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Synergistic and Antagonistic Herbicide Interactions for Control of Volunteer Corn in Glyphosate/Glufosinate/2,4-D-Resistant Soybean

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Abstract

Weed interference from glyphosate/glufosinate-resistant (GGR) volunteer corn can reduce soybean yield and quality. The recent release of glyphosate/glufosinate/2,4-D choline (GG2)-resistant soybean will allow for expanded POST herbicide mixture options for broad-spectrum weed control. Herbicide antagonism between ACCase-inhibiting graminicides and synthetic auxin herbicides has been confirmed for various grass weed species, including volunteer corn. Field experiments (total of 4) were carried out in 2021 and 2022 in southwestern Ontario to assess volunteer corn control with combinations of glufosinate, 2,4-D choline, or dicamba plus clethodim or quizalofop-p-ethyl applied POST to GG2-resistant soybean. Quizalofop-p-ethyl and quizalofop-p-ethyl + glufosinate controlled GGR volunteer corn 95 and 98%, respectively, 6 weeks after application (WAA); adding 2,4-D choline or dicamba to quizalofop-p-ethyl reduced control to ≤ 15%. Clethodim controlled GGR volunteer corn 81%, and the addition of glufosinate increased control to 97%; the co-application of 2,4-D choline or dicamba with clethodim reduced GGR volunteer corn control to 58 and 45%, respectively at 6 WAA. ACCase-inhibiting herbicides co-applied with glufosinate resulted in a synergistic improvement in GGR volunteer corn control while co-applications with synthetic auxin herbicides resulted in an antagonistic decrease in GGR volunteer corn control. Greater antagonism occurred when the synthetic auxin herbicides were co-applied with quizalofop-p-ethyl than clethodim. All mixtures of quizalofop-p-ethyl or clethodim with 2,4-D or dicamba resulted in unacceptable control of GGR volunteer corn.

Keywords: antagonism, E3 soybean, GG2-resistant soybean, herbicide interactions, synergism, volunteer corn

1. Introduction

Soybean (*Glycine max* L.) often follows corn (*Zea mays* L.) in a typical crop rotation in Ontario causing volunteer corn to become a common weed escape in soybean (Gaudin et al., 2015; Soltani et al., 2015). Volunteer corn may be the result of insect or disease infestation in corn, kernel loss, shatter loss from combine header, fallen ears, and tillage (Owen & Zelaya, 2005; Soltani et al., 2015). Volunteer corn can cause substantial soybean yield losses. Deen et al. (2006), and Alms et al. (2016) reported 60 and 71% soybean yield loss, respectively due to volunteer corn interference. Along with reduced yield, volunteer corn also reduces soybean harvestability and lowers soybean quality (Deen et al., 2006). Populations of volunteer corn can act as a host for corn pests, reducing the benefit of crop rotation for insect and disease management (Marquardt et al., 2012). Compared to other annual grass weeds, volunteer corn can grow taller and has wider leaves, which contributes to its competitiveness in soybean (Alms et al., 2016).

Currently, registered preemergence (PRE) herbicides do not offer satisfactory volunteer corn control in soybean; therefore, there is a reliance on post-emergent (POST) herbicides for control (Chahal et al., 2014). Volunteer glyphosate-resistant (GR) corn in GR soybean must be controlled with alternate POST herbicides to glyphosate. Effective volunteer corn control has been obtained with acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides (Alms et al., 2016; Chahal et al., 2014; Chahal & Jhala, 2015; Soltani et al., 2006; Soltani et al., 2015; Underwood et al., 2016). Sethoxydim has reduced efficacy on volunteer corn control in comparison to other ACCase-inhibiting herbicides available for the control of annual grasses in soybean (Chahal & Jhala, 2015; Soltani et al., 2006). Alms et al. (2016) reported 90% control of GR volunteer corn in soybean with clethodim applied at 51 g ai ha^{-1}. In other studies, clethodim applied at 30 and 60 g ai ha^{-1} controlled volunteer corn 88-

90% and 98%, respectively (Soltani et al., 2006; Soltani et al., 2015; Underwood et al., 2016). Soltani et al. (2006) reported 90 and 93% volunteer corn control with quizalofop-p-ethyl application at 18 g ai ha^{-1} and 36 g ai ha^{-1}, respectively. Improved GR volunteer corn control has been documented when an ACCase-inhibiting herbicide is co-applied with glufosinate (Alms et al., 2016).

