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Additive and synergistic interactions of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and photosystem II (PSII) inhibitors for the control of glyphosate-resistant horseweed (*Conyza canadensis*) in corn

Research Article

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Abstract

Glyphosate-resistant (GR) horseweed [*Conyza canadensis* (L.) Cronquist; syn.: *Erigeron canadensis* L.] interference can substantially reduce corn (*Zea mays* L.) yield. The complementary activity of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and photosystem II (PSII) inhibitors has been investigated for the control of several weed species, and in many cases has been synergistic; however, there is little information on the interaction of HPPD- and PSII-inhibiting herbicides for postemergence control of GR *C. canadensis* in corn. Four field trials were studied over 2 yr (2019, 2020) in Ontario, Canada, in commercial corn fields with natural infestations of GR *C. canadensis* to evaluate the level of GR *C. canadensis* control with three HPPD-inhibiting herbicides (mesotrione, tolpyralate, and topramezone) and three PSII-inhibiting herbicides (atrazine, bromoxynil, and bentazon) applied individually and in tank-mix combinations, and to document the interaction of the three HPPD inhibitors tank mixed with the three PSII inhibitors. Mesotrione, tolpyralate, and topramezone controlled GR *C. canadensis* 83%, 84%, and 72%, respectively, at 8 wk after application (WAA). Bromoxynil and bentazon controlled GR *C. canadensis* 71% and 79%, respectively, while atrazine provided only 31% control at 8 WAA. The joint application of atrazine, bromoxynil, or bentazon with mesotrione increased GR *C. canadensis* control from 83% to 100% at 8 WAA. Tolpyralate tank mixed with atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 96%, 98%, and 98%, respectively, which was comparable to the mesotrione tank mixes at 8 WAA. Topramezone plus atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 91%, 93%, and 95%, respectively, at 8 WAA. Interactions between HPPD and PSII inhibitors were synergistic for all combinations of mesotrione or tolpyralate with atrazine, bromoxynil, or bentazon. The interaction between topramezone and PSII inhibitors was additive. All nine tank mixes controlled GR *C. canadensis* >90%. This study concludes that bromoxynil or bentazon, instead of atrazine, can be co-applied with mesotrione, tolpyralate, or topramezone without compromising GR *C. canadensis* control in corn.

Introduction

Horseweed [*Conyza canadensis* (L.) Cronquist; syn.: *Erigeron canadensis* L.] is a fall- or spring-germinating annual weed species that can germinate under a myriad environmental conditions (Buhler and Owen 1997; Main et al. 2006; Nandula et al. 2006; Weaver 2001). The high fecundity of *C. canadensis*, along with the tendency of its seeds to germinate best when located on or near the soil surface, makes *C. canadensis* a particularly successful weed in no-tillage cropping systems (Brown and Whitwell 1988; Nandula et al. 2006; Regehr and Bazzaz 1979). An individual *C. canadensis* plant can produce 230,000 seeds, which then may be wind disseminated 500 km from the plant of origin by the aid of an attached pappus (Shields et al. 2006; Weaver 2001). This favorable seed dispersal mechanism and high fecundity of *C. canadensis* allow for its quick and pervasive expansion in agroecosystems.

Conyza canadensis has been reported to be one of the most common and troublesome weeds in corn (*Zea mays* L.) in several U.S. states and in Ontario, Canada (Van Wychen 2020). A glyphosate-resistant (GR) biotype of *C. canadensis* was first reported in a population in

Delaware, USA in 2000 (VanGessel 2001). Ten years later, GR *C. canadensis* was found in a population from Essex County, Ontario, Canada (Byker et al. 2013). GR biotypes of *C. canadensis* have since been identified in 30 counties across southern Ontario (Budd et al. 2018). GR *C. canadensis* is also widespread in the United States, where it has been reported in 25 states as of September 2021 (Heap 2021).

Several herbicides applied preplant provide effective control (>90%) of GR biotypes of *C. canadensis* in corn (Brown et al. 2016; Ford et al. 2014). There are fewer postemergence herbicide options in corn that provide >90% control of GR *C. canadensis*. In Ontario, GR *C. canadensis* can be controlled >90% with a postemergence application of dicamba, dicamba/atrazine, bromoxynil + atrazine, or tolpyralate + atrazine (Langdon et al. 2020; Mahoney et al. 2017; Metzger et al. 2019). Only four active ingredients (dicamba, atrazine, bromoxynil, and tolpyralate) are represented across these postemergence herbicide options for effective GR *C. canadensis* control. The incorporation of tillage or cover crops into a crop production system can suppress GR *C. canadensis*, but these two weed management strategies are not suitable in all corn-cropping systems for various agronomic and economic reasons (Chahal and Jhala 2019; Cholette et al. 2018). Suppression of GR *C. canadensis* can be achieved with previous crop residue left on the soil, but the suppression is often not commercially acceptable (Main et al. 2006). The use of herbicides can complement various cultural and mechanical control options for effective *C. canadensis* control. When GR *C. canadensis* is present, effective control is imperative, as interference by this weed biotype has been reported to reduce grain corn yields up to 69% (Ford et al. 2014). The competitiveness, difficulty of control, and geographic distribution of GR *C. canadensis* in corn emphasizes the need for efficacious postemergence herbicide options for its control in corn.

