufenacil (25 g ai ha^{-1}) was added to glyphosate/dicamba (1,800 g ae ha^{-1}). Based on Colby's equation, saflufenacil plus glyphosate plus dicamba was additive for control 2 and 4 WAA and antagonistic for control 8 WAA (Equation 1). The observed density and biomass were greater than the expected, indicating an antagonistic interaction (Equation 2). GR horseweed control was not improved when metribuzin was added to glyphosate plus dicamba 2, 4, or 8 WAA, and there was no reduction in density or biomass. The addition of metribuzin to glyphosate plus dicamba was additive for control 2, 4, and 8 WAA and for biomass reduction. The observed densities were greater than expected indicating an antagonistic interaction.

Glyphosate plus 2,4-D ester controlled GR horseweed 50%, 79%, and 77% at 2, 4, and 8 WAA, respectively; there was no decrease in density or biomass (Table 6). Similar control was observed by Byker et al. (2013a) at two site locations at 4 WAA, whereas Soltani et al. (2020b) observed only 53% GR horseweed control with glyphosate (900 g ae ha^{-1}) plus 2,4-D ester (500 g ae ha^{-1}) at 8 WAA. When saflufenacil was added to glyphosate plus 2,4-D ester, GR horseweed control increased by 43% at 2 WAA, and biomass was reduced 85%; however, there was no

Table 5. Main effects and interaction for glyphosate-resistant (GR) horseweed control 2, 4, and 8 wk after application (WAA), density, biomass, and soybean yield with metribuzin or saflufenacil-based mixes with Group 4 herbicides from four factorial trials conducted in southwestern Ontario, Canada in 2020 and 2021.[a,b]

Main effects	Rate	GR horseweed control 2 WAA	GR horseweed control 4 WAA	GR horseweed control 8 WAA	Density[c]	Biomass[c]	Soybean yield[d]
	g ai/ae ha^{-1}	%	%	%	No. plants m^{-2}	g m^{-2}	kg ha^{-1}
Group 4 herbicide		**	**	**	*	**	*
None	–	41	48	46	46	172	1,840 b
Pyraflufen-ethyl/2,4-D	532	80	85	85	27	68	2,340 ab
Halauxifen-methyl[e]	5	77	89	91	21	19	2,380 a
2,4-D ester	528	73	87	88	22	33	2,370 a
Dicamba	600	73	93	97	9	4	2,480 a
SE[b]		2	2	2	5	5	0
Metribuzin or saflufenacil		**	**	**	**	**	NS
None	–	34	55	59	67	87	2,080
Metribuzin	400	71	84	86	13	46	2,370
Saflufenacil[f]	25	94	97	98	5	7	2,390
SE		2	2	2	5	5	0
Group 4 herbicide × metribuzin or saflufenacil		**	**	**	**	**	NS

[a]** Statistically significant when P < 0.01; * significant when P < 0.05; NS, non-significant.
[b]Abbreviation: SE, standard error of the mean.
[c]Density and biomass were collected 8 WAA.
[d]Means accompanied by a different letter in a column (a–b) significantly differ based on Tukey-Kramer's LSD (α = 0.05).
[e]Treatments with halauxifen-methyl included the surfactant MSO (1% v/v).
[f]Treatments with saflufenacil included the surfactant Merge (1 L ha^{-1}).

improvement in control at 4 and 8 WAA and no decrease in density. Mahoney et al. (2016) reported similar results with a 98% decrease in GR horseweed biomass when saflufenacil (25 g ai ha^{-1}) was added to glyphosate (900 g ae ha^{-1}) plus 2,4-D ester (500 g ae ha^{-1}). Based on Colby's equation, the addition of saflufenacil to glyphosate plus 2,4-D ester was additive for control at 2 WAA (Equation 1) and for the reduction of density and biomass (Equation 2). The expected control values were greater than the observed control values at 4 and 8 WAA, indicating an antagonistic interaction. When metribuzin was added to glyphosate plus 2,4-D ester, GR horseweed control did not improve and there was no reduction in density and biomass. In contrast, Soltani et al. (2020b) observed a 32% and 36% improvement in GR horseweed control at 4 and 8 WAA, respectively, and a 90% and 88% decrease in GR horseweed density and biomass, respectively, when metribuzin (400 g ai ha^{-1}) was added to glyphosate (900 g ae ha^{-1}) plus 2,4-D ester (500 g ae ha^{-1}). Based on Colby's equation, metribuzin plus glyphosate plus 2,4-D ester was additive for control at 2 and 8 WAA and for the reduction in density and biomass. The observed control values were less than the expected control values at 4 WAA, indicating an antagonistic interaction.

