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The addition of very low rates of protoporphyrinogen oxidase–inhibiting herbicides to glufosinate does not improve control of glyphosate-resistant horseweed (*Erigeron canadensis*)

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Abstract

Recent research reported synergism between glufosinate plus very low rates of protoporphyrinogen oxidase (PPO)–inhibiting herbicides on select broadleaf weeds. Two field studies, each consisting of four trials, were conducted in 2020 and 2021 in commercial fields with glyphosate-resistant (GR) horseweed in Ontario, Canada. Study 1 evaluated GR horseweed control with glufosinate plus five PPO inhibitors at 5% of the label rate; study 2 evaluated what dose of saflufenacil is needed when co-applied with glufosinate to improve GR horseweed control. In study 1, glufosinate plus very low rates of PPO-inhibiting herbicides provided low GR horseweed control. At site 1, despite the synergistic increase in GR horseweed control with saflufenacil (1.25 g ai ha^{-1}) plus glufosinate (300 g ai ha^{-1}), the level of control did not exceed 42% at 2 and 4 wk after application (WAA); the interaction was additive at 8 WAA. The co-application of glufosinate (300 g ai ha^{-1}) with pyraflufen-ethyl (0.34 g ai ha^{-1}), pyraflufen-ethyl/2,4-D (26.4 g ai ha^{-1}), flumioxazin (5.35 g ai ha^{-1}), fomesafen (12 g ai ha^{-1}), or sulfentrazone (7 g ai ha^{-1}) resulted in an additive interaction for GR horseweed control at 2, 4, and 8 WAA. However, glufosinate plus pyraflufen-ethyl or sulfentrazone was antagonistic at 8 WAA. In study 2, similar doses of saflufenacil were required for 50%, 80%, and 95% GR horseweed control whether glufosinate was included in the mixture or not. Interactions between glufosinate (300 g ai ha^{-1}) plus saflufenacil at 1.56, 3.13, 6.25, and 12.5 g ai ha^{-1} were antagonistic at 2, 4, and 8 WAA at sites 1, 2, and 3; all other interactions were additive. The results of this research indicate there was little to no benefit of adding very low rates of PPO-inhibiting herbicides to glufosinate to improve GR horseweed control under field conditions.

Introduction

Horseweed is a weed commonly found in no-tillage crop production systems. Horseweed has an elongated emergence period (Nandula et al. 2006), is a prolific seed producer (Bhowmik and Bekech 1993; Davis et al. 2009), and produces wind-disseminated seeds that can move long distances (Shields et al. 2006). Horseweed seed is nondormant and can germinate within 0.3 cm of the soil surface (Buhler and Owen 1997), which makes it well-adapted to no-tillage crop production systems (Nandula et al. 2006). The ruderal nature of horseweed allows it to colonize undisturbed land, including fields with reduced or no tillage, vineyards, orchards, roadsides, or pastures (Weaver 2001). Horseweed emergence occurs primarily during two periods: April to May or September to October in Ontario, Canada (Koger et al. 2005; Tozzi and Van Acker 2014). Horseweed was the first broadleaf weed to evolve glyphosate resistance (VanGessel 2001) and is among the top 10 most troublesome and common weeds found in broadleaf crops in Canada and the United States (Van Wychen 2016). Ontario farmers consider horseweed to be one of the most challenging weeds to manage (Fraser 2019).

Soybean is a valuable crop in Canada. In the 2021 growing season, over 2 million hectares were seeded in Canada; nearly 1.2 million hectares were seeded in Ontario (SOY Canada 2021). If GR horseweed is left uncontrolled in soybean, up to 93% yield loss can result (Byker et al. 2013a). Mechanical control such as tillage can be used as a weed management strategy in conventional tillage systems, but in no-tillage systems, chemical control is commonly used. Postemergence herbicides provide limited and inconsistent GR horseweed control in

identity-preserved (IP, non-genetically modified) or GR soybean (Byker et al. 2013b). Improved GR horseweed control has been reported with preplant (PP) or preemergence herbicides in soybean (Byker et al. 2013b).

Glufosinate is a glutamine synthetase–inhibiting herbicide from the organophosphorus chemical family (Zhou et al. 2020). Glufosinate is a fast-acting, nonselective herbicide (Feng et al. 2010) with activity on young annual grass and broadleaf weeds (Dayan et al. 2009; Steckel et al. 1997). As glufosinate has contact activity, thorough spray coverage is crucial for effective weed control, especially when weed density is high (Eubank et al. 2008). The fast phytotoxicity of glufosinate has recently been attributed to the production of reactive oxygen species driven by light-dependent reactions, leading to lipid peroxidation of the cell membranes and subsequent plant death (Takano et al. 2019, 2020a). Studies have shown that glufosinate is most efficacious when applied at midday (Cánovas et al. 1986; Martinson et al. 2005; Sellers et al. 2004), under warm air temperatures (Kumaratilake and Preston 2005), high humidity (Coetzer et al. 2001), and full-sunlight conditions (Takano et al. 2019, 2020b).

Protoporphyrinogen oxidase (PPO)–inhibiting herbicides are also known as protox-inhibiting herbicides. PPO is an essential enzyme for catalyzing the synthesis of protoporphyrin IX from protoporphyrinogen IX and subsequent biosynthesis of heme and chlorophyll (Lermontova et al. 1997). The PPO enzyme has two isoforms, PPXI and PPXII, found in the chloroplasts and mitochondria, respectively (Dayan et al. 2018; Watanabe et al. 2001). PPO inhibition results in a buildup and leakage of protoporphyrinogen IX into the cytoplasm, where it is converted to protoporphyrin IX and forms singlet oxygen radicals in the presence of light (Matringe et al. 1989). The formation of singlet oxygen radicals and subsequent lipid peroxidation of the cell membranes drives the fast activity of the PPO-inhibitors (Dayan et al. 2019). PPO-inhibitors provide broad-spectrum control of young annual dicots and some monocot species (Hao et al. 2011).

Recent research by Takano et al. (2020c) demonstrated improved control of kochia [*Bassia scoparia* (L.) A.J. Scott] and Palmer amaranth [*Amaranthus palmeri* (S.) Watson] when glufosinate was mixed with very low rates (≤5% of field rates) of PPO-inhibiting herbicides. The mixture of glufosinate (420 g ai ha^{-1}) with pyraflufen-ethyl (0.2 g ai ha^{-1}), saflufenacil (1 g ai ha^{-1}), flumioxazin (2.5 g ai ha^{-1}), lactofen (4.2 g ai ha^{-1}), or fomesafen (7.1 g ai ha^{-1}) enhanced injury on kochia relative to glufosinate and the PPO-inhibiting herbicides applied alone. Glufosinate (420 g ai ha^{-1}) plus saflufenacil (1 g ai ha^{-1}) provided the greatest level of injury to kochia in field, greenhouse, and lab; the herbicides applied individually provided approximately 25% control of kochia, whereas the mixture improved control to approximately 80% (Takano et al. 2020c). Takano et al. (2020c) reported enhanced control of Palmer amaranth when glufosinate (280 g ai ha^{-1}) was mixed with saflufenacil (1 g ai ha^{-1}); the mixture required a lower effective rate to achieve 50% control compared to glufosinate and saflufenacil applied individually. In the same study, Takano et al. (2020c) reported that adding saflufenacil (1 g ai ha^{-1}) to glufosinate (280 g ai ha^{-1}) enhanced Palmer amaranth control during unfavorable environmental conditions relative to glufosinate applied alone. During favorable conditions (25 C air temperature and 70% relative humidity), 100% control of Palmer amaranth was reported with glufosinate and glufosinate plus saflufenacil, whereas <10% control was reported with saflufenacil applied alone. During unfavorable conditions (13 C air temperature and 30% relative humidity), <10% and 60% control was reported with saflufenacil and glufosinate, respectively, whereas 100% control was reported with glufosinate plus saflufenacil. This indicates that the addition of a very low rate of saflufenacil can improve glufosinate efficacy and can improve efficacy under unfavorable conditions on some weed species. Takano et al. (2020c) suggest the synergism between glufosinate plus PPO-inhibiting herbicides is a result of increased formation of protoporphyrin IX in the chlorophyll pathway when glutamine synthetase and PPO are inhibited simultaneously, resulting in increased formation of reactive oxygen species and catastrophic lipid peroxidation of cell membranes.