Photosystem II (PSII) and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors are commonly applied in tank mixes. Herbicides that inhibit the HPPD enzyme block the production of homogentisic acid, which prevents the synthesis of plastoquinone and tocopherols in susceptible plants (Schulz et al. 1993; Secor 1994; Tsegaye et al. 2002). A lack of plastoquinone and tocopherols limits the ability of a susceptible plant to quench reactive oxygen species, which leads to the destruction of plant cells (Kruk et al. 2005; Trebst et al. 2002). Mesotrione, topramezone, and tolpyralate are commonly used postemergence HPPD inhibitors in Ontario with different weed control spectrums (Kohrt and Sprague 2017; Metzger et al. 2018). PSII inhibitors such as atrazine, bentazon, and bromoxynil are complementary to HPPD inhibitors because of their interrelated modes of action (Abendroth et al. 2006; Armel et al. 2005; Creech et al. 2004; Kim et al. 1999). PSII inhibitors disrupt electron flow in the photosynthetic electron transport chain by competing with plastoquinone for the Q_B binding site on the D1 protein (Hess 2000). PSII inhibitors cause cell death by triggering a massive influx of reactive oxygen species, which induces lipid peroxidation (Hess 2000). Herbicides that inhibit HPPD therefore can increase the productivity of a PSII inhibitor to bind to the D1 protein by limiting the biosynthesis of plastoquinone when the herbicides are applied in conjunction (Armel et al. 2005). Also, because HPPD inhibitors induce a loss in quenching of reactive oxygen species and PSII inhibitors cause an influx of reactive oxygen species, greater herbicidal activity may occur with joint applications of the two herbicides (Armel et al. 2005; Creech et al. 2004). Therefore, PSII inhibitors, typically atrazine, and HPPD inhibitors are commonly tank mixed to improve the efficacy and broaden the weed control spectrum of a single herbicide application (Armel et al. 2005, 2008, 2009; Johnson et al. 2002; Kohrt and Sprague 2017; Metzger et al. 2018, 2019; Whaley et al. 2006).

When herbicides from distinct modes of action are co-applied, weed control with these tank-mix combinations can be termed "antagonistic," "additive," or "synergistic." Colby's equation is used to calculate the expected weed control of a herbicide mix based on the level of weed control from the component herbicides applied individually (Colby 1967). If the observed weed control from the tank mix is less, equal, or greater than expected, the interaction between the two herbicides is either antagonistic, additive, or synergistic, respectively (Colby 1967).

In many cases, the joint activity of HPPD and PSII inhibitors has been reported as synergistic or additive for the control of several weed species; however, the reported interaction between HPPD and PSII inhibitors can vary among HPPD inhibitors, PSII inhibitors, herbicide rates evaluated, weed species, and weed biotype resistance profiles (Abendroth et al. 2006; Hugie et al. 2008; Kohrt and Sprague 2017; Woodyard et al. 2009a). The synergy between postemergence-applied HPPD and PSII inhibitors has been documented for the control of waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], Palmer amaranth (*Amaranthus palmeri* S. Watson), redroot pigweed (*Amaranthus retroflexus* L.), wild radish (*Raphanus raphanistrum* L.), velvetleaf (*Abutilon theophrasti* Medik.), giant ragweed (*Ambrosia trifida* L.), common cocklebur (*Xanthium strumarium* L.), red morningglory (*Ipomoea coccinea* L.), common lambsquarters (*Chenopodium album* L.), and giant foxtail (*Setaria faberi* Herrm.) (Armel et al. 2007; Hugie et al. 2008; Kohrt and Sprague 2017; Walsh et al. 2012; Willemse et al. 2021; Woodyard et al. 2009a, 2009b). Additive interactions between HPPD and PSII inhibitors have also been reported for the control of *Amaranthus* spp., *A. theophrasti*, *A. trifida*, *C. album*, and *R. raphanistrum* (Hugie et al. 2008; Kohrt and Sprague 2017; Walsh et al. 2012; Willemse et al. 2021; Woodyard et al. 2009a, 2009b).

Most of the published literature on the interaction between HPPD and PSII inhibitors involves the use of atrazine, with few studies incorporating other PSII inhibitors such as bentazon and bromoxynil. Given that the use of atrazine has become more restricted due to atrazine detected in groundwater and surface water, identifying the interaction and level of control of GR *C. canadensis* with HPPD inhibitors when applied with atrazine-alternative PSII inhibitors is valuable (Graymore et al. 2001). Additionally, much of the peer-reviewed literature focuses on the interaction of HPPD and PSII inhibitors on *Amaranthus* spp., while the interaction has not been intensively studied for GR *C. canadensis* control in corn. Therefore, the two objectives of this study were to evaluate the level of GR *C. canadensis* control with HPPD and PSII inhibitors applied alone and in tank mixes and to classify the interaction of HPPD and PSII inhibitors for the control of GR *C. canadensis* in corn.

Materials and Methods

Four no-tillage field trials were conducted in 2019 and 2020 in commercial corn fields in southwestern Ontario, Canada (Table 1). In 2019, two trials were conducted near the community of Zone Centre on two separate fields located at 42.62°N, 81.94°W and 42.62°N, 81.95°W, with the trials being separated both geographically and temporally. In 2020, one field trial was near Highgate (42.55°N, 81.84°W) and another one was near Thamesville (42.53°N, 81.91°W). Populations of *C. canadensis*

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Table 1. Year, location, soil characteristics, corn planting dates, corn harvest dates, treatment application dates, corn development stages at treatment application, and glyphosate-resistant *Conyza canadensis* size and density at treatment application for four field trials in southwestern Ontario, Canada, in 2019 and 2020.