Multiple herbicides are often concomitantly applied to provide a broader spectrum of weed control. Combining multiple herbicides has the potential to result in antagonistic, additive, or synergistic interactions. Antagonistic interactions between aryloxyphenoxy propionate and cyclohexanedione families of ACCase-inhibiting herbicides, in combination with synthetic auxins, have been reported in the literature (Barnwell & Cobb, 1994; Blackshaw et al., 2006; Costa et al., 2014; Hall et al., 1982; Harre et al., 2020; Mueller et al., 1989; Underwood et al., 2016). The predominant method to minimize antagonism is to increase the rate of the antagonized herbicde (Green, 1989). Underwood et al. (2016) observed an antagonistic interaction with dicamba plus quizalofop-p-ethyl or clethodim mixture for GR volunteer corn control in soybean. Greater levels of antagonism were observed when an increased rate of dicamba was co-applied with a low rate of the graminicide (Underwood et al., 2016).

Three theories have been postulated on the cause of antagonism between ACCase-inhibiting and synthetic auxin herbicides. Firstly, the two herbicide groups may compete for the same binding site, acting as competitive inhibitors (Barnwell & Cobb, 1994). Secondly, Mueller et al. (1990) suggested that the co-application of ACCase-inhibitors with synthetic auxin herbicides causes an alteration in translocation of the ACCase-inhibitor however, others have reported that a decrease or change in herbicide uptake and translocation is not likely the basis for antagonism between diclofop-methyl and 2,4-D (Hall et al., 1982; Han et al., 2013). Thirdly, studies conducted by Han et al. (2013) observed that a pre-treatment of 2,4-D induces amplified metabolism rates of diclofop-methyl in ryegrass.

Soybean cultivars with stacked herbicide resistance to glyphosate, glufosinate, and 2,4-D choline (GG2-resistant soybean/E3) are now commercially available. The utilization of this technology allows for POST applications of these herbicides without damage to the soybean crop. Glufosinate or 2,4-D choline can be co-applied with an ACCase-inhibiting herbicide to control emerged weeds including GR volunteer corn. Considering previous research, this may cause reduced volunteer corn control due to antagonism between the mixture partners. Limited research has been conducted on the control of volunteer corn in GG2-resistant soybean. The aim of this study was to assess the interaction between 2,4-D choline, dicamba, or glufosinate with clethodim or quizalofop-p-ethyl applied POST for glyphosate/glufosinate-resistant (GGR) volunteer corn control in GG2-resistant soybean.

2. Materials and Methods

Two field experiments were conducted near Exeter (43.32°N, -81.50°W) in 2021 and two trials were conducted near London, (42.87°N, -81.13°W) Ontario in 2022; the trials at both locations were separated by herbicide application date. GGR corn seed was saved from the previous year's harvested corn crop. Seedbed preparation for soybean consisted of one pass of field cultivation followed by spreading corn seed produced from GGR hybrid in the previous year across the trial area. After spreading the GGR corn seed, a second cultivator pass incorporated the seed into the soil and was then packed with a roller prior to soybean seeding. GG2-resistant soybean was planted 4.0 cm deep at the approximate rate of 400,000 seeds ha^{-1}. Plots were 3 m in width (4 soybean rows spaced 75 cm apart) and 10 m in length at Exeter and 8 m in length at London. *S*-metolachlor/metribuzin (1943 g ai ha^{-1}) + flumetsulam (50 g ai ha^{-1}) PRE fb glyphosate (900 g ae ha^{-1}) POST was applied POST to control all other weed species. Other pertinent experiment information is presented in Table 1.