Glyphosate plus halauxifen-methyl controlled GR horseweed 40%, 63%, and 71% at 2, 4, and 8 WAA, respectively, and did not reduce density and biomass (Table 6). Similar control was observed by Soltani et al. (2020a) at 8 WAA, but contrasts with Zimmer et al. (2018), who observed 87% GR horseweed control using glyphosate (560 g ae ha^{-1}) plus halauxifen-methyl (5 g ai ha^{-1}) at 5 WAA. In the present study, when saflufenacil was added to glyphosate plus halauxifen-methyl, GR horseweed control improved by 57%, 36%, and 27% at 2, 4, and 8 WAA, respectively, and decreased GR horseweed density and biomass by 95% and 92%, respectively (Table 6). Similarly, Quinn et al. (2021) observed 91% GR horseweed control using glyphosate (900 g ae ha^{-1}) plus halauxifen-methyl (5 g ai ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) when applied PP in soybean at 8 WAA and a 97% and 98% decrease in density and biomass, respectively. Based on Colby's equation, the addition of saflufenacil to glyphosate plus halauxifen-methyl was additive for control 4 and 8 WAA and synergistic at 2 WAA; the interaction for density and biomass reduction was additive (Equations 1 and 2). When metribuzin was added to glyphosate plus halauxifen-methyl, GR horseweed control improved by 43%, 31%, and 25% at 2, 4, and 8 WAA, respectively, and decreased density and biomass 94% and 86%, respectively. Similar results were reported by Quinn et al. (2021), who observed 93% GR horseweed control in soybean at 8 WAA and a 98% and 99% reduction in density and biomass, respectively, with glyphosate (900 g ae ha^{-1}) plus halauxifen-methyl (5 g ai ha^{-1}) plus metribuzin (400 g ai ha^{-1}). Based on Colby's equation, the improvement in GR horseweed control 2, 4, and 8 WAA and the decrease in density and biomass was synergistic when metribuzin was added to glyphosate plus halauxifen-methyl (Equations 1 and 2).

Glyphosate plus pyraflufen-ethyl/2,4-D controlled GR horseweed 48%, 55%, and 52% at 2, 4, and 8 WAA, respectively; there was no reduction in density and biomass (Table 6). When saflufenacil was added to glyphosate plus pyraflufen-ethyl/2,4-D, GR horseweed control improved by 49%, 44%, and 47% at 2, 4, and 8 WAA, respectively, and decreased density and biomass by 97% and 95%, respectively. Based on Colby's equation, the improvement in GR horseweed control at 2 and 8 WAA from the addition of saflufenacil to glyphosate plus pyraflufen-ethyl/2,4-D was synergistic; the interactions for control at 4 WAA and density and biomass were additive. When metribuzin was added to glyphosate plus pyraflufen-ethyl/2,4-D, GR horseweed control improved by 36%, 34%, and 37% at 2, 4, and 8 WAA, respectively, and decreased density and biomass by 89% and 93%, respectively. Similarly, Soltani et al. (2020a) observed 95% GR horseweed control and a 98% and 97% density and biomass reduction, respectively, with glyphosate (900 g ae ha^{-1}) plus pyraflufen-ethyl/2,4-D (532 g ai ha^{-1}) plus metribuzin (400 g ai ha^{-1}) applied PP in soybean at 8 WAA. Based on the Colby's equation, the improvement in GR horseweed control 2, 4, and 8 WAA and the decrease in biomass was synergistic when metribuzin was added to glyphosate plus pyraflufen-ethyl/2,4-D; the interaction for density was additive.