Year	Nearest community	Soil characteristics[a]			Corn planting date	Corn harvest date	Treatment application information			
		Texture	OM	pH			Application date	Corn development stage	*C. canadensis* size[b]	*C. canadensis* density[c]
			%						cm	plants m^{-2}
2019	Zone Centre	Sand	2.4	7.0	June 4	November 7	June 18	V1	9	96
	Zone Centre	Sand	2.6	6.7	May 24	November 6	June 7	V1	9	117
2020	Highgate	Loamy sand	2.5	6.6	June 12	November 10	June 16	PRE[d]	10	120
	Thamesville	Loamy sand	2.1	5.5	June 5	November 11	May 26	PP[d]	8	245

[a]Soil characteristics were obtained from soil cores taken to a depth of 15 cm and subsequent analysis at A&L Canada Laboratories Inc. (2136 Jetstream Road, London, ON N5V 3P5, Canada). OM, organic matter; PP, preplant.
[b]Size determined as height of bolting plants or diameter of rosettes at time of treatment application. Mean of measurements taken in two randomly placed 0.25-m^2 quadrats in each nontreated control plot.
[c]Mean density of two stand counts in each nontreated control plot.
[d]Treatments applied before corn emergence, as weed size dictated application date.

Table 2. Herbicide active ingredient, rate, trade name, and manufacturer for the study of the interaction between 4-hydroxyphenylpyruvate dioxygenase and photosystem II inhibitors on the control of glyphosate-resistant *C. canadensis* in southwestern Ontario, Canada, in 2019 and 2020.

Herbicide active ingredient[a]	Rate	Trade name	Manufacturer
	g ai ha^{-1}		
Atrazine	560	AAtrex® Liquid 480	Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada, https://www.syngenta.ca
Bentazon	840	Basagran® Forté Herbicide Liquid	BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, https://www.basf.com/ca/en.html
Bromoxynil	280	Pardner® Herbicide	Bayer CropScience Inc., 160 Quarry Park Boulevard SE, Calgary, AB T2C 3G3, Canada, https://www.cropscience.bayer.ca/en
Mesotrione	100	Callisto® 480SC Herbicide	Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada, https://www.syngenta.ca
Tolpyralate	30	Shieldex® 400SC Herbicide	ISK Biosciences Corporation, 740 Auburn Road, Concord, OH 44077, USA, http://www.iskbc.com
Topramezone	12.5	Armezon® Herbicide	BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, https://www.basf.com/ca/en.html

[a]The adjuvant used depended on the herbicide: mesotrione included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada) at 0.2% v/v; tolpyralate included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA) at 0.5% v/v and urea ammonium nitrate (UAN 28-0-0) at 2.5% v/v; topramezone included Merge® (BASF Canada Inc., 100 Milverton Drive, Mississauga L5R 4H1, ON, Canada) at 0.5% v/v.

from these sites were previously confirmed as GR by greenhouse screening (unpublished data). All sites contained natural infestations of confirmed GR *C. canadensis*. GR corn hybrids, DKC45-65RIB® and DKC42-60RIB® (Bayer CropScience Canada, 160 Quarry Boulevard SE, Calgary, AB T2C 3G3, Canada) were planted at a seeding rate of approximately 80,000 seeds ha^{-1} in 2019 and 2020, respectively. Corn was planted approximately 4-cm deep in 75-cm row spacing. Plot dimensions were 8 m in length and 2.25 m wide. Trials were set up as randomized complete block designs with four replicated blocks in each trial. The trials were fertilized according to provincial recommendations for corn production in Ontario (OMAFRA 2017). Soil characteristics, corn planting and harvest dates, herbicide application dates, and GR *C. canadensis* size and density at application are listed in Table 1.

Glyphosate-susceptible *C. canadensis* and other competing weed species were removed before treatment application with an application of glyphosate (Roundup WeatherMax®, 540 g ae L^{-1}, Bayer CropScience Canada) at a rate of 900 g ae ha^{-1} to ensure a monoculture weed population of GR *C. canadensis*. No pesticide other than the herbicide treatments was applied for the rest of the study. The study was conducted as a two-factor factorial. Factor 1 included four levels of HPPD-inhibiting herbicides: nontreated control, mesotrione, tolpyralate, and topramezone. Factor 2 comprised the PSII-inhibiting herbicide factor: nontreated control, atrazine, bromoxynil, and bentazon. Detailed treatment information is presented in Table 2. All herbicide treatments were sprayed with a CO_2-powered backpack plot sprayer, outfitted with four ULD120-02 spray nozzles (Pentair, 375 5th Avenue NW, New Brighton, MN 55112, USA) at 50-cm spacing calibrated to deliver 200 L ha^{-1} spray volume at 240 kPa pressure. Herbicide treatments were sprayed when the GR *C. canadensis* in the nontreated control plots reached an average of approximately 10 cm in height or rosette diameter. The herbicide treatments were intended to be sprayed postemergence to the corn; however, in 2020, the herbicide treatments were sprayed before corn emergence in the interest of targeting 10-cm GR *C. canadensis*, because the control of GR *C. canadensis* was the research focus.