Table 1. Year, location, soil characteristics, glyphosate/glufosinate/2,4-D choline-resistant soybean planting, emergence, and harvest dates, and herbicide application for four field trials on control of glyphosate/glufosinate-resistant volunteer corn conducted in southwestern Ontario, Canada in 2021 and 2022

Year	Location	Soil characteristics			Soybean			Herbicide application			
		Texture	OM	pH	Planting date	Emergence date	Harvest date	Application date	Volunteer corn height	Volunteer corn growth stage	Volunteer corn density
			%						cm		plants m^{-2}
2021	Exeter	Loam	4.4	8.0	May 13	May 21	Nov 2	June 11	23	V4	11
	Exeter	Loam	4.4	8.0	May 13	May 21	Nov 2	June 14	28	V5	8
2022	London	Loam	3.3	6.7	Jun 15	Jun 22	Oct 11	Jul 7	25	V4	23
	London	Loam	3.3	6.7	Jun 15	Jun 22	Oct 11	Jul 11	31	V5	16

Note. Abbreviations: OM, organic matter.

Experiments were set up as a randomized complete block design with four replications. The treatments included quizalofop-p-ethyl (24 g ai ha^{-1}) or clethodim (30 g ai ha^{-1}) applied alone and in a mixture with glufosinate (500 g ai ha^{-1}), 2,4-D choline (817 g ae ha^{-1}), or dicamba (600 g ae ha^{-1}). Supplemental information regarding the herbicides utilized is presented in Table 2. Treatment applications were made after 9 am and prior to 10:30 am to reduce the time-of-day at application effect on weed control with glufosinate (Martinson et al., 2005; Montgomery et al., 2017; Takano & Dayan, 2020). A backpack sprayer equipped with pressurized CO_2, calibrated to deliver a water volume of 200 L ha^{-1} at 240 kPa was used to apply the herbicide treatments. The backpack sprayer was outfitted with four ULD 11002 spray nozzles spaced 50 cm apart producing a spray width of 2.0 m. Treatments were applied when most of the volunteer corn plants had emerged to reduce the effect of late-emerging plants impacting control ratings.

Table 2. Herbicide active ingredient, trade name, rate, manufacturer, and manufacturer address for herbicides used in experiments for glyphosate/glufosinate volunteer corn control in glyphosate/glufosinate/2,4-D-resistant soybean in Ontario in 2021 and 2022

Herbicide[a]	Trade name	Rate	Manufacturer	Manufacturer address
		g ai ha^{-1} or g ae ha^{-1}		
Glufosinate ammonium	Liberty® 200 SN	500	BASF Canada Inc.	100 Milverton Drive, Mississauga, Ontario, Canada, L5R 4H1 https://www.basf.com/ca/en.html
2,4-D choline	Enlist One™	817	Corteva Agriscience	215 2nd St. SW Calgary, Alberta, Canada, T2P 1M4 https://www.corteva.ca/
Dicamba	Engenia®	600	BASF Canada Inc.	100 Milverton Drive, Mississauga, Ontario, Canada, L5R 4H1 https://www.basf.com/ca/en.html
Quizalofop-p-ethyl	Assure II	24	AMVAC Chemical Corp.	4695 MacArthur Ct, Newport Beach, California, USA, 92660 https://www.amvac.com/
Clethodim	Select®	30	BASF Canada Inc.	100 Milverton Drive, Mississauga, Ontario, Canada, L5R 4H1 https://www.basf.com/ca/en.html

Note. [a]The recommended adjuvant was applied with each herbicide used: Glufosinate ammonium included ammonium sulfate (AMS) (Alpine Plant Foods, 30 Neville St, New Hamburg, Ontario, Canada, N3A 4G7) at 6.5 L ha^{-1}; Quizalofop-p-ethyl included Sure-Mix® (Corteva Agriscience; 215 2nd St. SW Calgary, Alberta, Canada, T2P 1M4) at 0.5% v/v; Clethodim included Amigo® (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, Colorado, USA, 80538) at 0.5% v/v.