There is limited research on glufosinate plus PPO-inhibiting herbicides for the control of GR horseweed. Eubank et al. (2008) reported up to 88% and 93% GR horseweed control with glufosinate (470 g ai ha^{-1}) plus flumioxazin (70 g ai ha^{-1}) and glufosinate (470 g ai ha^{-1}) plus sulfentrazone (360 g ai ha^{-1}), respectively, applied PP to soybean at 4 WAA. Budd et al. (2016a) reported 93% GR horseweed control with glyphosate (900 g ae ha^{-1}) plus glufosinate (500 g ai ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) applied PP to soybean at 8 WAA. Waggoner (2010) reported 83% GR horseweed control with glufosinate (450 g ai ha^{-1}) plus saflufenacil (25 g ai ha^{-1}) applied PP to cotton (*Gossypium hirsutum* L.) at 5 WAA. There are few studies investigating GR horseweed control with glufosinate plus PPO-inhibiting herbicides and no studies with very low rates of the PPO-inhibiting herbicides. Therefore, the objectives of this research were (i) to ascertain if the addition of very low rates of PPO-inhibiting herbicides to glufosinate will improve GR horseweed control applied PP to soybean, and (ii) to identify the effective dose of saflufenacil that will enhance GR horseweed control when mixed with glufosinate, applied PP, in soybean.

Materials and Methods

Experimental Methods

Two field studies, each consisting of four trials, were conducted in 2020 and repeated in 2021 in southwestern Ontario, Canada. The resistance level of the horseweed populations at each site was confirmed through greenhouse screening. Study 1 investigated GR horseweed control with glufosinate plus very low rates (5% of field rates) of PPO-inhibiting herbicides (pyraflufen-ethyl, pyraflufen-ethyl/2,4-D, saflufenacil, sulfentrazone, flumioxazin, or fomesafen). Study 2 investigated the effective dose of saflufenacil to enhance GR horseweed control when mixed with glufosinate.

In both studies, treatments were organized as a randomized complete block design with four blocks. Study 1 was a 2 × 7 factorial with 13 treatments plus a nontreated weedy control: Factor 1 was control and glufosinate, and factor 2 was control, pyraflufen-ethyl, pyraflufen-ethyl/2,4-D, flumioxazin, fomesafen, sulfentrazone, and saflufenacil. In study 2, saflufenacil was applied at various doses (1.56, 3.13, 6.25, 12.5, 25, 50, and 100 g ai ha^{-1}) and co-applied with glufosinate (1.56 + 300, 3.13 + 300, 6.25 + 300, 12.5 + 300, 25 + 300, 50 + 300, and 100 + 300 g ai ha^{-1}). All treatments with saflufenacil included Merge (surfactant blend [50%], petroleum hydrocarbons solvent [50%]) surfactant (1 L ha^{-1}). Both studies included a nontreated weedy control. Experimental plots were 2.25 m wide (three soybean rows spaced 75 cm apart) by 8 m long. Treatments were applied PP once the GR horseweed reached an approximate diameter/height of 10 cm. Treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 240 kPa. The boom measured 1.5 m wide and included four ultra–low dose flat-fan spray nozzles (Hypro, New Brighton, MN) spaced 50 cm apart, which produced a 2-m

spray width. A postemergence application of glyphosate (450 g ae ha^{-1}) was made to the research area at each site to remove other weed species. GR horseweed control was assessed using a 0 to 100% scale; 0% indicated no GR horseweed control, 100% indicated complete GR horseweed control (Canadian Weed Science Society 2018). Visible control ratings were conducted at 2, 4, and 8 WAA. At 8 WAA, two 0.25-m^2 quadrats were placed between the soybean rows approximately 1 m inward from the front and 1 m inward from the back of each plot. GR horseweed density was collected by counting all plants within each quadrat. GR horseweed biomass was determined for each plot by removing the aboveground portion of plants within each quadrat, drying the samples in a kiln to constant moisture, and taking the dry weights of each sample to ascertain GR horseweed biomass.

Glyphosate/dicamba-resistant soybean (DKB12-16; Bayer Crop Science Canada) was planted to approximately 400,000 seeds ha^{-1} at a 3.75-cm depth. Planting occurred 1 to 16 d after the PP applications (Table 1), depending on the site. Crop injury was assessed 2 and 4 wk after emergence using a 0 to 100% scale; 0% indicated no soybean injury, 100% indicated complete soybean necrosis (Canadian Weed Science Society 2018). Once the glyphosate/dicamba-resistant soybean reached harvest maturity, two soybean rows were harvested per treatment. Soybean moisture content and yield were recorded; before statistical analysis, the yield was adjusted to a 13.5% moisture content. Year, location, soil attributes, herbicide application dates, and soybean seeding and emergence dates are listed in Table 1. GR horseweed height and density at the time of application and the resistance profile for each site are listed in Table 2. Herbicides used in both studies are listed in Table 3.

Statistical Analysis

All analyses were conducted in SAS 9.4 (Statistical Analysis Systems Institute 2020). In study 1, PROC GLIMMIX was used to assess the data. There was a treatment-by-site interaction, so the sites were partitioned into different groups for the analysis; site 1 and sites 2, 3, and 4. The fixed effects included glufosinate, PPO-inhibiting herbicides, and glufosinate-by-PPO-inhibiting herbicides, and the random effects included site, block within the site, and the interaction of the site with glufosinate and PPO-inhibiting herbicides. Normality was confirmed after conducting the Shapiro-Wilk test and reviewing studentized residual plots. An arc-sine square root transformation was used for control at 2, 4, and 8 WAA, and a log-transformation (dist = log-normal) was used for density and biomass; the back-transformed means are presented in the current presentation. The Tukey-Kramer test was used to compare the least-square means.

In study 2, a nonlinear regression (PROC NLIN) was conducted in SAS 9.4 (Statistical Analysis Systems Institute 2020). The weedy control was removed from the analysis. Scatter plots were created to obtain a visual representation of the response curve for each parameter. The sum of squares reduction test was used to ascertain if the sites could be pooled together for the regression analysis (Schabenberger and Pierce 2002). The sum of squares reduction test investigates two models: the full model (assumes different responses under the four sites) and the separate model (assumes similar responses under the four sites). If $P \leq 0.05$, then the full model was used and the sites were partitioned accordingly; if $P \geq 0.05$, then the separate model was used, and the sites were pooled (Schabenberger and Pierce 2002).

All parameters were regressed against herbicide rate. A log-logistic model (Seefeldt 1995) with four parameters was the equation used for control at 2, 4, and 8 WAA:

$$Y = C + (D - C) \,/\, [1 + exp[-b(lnRate - lnI_{50})] \quad [1]$$

where C indicates the lower limit, D indicates the upper limit, b indicates the slope, and I_{50} is the rate half-way between C and D.

A log-logistic model with four parameters was the equation used for density and biomass:

$$Y = C + (D - C) \,/\, [1 + exp(b(lnRate - ln_{50})] \quad [2]$$

The parameters were the same as Equation 1, but b is positive to represent the direction of the response. Parameters calculated from the regression were used to determine the predicted doses (PD_{50}, PD_{80}, PD_{95}) of saflufenacil or saflufenacil plus glufosinate required for 50%, 80%, and 95% GR horseweed control or for a 50%, 80%, and 95% reduction of density or biomass. When the predicted dose of PD_{50}, PD_{80}, or PD_{95} could not be computed or if it was beyond the range of doses in this study, 'Non-est.' was used to represent the data in Tables 4, 5, 6, and 7.