Visible corn injury was evaluated at 1, 2, and 4 wk after application (WAA) on a 0% to 100% scale; 0% represented no visible corn injury, and 100% was complete corn death. Depending on the herbicide treatment, leaf bleaching, chlorosis, and necrosis were the symptoms observed on corn leaf tissue exposed at herbicide application. Visible GR *C. canadensis* control at 2, 4, and 8 WAA was assessed on a scale of 0% to 100% as a visual assessment of aboveground GR *C. canadensis* biomass reduction compared with aboveground GR *C. canadensis* biomass in the nontreated control within the corresponding replication. Depending on the herbicide treatment, the herbicides induced bleaching, chlorosis, and necrosis on GR *C. canadensis*. The density and aboveground biomass of GR *C. canadensis* was collected at 8 WAA by counting the GR *C. canadensis* plants and clipping them at the soil surface within two 0.25-m^2 quadrats placed indiscriminately in each plot. The clipped GR *C. canadensis* plants from each plot were placed in separate paper bags and kiln-dried at 60 C until the biomass reached constant moisture. The GR *C. canadensis* dry biomass contents of each bag were measured with an analytical scale. Grain corn yield and harvest moisture were recorded at corn harvest maturity by combining two corn rows in each plot using a small plot research combine. Grain corn yields were corrected to 15.5% moisture before statistical analysis of yield data.

Statistical Analysis

All response parameters were subject to mixed model variance analysis within PROC GLIMMIX in SAS v. 9.4 (SAS Institute, 100 SAS Campus Drive, Cary, NC 27513, USA). The variance was subdivided into the fixed effects of HPPD inhibitor (Factor 1), PSII inhibitor (Factor 2), and the interaction between the two factors. The random effects included the environment (a collective term for the trial year and location combinations), replicated block within environment, and the interaction of environment with Factors 1 and 2. The significance of random effects was ascertained with a log-likelihood ratio test, and fixed effects with an *F*-test, with $\alpha = 0.05$ set for all tests. All data were pooled across environments, except for corn injury data, because herbicide treatments were applied postemergence in 2019 and before corn emergence in 2020. Studentized residual plots and the Shapiro-Wilk test for normality were analyzed to ensure that the residuals were random, independent of treatment and design effects, normally distributed, homogeneous, and had a mean of zero. A gaussian distribution was used to analyze corn injury and yield data. GR *C. canadensis* control data at all assessment timings were arcsine square-root transformed to meet the assumptions of variance analysis. Control data were back-transformed from the analysis scale for the presentation of results. A lognormal distribution was used to analyze GR *C. canadensis* density and dry biomass data. For the presentation of GR *C. canadensis* density and dry biomass, the least-square means were back-transformed using the omega method of back-transformation (M Edwards, Ontario Agricultural College Statistics Consultant, University of Guelph, personal communication). The main effects (HPPD inhibitor or PSII inhibitor) least-square means were only assessed when the interaction between HPPD and PSII inhibitors was not significant. When the interaction between HPPD and PSII inhibitors was significant, the simple effects for each factor were presented. The Tukey-Kramer multiple-range test was used to separate main and simple effect least-square means at a type I error of $\alpha = 0.05$.

Colby's equation (Equation 1) was used to compute the expected visible GR *C. canadensis* control and corn injury for each herbicide tank mix within each block by utilizing the observed values for HPPD inhibitor alone (X) and PSII inhibitor alone (Y).

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

Expected GR *C. canadensis* density and dry biomass were also calculated for each herbicide tank mix within each replication in each trial by using the adjusted Colby's equation, which incorporates the value from the nontreated control (Z) within the replication (Equation 2).

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

The observed and expected values for GR *C. canadensis* control, density, dry biomass, and corn injury were compared using a two-tailed *t*-test. Statistically similar observed and expected values were reported as additive interactions. Antagonistic or synergistic interactions occurred when the observed and expected values were statistically different. Data analysis was conducted with a significance level set to $\alpha = 0.05$; significance levels of $\alpha = 0.01$ are also presented in Tables 4 and 6.

Results and Discussion

GR Conyza canadensis Control

There was an interaction between HPPD- and PSII-inhibiting herbicides for GR *C. canadensis* control at 2, 4, and 8 WAA; therefore, the effect of HPPD inhibitor was analyzed by each PSII inhibitor and the effect of PSII inhibitor was analyzed by each HPPD inhibitor (Table 3).

At 2 WAA, the HPPD inhibitors mesotrione, tolpyralate, and topramezone controlled GR *C. canadensis* 79%, 79%, and 70%, respectively (Table 4). In a previous study, tolpyralate controlled GR *C. canadensis* 85% at 2 WAA (Metzger et al. 2019). The PSII inhibitors bromoxynil and bentazon controlled GR *C. canadensis* 39 and 43 percentage points greater than atrazine, respectively, at 2 WAA (Table 4). The application of atrazine, bromoxynil, or bentazon with mesotrione improved GR *C. canadensis* control to 98%, 100%, and 99%, respectively (Table 4). The co-application of bromoxynil or bentazon with tolpyralate increased the control of GR *C. canadensis* to 99% and 98%, respectively (Table 4). Similarly, bromoxynil or bentazon addition to topramezone improved GR *C. canadensis* control by 22 percentage points (Table 4). The addition of atrazine to tolpyralate or topramezone did not increase GR *C. canadensis* control at 2 WAA (Table 4). In contrast, the addition of atrazine to tolpyralate increased GR *C. canadensis* control at 2 WAA in a study by Metzger et al. (2019). The addition of an HPPD inhibitor to atrazine, bromoxynil, or bentazon improved GR *C. canadensis* except for the addition of topramezone to bentazon (Table 4). The tank mix of mesotrione + atrazine controlled GR *C. canadensis* 14 percentage points greater than topramezone + atrazine (Table 4). Additionally, the tank mix of mesotrione + bromoxynil controlled GR *C. canadensis* 8 percentage points greater than topramezone + bromoxynil at 2 WAA (Table 4).