Soybean injury (%) was assessed 1, 2, and 4 weeks after application (WAA) on a scale of 0-100 (0 = no soybean injury and 100 = total plant death). Volunteer corn control ratings were conducted 2, 4, and 6 WAA on a scale of 0-100% (0 = no control and 100 = total control, compared to the nontreated control). Density and dry weight (aboveground biomass) of volunteer corn plants were determined 6 WAA based on two randomly placed 0.5 m^2 quadrats in 2021 and 0.25 m^2 quadrats in 2022 within each plot. Within each quadrat, the volunteer corn plants were counted, and then cut at the base of the plant, placed in paper bags, and dried in a dryer. Upon reaching constant moisture, volunteer corn dry weight was recorded. After all data were collected, a blanket application of quizalofop-p-ethyl (72 g ai ha^{-1}) + SureMix (0.5% v/v) was made to reduce the confounding effect of corn grain on soybean yield data by controlling all remaining volunteer corn plants. Soybean was harvested with a small-plot combine at maturity. Soybean yield was adjusted to 13.0% moisture. Soybean yield from 2022 was omitted from the analysis due to uncontrolled volunteer corn resulting in soybean yield samples contaminated with corn seed.

2.1 Statistical Analysis

All response parameters were analyzed in SAS 9.4 using the PROC GLIMMIX procedure for ANOVA analysis. Variances were divided into fixed effects (treatment) and random effects [environment (including both location and year), and replication within environment]. The significance of the fixed effects was ascertained with an F-test and the random effects using a log-likelihood ratio test, $\alpha = 0.05$ was used for all tests. Data were pooled across environments. Analysis of the studentized residual plots and the Shapiro-Wilk test for normality was conducted to make sure the data met the assumptions of ANOVA. When necessary, transformations including arcsine square root were performed to control data. Volunteer corn density and dry weight were transformed using a log-normal distribution. All transformed data were back-transformed for the presentation of the results. The Tukey-Kramer test was used with a significance of $P = 0.05$. All treatments with zero variance were excluded from the analysis. Comparisons of each treatment with zero were conducted using the P-value given in the LSMEANS table.

To calculate the expected visible GGR volunteer corn control values within each block, Colby's equation was used (Colby, 1967). The equation utilized observed values for the ACCase-inhibitor applied alone (X) and either glufosinate, 2,4-D choline, or dicamba (Y).

$$\text{Expected} = (X + Y) - [(X \times Y)/100] \quad (1)$$

An adjusted Colby's equation was utilized to calculate expected GGR volunteer corn density and dry weight within each block. The altered Colby's equation utilizes the observed value from the nontreated control (Z) in each block.

$$\text{Expected} = [(X \times Y)/Z] \quad (2)$$

The expected values were analyzed using the same statistical tests and when required the same transformations were applied as the control data. A two-tailed t-test was utilized to compare the observed and expected values for GGR volunteer corn control, density, and dry biomass. Herbicide interactions were classified as antagonistic or synergistic when the observed and expected values were statistically different. A minimum statistical significance level of $P = 0.05$ was used.

3. Results and Discussion

3.1 Soybean Injury

Applications of clethodim, quizalofop-p-ethyl, glufosinate, or 2,4-D choline caused minimal GG2-resistant soybean injury (< 6%). Applications of dicamba to non-dicamba-resistant soybean resulted in 100% crop death (data not presented).