The fit of the model was determined by calculating the root mean squared error (RMSE) (Equation 3) and the modeling efficiency (ME) (Equation 4) (Mayer and Butler 1993):

$$RMSE = \sqrt{\frac{RSS}{n - p - 1}} \quad [3]$$

where RSS represents the residual sum of squares, n represents the quantity of observations used, p represents the quantity of parameters from the model.

$$ME = 1 - \left[\sqrt{\frac{\sum_{i=1}^{n} (O_i - P_i)^2}{\sum_{i=1}^{n} (\overline{O}_i - i)^2}} \right] \quad [4]$$

where n represents the number of observations used, O_i is the observed value, $\bar{O}_i$ is the observed value of the mean, P_i is the predicted value. RMSE and ME values closer to 1 indicate a better fit to the model.

Colby's equation (Equation 5) is a common method to evaluate herbicide interactions and was used in both studies to calculate the expected control means. The observed control means were used in the equation (A, Glufosinate and B, PPO-inhibiting herbicide).

$$Expect = (A + B) - \left(\frac{A \times B}{100} \right) \quad [5]$$

An altered Colby's equation (Equation 6) was used to calculate the expected density and biomass data (W, nontreated weedy control mean).

$$Expected = \left(\frac{A \times B}{W} \right) \quad [6]$$

A paired t-test ($P < 0.05$) was used to compare the observed and expected means. If the observed mean was greater than or less than the expected mean, then the interaction was considered synergistic or antagonistic, respectively. If the observed mean was similar to the expected, the interaction was considered additive. If the observed biomass or density means were less than or greater than the expected means, then the interaction was considered synergistic or antagonistic, respectively (Colby 1967).

Table 1. Site, year, nearest town to the site location, location coordinates, soil traits, treatment spray date, and soybean seeding and emergence dates for field trials conducted in Ontario, Canada in 2020 and 2021.

			Soil traits						Agronomic information			
Site	Year	Location	Texture	Sand	Silt	Clay	Organic matter	pH	Study 1: Treatment spray date	Study 2: Treatment spray date	Soybean seeding date	Soybean emergence date
				%								
S1	2020	Ridgetown (42.46° N, 81.85° W)	Sandy loam	75	17	7	1.9	7.1	May 26	May 28	June 5	June 11
S2	2020	Moraviantown (42.55° N, 81.84° W)	Loamy sand	87	7	5	2.5	6.6	June16	June 12	June 18	July 2
S3	2021	Kintyre (42.56° N, 81.77° W)	Sandy loam	53	29	18	4.4	6.9	May 18	May 13	May 19	May 25
S4	2021	Bothwell (42.62° N, 81.91° W)	Loamy sand	85	11	4	3.3	6.8	May 31	May 27	June 12	June 18

Table 2. Site, year, location, glyphosate-resistant (GR) horseweed height and density at the time of the preplant application, and the resistance profile for site locations in Ontario, Canada in 2020 and 2021.

			GR horseweed				Resistance	
Site	Year	Location	Study 1 height	Study 2 height	Study 1 density	Study 2 density	Glyphosate	Cloransulam-methyl
			cm		No. plants m^{-2}		%	
S1	2020	Ridgetown	7	8	748	786	100	99
S2	2020	Moraviantown	10	9	19	207	79	100
S3	2021	Kintyre	8	7	78	123	98	85
S4	2021	Bothwell	9	8	92	133	–	–

Table 3. The herbicides and surfactants that were used in both studies conducted in Ontario, Canada in 2020 and 2021.

Active ingredient	Trade name	Manufacturer
Herbicides		
Glufosinate	Liberty	BASF Canada Inc., Mississauga, ON
Pyraflufen-ethyl	NUP 6D 04	Nufarm Canada, Calgary, AB.
Pyraflufen-ethyl/2,4-D	Blackhawk	Nufarm Canada
Fomesafen[a]	Reflex	Nufarm Canada
Flumioxazin	Valtera	Syngenta Canada Inc. Guelph, ON
Sulfentrazone	Authority	FMC Canada, Mississauga, ON.
Saflufenacil[b]	Eragon LQ	BASF Canada Inc. Mississauga, ON
Surfactants		
Surfactant/solvent	Turbocharge	Syngenta Canada Inc.
Oil/surfactant blend	Merge	BASF Canada Inc.

[a]All treatments with fomesafen included the surfactant Turbocharge, 0.5% v/v.
[b]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Results and Discussion

Study 1: Control of Glyphosate-Resistant Horseweed with Glufosinate Plus Very Low Rates of PPO-Inhibiting Herbicides

A treatment-by-site interaction was significant for GR horseweed visible control, density, biomass, and soybean yield, mainly because site 1 responded differently than the other sites; therefore, sites 2, 3, and 4 were pooled for the analyses, and site 1 was analyzed separately. Sites 1, 2, 3, and 4 had 748, 19, 78, and 92 plants per m^{-2}, respectively, within the nontreated weedy control at the time of herbicide application (Table 2). Because glufosinate is a contact herbicide and has minimal translocation in plants, thorough coverage is essential to achieve acceptable weed control (Anonymous 2021). Previous research reported poor weed control with glufosinate on high weed densities (Steckel et al. 1997; Tharp and Kells 2002). The high GR horseweed densities, especially at site 1, could have contributed to the low GR horseweed control at this site.

Site 1

There was no interaction between glufosinate and the PPO-inhibiting herbicides on GR horseweed control or for GR horseweed density or biomass at site 1, so the main effects are presented (Table 4). Averaged across the PPO-inhibiting herbicides, glufosinate controlled GR horseweed 18% and 14% at 2 and 4 WAA, respectively, and reduced biomass 14%. The application of glufosinate (300 g ai ha^{-1}) did not improve GR horseweed control at 8 WAA, and there was no reduction in density. When averaged across glufosinate rates, pyraflufen-ethyl, pyraflufen-ethyl/2,4-D, flumioxazin, fomesafen, and sulfentrazone applied at 5% of the label rate did not improve GR horseweed control at 2, 4, and 8 WAA and did not reduce GR horseweed density and biomass. When averaged across glufosinate rates, saflufenacil (1.25 g ai ha^{-1}) controlled GR horseweed 29% and 24% 2 and 4 WAA, respectively; there was no improvement in control at 8 WAA and no decrease in density and biomass. The Colby's equation and a paired t-test suggested there was a synergistic increase in GR horseweed control when glufosinate (300 g ai ha^{-1}) was co-applied with saflufenacil (1.25 g ai ha^{-1}) at 2 and 4 WAA; however, synergism was temporary and the interaction was additive at 8 WAA. Glufosinate (300 g ai ha^{-1}) plus pyraflufen (0.34 g ai ha^{-1}) or sulfentrazone (7 g ai ha^{-1}) was antagonistic at 8 WAA; all remaining interactions were additive (Table 5). In the present study, when saflufenacil (1.25 g ai ha^{-1}) was averaged across glufosinate rates (0 and 300 g ai ha^{-1}), the level of GR horseweed control was much lower at 29% and 24% at 2 and 4 WAA, respectively (Table 4).