The addition of any PSII inhibitor to the HPPD inhibitors improved GR *C. canadensis* control by 13 to 22 percentage points at 4 WAA (Table 4). Metzger et al. (2019) also observed that adding atrazine to tolpyralate increased GR *C. canadensis* control 13 percentage points at 4 WAA. GR *C. canadensis* control with atrazine, bromoxynil, and bentazon was improved 59 to 69, 20 to 27, and 15 to 22 percentage points, respectively, with the addition of an HPPD inhibitor (Table 4). Similarly, Armel et al. (2009) reported a 63 percentage point increase in glyphosate-susceptible *C. canadensis* control when mesotrione was added to atrazine at 3 WAA in no-tillage corn. Mesotrione tank mixed with atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 7 to 10 percentage points greater than topramezone tank mixed with atrazine, bromoxynil, or

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Table 3. Least-square means and significance of main effects and interaction for glyphosate-resistant *Conyza canadensis* control (2, 4, and 8 wk after application), density, and dry biomass in corn after the application of HPPD, PSII, and HPPD + PSII inhibitors across four field trials in southwestern Ontario, Canada, in 2019 and 2020.[a]

Main effects	Rate	Control			Density[b]	Dry biomass[b]
		2 WAA	4 WAA	8 WAA		
	—g ai ha^{-1}—		%		—plants m^{-2}—	—g m^{-2}—
HPPD inhibitor[c]						
No tank-mix partner	—	40	39	39	74 c	242.7 c
Mesotrione	100	97	98	98	2 a	2.3 a
Tolpyralate	30	94	94	95	7 ab	5.1 a
Topramezone	12.5	85	87	89	16 b	21.0 b
SE		1.8	1.8	1.8	4.2	9.2
HPPD inhibitor P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PSII inhibitor						
No tank-mix partner	—	51	54	55	65 c	124.6 c
Atrazine	560	82	84	86	16 b	35.1 b
Bromoxynil	280	94	94	94	7 ab	10.8 a
Bentazon	840	94	94	95	5 a	9.0 a
SE		1.8	1.8	1.8	4.2	9.2
PSII inhibitor P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction						
HPPD inhibitor × PSII inhibitor P-value		<0.0001	<0.0001	<0.0001	0.5393	0.1778

[a]Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; PSII, photosystem II; WAA, weeks after application.
[b]Means within the same main effect and column followed by the same lowercase letter are not statistically different according to the Tukey-Kramer multiple-range test ($P < 0.05$).
[c]The adjuvant used depended on the herbicide: mesotrione included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada) at 0.2% v/v; tolpyralate included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA) at 0.5% v/v and urea ammonium nitrate (UAN 28-0-0) at 2.5% v/v; topramezone included Merge® (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 0.5% v/v.

bentazon (Table 4). Mesotrione or tolpyralate tank mixed with atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 99% to 100% and 96% to 98%, respectively (Table 4). In two previous studies conducted in Ontario, tolpyralate + atrazine controlled GR *C. canadensis* 99% and 96% at 4 WAA (Langdon et al. 2020; Metzger et al. 2019).

At 8 WAA, the addition of an HPPD inhibitor to atrazine, bromoxynil, or bentazon increased GR *C. canadensis* control 60 to 69, 22 to 29, and 16 to 21 percentage points, respectively (Table 4). Atrazine applied alone controlled GR *C. canadensis* nearly 30% at all visible control intervals (Table 4). Similarly, Mahoney et al. (2017) observed 37% control of GR *C. canadensis* at 8 WAA with atrazine applied alone. Atrazine tank mixed with mesotrione, tolpyralate, or topramezone controlled GR *C. canadensis* 100%, 96%, and 91%, respectively (Table 4). In previous studies, tolpyralate + atrazine controlled GR *C. canadensis* 97% and 98% at 8 WAA (Langdon et al. 2020; Metzger et al. 2019). When the PSII inhibitor tank-mix partner was atrazine or bromoxynil, mesotrione control of GR *C. canadensis* was 9 and 7 percentage points greater, respectively, than topramezone (Table 4). In a previous study on GR *C. canadensis* control, mesotrione + atrazine and topramezone + atrazine controlled GR *C. canadensis* 76% and 67%, respectively, at 8 WAA (Mahoney et al. 2017). The differences in control between this study and the study by Mahoney et al. (2017) could be because those authors sprayed 15-cm GR *C. canadensis*, while 10-cm GR *C. canadensis* was treated in the present study. GR *C. canadensis* control with mesotrione + bromoxynil and tolpyralate + bromoxynil was 100% and 98%, respectively (Table 4). The tank mixes of mesotrione, tolpyralate, or topramezone with bentazon controlled GR *C. canadensis* 100%, 98%, and 95%, respectively, with no statistical differences detected between these tank-mix combinations (Table 4).

