Interaction between 4-hydroxyphenylpyruvate dioxygenase–inhibiting and reactive oxygen species–generating herbicides for the control of annual weed species in corn

John C. Fluttert¹, Nader Soltani², Mariano Galla³, David C. Hooker⁴, Darren E. Robinson⁵ and Peter H. Sikkema⁵

¹Graduate Student, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; ²Adjunct Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada; ³Product Development and Technical Service Representative, ISK Biosciences Inc., Concord, OH, USA; ⁴Associate Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada and ⁵Professor, Department of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada

Abstract

The complementary modes of action of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and photosystem II (PSII) inhibitors have been credited for the synergistic weed control improvement of several species. Recent research discovered that reactive oxygen species (ROS) generation and subsequent lipid peroxidation is the cause of cell death by the glutamine synthetase inhibitor glufosinate. Therefore, a basis for synergy exists between glufosinate and HPPD inhibitors, but the interaction has not been well reported. Four field experiments were conducted in Ontario, Canada, in 2020 and 2021 to determine the interaction between HPPD-inhibiting (mesotrione and tolpyralate) and ROS-generating (atrazine, bromoxynil, bentazon, and glufosinate) herbicides on control of annual weed species in corn (Zea mays L.). The ROS generators were synergistic with the HPPD inhibitors and provided ≥95% control of velvetleaf (Abutilon theophrasti Medik.), except for tolpyralate + glufosinate, which was additive at 8 wk after application (WAA) and provided 87% control. Tank mixes of HPPD inhibitors plus ROS generators were synergistic for the control of common ragweed (Ambrosia artemisifolia L.), except for tolpyralate + glufosinate, which was antagonistic at 8 WAA. Tolpyralate + glufosinate was antagonistic for the control of barnyardgrass [Echinochloa crus-galli (L.) P. Beauv.] and Setaria spp. at 8 WAA. Common lambsquarters (Chenopodium album L.) control at 8 WAA was synergistic and ≥95% with mesotrione plus atrazine, bromoxynil, or glufosinate and with tolpyralate plus bromoxynil or bentazon. Herbicide tank mixes were generally additive for the control of wild mustard (Sinapis arvensis L.) at 8 WAA, except for the synergistic tank mixes of tolpyralate plus atrazine or bromoxynil; however, each tank mix provided 97% to 100% control of S. arvensis. Results from this study demonstrate that co-application of ROS generators with mesotrione or tolpyralate controlled all broadleaf weed species >90% at 8 WAA, with the exceptions of A. artemisifolia and C. album control with tolpyralate + glufosinate. Mesotrione plus PSII inhibitors controlled E. crus-galli and Setaria spp. 48 to 68 percentage points less than tolpyralate plus the respective PSII inhibitor at 8 WAA; however, mesotrione + glufosinate and tolpyralate + glufosinate controlled the grass weed species similarly.

Introduction

Weed interference can reduce corn (Zea mays L.) yield. In a recent meta-analysis, uncontrolled weeds reduced corn yield by 50% on average in the primary corn-producing regions of the United States and Canada (Soltani et al. 2016). At varying weed densities, velvetleaf (Abutilon theophrasti Medik.), common ragweed (Ambrosia artemisifolia L.), common lambsquarters (Chenopodium album L.), barnyardgrass [Echinochloa crus-galli (L.) P. Beauv.], giant foxtail (Setaria faberi Herrm.), and green foxtail [Setaria viridis (L.) P. Beauv.] interference reduced corn yield 32%, 80%, 58%, 35%, 18%, and 18%, respectively (Beckett et al. 1988; Bosnic and Swanton 1997; Scholes et al. 1995; Sibuga and Bandeen 1980; Weaver 2001). These weed species are widespread and problematic in corn production in the United States and Ontario, Canada (Van Wychen 2020). A timely, effective postemergence herbicide application can prevent corn yield loss from weed interference (Carey and Kells 1995; Myers et al. 2005).

Photosystem II (PSII)- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides are commonly tank mixed for broad-spectrum postemergence weed control in corn
(Armel et al. 2008b; Johnson et al. 2002; Kohrt and Sprague 2017; Metzger et al. 2018; Whaley et al. 2006). Mesotrione and tolpyralate are HPPD inhibitors commonly applied postemergence in corn. Although the two herbicides have different weed control spectrums, both herbicides impede the production of homogentisic acid in susceptible plants (Metzger et al. 2018; Pallett et al. 1998; Schulz et al. 1993; Secor 1994). The lack of homogentisic acid inhibits the production of plastoquinone and tocopherols, which are needed for dissipation of reactive oxygen species (ROS) formed by the plant (Kruk et al. 2005; Pallett et al. 1998; Schulz et al. 1993; Trebst et al. 2002; Tsegaye et al. 2002). Cell destruction and plant death follow, as the plant can no longer quench ROS (Kruk et al. 2005; Trebst et al. 2002). The mode of action of PSII inhibitors is complementary with HPPD inhibitors. PSII inhibitors such as atrazine, bentazon, and bromoxynil occupy the QB binding site on the D1 protein, which causes a build-up of ROS by displacing plastoquinone in the photosynthetic electron transport chain (Hess 2000). The buildup of ROS overloads the quenching capabilities of the carotenoid system and causes lipid peroxidation and subsequent plant death (Hess 2000). Synergy between the HPPD and PSII inhibitors can occur when co-applied, because (1) the HPPD inhibitors increase the binding efficiency of the PSII inhibitors to the D1 protein by plastoquinone depletion, and (2) the lack of ROS-quenching capabilities induced by the HPPD inhibitors amplifies cell membrane destruction by ROS generation from PSII inhibitors (Abendroth et al. 2006; Armel et al. 2005; Creech et al. 2004; Kim et al. 1999).

Synergistic, additive, or antagonistic interactions can occur when two herbicides from different modes of action are co-applied (Colby 1967). Synergistic, additive, or antagonist interactions for weed control occur when the observed weed control is greater, equal, or less than expected, respectively (Colby 1967). The synergy between HPPD and PSII inhibitors has been reported for control of several weed species in corn; however, additive interactions are also common between the two herbicide sites of action (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).