3.2 Volunteer Corn Control

The volunteer corn used in this study was GGR; therefore, applications of glufosinate, 2,4-D choline, or dicamba caused 0% volunteer corn control at all assessment timings (Table 3). Quizalofop-p-ethyl applied alone or co-applied with glufosinate and clethodim co-applied with glufosinate controlled GGR volunteer corn 88-95% at 2 WAA. Clethodim alone controlled GGR volunteer corn 81%, similar to the aforementioned herbicides, and to clethodim + dicamba. The mixture of quizalofop-p-ethyl with 2,4-D choline or dicamba decreased GGR volunteer corn control by 77 and 68 percentage points, respectively, compared to quizalofop-p-ethyl alone. The mixture of a synthetic auxin herbicide with clethodim resulted in greater GGR volunteer corn control than quizalofop-p-ethyl mixture with a synthetic auxin herbicide. At 2 WAA, there was a decrease of 29 percentage points in GGR volunteer corn control with clethodim mixture with 2,4-D choline compared to clethodim application alone.

Table 3. Glyphosate/glufosinate-resistant volunteer corn control and nonorthogonal contrasts 2, 4, and 6 weeks after herbicide application in glyphosate/glufosinate/2,4-D choline-resistant soybean from four field trials in southwestern Ontario, Canada in 2021 and 2022

Treatment[d]	Rate	Control[ab]											
		2 WAA				4 WAA				6 WAA			
		Observed		Expected[c]		Observed		Expected		Observed		Expected	
	g ai ha^{-1}	%											
Nontreated control	-	0	e			0	e			0	f		
Glufosinate	500	0	e			0	e			0	f		
2,4-D choline	817	0	e			0	e			0	f		
Dicamba	600	0	e			0	e			0	f		
Quizalofop-p-ethyl	24	89	a			94	ab			95	ab		
Quizalofop-p-ethyl+glufosinate	24+500	95	a	89	**	98	a	94	*	98	a	95	*
Quizalofop-p-ethyl+2,4-D choline	24+817	12	d	89	**	11	d	94	**	15	e	95	**
Quizalofop-p-ethyl+dicamba	24+600	21	d	89	**	18	d	94	**	14	e	95	**
Clethodim	30	81	ab			80	bc			81	bc		
Clethodim+glufosinate	30+500	88	a	81	**	95	ab	80	**	97	ab	81	**
Clethodim+2,4-D choline	30+817	52	c	81	**	56	c	80	**	58	dc	81	**
Clethodim+dicamba	30+600	53	bc	81	**	54	c	80	**	45	d	81	**
Contrasts													
ACCase-inhibitor vs. ACCase-inhibitor+glufosinate		85 vs. 92 NS				88 vs. 97**				89 vs. 98**			
ACCase-inhibitor vs. ACCase-inhibitor+synthetic auxin		85 vs. 33**				88 vs. 33**				89 vs. 31**			
ACCase-inhibitor+2,4-D choline vs. ACCase-inhibitor+dicamba		30 vs. 36 NS				31 vs. 35 NS				35 vs. 28 NS			

Note. Abbreviations: WAA, weeks after application.

[a] Means within the same column followed by the same lowercase letter are not statistically different according to the Tukey-Kramer multiple range test (P < 0.05).

[b] Percent control data (0%) of the nontreated control, glufosinate, 2,4-D choline, and dicamba treatments were not included in statistical analysis.

[c] Expected values calculated from the Colby's equation.

[d] The recommended adjuvant was applied with each herbicide used: Glufosinate ammonium included ammonium sulfate (AMS) at 6.5 L ha^{-1}; Quizalofop-p-ethyl included Sure-Mix® at 0.5% v/v; Clethodim included Amigo® at 0.5% v/v.

*Indicates significant differences of P < 0.05.

**Indicates significant differences of P < 0.01.

At 4 WAA, quizalofop-p-ethyl mixture with glufosinate controlled GGR volunteer corn 98%. Quizalofop-p-ethyl alone and clethodim mixture with glufosinate provided 94-95% control, similar to both quizalofop-p-ethyl + glufosinate, and clethodim alone which provided 80% control. Similar results were reported by Soltani et al. (2006) who observed > 86% control on GR volunteer corn with quizalofop-p-ethyl or clethodim. Mixtures of quizalofop-p-ethyl with both 2,4-D choline or dicamba caused only 11-18% control of GGR volunteer corn. The co-application of clethodim with either synthetic auxin provided 54-56% control.