Table 4. Main effects for glyphosate-resistant (GR) horseweed control 2, 4, and 8 wk after application (WAA), density, biomass, and soybean yield for glufosinate plus protoporphyrin oxidase (PPO) inhibitors from study 1 conducted in Ontario, Canada, in 2020 and 2021.[a–c]

		GR horseweed control											
		2 WAA		4 WAA		8 WAA		Density[d]		Biomass[d]		Soybean yield	
Main effects	Dose	S1	S2, S3, S4	S1	S2, S3, S4	S1	S2, S3, S4	S1	S2, S3, S4	S1	S2, S3, S4	S1	S2, S3, S4
	g ai/ae ha^{-1}	%						No. plants m^{-2}		g m^{-2}		kg ha^{-1}	
Glufosinate		*	NS	*	NS	NS	NS	NS	NS	*	*	NS	NS
None	–	1b	18	1b	17	1	13	1031	45	310b	155b	190	1,390
Glufosinate	300	18a	50	14a	46	4	42	828	5	268a	94a	430	1,540
PPO inhibitors		*	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
None	–	4b	12b	4b	11	3	11	1020	49	253	114	300	1,480
Pyraflufen-ethyl	0.34	4b	34ab	4b	29	2	22	1065	38	276	117	270	1,390
Pyraflufen-ethyl/2,4-D	26.4	5b	42a	4b	35	2	29	982	41	274	125	290	1,340
Flumioxazin	5.35	3b	40ab	3b	35	1	29	990	32	344	112	320	1,470
Fomesafen[e]	12	3b	35ab	3b	33	3	28	1036	34	301	165	180	1,440
Sulfentrazone	7	4b	34ab	4b	35	1	30	788	44	321	122	260	1,610
Saflufenacil[f]	1.25	29a	42a	24a	40	4	39	620	30	260	117	620	1,460
Glufosinate × PPO inhibitors		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Abbreviations: NS, nonsignificant; S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]* Significant at $P < 0.05$.
[c]Means accompanied by a different letter in a column (a–b) significantly differ based on Tukey-Kramer's LSD ($\alpha = 0.05$).
[d]Density and biomass were collected 8 WAA.
[e]All treatments with fomesafen included the surfactant Turbocharge, 0.5% v/v.
[f]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Table 5. Observed and predicted means for glyphosate-resistant (GR) horseweed control 2, 4, and 8 wk after application (WAA) for glufosinate plus protoporphyrin oxidase (PPO) inhibitors from study 1 conducted in Ontario, Canada in 2020 and 2021.[a–c]

		GR horseweed control											
		2 WAA				4 WAA				8 WAA			
Treatments	Dose	S1		S2, S3, S4		S1		S2, S3, S4		S1		S2, S3, S4	
	g ai/ae ha^{-1}	%											
Weedy control	–	0		0		0		0		0		0	
Pyraflufen-ethyl	0.34	0		19		0		16		1		6	
Pyraflufen-ethyl/2,4-D	26.4	0		28		1		24		1		16	
Flumioxazin	5.35	0		31		1		27		0		21	
Fomesafen[d]	12	0		21		0		20		3		16	
Sulfentrazone	7	0		23		1		25		0		25	
Saflufenacil[e]	1.25	14		28		8		25		1		26	
Glufosinate	300	16		41		15		38		10		37	
Glufosinate + pyraflufen-ethyl	300 + 0.34	13	(16)	51	(47)	13	(15)	43	(47)	1	(13)*	43	(44)
Glufosinate + pyraflufen-ethyl/2,4-D	300 + 26.4	19	(16)	57	(51)	10	(16)	48	(50)	4	(12)	45	(48)
Glufosinate + flumioxazin	300 + 5.35	12	(16)	50	(54)	6	(17)	42	(54)	1	(10)	38	(53)
Glufosinate + fomesafen[d]	300 + 12	12	(16)	51	(48)	10	(15)	47	(50)	3	(13)	42	(50)
Glufosinate + sulfentrazone	300 + 7	14	(16)	46	(50)	9	(17)	46	(51)	1	(10)*	37	(54)
Glufosinate + saflufenacil[e]	300 + 1.25	46	(30)*	57	(48)	45	(24)*	48	(49)	12	(11)	54	(49)

[a]Abbreviations: S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Values in parentheses are the expected means calculated by Colby's equation.
[c]*Significant at $P < 0.05$ based on a paired t-test conducted on observed and expected values.
[d]All treatments with fomesafen included the surfactant Turbocharge, 0.5% v/v.
[e]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

In contrast to the current study, Bolte (2015) reported a minimum of 29% GR horseweed control with glufosinate (594 g ai ha^{-1}) at 5 WAA. In contrast to the current study, Takano et al. (2020c) reported enhanced control of Palmer amaranth when glufosinate (280 g ai ha^{-1}) was mixed with a very low rate of saflufenacil (1 g ai ha^{-1}); the mixture required a lower rate to reach 50% control compared to glufosinate and saflufenacil applied individually. Synergism was also reported between glufosinate (420 g ai ha^{-1}) plus a very low rate of saflufenacil (1 g ai ha^{-1}) for kochia control (Takano et al. 2020c). The herbicides applied individually provided approximately 25% control of kochia, whereas the mixture improved control to approximately 80% (Takano et al. 2020c). In the present study, when saflufenacil (1.25 g ai ha^{-1}) was averaged across glufosinate rates (0 and 300 g ai ha^{-1}), the level of GR horseweed control was much lower at 29% and 24% at 2 and 4 WAA, respectively (Table 4). Takano et al. (2020c) reported enhanced control of kochia when glufosinate (420 g ai ha^{-1}) was mixed with pyraflufen (0.2 g ai ha^{-1}). However, in the current study, this mixture was antagonistic on GR horseweed at 8 WAA (Table 5), possibly suggesting that this interaction is species-specific.

Table 6. Regression parameters and predicted dose of saflufenacil for 50%, 80%, and 95% GR horseweed control at 2, 4, and 8 wk after application (WAA) and the predicted dose (*PD*) to achieve a 50%, 80%, and 95% reduction in GR horseweed density or biomass from study 2 conducted in Ontario, Canada in 2020 and 2021.[a]

			Regression parameters (± SE)[b,c]						Predicted dose[d]				
Site no.	ME	RMSE	*c*		*d*		*b*		I_{50}		PD_{50}	PD_{80}	PD_{95}
											g ai ha^{-1}		
Saflufenacil alone at 2 WAA[e]													
S1	0.9	6.7	58	(3.2)	98	(3.7)	3.0	(1.3)	22	(3.3)	Non-est.	23.5	51.9
S2, S3	0.6	18.6	25	(14.0)	100	(0)	1.4	(0.5)	8	(3.2)	4.9	16.5	53.0
S4	0.8	9.7	0	(0)	53	(3.8)	1.8	(0.5)	5	(1.0)	23.4	Non-est.	Non-est.
Saflufenacil alone at 4 WAA[e]													
S1	0.8	6.4	57	(7.9)	100	(0)	0.9	(0.3)	16	(8.2)	Non-est.	19.4	129.2
S2, S3	0.8	14.2	6	(29.1)	100	(0)	1.1	(0.4)	4	(2.4)	3.5	11.6	43.5
S4	0.9	10.7	0	(0)	95	(6.5)	1.5	(0.3)	10	(1.7)	11.1	31.5	Non-est.
Saflufenacil alone at 8 WAA[e]													
S1	0.8	7.7	60	(3.8)	98	(8.2)	2.3	(1.3)	31	(9.2)	Non-est.	32.5	89.7
S2, S3	0.8	14.4	18	(10.3)	100	(0)	1.7	(0.4)	7	(1.6)	5.3	13.7	36.4
S4	0.5	27.0	0	(0)	95	(5.5)	1.7	(0.3)	9	(1.3)	9.7	24.5	Non-est.
Density[c]													
S1	0.6	48.7	12	(78.0)	306	(0)	5.9	(20.9)	23	(9.5)	22.4	28.4	37.0
S2, S3, S4	0.7	45.8	0	(0)	200	(0)	1.3	(0.3)	23	(9.1)	2.0	8.1	39.1
Biomass[c]													
S1	0.5	46.0	7	(25.9)	115	(16.1)	7.4	(19.3)	42	(20.7)	41.2	50.1	61.9
S2, S3, S4	0.6	49.4	0	(0)	246	(56.1)	1.3	(0.4)	6	(3.0)	6.2	18.5	63.7