The cause of cell death by the glutamine synthetase inhibitor glufosinate has recently been elucidated. The cause of cell death by glufosinate was assumed to be due to inhibition of carbon assimilation or ammonia accumulation after glufosinate application to susceptible plants (Wild et al. 1987). Takano et al. (2019) discovered that ammonia accumulation and carbon assimilation inhibition are secondary effects of glutamine synthetase inhibition. The cause of phytotoxicity by glufosinate is due to the production of ROS, which causes lipid peroxidation of cell membranes and cell death (Takano et al. 2019). The recent finding of the cause of cell death by glufosinate suggests that the mode of action may be complementary to herbicides that reduce the quenching of ROS, such as HPPD inhibitors (Takano and Dayan 2020).

The interaction between HPPD- and PSII-inhibiting herbicides has been documented for the control of several weed species; however, the interaction of the two herbicides has not been comprehensively reported with tolpyralate, bentazon, or bromoxynil. Additionally, the recent discovery of the cause of phytotoxicity induced by glufosinate suggests that a basis for synergy exists between HPPD-inhibiting herbicides and glufosinate; however, an evaluation of the interaction between these two herbicides remains largely unexplored. Determining the level of control and interaction between HPPD-inhibiting and several ROS-generating (collective term for PSII inhibitors and glutosinate) herbicides on several weed species in corn is valuable, especially in regions where atrazine use is restricted or prohibited. Therefore, the objectives of this study were to determine: (1) the level of weed control with HPPD inhibitors and ROS generators applied alone and in combination and (2) to evaluate the type of interaction between HPPD inhibitors and ROS generators for control of several annual broadleaf and grass weed species in corn.

### Materials and Methods

Four field experiments were conducted in two growing seasons (2020 and 2021) at University of Guelph research sites in Ridgetown, ON, Canada (Ridgetown Campus, 42.45°N, 81.88°W) and near Exeter, ON, Canada (Huron Research Station, 43.32°N, 81.50°W) (Table 1). Fields were managed under conventional tillage practices. Fields were fertilized before planting to meet corn requirements. Corn was planted at a population of approximately 85,000 seeds ha$^{-1}$ to a seed depth of approximately 5 cm in rows spaced 75 cm apart. A glyphosate/glufosinate-resistant corn hybrid, DKC42-04RIB® (Bayer CropScience Canada, 160 Quarry Boulevard SE, Calgary, AB T2C 3G3, Canada) was planted at the Huron Research Station in 2020 and 2021. Glyphosate/glufosinate-resistant corn hybrids, DKC42-60RIB® and DKC39-97RIB®, were planted at Ridgetown Campus in 2020 and 2021, respectively. Plots were 10-m long at the Huron Research Station and 8-m long at Ridgetown Campus. All plots were 3 m (4 corn rows) in width. Experiments were organized as a randomized complete block design with four blocks in each experiment. Detailed soil information, corn planting and harvest dates, herbicide application dates, and corn developmental stages are presented in Table 1.

### Table 1. Year, location, soil characteristics, corn planting and harvest dates, herbicide application dates, and corn developmental stages at application for four field trials in Ontario, Canada, in 2020 and 2021.

| Year       | Research site              | Texture          | OM (%) | pH  | Corn planting date | Corn harvest date | Application date | Corn developmental stage |
|------------|----------------------------|------------------|--------|-----|--------------------|-------------------|-------------------|------------------------|
| 2020       | Ridgetown Campus           | Sandy clay loam  | 3.1    | 7.0 | May 26             | November 5        | June 19           | V4                     |
|            | Huron Research Station     | Loam             | 3.6    | 7.9 | May 14             | October 26        | June 12           | V5                     |
| 2021       | Ridgetown Campus           | Sandy clay loam  | 2.7    | 6.7 | May 14             | October 1         | June 16           | V5                     |
|            | Huron Research Station     | Clay loam        | 4.4    | 7.9 | April 27           | November 10       | June 7            | V5                     |

*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) were used to determine soil characteristics. OM, organic matter.
The study was arranged as a two-factor factorial. Factor A included three levels of HPPD-inhibiting herbicides: nontreated control, mesotrione, and tolpyralate. Factor B included five levels of ROS-generating herbicides: nontreated control, atrazine, bromoxynil, bentazon, and glufosinate. Herbicide specifics are presented in Table 2. All herbicide treatments were applied with a CO₂-pressurized backpack sprayer calibrated to deliver 200 L ha⁻¹ at 240 kPa through four ULD120-02 spray nozzles (Pentair, 375 5th Avenue NW, New Brighton, MN 55112, USA) at 50-cm spacing on the spray boom, producing a 2-m spray width. Herbicide treatments were applied postemergence when the natural weed population in the nontreated control reached an average height of 15 cm. Sites contained natural infestations of A. theophrasti, A. artemisiafolia, C. album, wild mustard (Sinapis arvensis L.), Setaria spp., and E. crus-galli. Setaria spp. were grouped, because sites contained a heterogeneous population of S. viridis and S. faberi, and it was difficult to accurately distinguish between those species for data collection. Herbicide efficacy of atrazine, bromoxynil, bentazon, and glufosinate can be affected by the time of day of application (Doran and Andersen 1976; Montgomery et al. 2017; Stewart et al. 2009). In contrast, a lack of time of day of application effect on tolpyralate + atrazine efficacy has been reported for the control of several of the weed species investigated in this study (Langdon et al. 2021). Therefore, all herbicide treatments were applied within the period of 0900 to 1030 hours Eastern Daylight Saving Time to have consistent data among site-years.