According to the two-tailed *t*-test comparisons of observed and expected control, the tank mixes of mesotrione or tolpyralate with atrazine, bromoxynil, or bentazon were synergistic for the control of GR *C. canadensis* at 2, 4, and 8 WAA (Table 4). Ditschun et al. (2016) also documented synergy between HPPD and PSII inhibitors for GR *C. canadensis* control, but that study was conducted with isoxaflutole and metribuzin in no-crop field trials. As per Colby's equation, the tank mixes of mesotrione plus atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 11, 6, and 4 percentage points greater than expected at 8 WAA, respectively (Table 4). The synergy between mesotrione and atrazine has also been recorded for the control of *A. palmeri*, *A. tuberculatus*, *A. retroflexus*, *A. trifida*, *A. theophrasti*, *C. album*, *I. coccinea*, *R. raphanistrum*, *X. strumarium*, and *S. faberi* (Armel et al. 2007; Hugie et al. 2008; Kohrt and Sprague 2017; Walsh et al. 2012; Woodyard et al. 2009a, 2009b). In addition, synergy has also been detected between mesotrione and bromoxynil for the control of *A. palmeri*, *A. tuberculatus*, *A. retroflexus*, *A. theophrasti*, *A. trifida*, and *C. album* in previous studies (Abendroth et al. 2006; Hugie et al. 2008; Woodyard et al. 2009a). A study on *A. tuberculatus* control documented synergy between mesotrione plus bromoxynil or bentazon, but additive interactions with mesotrione + atrazine (Willemse et al. 2021). Tolpyralate plus atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 6, 4, and 2 percentage points greater than expected at 8 WAA, respectively (Table 4). Willemse et al. (2021) reported synergy with tolpyralate + bromoxynil, but not with tolpyralate plus atrazine or bentazon for the control of *A. tuberculatus*. Kohrt and Sprague (2017) also did not find synergy between tolpyralate and atrazine for the control of *A. palmeri*. In contrast to mesotrione and tolpyralate tank mixes, the interaction of topramezone with atrazine, bentazon, or bromoxynil was additive for the control of GR *C. canadensis* at all assessment timings (Table 4). Similarly, synergy was generally not documented between topramezone and PSII inhibitors in previous studies focusing on *A. palmeri* and *A. tuberculatus* control (Kohrt and Sprague 2017; Willemse et al. 2021).

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Table 4. Glyphosate-resistant *Conyza canadensis* control (2, 4, and 8 wk after application), density, and dry biomass in corn after the application of HPPD, PSII, and HPPD + PSII inhibitors from four field trials in southwestern Ontario, Canada, in 2019 and 2020.[a]

Herbicide treatment[b]	No tank-mix partner[c]	Mesotrione[d]	Tolpyralate[d]	Topramezone[d]	SE
Control at 2 WAA			%		
No tank-mix partner	0 c Y	79 b Z	79 b Z	70 b Z	4.4
Atrazine	34 b X	98 a Z (86)**	94 ab YZ (86)**	84 ab Y (81)	3.4
Bromoxynil	73 a X	100 a Z (93)**	99 a YZ (92)**	92 a Y (90)	2.2
Bentazon	77 a Y	99 a Z (94)*	98 a Z (94)**	92 a YZ (92)	1.7
SE	4.2	1.6	1.7	2.2	
Control at 4 WAA					
No tank-mix partner	0 c Y	82 b Z	83 b Z	71 b Z	4.5
Atrazine	30 b X	99 a Z (87)**	96 a YZ (89)**	89 a Y (80)	3.6
Bromoxynil	73 a X	100 a Z (94)**	98 a YZ (94)**	93 a Y (91)	2.2
Bentazon	78 a X	100 a Z (95)**	97 a YZ (95)*	93 a Y (93)	1.7
SE	4.3	1.3	1.5	2.2	
Control at 8 WAA					
No tank-mix partner	0 c Y	83 b Z	84 b Z	72 b Z	4.6
Atrazine	31 b X	100 a Z (89)**	96 a YZ (90)*	91 a Y (81)	3.7
Bromoxynil	71 a X	100 a Z (94)**	98 a YZ (94)**	93 a Y (91)	2.2
Bentazon	79 a Y	100 a Z (96)**	98 a Z (96)*	95 a Z (94)	1.8
SE	4.3	1.3	1.3	2.1	
Density			plants m^{-2}		
No tank-mix partner	155	15	26	60	10.8
Atrazine	67	1 (12)**	6 (13)	10 (32)*	10.6
Bromoxynil	38	0 (6)**	2 (8)	7 (18)	4.0
Bentazon	20	0 (4)*	3 (4)	8 (10)	2.7
SE	13.8	1.7	2.6	5.2	
Dry biomass			g m^{-2}		
No tank-mix partner	464.9	24.8	18.6	54.4	30.3
Atrazine	236.1	0.6 (29.2)**	3.4 (15.0)	19.1 (28.6)	15.3
Bromoxynil	78.2	0.0 (5.4)**	1.8 (4.8)	12.7 (7.5)	6.6
Bentazon	58.4	0.0 (2.9)**	2.7 (3.0)	10.7 (6.2)	6.4
SE	30.0	1.7	1.7	5.3	

[a]Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; PSII, photosystem II; WAA, weeks after application.
[b]The adjuvant used depended on the herbicide: mesotrione included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada) at 0.2% v/v; tolpyralate included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA) at 0.5% v/v and urea ammonium nitrate (UAN 28-0-0) at 2.5% v/v; topramezone included Merge® (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 0.5% v/v.
[c]Means followed by the same lowercase letter within the same column and response parameter or means followed by the same uppercase letter within a row are not statistically different according to the Tukey-Kramer multiple-range test ($P < 0.05$).
[d]Values in parentheses are expected values calculated from Colby's equation. Asterisks (*)indicate significant differences of *$P < 0.05$ and **$P < 0.01$, respectively, between observed and expected values based on a two-tailed *t*-test.