[a]Abbreviations: ME, modeling efficiency; Non-est., non-estimable; RMSE, root mean squared error; SE, standard error; S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Regression parameters control: $Y = c + (d - c)/[1 + exp(-b(lnRate - lnI_{50})]$; c = lower asymptote, d = upper asymptote, b = slope, I_{50} = effective dose to achieve a 50% response (see Equation 1); values in parentheses represent the standard errors of each regression parameter.
[c]Regression parameters density and biomass, inverse of Equation 1, $Y = c + (d - c)/[1 + exp(b(lnRate - lnI_{50})]$ (see Equation 2).
[d]PD_x, predicted dose to achieve X% GR horseweed control or the predicted dose to reduce GR horseweed density or biomass by X.
[e]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Table 7. Regression parameters and predicted dose of saflufenacil when mixed with glufosinate for 50%, 80%, and 95% GR horseweed control at 2, 4, and 8 wk after application (WAA) and the predicted dose to achieve a 50%, 80%, and 95% reduction in GR horseweed density or biomass from study 2 conducted in Ontario, Canada in 2020 and 2021.[a]

			Regression parameters (± SE)[b, c]						Predicted dose[d]				
Site no.	ME	RMSE	*c*		*d*		*b*		I_{50}		PD_{50}	PD_{80}	PD_{95}
											g ai ha^{-1}		
Glufosinate + saflufenacil at 2 WAA[e]													
S1, S2, S3	0.6	15.1	38	(14.3)	100	(0)	1.0	(0.3)	8	(4.4)	2.0	15.5	78.8
S4	0.7	9.6	0	(0)	64	(6.8)	0.9	(0.3)	3	(1.1)	12.9	Non-est.	Non-est.
Glufosinate + saflufenacil at 4 WAA[e]													
S1, S2, S3	0.6	12.9	52	(8.0)	100	(5.9)	1.4	(0.7)	10	(3.1)	Non-est.	12.9	48.6
S4	0.8	13.2	5	(30.7)	100	(0)	0.9	(0.3)	7	(6.2)	6.1	31.0	185.5
Glufosinate + saflufenacil at 8 WAA[e]													
S1, S2, S3, S4	0.7	13.3	34	(9.0)	100	(5.9)	1.4	(0.6)	9	(2.3)	4.0	16.5	55.0
Density[c]													
S1	0.4	56.8	104	(77.0)	986	(89.5)	33.5	(96.3)	7	(8.2)	6.9	7.3	7.6
S2, S3, S4	0.5	32.1	0	(0)	200	(0)	1.3	(0.3)	4	(0.8)	9.7	18.1	36.4
Biomass[c]													
S1, S2, S4	0.6	49.5	55	(9.2)	211	(14.1)	2.8	(1.0)	9	(1.4)	7.1	13.1	23.6
S3	0.6	55.7	17	(7.5)	150	(0)	2.2	(0.8)	2	(0.4)	2.1	4.1	8.6

[a]Abbreviations: ME, modeling efficiency; Non-est., non-estimable; RMSE, root mean squared error; SE, standard error; S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Regression parameters control: $Y = c + (d - c)/[1 + exp(-b(lnRate - lnI_{50})]$; c = lower asymptote, d = upper asymptote, b = slope, I_{50} = effective dose to achieve a 50% response (see Equation 1); values in parentheses represent the standard errors of each regression parameter.
[c]Regression parameters density and biomass, inverse of Equation 1, $Y = c + (d - c)/[1 + exp(b(lnRate - lnI_{50})]$ (see Equation 2).
[d]PD_x, predicted dose to achieve X% GR horseweed control or the predicted dose to reduce GR horseweed density or biomass by X.
[e]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Sites 2, 3, and 4

There was no interaction between glufosinate and the PPO-inhibiting herbicides on GR horseweed control or for GR horseweed density or biomass at sites 2, 3, and 4, so the main effects are presented (Table 4). Averaged across PPO-inhibiting herbicides, glufosinate did not improve GR horseweed control at 2, 4, and 8 WAA, and there was no reduction in density; averaged across PPO-inhibiting herbicides, glufosinate decreased GR horseweed biomass 39%. Similarly, Bolte (2015) reported a 37% decrease in GR horseweed biomass with glufosinate (594 g ai ha^{-1}) compared to the untreated control. Averaged across glufosinate rates, the PPO-inhibiting herbicides at 5% of the label rate did not improve GR horseweed control at 4 and 8 WAA, and there was no reduction in density or biomass.

Averaged across glufosinate rates, pyraflufen-ethyl/2,4-D and saflufenacil controlled GR horseweed 42%. The Colby's equation and a paired t-test suggested all interactions were additive (Table 5).

Soybean Injury and Yield

Soybean injury was minimal (≤5%) at all sites (data not presented). There was no interaction between glufosinate and the PPO-inhibiting herbicides on soybean yield for site 1 or sites 2, 3, and 4, so the main effects are presented (Table 4). There were no differences in soybean yield from the main effect of glufosinate rate or PPO-inhibiting herbicide at site 1 or sites 2, 3, and 4.

Study 2: Biologically Effective Dose of Saflufenacil Alone and Glufosinate Plus Saflufenacil for the Control of Glyphosate-Resistant Horseweed

Saflufenacil Alone

Based on the sums of squares reduction test, the sites were partitioned into groups for the analysis of GR horseweed control at 2, 4, and 8 WAA and for density and biomass reduction with saflufenacil applied alone.

At 2 WAA, the predicted doses of saflufenacil for 50% GR horseweed control at sites 2 and 3, and site 4 were 4.9 and 23.4 g ai ha^{-1}, respectively. At site 1 the dose could not be estimated (Table 6). Similar to the control observed at site 2 and 3, 42% GR horseweed control with 1.25 g ai ha^{-1} saflufenacil was reported in study 1 at 2 WAA. The predicted doses of saflufenacil for 80% GR horseweed control at site 1, and sites 2 and 3 were 23.5 and 16.5 g ai ha^{-1}, respectively; at site 4 the dose could not be estimated. The predicted doses of saflufenacil for 95% GR horseweed control at site 1, and sites 2 and 3 were 51.9 and 53.0 g ai ha^{-1}, respectively; at site 4 the dose could not be estimated.

At 4 WAA, the predicted doses of saflufenacil for 50% GR horseweed control at sites 2 and 3, and site 4 were 3.5 and 11.1 g ai ha^{-1}, respectively; at site 1 the dose could not be estimated (Table 6). The predicted doses of saflufenacil for 80% GR horseweed control at site 1, sites 2 and 3, and site 4 were 19.4, 11.6, and 31.5 g ai ha^{-1}, respectively. The predicted doses of saflufenacil for 95% GR horseweed control at site 1 and sites 2 and 3 were 129.2 and 43.5 g ai ha^{-1}, respectively; at site 4 the dose could not be estimated. In contrast, Budd et al. (2016b) reported 95% GR horseweed control with 15 g ai ha^{-1} of saflufenacil.

At 8 WAA, the predicted doses of saflufenacil for 50% GR horseweed control at sites 2 and 3, and 4 were 5.3 and 9.7 g ai ha^{-1}, respectively; at site 1 the dose could not be estimated (Table 6). The predicted doses of saflufenacil for 80% GR horseweed control at site 1, sites 2 and 3, and site 4 were 32.5, 13.7, and 24.5 g ai ha^{-1}, respectively. The predicted doses of saflufenacil for 95% GR horseweed control at site 1 and sites 2 and 3 were 89.7 and 36.4 g ai ha^{-1}, respectively; at site 4 the dose could not be estimated.