Visible weed control was evaluated by species at 2, 4, and 8 wk after application (WAA) on a scale of 0% to 100% for each weed species as an assessment of aboveground weed biomass reduction relative to the nontreated control. At 1, 2, and 4 WAA, corn injury (aggregate of visible chlorosis and necrosis) was assessed on a 0% to 100% scale; 0% indicated no visible corn injury, and 100% signified complete corn death. Immediately after weed assessment at 8 WAA, density was determined for each weed species by counting the number of weeds by species within two randomly placed 0.5-m² quadrats per plot. The weeds were clipped at the soil surface, separated by species into paper bags, and placed in a kiln drier until the weed biomass reached constant moisture. Dry biomass data for each weed species were recorded by weighing the dried biomass on an analytical scale. At corn harvest maturity, the center two rows of each plot were mechanically harvested with a small plot combine to obtain grain corn yield weight and harvest moisture. Statistical analysis of corn yield was run on grain yields corrected to 15.5% moisture.

### Statistical Analysis

Weed control, weed density, weed dry biomass, corn injury, and corn yield data were analyzed using a generalized linear mixed model in SAS v. 9.4 (SAS Institute, 100 SAS Campus Drive, Cary, NC 27513, USA). The variance was partitioned into the fixed effects of HPPD inhibitor (Factor A), ROS generator (Factor B), and the interaction between the two herbicides. The significance of fixed effects was determined with an F-test at a significance level of α = 0.05. Environment (site and year combinations), replication within the environment, and the interaction of the environment with Factors A and B were the random effects. Random effects significance was determined using a restricted log-likelihood test with a type I error declared at α = 0.05. Data for each response parameter were pooled across environments. Weed control data were analyzed by weed species. *Abutilon theophrasti*, *A. artemisiifolia*, *C. album*, *S. arvensis*, and *Setaria* spp. control and corn injury data at all assessment timings were arcsine square-root transformed. Data were back-transformed for the presentation of results. All weed density and dry biomass data were analyzed using a lognormal distribution with PROC GLIMMIX. The omega method of back-transformation (M Edwards, Ontario Agricultural College Statistics Consultant, University of Guelph, personal communication) was used to back-transform the density and dry biomass data for the presentation of results. *Echinochloa crus-galli* control and corn yield data were not transformed and were analyzed using a Gaussian distribution. The distributions and transformations chosen were those that best met the assumptions of the analysis by visual inspection of studentized residual plots and the Shapiro-Wilk statistic. The assumptions of the variance analysis were that the residuals were random, independent of treatment and design effects, homogeneous, and

### Table 2. Herbicide active ingredient, mode of action, rate, trade name, and manufacturer for the study of the interaction between 4-hydroxyphenylpyruvate dioxygenase–inhibiting and reactive oxygen species–generating herbicides on the control of annual weed species in Ontario, Canada, in 2020 and 2021.

| Herbicide active ingredient | Mode of action | Rate | Trade name | Manufacturer |
|-----------------------------|----------------|------|------------|--------------|
| Atrazine                    | PSII inhibitor | 840  | Basagran® Forté | BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, [https://www.basf.com/ca/en.html](https://www.basf.com/ca/en.html) |
| Bentazon                    | PSII inhibitor | 300  | Liberty® 200 SN | BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, [https://www.basf.com/ca/en.html](https://www.basf.com/ca/en.html) |
| Bromoxynil                  | PSII inhibitor | 300  | Callisto® 480SC | BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, [https://www.basf.com/ca/en.html](https://www.basf.com/ca/en.html) |
| Glufosinate                 | GS inhibitor  | 300  | Shieldex® 400SC | BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada, [https://www.basf.com/ca/en.html](https://www.basf.com/ca/en.html) |
| Mesotrione                  | HPPD inhibitor | 100  | Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada, [https://www.syngenta.ca](https://www.syngenta.ca) |
| Tolpyralate                 | HPPD inhibitor | 30   | Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada, [https://www.syngenta.ca](https://www.syngenta.ca) |

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*aAppropriate adjuvants were used with each treatment: 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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹.*

*bAbbreviations: GS, glutamine synthetase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; PSII, photosystem II.*
Table 3. Least-squares means and significance of main effects and interaction for Abutilon theophrasti control (at 2 and 4 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.a

| Main effects                          | Rate     | Controlb | Density | Dry biomass |
|--------------------------------------|----------|----------|---------|-------------|
|                                      | 2 WAA    | 4 WAA    | —plants m⁻²— | —g m⁻²—   |
| HPPD inhibitor                       | —g ai ha⁻¹— | ——— | ——— | ——— | ——— |
| No tank-mix partner                  | 20 b     | 21 b     | 4 b     | 5.0 b       |
| Mesotrione                           | 100      | 96 a     | 99 a    | 0 a         | 0.0 a |
| Tolpyralate                          | 30       | 88 a     | 89 a    | 1 a         | 0.4 a |
| SE                                   | 3.1      | 3.1      | 0.3     | 0.4         |
| HPPD inhibitor P-value               | 0.0039   | 0.0185   | 0.0082  | 0.0089      |
| ROS generator                        |          |          |         |             |
| No tank-mix partner                  | —        | 35 d     | 42 c    | 2           | 1.9  |
| Atrazine                             | 280      | 79 b     | 80 ab   | 1           | 1.4  |
| Bromoxynil                           | 280      | 87 a     | 87 a    | 1           | 1.1  |
| Bentazon                             | 840      | 84 a     | 87 a    | 1           | 0.4  |
| Glufosinate                          | 300      | 71 c     | 76 b    | 2           | 2.5  |
| SE                                   | 3.1      | 3.1      | 0.3     | 0.4         |
| ROS generator P-value                | 0.0001   | 0.0012   | 0.2310  | 0.1875      |
| Interaction                          |          |          |         |             |
| HPPD inhibitor x ROS generator P-value | 0.0570 | 0.0663   | 0.8984  | 0.1406      |

aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.
bMeans within the same main effect and column followed by the same lowercase letter are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

Expected weed control and corn injury for each herbicide tank mix within each block were calculated with Colby’s equation (Equation 1) by using the observed values for HPPD inhibitor alone (X) and ROS generator alone (Y).

Expected = (X + Y) − [(X × Y)/100]   [1]

The modified Colby’s equation (Equation 2), which includes the value from the nontreated control (Z) within each block, was used to calculate the expected weed density and dry biomass for each herbicide tank mix.