GR Conyza canadensis Density and Dry Biomass

No interaction effect was detected between the two herbicide factors for GR *C. canadensis* density and aboveground dry biomass at 8 WAA, so the main effects are analyzed (Table 3). Mesotrione, tolpyralate, and topramezone reduced the density of GR *C. canadensis* by 97%, 91%, and 78%, respectively, when averaged across the PSII inhibitors (Table 3). The density reduction was greater with mesotrione than topramezone; the density reduction with tolpyralate was intermediate and similar to both (Table 3). Atrazine, bromoxynil, and bentazon reduced the density of GR *C. canadensis* by 75%, 89%, and 92%, respectively, when averaged across the HPPD inhibitors (Table 3). Bentazon reduced the density of GR *C. canadensis* more than atrazine; the density reduction with bromoxynil was intermediate and similar to both (Table 3). The co-application of mesotrione with the PSII inhibitors and the tank mix of topramezone + atrazine synergistically decreased GR *C. canadensis* density (Table 4).

Averaged across the PSII inhibitors, all the HPPD inhibitors reduced the dry biomass of GR *C. canadensis* 91% to 99% (Table 3). Topramezone reduced GR *C. canadensis* dry biomass less than mesotrione and tolpyralate (Table 3). The PSII inhibitors atrazine, bromoxynil, and bentazon reduced GR *C. canadensis* dry biomass 72%, 91%, and 93%, respectively (Table 3). Atrazine reduced GR *C. canadensis* dry biomass less than bromoxynil and bentazon (Table 3). The interaction between mesotrione and the three PSII inhibitors was synergistic for the reduction of the aboveground dry biomass of GR *C. canadensis* (Table 4). In contrast, the interactions between tolpyralate or topramezone and atrazine, bromoxynil, or bentazon were all additive for the reduction of dry biomass of GR *C. canadensis* (Table 4).

Corn Injury and Grain Yield

No visible corn injury was observed at both trials in 2020 at 1, 2, and 4 WAA. Corn was not emerged at the time of application in 2020, because GR *C. canadensis* reached an average height or rosette diameter of 10 cm before corn emergence. Therefore, corn injury was only analyzed for the trials in 2019. Corn injury at 42.62°N, 81.95°W was 0% for all treatments at 2 WAA, so these data were removed from analysis, and only injury data from 42.62°N, 81.94°

Table 5. Least-square means and significance of main effects and interaction for corn injury (1 and 2 wk after application) and corn grain yield after the application of HPPD, PSII, and HPPD + PSII inhibitors from four field trials in southwestern Ontario, Canada, in 2019 and 2020.[a]

Main effects	Rate	Corn injury 1 WAA[b]	Corn injury 2 WAA[c]	Corn grain yield
	g ai ha^{-1}	%	%	kg ha^{-1}
HPPD inhibitor[d]				
No tank-mix partner	—	2	1	7,600
Mesotrione	100	2	1	10,100
Tolpyralate	30	5	1	9,800
Topramezone	12.5	2	1	9,800
SE		0.3	0.1	160
HPPD inhibitor P-value		0.2149	0.0015	0.0081
PSII inhibitor				
No tank-mix partner	—	1	0	8,600
Atrazine	560	1	1	9,600
Bromoxynil	280	7	1	9,700
Bentazon	840	3	1	9,400
SE		0.3	0.1	160
PSII inhibitor P-value		0.2856	<0.0001	0.1411
Interaction				
HPPD inhibitor x PSII inhibitor P-value		0.7014	<0.0001	<0.0001

[a]Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; PSII, photosystem II; WAA, weeks after application.
[b]Corn injury at 1 WAA in 2020 is omitted, because no corn injury was observed.
[c]Corn injury at 2 WAA in 2020 and at 42.62°N, 81.95°W in 2019 is omitted, because no corn injury was observed.
[d]The adjuvant used depended on the herbicide: mesotrione included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada) at 0.2% v/v; tolpyralate included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA) at 0.5% v/v and urea ammonium nitrate (UAN 28-0-0) at 2.5% v/v; topramezone included Merge® (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 0.5% v/v.

Table 6. Corn injury (1 and 2 wk after application) and corn grain yield after the application of HPPD, PSII, and HPPD + PSII inhibitors from four field trials in southwestern Ontario, Canada, in 2019 and 2020.[a]

Herbicide treatment[b]	No tank-mix partner[c]	Mesotrione[d]	Tolpyralate[d]	Topramezone[d]	SE
Corn injury at 1 WAA[e]		%			
No tank-mix partner	0	0	2	0	0.2
Atrazine	0	0 (0)[d]	3 (2)	1 (0)*	0.3
Bromoxynil	6	6 (6)	8 (8)	7 (6)	0.7
Bentazon	1	3 (1)*	7 (3)**	2 (1)	0.6
SE	0.5	0.6	0.7	0.6	
Corn injury at 2 WAA[f]					
No tank-mix partner	0 a Z	0 a Z	0 a Z	0 a Z	0.0
Atrazine	0 a Z	0 a Z (0)	1 b Y (0)*	1 b Y (0)**	0.2
Bromoxynil	1 b Z	1 b Z (1)	1 b Z (1)	1 b Z (1)	0.1
Bentazon	1 b Z	2 c Y (1)	2 b Y (1)	1 b YZ (1)	0.1
SE	0.2	0.2	0.2	0.1	
Corn grain yield		kg ha^{-1}			
No tank-mix partner	4,900 b Y	10,100 a Z	9,700 a Z	9,600 a Z	390
Atrazine	7,800 a Y	10,000 a Z	10,400 a Z	10,000 a Z	310
Bromoxynil	8,700 a Z	10,500 a Z	9,300 a Z	10,200 a Z	290
Bentazon	9,000 a Z	9,700 a Z	9,700 a Z	9,400 a Z	290
SE	340	270	310	270	