Clethodim and quizalofop-p-ethyl controlled GGR volunteer corn 81 and 95%, respectively at 6 WAA; the mixture of glufosinate with clethodim or quizalofop-p-ethyl provided 98 and 97% control, respectively. Similarly, Alms et al. (2016) reported ≥ 96% GR volunteer corn control with glufosinate mixture with quizalofop-p-ethyl. Quizalofop-p-ethyl co-applied with 2,4-D choline or dicamba decreased in volunteer control by 80 and 81 percentage points, respectively; in contrast, clethodim co-applied with 2,4-D choline or dicamba decreased volunteer control 23 and 36 percentage points, respectively.

The results from this study indicate synergistic interactions when quizalofop-p-ethyl or clethodim were concomitantly applied with glufosinate for GGR volunteer corn control at all assessment timings. Alms et al. (2016) reported improved GR volunteer corn control when glufosinate was combined with quizalofop-p-ethyl or clethodim compared to glufosinate alone. Comparing observed and expected volunteer corn control values indicated antagonistic interactions between mixtures of quizalofop-p-ethyl or clethodim and 2,4-D choline or dicamba across all assessment timings. Underwood et al. (2016) also observed an antagonistic response with quizalofop-p-ethyl (24 g ai ha^{-1}) or clethodim (30 g ai ha^{-1}) mixed with dicamba (600 g ai ha^{-1}) for GR volunteer corn control; however, the control was greater than in this study. Underwood et al. (2016) observed 74 and 68% control of GR volunteer corn control with quizalofop-p-ethyl or clethodim + dicamba which was 20 and 11% lower than the expected values, respectively. In this study, the co-application of dicamba with quizalofop-p-ethyl or clethodim reduced GGR volunteer corn by 83 and 24% from the expected values, respectively at 4 WAA. Differences in the level of antagonism observed between studies may be due to environmental differences and corn size at application timing.

Based on nonorthogonal contrasts, there was an improvement in volunteer corn control of 9% when the ACCase-inhibiting herbicides were co-applied with glufosinate at 4 and 6 WAA. When the ACCase-inhibiting herbicides were co-applied with the synthetic auxin herbicides, control was reduced at all assessment timings. The GGR volunteer corn control was not different among the two synthetic auxin herbicides when co-applied with the ACCase-inhibitors.

3.3 Volunteer Corn Density and Dry Biomass

Glufosinate, 2,4-D choline, and dicamba caused no reduction in the GGR volunteer corn density (Table 4). Quizalofop-p-ethyl reduced GGR volunteer corn density by 90%; there was no further decrease in GGR volunteer corn density when quizalofop-p-ethyl was mixed with glufosinate. In contrast, when quizalofop-p-ethyl was mixed with 2,4-D choline or dicamba, the GGR volunteer corn density was similar to the nontreated control. Clethodim reduced GGR volunteer corn density 60%; there was a further decrease of 20% when clethodim was co-applied with glufosinate. The clethodim mixture with 2,4-D choline or dicamba resulted in GGR volunteer corn density similar to the nontreated control.

Table 4. Glyphosate/glufosinate-resistant volunteer corn density, dry biomass, and nonorthogonal contrasts after herbicide application in glyphosate/glufosinate/2,4-D choline-resistant soybean from four field trials in southwestern Ontario, Canada in 2021 and 2022