Expected = [(X × Y)/Z]   [2]

Two-sided t-tests were used to compare the observed values and calculated expected values for weed control, weed density, weed dry biomass, and corn injury. Additive interactions were declared when the observed and expected values were similar. Synergistic or antagonistic interactions occurred when the observed and expected values were significantly different at α = 0.05; for the presentation of results, α = 0.01 was also noted.

Results and Discussion

Abutilon theophrasti

Abutilon theophrasti was present at Ridgetown Campus in 2020 and 2021, so the results presented are pooled from the two experiments. The interaction between HPPD inhibitors and ROS generators was not significant for A. theophrasti control at 2 and 4 WAA, density, and dry biomass, so the main effects are presented (Table 3). When averaged across ROS generators, mesotrione and tolpyralate provided 96% to 99% and 88% to 89% control of A. theophrasti, respectively, at 2 and 4 WAA. Bromoxynil and bentazon controlled A. theophrasti more than atrazine; glufosinate provided lower A. theophrasti control than the PSII inhibitors when averaged across the HPPD inhibitors at 2 WAA. At 4 WAA, bromoxynil and bentazon provided 87% control of A. theophrasti, which was greater than control by glufosinate; atrazine provided intermediate control and was similar to the other ROS generators when averaged across the HPPD inhibitors. When averaged across the ROS generators, mesotrione and tolpyralate caused 100% and 75% density reduction of A. theophrasti, respectively (Table 4). Bethke et al. (2013) also reported <40% control of A. theophrasti with glufosinate. Bentazon provided 49% control of A. theophrasti at 8 WAA, which was greater than control by atrazine and glufosinate but similar to bromoxynil control. Mesotrione controlled A. theophrasti 89% at 8 WAA; control improved to 99%
to 100% with the addition of atrazine, bromoxynil, bentazon, or glufosinate. The tank mixes of mesotrione plus atrazine, bromoxynil, bentazon, or glufosinate were synergistic for the control of *Ambrosia artemisiifolia* at 2, 4, and 8 WAA. Previous research has also reported synergistic interactions with mesotrione + atrazine and mesotrione + bromoxynil for the control of *A. theophrasti* (Abendroth et al. 2006; Woodyard et al. 2009b). At 8 WAA, control of *Ambrosia artemisiifolia* was 38 to 40 percentage points greater than mesotrione or tolpyralate at 8 WAA; there was no difference in control between mesotrione and tolpyralate when co-applied with an ROS generator. The addition of atrazine, bromoxynil, bentazon, or glufosinate to mesotrione improved control of more than mesotrione at 8 WAA (Table 5). Glufosinate provided 94% control of *Ambrosia artemisiifolia* at 4 WAA. Tolpyralate controlled *Ambrosia artemisiifolia* 28 percentage points more than mesotrione at 8 WAA (Table 5). Glufosinate provided 31 to 40 percentage points greater control of *Ambrosia artemisiifolia* than the PSII inhibitors at 8 WAA. Mesotrione or tolpyralate plus ROS generators provided 81% to 99% control of *Ambrosia artemisiifolia* at 8 WAA; there was no difference in control between mesotrione and tolpyralate when co-applied with an ROS generator. The addition of atrazine, bromoxynil, bentazon, or glufosinate to mesotrione improved *Ambrosia artemisiifolia* control 40 to 48 percentage points at 8 WAA. Similarly, Whaley et al. (2006) reported that the addition of atrazine to mesotrione improved *Ambrosia artemisiifolia* control 38 to 57 percentage points. In a previous study, mesotrione + glufosinate provided 94% control of *Ambrosia artemisiifolia*, which is comparable to the 92% control reported in this study (Armel et al. 2008a). At 8 WAA, the addition of atrazine or bromoxynil to tolpyralate improved *Ambrosia artemisiifolia* control 98% and 96%, respectively. At 8 WAA, there was no increase in *Ambrosia artemisiifolia* control with the addition of bentazon or glufosinate to tolpyralate. The co-application of ROS generators plus mesotrione synergistically controlled *Ambrosia artemisiifolia*. Similarly, the tank mixes of the PSII inhibitors with tolpyralate were synergistic for the control of *Ambrosia artemisiifolia*. In contrast, the tank mix of tolpyralate + glufosinate was antagonistic for the control of several broadleaf weed species (Besançon et al. 2018;
Bethke et al. (2013; Meyer and Norsworthy 2019). The antagonism has been attributed to reduced absorption and translocation of glyphosate when tank mixed with glufosinate (Besançon et al. 2018; Meyer and Norsworthy 2019). It is plausible that glufosinate induced similar antagonistic mechanisms on tolpyralate to cause the antagonistic interaction; however, this remains to be investigated.

The interaction between HPPD inhibitors and ROS generators was significant for *A. artemisiifolia* density (P = 0.0364) and dry biomass (P = 0.0376). No ROS generator or HPPD inhibitor applied alone reduced the *A. artemisiifolia* density in comparison to the nontreated control (Table 5). The addition of mesotrione or tolpyralate to the PSII inhibitors reduced *A. artemisiifolia* density more than the PSII inhibitors applied alone. In contrast, the addition of mesotrione or tolpyralate to glufosinate did not improve the reduction in *A. artemisiifolia* density. Except for tolpyralate + bentazon, the tank mixes of mesotrione or tolpyralate with atrazine, bromoxynil, or bentazon were synergistic for the reduction in *A. artemisiifolia* density. Consistent with the density data, no ROS generator decreased the dry biomass of *A. artemisiifolia*. In contrast, tolpyralate applied alone reduced the *A. artemisiifolia* dry biomass by 91%. In agreement with *A. artemisiifolia* control and density data, the addition of mesotrione or tolpyralate to the PSII inhibitors reduced dry biomass of *A. artemisiifolia* compared with the PSII inhibitors applied alone. The reduction in *A. artemisiifolia* dry biomass was synergistic with mesotrione plus atrazine, bromoxynil, or bentazon. The reduction in dry biomass of *A. artemisiifolia* was synergistic with tolpyralate + atrazine, but additive with tolpyralate plus bromoxynil or bentazon. Consistent with the control data, the tank mix of tolpyralate + glufosinate was antagonistic for *A. artemisiifolia* dry biomass reduction.