The predicted doses of saflufenacil to reduce GR horseweed density by 50%, 80%, and 95% at site 1 were 22.4, 28.4, and 37.0 g ai ha$^{-1,}$ and to reduce biomass were 41.2, 50.1, and 61.9 g ai ha^{-1}, respectively (Table 6). A similar trend was observed at sites 3 ,2, and 4, although much lower doses were predicted for a 50% and 85% reduction. Budd et al. (2016b) reported a 95% decrease in GR horseweed density and biomass with 22 and 36 g ai ha^{-1} of saflufenacil, respectively.

Glufosinate Plus Saflufenacil

Based on the sums of squares reduction test, the sites were partitioned into groups for the analysis of GR horseweed control at 2 and 4 WAA, and for density and biomass with glufosinate plus saflufenacil. The sites were pooled for the analysis of GR horseweed control with glufosinate plus saflufenacil at 8 WAA.

At 2 WAA, the predicted doses of saflufenacil when applied with glufosinate for 50%, 80%, and 95% GR horseweed control were 2.0, 15.5, and 78.8 g ai ha^{-1}, respectively (Table 7). At sites 1, 2, and 3, the predicted doses for 80% and 95% control could not be estimated.

At 4 WAA, the predicted dose of saflufenacil when applied with glufosinate for 50% GR horseweed control at site 4 was 6.1 g ai ha^{-1}; at sites 1, 2, and 3 the dose could not be estimated (Table 7). The predicted doses of saflufenacil required when mixed with glufosinate for 80% GR horseweed control at sites 1, 2, 3, and site 4 were 12.9 and 31.0 g ai ha^{-1} and for 95% control were 48.6 and 185.5 g ai ha^{-1}, respectively.

At 8 WAA, the predicted doses of saflufenacil when applied with glufosinate for 50%, 80%, and 95% GR horseweed control were 4.0, 16.5, and 55 g ai ha^{-1}, respectively (Table 7). Budd et al. (2016a) reported 93% GR horseweed control when saflufenacil (25 g ai ha^{-1}) plus glufosinate (500 g ai ha^{-1}) was mixed with glyphosate (900 g ae ha^{-1}) at 8 WAA.

Colby's equation and a paired t-test suggested all interactions were antagonistic or additive (Table 8). At 2 and 4 WAA, glufosinate plus 1.56, 3.13, 6.25, 12.5, or 25 g ai ha^{-1} of saflufenacil was antagonistic at sites 1, 2, and 3. At 8 WAA, glufosinate plus 1.56, 3.13, 6.25, or 12.5 g ai ha^{-1} of saflufenacil was antagonistic. All remaining interactions at sites 1, 2, and 3 and all interactions at site 4 were additive. In contrast, Takano et al. (2020c) reported a synergistic response between a very low dose of saflufenacil (1 g ai ha^{-1}) plus glufosinate (280 g ai ha^{-1}) or glufosinate (420 g ai ha^{-1}) on Palmer amaranth or kochia, respectively. Jhala et al. (2013) reported saflufenacil (37 to 50 g ai ha^{-1}) plus glufosinate (1,000 to 1,033 g ai ha^{-1}) had an additive effect on broadleaf weeds in citrus. Damalas (2004) and Takano et al. (2020c) suggest that synergism is weed species-dependent and is more commonly observed in broadleaf weeds compared to grasses. This may be the case in the present study, in that synergism was not reported in GR horseweed but has been reported on other broadleaf weeds (Takano et al. 2020c).

The predicted doses of saflufenacil when co-applied with glufosinate to reduce GR horseweed density by 50%, 80%, and 95% at site 1 were 6.9, 7.3, and 7.6 g ai ha^{-1}, and at sites 2, 3, and 4 were 9.7, 18.1, and 36.4 g ai ha^{-1}, respectively. The Colby's equation and a paired t-test suggested glufosinate plus 50 and 100 g ai ha^{-1} of saflufenacil was antagonistic at site 1 and glufosinate plus 12.5, 25, 50, and 100 g ai ha^{-1} of saflufenacil was also antagonistic at sites 3 ,2, and 4 (Table 9). All remaining interactions were additive.

The predicted doses of saflufenacil when co-applied with glufosinate to reduce GR horseweed biomass by 50%, 80%, and 95% at sites 1, 2, and 4 were 7.1, 13.1, and 23.6 g ai ha^{-1} and at site 3 were 2.1, 4.1, and 8.6 g ai ha^{-1}, respectively. Colby's equation and a paired t-test suggested glufosinate plus 50 and 100 g ai ha^{-1} of saflufenacil was antagonistic at sites 1, 2, and 4; remaining interactions at sites 1, 2, and 4 and all interactions at site 3 were additive (Table 9).

Similar predicted doses of saflufenacil were needed to achieve 50%, 80%, and 90% GR horseweed control and to reduce GR horseweed density by 50%, 80%, and 95% whether glufosinate was included in the mixture or not. Lower doses of saflufenacil were predicted to reduce GR horseweed biomass when saflufenacil was co-applied with glufosinate compared to saflufenacil applied alone.

Table 8. Observed and predicted means for glyphosate-resistant (GR) horseweed control 2, 4, and 8 wk after application (WAA) for glufosinate plus saflufenacil from study 2 conducted in Ontario, Canada in 2020 and 2021.[a–c]

		GR horseweed control									
		2 WAA				4 WAA				8 WAA	
Treatment	Dose	S1, S2, S3		S4		S1, S2, S3		S4		S1, S2, S3, S4	
	g ai/ae ha^{-1}	%									
Weedy control	–	0		0		0		0		0	
Saflufenacil[d]	1.56	36		3		35		0		22	
Saflufenacil	3.13	50		13		57		10		33	
Saflufenacil	6.25	52		38		62		40		50	
Saflufenacil	12.5	72		36		79		46		69	
Saflufenacil	25	87		53		90		79		85	
Saflufenacil	50	93		50		94		85		93	
Saflufenacil	100	99		57		98		93		97	
Glufosinate	300	60		23		65		33		53	
Glufosinate + saflufenacil	300 + 1.56	46	(74)*	20	(25)	54	(77)*	24	(33)	38	(63)*
Glufosinate + saflufenacil	300 + 3.13	62	(80)*	35	(33)	63	(85)*	41	(40)	47	(69)*
Glufosinate + saflufenacil	300 + 6.25	63	(81)*	36	(52)	66	(87)*	48	(60)	56	(77)*
Glufosinate + saflufenacil	300 + 12.5	78	(89)*	52	(51)	82	(93)*	65	(64)	76	(85)*
Glufosinate + saflufenacil	300 + 25	82	(95)*	58	(64)	87	(97)*	77	(86)	83	(93)
Glufosinate + saflufenacil	300 + 50	96	(97)	56	(62)	97	(98)	84	(90)	94	(97)
Glufosinate + saflufenacil	300 + 100	97	(100)	63	(67)	97	(99)	95	(95)	97	(99)

[a]Abbreviations: S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Values in parentheses are the expected means calculated by the Colby's equation.
[c]*Significant at $P < 0.05$ based on a paired t-test conducted on observed and expected values.
[d]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Table 9. Observed and predicted means for density and biomass for glufosinate plus saflufenacil from study 2 conducted in Ontario, Canada in 2020 and 2021.[a–c]

		Density[d]				Biomass[d]			
Treatment	Dose	S1		S2, S3, S4		S1, S2, S4		S3	
	g ai/ae ha^{-1}	No. plants m^{-2}				g m^{-2}			
Weedy control	–	1,128		240		237		108	
Saflufenacil[e]	1.56	248		168		224		108	
Saflufenacil	3.13	–		108		221		38	
Saflufenacil	6.25	515		75		158		37	
Saflufenacil	12.5	292		52		103		34	
Saflufenacil	25	117		14		79		14	
Saflufenacil	50	24		5		19		12	
Saflufenacil	100	3		3		7		0	
Glufosinate	300	515		60		196		84	
Glufosinate + saflufenacil	300 + 1.56	986	(113)	84	(42)	188	(185)	120	(184)
Glufosinate + saflufenacil	300 + 3.13	–		90	(27)	207	(183)	78	(30)
Glufosinate + saflufenacil	300 + 6.25	967	(235)	67	(19)	178	(131)	44	(29)
Glufosinate + saflufenacil	300 + 12.5	64	(133)	33	(13)*	100	(85)	12	(26)
Glufosinate + saflufenacil	300 + 25	289	(53)	18	(4)*	79	(65)	7	(11)
Glufosinate + saflufenacil	300 + 50	51	(11)*	8	(1)*	23	(16)*	3	(9)
Glufosinate + saflufenacil	300 + 100	11	(1)*	2	(1)*	8	(6)*	0	(0)

[a]Abbreviations: S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Values in parentheses are the expected means calculated by the Colby's equation.
[c]*Significant at $P < 0.05$ based on a paired t-test conducted on observed and expected values.
[d]Density and biomass were collected 8 wk after the preplant application.
[e]All treatments with saflufenacil included the surfactant Merge, 1 L ha^{-1}.