[a]Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; PSII, photosystem II; WAA, weeks after application.
[b]The adjuvant used depended on the herbicide: mesotrione included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada) at 0.2% v/v; tolpyralate included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA) at 0.5% v/v and urea ammonium nitrate (UAN 28-0-0) at 2.5% v/v; topramezone included Merge® (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 0.5% v/v.
[c]Means followed by the same lowercase letter within the same column and response parameter or means followed by the same uppercase letter within a row are not statistically different according to the Tukey-Kramer multiple-range test ($P < 0.05$).
[d]Values in parentheses are expected values calculated from Colby's equation. Asterisks (*) indicate significant differences of *$P < 0.05$ and **$P < 0.01$, respectively, between observed and expected values based on a two-tailed *t*-test.
[e]Corn injury at 1 WAA in 2020 is omitted, because no corn injury was observed.
[f]Corn injury 2 WAA in 2020 and at 42.62°N, 81.95°W in 2019 is omitted, because no corn injury was observed.

W are presented 2 WAA. No corn injury was observed at 4 WAA at both trials in 2019.

There was an interaction effect between the two herbicide factors for corn injury at 2 WAA and corn yield at maturity; therefore, the effect of HPPD inhibitor was analyzed by each PSII inhibitor and the effect of PSII inhibitor was analyzed by each HPPD inhibitor (Table 5).

There was no corn injury observed at 2 WAA when mesotrione, tolpyralate, and topramezone were applied alone (Table 6). Bromoxynil and bentazon caused 1% corn injury at 2 WAA, while atrazine did not injure corn (Table 6). Adding mesotrione to atrazine did not increase corn injury, but the addition of tolpyralate or topramezone to atrazine injured corn 1% (Table 6). Corn injury with atrazine plus tolpyralate or topramezone was synergistic (Table 6). In contrast, the addition of any HPPD inhibitor to bromoxynil did not accentuate corn injury (Table 6). The addition of mesotrione or tolpyralate to bentazon increased corn injury to 2%; however, the addition of topramezone to bentazon did not increase corn injury (Table 6). The addition of atrazine, bromoxynil, or bentazon increased the level of corn injury at 2 WAA similarly when applied with tolpyralate or topramezone (Table 6). In contrast, bentazon was the most injurious tank mix partner with mesotrione (Table 6).

In this study, GR *C. canadensis* interference decreased corn yield up to 53% (highest-yielding treatment compared with the nontreated control) (Table 6). Reduced GR *C. canadensis* interference with mesotrione, tolpyralate, or topramezone treatments increased corn yield 106%, 98%, and 96%, respectively (Table 6). Reduced GR *C. canadensis* interference with atrazine, bromoxynil, or bentazon increased corn yield 59%, 78%, and 84%, respectively (Table 6). The addition of mesotrione, tolpyralate or topramezone to atrazine increased corn yield 28% to 33% (Table 6). The improved GR *C. canadensis* control with the co-application of HPPD inhibitors and atrazine may explain the increased corn yield with these tank mixes compared with atrazine alone. In contrast, the addition of an HPPD inhibitor to bromoxynil or bentazon did not improve corn yield (Table 6). The use of a PSII inhibitor with mesotrione, tolpyralate, or topramezone did not increase the yield of corn relative to the HPPD inhibitors applied alone (Table 6). Similarly, Metzger et al. (2019) found that reduced GR *C. canadensis* interference with tolpyralate and tolpyralate + atrazine resulted in higher corn yield than the nontreated control, but the two herbicide treatments did not differ in respect to corn yield.

In summary, nine tank mixes of HPPD inhibitors + PSII inhibitors controlled GR *C. canadensis* >90% at 8 WAA. Among these tank mixes, mesotrione or tolpyralate plus atrazine, bromoxynil, or bentazon controlled GR *C. canadensis* 96% to 100%, while topramezone tank mixes controlled GR *C. canadensis* 91% to 95% at 8 WAA. These results increase the known available herbicide options for excellent (>90%) GR *C. canadensis* control in corn. Bromoxynil or bentazon can be used in place of atrazine when co-applied with mesotrione, tolpyralate, or topramezone with no compromise in GR *C. canadensis* control. This is particularly advantageous in geographic regions where the use of atrazine is prohibited or restricted. The tank mixes of mesotrione or tolpyralate plus atrazine, bromoxynil, or bentazon were all synergistic for the control of GR *C. canadensis* at 2, 4, and 8 WAA; however, topramezone tank mixed with atrazine, bromoxynil, or bentazon was additive for the control of GR *C. canadensis* at all assessment timings. These findings expand the understanding of the interaction between HPPD and PSII inhibitors over several active ingredients within each herbicide mode of action. To our knowledge, this is the first report of the interaction of mesotrione, tolpyralate, or topramezone with atrazine, bromoxynil, or bentazon for GR *C. canadensis* control. The judicious use of effective herbicide tank mixes with cultural and mechanical weed control tactics may help to maintain the efficacious long-term use of the herbicide tank mixes for GR *C. canadensis* control in corn.

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