**Chenopodium album**

Results of *C. album* are pooled results from four experiments. The interaction between HPPD inhibitors and ROS generators was significant for *C. album* control at 2, 4, and 8 WAA (P < 0.0001). Tolpyralate and mesotrione controlled *C. album* similarly at 2 and 4 WAA (Table 6). The addition of mesotrione or tolpyralate to the ROS generators improved *C. album* control compared with the ROS generators applied alone at 2 and 4 WAA. Poor control (35% to 61%) of *C. album* with atrazine, bromoxynil, bentazon, and glufosinate is consistent with previous research (Bethke et al. 2013; Woodyard et al. 2009a). Mesotrione + glufosinate controlled *C. album* more than tolpyralate + glufosinate at 2 and 4 WAA. Mesotrione tank mixed with any ROS generator synergistically controlled *C. album* 94% to 99% at 2 and 4 WAA. Tolpyralate tank mixed with glufosinate was additive for the control of *C. album* at 2 and 4 WAA, but tank mixes of tolpyralate with the PSII inhibitors were synergistic.

At 8 WAA, mesotrione controlled *C. album* 12 percentage points more than tolpyralate (Table 7). At 8 WAA, the addition of atrazine or bromoxynil to mesotrione improved control to 99% to 100%; however, addition of bentazon or glufosinate to mesotrione did not improve *C. album* control compared with mesotrione applied alone. Similarly, Armel et al. (2003) documented >90% control of *C. album* with mesotrione applied alone. The tank mixes of mesotrione plus atrazine, bromoxynil, or

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**Table 5. Ambrosia artemisiifolia** control (at 2, 4, and 8 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada in 2020 and 2021.a

| Herbicide treatment | No tank-mix partnerb | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|---------------------|-----------------------|----------|------------|----------|-------------|----|
| Control at 2 WAA    |                       |          |            |          |             |    |
| No tank-mix partner | 0 c X                 | 27 c Y   | 34 b Y     | 32 b Y   | 73 b Z      | 2.9|
| Mesotrione          | 43 b X                | 85 b Y (59)** | 97 a Z (63)** | 83 a Y (62)** | 90 a YZ (85)** | 2.3|
| Tolpyralate         | 70 a X                | 95 a Z (78)** | 95 a Z (81)** | 89 a YZ (80)** | 84 a YZ (92)** | 1.2|
| SE                  | 4.3                   | 4.5       | 4.4        | 3.9       | 1.5         |    |
| Control at 4 WAA    |                       |          |            |          |             |    |
| No tank-mix partner | 0 c X                 | 29 c Y   | 37 b Y     | 33 b Y   | 70 b Z      | 3.0|
| Mesotrione          | 47 b X                | 88 b Y (63)** | 98 a Z (67)** | 87 a Y (64)** | 91 a YZ (84)** | 2.2|
| Tolpyralate         | 77 a X                | 97 a Z (84)** | 96 a Z (86)** | 93 a YZ (85)** | 82 ab YX (93)** | 1.2|
| SE                  | 4.7                   | 4.5       | 4.3        | 4.2       | 2.1         |    |
| Control at 8 WAA    |                       |          |            |          |             |    |
| No tank-mix partner | 0 c X                 | 28 b Y   | 37 b Y     | 32 b Y   | 68 b Z      | 3.1|
| Mesotrione          | 51 b Y                | 91 a Z (65)** | 99 a Z (69)** | 91 a Z (67)** | 92 a YZ (84)** | 2.2|
| Tolpyralate         | 79 a Y                | 98 a Z (85)** | 96 a Z (87)** | 94 a YZ (86)** | 81 ab Y (94)** | 1.4|
| SE                  | 4.9                   | 4.7       | 4.5        | 4.4       | 2.5         |    |
| Density             |                       |          |            |          |             |    |
| No tank-mix partner | 36 a Z                | 22 b Z   | 19 b Z     | 30 b Z   | 11 a Z      | 2.5|
| Mesotrione          | 20 a Y                | 4 a YZ (13)* | 1 a Z (14)** | 4 a YZ (21)* | 7 a YZ (8)   | 1.5|
| Tolpyralate         | 15 a Y                | 2 a YZ (8)* | 3 a Z (9)* | 4 a YZ (10) | 10 a YZ (5) | 1.6|
| SE                  | 3.5                   | 1.9       | 2.0        | 2.5       | 2.5         |    |
| Dry biomass         |                       |          |            |          |             |    |
| No tank-mix partner | 281.5 b Z             | 272.4 b Z | 282.9 b Z | 352.5 b Z | 74.8 a Z    | 36.5|
| Mesotrione          | 129.9 ab Y            | 185.4 a YZ (96.4)* | 1.4 a Z (113.5)** | 25.4 a YZ (130.8)* | 37.5 a YZ (36.6) | 16.2|
| Tolpyralate         | 24.4 a YZ             | 1.8 a YZ (16.6)* | 7.3 a YZ (23.1) | 12.4 a YZ (19.9) | 66.5 a YZ (8.8)* | 9.6|
| SE                  | 42.9                  | 30.8      | 30.3       | 34.6      | 24.8        |    |

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*aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.

bAppropriate adjuvants were used with each treatment: mesotrione included Agralis 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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹.

cMeans followed by the same lowercase letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

dValues in parentheses are expected values calculated from Colby’s equation (Equation 1). Asterisks indicate significant differences between observed and expected values based on a two-sided t-test: *P < 0.05; **P < 0.01.

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Glufosinate and ROS generators were synergistic for *C. album* control at 8 WAA. Woodward et al. (2009a) also reported that mesotrione plus atrazine or bromoxynil was synergistic for the control of *C. album*. Mesotrione + bentazon was additive for the control of *C. album* at 8 WAA. The addition of a PSII inhibitor to tolpyralate increased *C. album* control to 95% to 97%; there was no increase in *C. album* control when glufosinate was added to tolpyralate. In contrast, Metzger et al. (2018) did not report a benefit of adding atrazine to tolpyralate for *C. album* control at 8 WAA. Tolpyralate + bromoxynil controlled *C. album* more than tolpyralate + glufosinate at 8 WAA. Tolpyralate plus bentazon or bromoxynil was synergistic for the control of *C. album* at 8 WAA; however, tolpyralate plus atrazine or glufosinate was additive for the control of *C. album*.