Treatment[c]	Rate	Volunteer corn density				Volunteer corn dry biomass			
		Observed[a]		Expected[b]		Observed		Expected	
	g ai ha^{-1}	--------- plants m^{-2} --------				--------------- g m^{-2} --------------			
Nontreated control	-	10	c			433	ef		
Glufosinate	500	10	c			463	ef		
2,4-D choline	817	11	c			385	ef		
Dicamba	600	9	c			558	f		
Quizalofop-p-ethyl	24	1	a			26	ab		
Quizalofop-p-ethyl+glufosinate	24+500	1	a	1		7	a	30	
Quizalofop-p-ethyl+2,4-D choline	24+817	6	bc	1	**	280	def	32	**
Quizalofop-p-ethyl+dicamba	24+600	6	bc	1	**	363	def	43	**
Clethodim	30	4	b			47	bc		
Clethodim+glufosinate	30+500	2	a	4	**	9	a	82	
Clethodim+2,4-D choline	30+817	7	bc	5		120	cd	50	*
Clethodim+dicamba	30+600	6	bc	3	**	163	de	66	**
Contrasts									
ACCase-inhibitor vs. ACCase-inhibitor+glufosinate		3 vs. 1**				61 vs. 12**			
ACCase-inhibitor vs. ACCase-inhibitor+synthetic auxin		3 vs. 6 **				61 vs. 259**			
ACCase-inhibitor+2,4-D choline vs. ACCase-inhibitor+dicamba		7 vs. 6 NS				228 vs. 293 NS			

Note. Abbreviations: WAA, weeks after application.

[a]Means within the same column followed by the same lowercase letter are not statistically different according to the Tukey-Kramer multiple range test ($P < 0.05$).

[b]Expected values calculated from the Colby's equation.

[c]The recommended adjuvant was applied with each herbicide used: Glufosinate ammonium included ammonium sulfate (AMS) at 6.5 L ha^{-1}; Quizalofop-p-ethyl included Sure-Mix® at 0.5% v/v; Clethodim included Amigo® at 0.5% v/v.

*Indicates significant differences of $P < 0.05$.

**Indicates significant differences of $P < 0.01$.

Glufosinate, 2,4-D choline, and dicamba caused no reduction in GGR volunteer corn biomass (Table 5). Quizalofop-p-ethyl reduced GGR volunteer corn biomass by 94%; when co-applied with glufosinate GGR volunteer corn biomass decreased by 98%. Similarly, Underwood et al. (2016) observed a 90% decrease in GR volunteer corn dry biomass with quizalofop-p-ethyl (24 g ai ha^{-1}). In contrast, the mixture of quizalofop-p-ethyl with 2,4-D choline or dicamba resulted in GGR volunteer corn biomass comparable to the nontreated control. Clethodim reduced GGR volunteer corn biomass by 89%; a further decrease in GGR volunteer corn biomass of 9% was observed when clethodim was mixed with glufosinate. In contrast, the mixture of clethodim with 2,4-D choline or dicamba decreased volunteer corn dry biomass by 72% and 62%, respectively; the biomass with the co-application of clethodim and dicamba was similar to the nontreated control.

Table 5. Glyphosate/glufosinate/2,4-D choline-resistant soybean yield and nonorthogonal contrasts from two trials in southwestern Ontario, Canada in 2021

Treatment[c]	Rate	Soybean yield[ab]
	g ai ha^{-1}	t ha^{-1}
Nontreated control	-	3.2 d
Glufosinate	500	3.2 d
2,4-D choline	817	3.3 cd
Dicamba	600	-
Quizalofop-p-ethyl	24	4.2 ab
Quizalofop-p-ethyl+glufosinate	24+500	4.5 a
Quizalofop-p-ethyl+2,4-D choline	24+817	3.2 d
Quizalofop-p-ethyl+dicamba	24+600	-
Clethodim	30	4.0 ab
Clethodim+glufosinate	30+500	4.4 a
Clethodim+2,4-D choline	30+817	3.8 bc
Clethodim+dicamba	30+600	-
Contrasts		
ACCase-inhibitor vs. ACCase-inhibitor+glufosinate		4.1 vs. 4.5**
ACCase-inhibitor vs. ACCase-inhibitor+2,4-D choline		4.1 vs. 3.5**

Note. Abbreviations: WAA, weeks after application.