Soybean Injury and Yield

Treatments with saflufenacil at 100 g ai ha^{-1} had up to 12% soybean injury (data not presented). Injury symptoms included chlorosis, necrosis, and stunting. Despite the injury, treatments with saflufenacil (100 g ai ha^{-1}) yielded similarly to the other treatments. Similarly, Soltani et al. (2010) reported 6% and 22% injury when saflufenacil was applied at 100 and 200 g ai ha^{-1}, respectively 4 wk after emergence; however, yield reduction was less than 5%.

Based on the sums of squares reduction test, the sites were analyzed individually for soybean yield with saflufenacil and when saflufenacil was co-applied with glufosinate (Table 10). At sites 2 and 4, the predicted doses of saflufenacil to achieve a 50%, 80%, and 95% soybean yield compared to the highest yielding treatment were 0.5 to 0.6, 6.4, and 20.5 to 20.6 g ai ha^{-1}, respectively; the predicted doses were much higher at site 1 and much lower at site 3. At site 1, the predicted doses of saflufenacil when co-applied with glufosinate for a 50%, 80%, and 95% soybean yield relative to the highest yielding treatment were 4.6, 13.8, and 24.6 g ai ha^{-1}, respectively. The predicted dose of saflufenacil when co-applied with glufosinate for 50% soybean yield could not be estimated at sites 2, 3, and 4. The predicted dose of saflufenacil when co-applied with glufosinate for 80% soybean yield could not be

Table 10. Regression parameters and predicted dose of saflufenacil or saflufenacil plus glufosinate to achieve a 50%, 80%, and 95% soybean yield relative to the highest yielding treatment at each site from study 2 conducted in Ontario, Canada in 2020 and 2021.[a]

			Regression parameters (± SE)[b]								Predicted dose[c]		
Site no.	ME	RMSE	*c*		*d*		*b*		I_{50}		PD_{50}	PD_{80}	PD_{95}
											g ai ha^{-1}		
Saflufenacil alone yield													
S1	0.9	0.1	2	(0.1)	4	(0.1)	3.4	(1.7)	26	(3.6)	Non-est.	21.1	41.3
S2	0.6	0.2	1	(0.5)	1	(0.3)	3.9	(9.3)	4	(3.5)	0.5	6.4	20.6
S3	0.7	0.3	2	(0.7)	2	(0.5)	2.0	(4.9)	5	(2.2)	Non-est.	1.8	9.5
S4	0.9	0.1	1	(0.6)	3	(0.2)	1.5	(1.4)	5	(3.7)	0.6	6.4	20.5
Glufosinate + saflufenacil yield													
S1	0.9	0.1	2	(0.2)	4	(0.1)	3.0	(1.1)	12	(1.5)	4.6	13.8	24.6
S2	0.5	0.3	1	(0.3)	1	(0.2)	3.6	(5.7)	6	(0.6)	Non-est.	7.0	11.6
S3	0.5	0.4	2	(0.3)	2	(0.2)	2.9	(5.5)	5	(3.3)	Non-est.	Non-est.	5.4
S4	0.7	0.3	2	(0.6)	3	(0.2)	2.8	(5.3)	4	(3.8)	Non-est.	4.1	8.5

[a]Abbreviations: ME, modeling efficiency; Non-est., non-estimable; RMSE, root mean squared error; SE, standard error; S1, site 1; S2, site 2; S3, site 3; S4, site 4.
[b]Regression parameters yield: $Y = c + (d - c)/[1 + exp(-b(lnRate - lnI_{50})]$; c = lower asymptote, d = upper asymptote, b = slope, I_{50} = effective dose to achieve a 50% response (see Equation 1); values in parentheses represent the standard errors of each regression parameter.
[c]PD_x, predicted dose to achieve X% GR horseweed control or the predicted dose to reduce GR horseweed density or biomass by X.

estimated at site 3. Similar doses of saflufenacil were required to maintain a 50%, 80%, and 95% soybean yield in contrast to the highest yielding treatment whether glufosinate was included or not.

In summary, there was no benefit of adding very low rates of PPO-inhibiting herbicides (pyraflufen-ethyl, pyraflufen-ethyl/2,4-D, sulfentrazone, flumioxazin, or fomesafen) to glufosinate to improve the control of GR horseweed. Despite the synergistic increase in GR horseweed control with the addition of saflufenacil at 5% of the label rate to glufosinate, the level of control did not exceed 42% at 2 and 4 WAA. Overall, the co-application of glufosinate plus low doses of PPO-inhibiting herbicides did not enhance the control of GR horseweed. Similar doses of saflufenacil were needed to achieve 50%, 80%, and 90% GR horseweed control and to reduce GR horseweed density by 50%, 80%, and 95% when applied alone or co-applied with glufosinate. In contrast to study 1, there was an antagonistic response when glufosinate was co-applied with saflufenacil at 1.56, 3.13, 6.25, and 12.5 g ai ha^{-1} at 2, 4, and 8 WAA at sites 1, 2, and 3; antagonism also occurred with glufosinate plus 25 g ai ha^{-1} of saflufenacil at 2 and 4 WAA. Lower doses of saflufenacil were predicted to reduce GR horseweed biomass when saflufenacil was co-applied with glufosinate compared to saflufenacil applied alone. Previous research indicated that glufosinate plus very low doses of PPO-inhibiting herbicides can lead to enhanced herbicidal activity on certain broadleaf weeds (Takano et al. 2020c). This study suggests this interaction may be species-specific, in that glufosinate applied with low doses of PPO-inhibiting herbicides does not result in a synergistic improvement in GR horseweed control.