There was no interaction between HPPD inhibitors and ROS generators for the density and dry biomass reduction of *C. album*; therefore, the main effects are discussed (Table 6). When averaged across the ROS generators, mesotrione reduced *C. album* density by 94%, which was greater than the 61% reduction by tolpyralate. When averaged across the ROS generators, tolpyralate did not reduce *C. album* dry biomass; however, mesotrione reduced the dry biomass by 98%. When averaged across the HPPD inhibitors, atrazine, bromoxynil, and bentazon reduced the density of *C. album* by 67%, 50%, and 42%, respectively; glufosinate did not reduce the density of *C. album*.

**Sinapis arvensis**

Sinapis arvensis results presented are the pooled results from the Huron Research Station in 2020 and 2021. The interaction between HPPD inhibitors and ROS generators was significant for *S. arvensis* control at 2, 4, and 8 WAA (P = 0.0002 at 2 WAA; P < 0.0001 at 4 and 8 WAA). Glufosinate controlled *S. arvensis* more than atrazine at 2 WAA, whereas bromoxynil and bentazon provided intermediate control (Table 8). Mesotrione provided 50 percentage points greater control of *S. arvensis* than tolpyralate at 2 WAA. At 2 WAA, mesotrione + atrazine controlled *S. arvensis* more than tolpyralate + atrazine. The addition of either HPPD inhibitor to bromoxynil improved *S. arvensis* control similarly at 2 WAA. At 2 WAA, *S. arvensis* control was not improved at any assessment timing with the addition of an HPPD inhibitor to bentazon or glufosinate. *Sinapis arvensis* control with mesotrione was improved by the addition of atrazine or glufosinate, but not by the addition of bromoxynil or bentazon at 2 WAA. The addition of an ROS generator to tolpyralate improved control to 94% to 96% at 2 WAA. The tank mixes of mesotrione + atrazine and tolpyralate + atrazine or bromoxynil were synergistic at 2 WAA for *S. arvensis* control.

Glufosinate and bentazon controlled *S. arvensis* more than atrazine at 4 WAA; bromoxynil control was intermediate and similar to that of the other ROS generators (Table 8). Mesotrione provided 60 percentage points greater control of *S. arvensis* than tolpyralate at 4 WAA. The addition of mesotrione or tolpyralate to the ROS generators controlled *S. arvensis* 100% and 96% to 98%, respectively, at 4 WAA. Additionally, mesotrione tank mixed with each ROS generator controlled *S. arvensis* more than tolpyralate tank mixed with the respective ROS generator at 4 WAA. The co-application of mesotrione with atrazine, bromoxynil, or bentazon resulted in a synergistic increase in *S. arvensis* control at 4 WAA. In contrast, tolpyralate + bentazon was additive for the control of *S. arvensis* at 4 WAA, while tolpyralate plus atrazine or bromoxynil was synergistic.

At 8 WAA, mesotrione controlled *S. arvensis* 60 percentage points more than tolpyralate (Table 8). Metzger et al. (2018) also reported poor *S. arvensis* control with tolpyralate applied alone. At 8 WAA, the addition of an ROS generator to mesotrione did not improve *S. arvensis* control. Control of *S. arvensis* with mesotrione plus atrazine, bromoxynil, bentazon, or glufosinate was 100% at 8 WAA. Metzger et al. (2018) also documented 100% *S. arvensis* control with mesotrione + atrazine at 8 WAA. The high level of *S. arvensis* control by mesotrione and the ROS generators applied alone is likely the reason for the inability to report synergy between mesotrione and ROS generators for the control of *S. arvensis* at 8 WAA. The addition of an ROS generator to tolpyralate improved *S. arvensis* control from 38% to 97% to 99%. Synergy with tolpyralate plus atrazine or bromoxynil occurred for the control of *S. arvensis* at 8 WAA.

**Sinapis arvensis** density and dry biomass had a significant interaction between HPPD inhibitor and ROS generator, so each herbicide factor was analyzed by the other herbicide factor (P = 0.0334 for density; P = 0.0040 for dry biomass). The ROS generators reduced *S. arvensis* density 81% to 99% (Table 8). Tolpyralate did not reduce the density of *S. arvensis* relative to the nontreated control, but mesotrione reduced density 97%. Consistent with *S. arvensis* control at 4 and 8 WAA, the addition of an ROS generator did not improve *S. arvensis* density reduction with mesotrione. In contrast, the addition of the ROS generators to tolpyralate improved the reduction in *S. arvensis* density to levels similar to those seen with mesotrione tank mixes. The dry biomass of *S. arvensis* was reduced 93% to 100% with the use of ROS generators applied individually. Tolpyralate did not reduce *S. arvensis* dry biomass. Mesotrione and its respective tank mixes reduced

### Table 6. Least-squares means and significance of main effects and interaction for *Chenopodium album* density and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.

| Main effects | Rate | Density | Dry biomass |
|--------------|------|---------|-------------|
|              | g ai ha⁻¹ | plants m⁻² | g m⁻² |
| **HPPD inhibitor** | | | |
| No tank-mix partner | — | 18 c | 52.3 b |
| Mesotrione | 100 | 1 a | 1.2 a |
| Tolpyralate | 30 | 7 b | 10.0 ab |
| SE | — | 0.8 | 4.4 |
| HPPD inhibitor P-value | 0.0007 | 0.0166 |
| **ROS generator** | | | |
| No tank-mix partner | — | 12 c | 15.8 |
| Atrazine | 280 | 4 a | 7.6 |
| Bromoxynil | 280 | 6 ab | 12.7 |
| Bentazon | 840 | 7 ab | 12.8 |
| Glufosinate | 300 | 10 bc | 20.8 |
| SE | — | 0.8 | 4.4 |
| ROS generator P-value | 0.0126 | 0.1177 |
| **Interaction** | | | |
| HPPD inhibitor x ROS generator | 0.4051 | 0.0643 |

*Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.*

*Means within the same main effect and column followed by the same lowercase letter are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).*
Table 7. Chenopodium album control (at 2, 4, and 8 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.a

| Herbicide treatment | No tank-mix partnerc | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|---------------------|----------------------|----------|------------|----------|-------------|----|
| **Control at 2 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 b X                | 54 b Z   | 35 b Y     | 40 b YZ  | 47 c YZ     | 2.4|
| Mesotrione & Tolpyralate | 77 a Y & 72 a Y | 98 a Z (90)** & 94 a Z (88)** | 98 a Z (86)** & 95 a Z (82)** | 94 a Z (86)** & 97 a Z (89)** | 11.1|
| SE                  | 5.2                  | 2.9      | 4.3        | 3.8      | 3.5         |    |
| **Control at 4 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 b X                | 61 b Z   | 37 b Y     | 40 b Y   | 37 c Y      | 2.6|
| Mesotrione & Tolpyralate | 87 a Y & 78 a Y | 99 a Z (95)** & 95 a YZ (91)* | 99 a Z (92)** & 96 a YZ (86)** | 96 a YZ (93)** & 97 a Z (92)** | 0.7|
| SE                  | 5.7                  | 2.7      | 4.3        | 3.9      | 4.1         |    |
| **Control at 8 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 c X                | 67 b Z   | 42 b Y     | 43 b Y   | 34 c Y      | 3.0|
| Mesotrione & Tolpyralate | 92 a Y & 80 b X | 99 a Z (97)** & 95 a YZ (93) | 100 a Z (96)** & 97 a YZ (96) | 99 a YZ (95)** & 86 b XY (86) | 1.0|
| SE                  | 6.0                  | 2.3      | 4.3        | 3.9      | 4.3         |    |
| **Density**         |                      |          |            |          |             |    |
| No tank-mix partner | 25 s d               | 11 s     | 16 s       | 16 s     | 24 s        | 2.0|
| Mesotrione & Tolpyralate | 2 & 11 | 1 (1) & 4 (7) | 1 (1) & 4 (10) | 2 (2) & 5 (12) | 2.0 & 0.4 |
| SE                  | 2.4                  | 1.1      | 1.4        | 1.4      | 2.5         |    |
| **Dry biomass**     |                      |          |            |          |             |    |
| No tank-mix partner | 75.3                 | 23.0     | 70.4       | 39.8     | 84.5        | 11.7|
| Mesotrione & Tolpyralate | 5.0 & 8.6 | 0.7 (0.4) & 1.8 (0.4) | 1.8 (0.4) & 3.6 (0.9) | 0.5 (1.5) & 0.5 (1.5) | 1.0 & 1.0 |
| SE                  | 13.2                 | 4.3      | 8.6        | 8.7      | 11.5        |    |

aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.
bAppropriate adjuvants were used with each treatment: 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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹.
cMeans followed by the same lowercase letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

Table 8. Sinapis arvensis control (at 2, 4, and 8 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.a

| Herbicide treatment | No tank-mix partnerc | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|---------------------|----------------------|----------|------------|----------|-------------|----|
| **Control at 2 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 c X                | 74 b Y   | 79 b Y     | 94 a Y   | 96 a Z      | 5.7|
| Mesotrione & Tolpyralate | 84 a Y & 34 b Y | 99 a Z (96)* & 94 b Z (83)** | 99 a YZ (97) & 96 a Z (87)** | 99 a YZ (99) & 95 a Z (97) | 1.4|
| SE                  | 7.3                  | 2.6      | 2.2        | 0.8      | 0.9         |    |
| **Control at 4 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 c X                | 80 c Y   | 88 c Y     | 96 b Z   | 97 b Z      | 5.9|
| Mesotrione & Tolpyralate | 97 a Z & 37 b Y | 100 a Z (99)* & 96 b Z (88)** | 100 a Z (100)* & 98 b Z (98) | 100 a Z (100)* & 98 b Z (98) | 0.6|
| SE                  | 8.3                  | 2.1      | 1.3        | 0.5      | 0.6         |    |
| **Control at 8 WAA** |                      |          |            |          |             |    |
| No tank-mix partner | 0 c X                | 84 b Y   | 90 b Y     | 98 a Z   | 98 a Z      | 6.0|
| Mesotrione & Tolpyralate | 98 a Z & 38 b Y | 100 a Z (100) & 98 a Z (90)** | 100 a Z (100) & 99 a Z (94)** | 100 a Z (100) & 99 a Z (99) | 0.4|
| SE                  | 8.5                  | 1.8      | 1.2        | 0.3      | 0.5         |    |
| **Density**         |                      |          |            |          |             |    |
| No tank-mix partner | 67 b Y               | 13 b Z   | 5 b Z      | 2 a Z    | 1 a Z       | 5.7|
| Mesotrione & Tolpyralate | 2 a Z & 45 b Y | 0 a Z (1) & 2 ab Z (11) | 0 a Z (0) & 1 ab Z (2) | 0 a Z (1) & 3 a Z (2) | 1.4|
| SE                  | 10.2                 | 1.9      | 0.9        | 0.5      | 0.6         |    |
| **Dry biomass**     |                      |          |            |          |             |    |
| No tank-mix partner | 84.1 b Y             | 5.5 b Z  | 2.6 a Z    | 0.5 a Z  | 0.2 a Z     | 6.5|
| Mesotrione & Tolpyralate | 0.3 a Y & 34.6 b Y | 0.0 a Z (0.0) & 0.7 ab Z (2.5) | 0.0 a Z (0.0) & 1.1 a Z (0.9) | 0.0 a Z (0.0) & 0.7 a Z (0.4) | 0.1|
| SE                  | 10.0                 | 0.8      | 0.6        | 0.1      | 0.4         |    |

aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.
bAppropriate adjuvants were used with each treatment: 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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹.
cMeans followed by the same lowercase letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

Values in parentheses are expected values calculated from Colby’s equation (Equation 1). Asterisks indicate significant differences between observed and expected values based on a two-sided t-test: *P < 0.05; **P < 0.01.
S. arvensis dry biomass 100%. When ROS generators were added to tolpyralate, the dry biomass reduction of S. arvensis was similar to that of mesotrione and the respective tank-mix partner. All tank-mix combinations had an additive effect for density and dry biomass reduction of S. arvensis.