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Table 6. The level of glyphosate-resistant horseweed control 2, 4, and 8 wk after application (WAA), density, and biomass from four factorial trials in southwestern Ontario, Canada in 2020 and 2021.[a,b,c,d]

Group 4 herbicide	Metribuzin or saflufenacil: None	Metribuzin	Saflufenacil[e]	SE
Weed control 2 WAA	——————%——————			
None	0b X	43b Y	91a Z	6
Pyraflufen-ethyl/2,4-D	48a Y	84a Z (69)**	97a Z (88)*	4
Halauxifen-methyl[c]	40a Y	83a Z (65)*	97a Z (87)*	4
2,4-D ester	50a Y	69a Y (70)	93a Z (89)	4
Dicamba	48a Y	70a Y (68)	94a Z (88)	4
SE	3	3	2	
Weed control 4 WAA				
None	0c X	51b Y	96a Z	6
Pyraflufen-ethyl/2,4-D	55b Y	89a Z (74)*	99a Z (96)	4
Halauxifen-methyl[f]	63b Y	94a Z (79)*	99a Z (96)	3
2,4-D ester	79ab Z	85a Z (89)*	96a Z (98)**	2
Dicamba	87a Z	91a Z (90)	98a Z (98)	2
SE	4	3	1	
Weed control 8 WAA				
None	0c X	46b Y	95a Z	6
Pyraflufen-ethyl/2,4-D	52b Y	89a Z (73)*	99a Z (94)*	4
Halauxifen-methyl[f]	71b Y	96a Z (86)**	98a Z (97)	2
2,4-D ester	77b Z	88ab Z (86)	96a Z (97)*	2
Dicamba	96a Z	96ab Z (98)	98a Z (100)**	1
SE	4	3	1	
Density	——————No. plants m^{-2}——————			
None	130b Y	28a ZY	8a Z	17
Pyraflufen-ethyl/2,4-D	96b Y	11a ZY (43)	3a Z (7)	12
Halauxifen-methyl[f]	77b Y	5a ZY (23)*	4a Z (10)	10
2,4-D ester	63ab Z	13a Z (16)	6a Z (6)	10
Dicamba	9a Z	15a Z (5)*	6a Z (1)*	2
SE	13	2	1	
Biomass	——————g m^{-2}——————			
None	188b Z	206b Z	25a Y	16
Pyraflufen-ethyl/2,4-D	114b Z	8a Y (97)*	6a Y (19)	10
Halauxifen-methyl[f]	50b Z	7a Y (30)*	4a Y (15)	5
2,4-D ester	53b Z	38a Z (51)	8a Z (12)	5
Dicamba	3a Z	8a Z (2)	2a Z (1)*	1
SE	9	10	3	

[a]Abbreviations: SE, standard error of the mean.
[b]Means accompanied with a different letter in a column (a–c) or row (X–Z) within each section significantly differ based on Tukey-Kramer's LSD (α = 0.05).
[c]** Significant at P < 0.01; * significant at P < 0.05 based on a t-test comparing observed and expected values.
[d]Values in parentheses represent the expected values from Colby's analysis.
[e]Treatments with saflufenacil included the surfactant Merge (1 L ha^{-1}).
[f]Treatments with halauxifen-methyl included the surfactant MSO (1% v/v).

Glyphosate plus saflufenacil controlled GR horseweed 91%, 96%, and 95% at 2, 4, and 8 WAA, respectively, and decreased density and biomass 94% and 87%, respectively (Table 6). Eubank et al. (2013) reported similar findings at 3 WAA, but the results from this study are in contrast to Ikley (2012), who observed 57% GR horseweed control with glyphosate (874 g ae ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) at 5 WAA. There was no improvement in GR horseweed control when dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D were added to glyphosate plus saflufenacil 2, 4, or 8 WAA, and there was no decrease in density or biomass. Similarly, Budd et al. (2016) observed no improvement in GR horseweed control at 4 and 8 WAA and no reduction in density when dicamba (600 g ae ha^{-1}) or 2,4-D ester (500 g ae ha^{-1}) were added to glyphosate (900 g ae ha^{-1}) plus saflufenacil (25 g ai ha^{-1}). In the present study, all mixes that included saflufenacil provided the fastest GR horseweed control, with greater than 90% control at 2 and 4 WAA.

Glyphosate plus metribuzin controlled GR horseweed 43%, 51%, and 46% at 2, 4, and 8 WAA, respectively; density and biomass were not reduced (Table 6). Similar results were reported by Eubank et al. (2008) but are in contrast to Byker et al. (2013a), who observed a minimum of 97% GR horseweed control using glyphosate (900 g ae ha^{-1}) plus metribuzin (1,120 g ai ha^{-1}) at 8 WAA. The improved GR horseweed control in the Byker et al. (2013a) study can be ascribed to the greater rate of metribuzin used in that study. When dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D were added to glyphosate plus metribuzin, GR horseweed control improved by 27%, 26%, 40%, and 41% at 2 WAA, 40%, 34%, 43%, and 38% at 4 WAA, and 50%, 42%, 50%, and 43% at 8 WAA, respectively. When dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D were added to glyphosate plus metribuzin, GR horseweed biomass was reduced by 96%, 82%, 97%, and 96%, respectively; however, there was no decrease in GR horseweed density.