[a]Means within the same column followed by the same lowercase letter are not statistically different according to the Tukey-Kramer multiple range test (P < 0.05).

[b]Yield data from treatments containing dicamba (0 yield) were excluded from statistical analysis.

[c]The recommended adjuvant was applied with each herbicide used: Glufosinate ammonium included ammonium sulfate (AMS) at 6.5 L ha^{-1}; Quizalofop-p-ethyl included Sure-Mix® at 0.5% v/v; Clethodim included Amigo® at 0.5% v/v.

*Indicates significant differences of P < 0.05.

**Indicates significant differences of P < 0.01.

When clethodim was co-applied with glufosinate, the observed volunteer corn density was lower than the expected density which shows a synergistic effect compared to clethodim applied alone. When quizalofop-p-ethyl was co-applied with either synthetic auxin and when clethodim was co-applied with dicamba, the observed values for both GGR volunteer corn density and dry biomass were greater than the expected values indicating an antagonistic effect. When clethodim was applied with 2,4-D choline, the observed volunteer corn dry biomass was greater than expected, an antagonistic interaction.

Contrast analysis showed a synergistic reduction in GGR volunteer corn density and dry biomass when the ACCase-inhibiting herbicides were co-applied with glufosinate in comparison with the ACCase-inhibiting herbicides alone. In contrast, there was an antagonistic increase in GGR volunteer corn density and dry biomass with co-applications of synthetic auxin and ACCase-inhibiting herbicides.

3.4 Soybean Yield

GGR volunteer corn interference lowered soybean yields by up to 29% (highest yielding treatment compared to the nontreated control) in this study. GGR volunteer corn interference with glufosinate and 2,4-D choline produced soybean yield that was similar to the nontreated control, consistent with the control ratings. Reduced GGR volunteer corn interference with quizalofop-p-ethyl, clethodim, and the mixture of the ACCase-inhibiting herbicides with glufosinate provided the highest and similar GG2-resistant soybean yield. The mixture of quizalofop-p-ethyl with 2,4-D choline provided a similar soybean yield to the nontreated control. As expected, all treatments containing dicamba resulted in the death of the GG2-resistant soybean, therefore all dicamba treatments were removed from statistical analysis. Nonorthogonal contrasts indicate improved soybean yield when glufosinate is co-applied with an ACCase-inhibiting herbicide and decreased soybean yield when 2,4-D choline was mixed with an ACCase-inhibiting herbicide.

4. Conclusions

The ACCase-inhibiting herbicides quizalofop-p-ethyl and clethodim provided good control of GGR volunteer corn in GG2-resistant soybean. When co-applied with glufosinate these ACCase-inhibiting herbicides show a synergistic interaction resulting in improved GGR volunteer corn control. The authors hypothesize that the improved GGR volunteer corn control may be due to improved herbicide absorption resulting from the adjuvant system in the commercial glufosinate formulation. In this study, there was a synergistic increase in GGR volunteer corn control with glufosinate mixture with the systemic ACCase-inhibiting herbicides which is likely due to improved herbicide absorption.

The mixture of 2,4-D choline or dicamba with quizalofop-p-ethyl or clethodim caused an antagonistic response and reduced GGR volunteer corn control. Numerically, there were greater levels of antagonism when quizalofop-p-ethyl was mixed with both synthetic auxin herbicides than when co-applied with clethodim. All antagonistic mixtures resulted in commercially unacceptable control of GGR volunteer corn. Increased doses of the antagonized herbicide can overcome herbicide antagonism. Further research is needed to ascertain the effect of ACCase-inhibitor rate when co-applied with 2,4-D choline in GG2-resistant soybean. This study concludes that the mixture of quizalofop-p-ethyl or clethodim with 2,4-D choline or dicamba at the rates evaluated results in an antagonistic interaction and unacceptable GGR volunteer control in GG2-resistant soybean.

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