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References

Anonymous (2021) Liberty® 150 SN Herbicide. BASF Canada Inc. Mississauga, ON. https://agriculture.basf.ca/content/dam/cxm/agriculture/canada/english/agriculture/west/products/documents/product-documents/Liberty150SN_Product_Label.pdf. Accessed: November 26, 2021

Bhowmik PC, Bekech MM (1993) Horseweed (*Conyza canadensis*) seed production, emergence, and distribution in no-tillage and conventional tillage corn (*Zea mays*). Agronomy 1:67–71

Bolte JD (2015) Emergence and control of horseweed (*Conyza canadensis*). M.S. Thesis, University of Missouri–Columbia, Columbia, MO. Pp 62–64

Bruce JA, Kells JJ (1990) Horseweed (*Conyza canadensis*) control in no-tillage soybeans (*Glycine max*) with preplant and preemergence herbicides. Weed Technol 4:642–647

Budd CM, Soltani N, Robinson DE, Hooker DC, Miller RT, Sikkema PH (2016a) Control of glyphosate-resistant Canada fleabane with saflufenacil plus tankmix partners in soybean. Can J Plant Sci 96:989–994

Budd CM, Soltani N, Robinson DE, Hooker DC, Miller RT, Sikkema PH (2016b) Improving the consistency of glyphosate-resistant Canada fleabane (*Conyza canadensis*) control with saflufenacil: distribution and control in soybean (*Glycine max*). M.Sc. thesis, University of Guelph, Guelph, ON. 51 pp

Buhler DD, Owen MDK (1997) Emergence and survival of horseweed. Weed Sci 45:98–101

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013a) Control of glyphosate-resistant Canada fleabane [*Conyza canadensis* (L.) Cronq.] with preplant herbicide tankmixes in soybean [*Glycine max*.(L). Merr.]. Can J Plant Sci 93:659–667

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013b) Glyphosate-resistant Canada fleabane [*Conyza canadensis* (L). Cronq.]: dose response to glyphosate and control with postemergence herbicides in soybean in Ontario. Can J Plant Sci 93:1187–1193

Canadian Weed Science Society (2018) Description of 0–100 rating scale for herbicide efficacy and crop phytotoxicity. https://weedscience.ca/cwss_scm-rating-scale/. Accessed: November 23, 2021

Cánovas FM, Avila C, Botella JR, Valpuesta V, de Castro IN (1986) Effect of light–dark transition on glutamine synthetase activity in tomato leaves. Physiol Plant 66:648–652

Coetzer E, Al-Khatib K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci 49:8–13

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

Damalas CA (2004) Herbicide tank mixtures: common interactions. Int J Agric Biol 6:209–212

Dauer JT, Mortensen DA, Humston R (2006) Controlled experiments to predict horseweed (*Conyza canadensis*) dispersal distances. Weed Sci 54:484–489

Davis VM, Kruger GR, Stachler JM, Loux MM, Johnson WG (2009) Growth and seed production of horseweed (*Conyza canadensis*) populations resistant to glyphosate, ALS-inhibiting, and multiple (glyphosate + ALS-inhibiting) herbicides. Weed Sci 57:494–504

Dayan FE, Barker A, Tranel PJ (2018) Origins and structure of chloroplastic and mitochondrial plant protoporphyrinogen oxidases: implications for the evolution of herbicide resistance. Pest Manag Sci 74:2226–2234

Dayan FE, Barker AL, Bough R, Ortiz M, Takano HK, Duke SO (2019) Herbicide mechanisms of action and resistance. Pages 36–48 in Moo-Young M, ed, Comprehensive Biotechnology, 3rd Edn. Oxford: Pergamon Press

Dayan FE, Cantrell CL, Duke SO (2009) Natural products in crop protection. Bioorg Med Chem 17:4022–4034

Eubank TW, Poston DH, Nandula VK, Koger CH, Shaw DR, Reynolds DB (2008) Glyphosate-resistant horseweed (*Conyza canadensis*) control using glyphosate-, paraquat-, and glufosinate-based herbicide programs. Weed Technol 22:16–21

Feng PC, Cajacob CA, Martino-Catt SJ, Cerny RE, Elmore GA, Heck GR, Padgette SR (2010) Glyphosate-Resistant Crops: Developing the Next Generation Products. Hoboken, NJ: Wiley. Pp 45–66

Fraser K (2019) Stratus survey, one million acres of glyphosate-resistant weeds in Canada. http://www.stratusresearch.com/newsroom/one-million-acres-of-glyphosate-resistant-weeds-in-canada-stratus-survey/. Accessed: November 9, 2021

Hao GF, Zuo Y, Yang SH, Yang GF (2011) Protoporphyrinogen oxidase inhibitor: an ideal target for herbicide discovery. Chimia 65:961–969

Jhala AJ, Ramirez AH, Singh M (2013) Tank mixing saflufenacil, glufosinate, and indaziflam improved burndown and residual weed control. Weed Technol 27:422–429

Koger CH, Poston DH, Eubank TW (2005) Factors affecting germination of horseweed (*Conyza canadensis*). Publication No. 176440. Proceedings Southern Weed Science Society

Kumaratilake AR, Preston C (2005) Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). Weed Sci 53:10–16

Lermontova I, Kruse E, Mock HP, Grimm B (1997) Cloning and characterization of plastidal and mitochondrial isoform of tobacco protoporphyrinogen IX oxidase. PNAS 94:8895–8900

Martinson KB, Durgan BR, Gunsolus JL Sothern RB (2005) Time of day of application effect on glyphosate and glufosinate efficacy. Crop Manag 4:1–7

Matringe M, Camadro JM, Labbe P, Scalla R (1989) Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. Biochem J 260:231–235

Mayer DG, Butler DG (1993) Statistical validation. Ecol Modell 68:21–32

Nandula VK, Eubank TW, Poston DH, Koger CH, Reddy KH (2006) Factors affecting germination of horseweed (*Conyza canadensis*). Weed Sci 54:898–902

Schabenberger O, Pierce FJ (2002) Contemporary statistical models for the plant and soil sciences. Boca Raton, FL: CRC Press. 213 pp

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:21

Sellers BA, Smeda RJ, Li J (2004) Glutamine synthetase activity and ammonium accumulation is influenced by time of glufosinate application. Pestic Biochem Physiol 78:9–20

Shields EJ, Dauer JT, VanGessel MJ, Neumann G (2006) Horseweed (*Conyza canadensis)* seed collected in the planetary boundary layer. Weed Sci 54:1063–1067

Soltani N, Shropshire C, Sikkema PH (2010) Sensitivity of leguminous crops to saflufenacil. Weed Technol 24:143–146

SOY Canada (2021) Seeded area: Acres. https://soycanada.ca/statistics/seeded-area-acres/. Accessed: October 29, 2021

Statistical Analysis Systems (2020) The SAS System, Version 9.2. Cary, NC: Statistical Analysis Systems Institute

Steckel GJ, Wax LM, Simmons FW, Phillips WH (1997) Glufosinate efficacy on annual weeds is influenced by rate and growth stage. Weed Technol 11:484–488

Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2019) Reactive oxygen species trigger the fast action of glufosinate. Planta 249:1837–1849

Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2020a) A novel insight into the mechanism of action of glufosinate: how reactive oxygen species are formed. Photosyn Res 144:361–372

Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2020b) Glufosinate-ammonium: a review of the current state of knowledge. Pest Manag Sci 76:3911–3925

Takano HK, Beffa R, Preston C, Westra P, Dayan FE (2020c) Glufosinate enhances the activity of protoporphyrinogen oxidase inhibitors. Weed Sci 68:324–332

Tharp BE, Kells JJ (2002) Residual herbicides used in combination with glyphosate and glufosinate in corn (*Zea mays*). Weed Technol 16:274–281

Tozzi E, Van Acker RC (2014) Effects of seedling emergence timing on the population dynamics of horseweed (*Conyza canadensis* var. *canadensis*). Weed Sci 62:451–456

VanGessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Sci 49:703–705

Van Wychen L (2016) Survey of the most common and troublesome weeds in broadleaf crops, fruits and vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wp-content/uploads/2016-Weed-Survey_Broadleaf-crops.xlsx. Accessed: November 9, 2021

Waggoner BS (2010) Control of glyphosate resistant horseweed (*Conyza canadensis*) with saflufenacil and tank-mixture partners. Master's Thesis, University of Tennessee, TN. 22 p

Watanabe N, Che FS, Iwano M, Takayama S, Yoshida S (2001) Dual targeting of spinach protoporphyrinogen oxidase II to mitochondria and chloroplasts by alternative use of two in-frame initiation codons. J Biol Chem 276:20474–20481

Weaver SE (2001) The biology of Canadian weeds. 115. *Conyza canadensis*. Can J Plant Sci 81:867–875

Zhou C, Luo X, Chen N, Zhang L, Gao J (2020) C–P natural products as next-generation herbicides: chemistry and biology of glufosinate. J Agric Food Chem 68:3344–3353