Table 7. The consistency of glyphosate-resistant horseweed control 2, 4, and 8 wk after application (WAA) from four factorial trials in southwestern Ontario, Canada in 2020 and 2021.

Group 4 herbicide	Saflufenacil or metribuzin: None	Metribuzin	Saflufenacil[a]
Consistency of control 2 WAA	——Coefficient of variation——		
None	–	87.1	49.6
Pyraflufen-ethyl/2,4-D	81.6	54.1	44.9
Halauxifen-methyl[b]	90.6	54.7	45.1
2,4-D ester	80.0	63.4	47.9
Dicamba	81.5	62.8	47.4
Consistency of control 4 WAA			
None	–	67.9	38.9
Pyraflufen-ethyl/2,4-D	64.9	42.9	36.3
Halauxifen-methyl[b]	57.2	39.2	36.5
2,4-D ester	45.4	43.9	37.9
Dicamba	41.8	40.4	36.6
Consistency of control 8 WAA			
None	–	63.3	37.7
Pyraflufen-ethyl/2,4-D	60.9	41.1	34.6
Halauxifen-methyl[b]	50.5	35.7	33.8
2,4-D ester	44.8	39.9	35.6
Dicamba	37.7	36.2	34.5

[a]Treatments with saflufenacil included the surfactant Merge (1 L ha^{-1}).
[b]Treatments with halauxifen-methyl included the surfactant MSO (1 % v/v).

Glyphosate-Resistant Horseweed Consistency in Control

At 2, 4, and 8 WAA, adding saflufenacil or metribuzin to glyphosate plus dicamba, 2,4-D ester, halauxifen-methyl, or pyraflufen-ethyl/2,4-D improved the consistency of GR horseweed control (Table 7). The addition of saflufenacil consistently reduced the CV more than metribuzin, indicating improved consistency of GR horseweed control with saflufenacil-based mixes relative to metribuzin-based mixes. Similar to the current study, Budd et al. (2016) observed that when a third herbicide such as metribuzin (400 g ai ha^{-1}) was added into the tank with glyphosate (900 g ae ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) applied PP in soybean, there was improved consistency of GR horseweed control. Soltani et al. (2020b) also reported improved consistency of GR horseweed control when metribuzin (400 g ai ha^{-1}) was added to glyphosate (900 g ae ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) or 2,4-D ester (500 g ae ha^{-1}).

Soybean Yield

Soybean yield was reduced up to 26% due to GR horseweed interference. The main effects are presented (Table 5), as there was no interaction between Factor One (control, dicamba, 2,4-D ester, halauxifen-methyl, and pyraflufen-ethyl/2,4-D) and Factor Two (control, saflufenacil, and metribuzin) on soybean yield. When averaged across Factor Two, reduced GR horseweed interference with the application of dicamba, 2,4-D ester, or halauxifen-methyl resulted in a soybean yield increase of 38% to 40% (Table 5). Similar soybean yields were found across all herbicide treatments. Soltani et al. (2020a) reported a similar soybean yield loss due to GR horseweed interference. In contrast, Eubank et al. (2008) reported a high soybean yield reduction of 97% from GR horseweed interference.

In conclusion, the addition of saflufenacil or metribuzin to glyphosate plus halauxifen-methyl or pyraflufen-ethyl/2,4-D applied PP in soybean improved the level and consistency of GR horseweed control at 8 WAA. GR horseweed control was not improved when saflufenacil or metribuzin was added to glyphosate plus dicamba or 2,4-D ester, though the consistency of control was improved. When saflufenacil was added to glyphosate plus halauxifen-methyl or pyraflufen-ethyl/2,4-D, there was reduced GR horseweed density and biomass. When metribuzin was added to glyphosate plus halauxifen-methyl or pyraflufen-ethyl/2,4-D, there was reduced GR horseweed biomass. However, GR horseweed control was improved with the addition of saflufenacil or metribuzin to glyphosate plus halauxifen-methyl or pyraflufen-ethyl/2,4-D; all treatments including saflufenacil resulted in the highest level and most consistent control.

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