**Echinochloa crus-galli**

Echinochloa crus-galli results are pooled across four experiments. The interaction between HPPD inhibitors and ROS generators was significant for E. crus-galli control at 2, 4, and 8 WAA (P < 0.0001). Tolpyralate provided 64% control of E. crus-galli at 2 WAA, whereas mesotrione did not provide control (Table 9). Glufosinate was the only ROS generator applied alone that provided E. crus-galli control at 2 WAA. Previous studies have reported <20% control or fresh weight reduction of E. crus-galli with PSII inhibitors (Jordan et al. 1993; Minton et al. 1989).

At 2 WAA, the addition of mesotrione to atrazine, bromoxynil, or bentazon improved control of E. crus-galli, but control was not acceptable despite some observed synergistic responses. Glufosinate was the best tank-mix partner with mesotrione and its tank mixes with PSII inhibitors was not acceptable despite some observed synergistic responses. Glufosinate was the best tank-mix partner with mesotrione for control of E. crus-galli at 4 and 8 WAA. In contrast to tolpyralate, mesotrione did not provide E. crus-galli control at 4 and 8 WAA. At 4 and 8 WAA, control of E. crus-galli with glufosinate was not improved with the addition of either HPPD inhibitor. In contrast, the addition of mesotrione to atrazine synergistically improved E. crus-galli control to 23%. The level of E. crus-galli control provided by mesotrione and its tank mixes with PSII inhibitors was not acceptable despite some observed synergistic responses. Glufosinate was the best tank-mix partner with mesotrione for control of E. crus-galli at 4 and 8 WAA. At 4 and 8 WAA, control of E. crus-galli with glufosinate was not improved with the addition of either HPPD inhibitor. In contrast, the addition of mesotrione to atrazine synergistically improved E. crus-galli control to 23%. The level of E. crus-galli control provided by mesotrione and its tank mixes with PSII inhibitors was not acceptable despite some observed synergistic responses. Glufosinate was the best tank-mix partner with mesotrione for control of E. crus-galli at 4 and 8 WAA. In contrast to mesotrione, the addition of an ROS generator to tolpyralate did not improve E. crus-galli control at 4 and 8 WAA. Tolpyralate + glufosinate was antagonistic for the control of E. crus-galli, but control was similar to control with tolpyralate alone and with tolpyralate plus the other ROS generators. Uptake and translocation of glyphosate in E. crus-galli can be reduced when tank mixed with glufosinate, so it is possible that glufosinate induces similar antagonistic effects on tolpyralate (Meyer and Norsworthy 2019; Meyer et al. 2020). Future work should investigate whether glufosinate reduces the uptake and translocation of tolpyralate as a mechanism of antagonism in E. crus-galli.

Table 9. Echinochloa crus-galli control (at 2, 4, and 8 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides generators from field trials in Ontario, Canada, in 2020 and 2021.

| Herbicide treatmentb | No tank-mix partnerc | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|----------------------|----------------------|----------|------------|----------|-------------|----|
| **Control at 2 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 b Y                | 1 c Y    | 1 c Y      | 2 c Y    | 68 a Z      | 3.1|
| Mesotrione           | 16 b Y               | 25 b Y (16)** | 27 b Y (17)** | 26 b Y (17)** | 76 a Z (74) | 2.9|
| Tolpyralate          | 64 a Z               | 75 a Z (65)** | 74 a Z (65)* | 72 a Z (65)* | 82 a Z (88)** | 1.1|
| SE                   | 4.2                  | 4.8      | 4.7        | 4.5      | 1.9         |    |
| **Control at 4 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 b Y                | 0 c Y    | 0 b Y      | 0 b Y    | 65 a Z      | 3.0|
| Mesotrione           | 13 b Y               | 23 b Y (13)** | 18 b Y (14)* | 18 b Y (13) | 70 a Z (71) | 2.8|
| Tolpyralate          | 67 a Z               | 72 a Z (67) | 71 a Z (67) | 72 a Z (67) | 78 a Z (87)** | 1.8|
| SE                   | 4.5                  | 4.7      | 4.7        | 4.8      | 2.4         |    |
| **Control at 8 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 b Y                | 0 c Y    | 0 b Y      | 0 b Y    | 62 a Z      | 2.9|
| Mesotrione           | 12 b Y               | 23 b Y (12)** | 17 b Y (12)* | 16 b Y (12) | 68 a Z (67) | 2.8|
| Tolpyralate          | 68 a Z               | 71 a Z (68) | 72 a Z (68) | 72 a Z (68) | 77 a Z (86)** | 2.0|
| SE                   | 4.7                  | 4.7      | 4.9        | 4.9      | 2.5         |    |
| **Density**          |                      |          |            |          |             |    |
| No tank-mix partner  | 27                   | 49       | 25         | 33       | 66          | 7.5|
| Mesotrione           | 15                   | 18 (13)  | 13 (20)    | 18 (14)  | 34 (14)*    | 3.0|
| Tolpyralate          | 63                   | 24 (174) | 22 (98)    | 18 (91)  | 46 (231)    | 5.4|
| SE                   | 8.0                  | 5.2      | 4.4        | 4.4      | 11.4        |    |
| **Dry biomass**      |                      |          |            |          |             |    |
| No tank-mix partner  | 34.1                 | 53.7     | 25.0       | 37.4     | 50.8        | 5.5|
| Mesotrione           | 16.9                 | 21.4 (10.7) | 21.2 (19.5) | 11.2 (8.1) | 31.9 (8.8)** | 4.7|
| Tolpyralate          | 75.0                 | 22.9 (80.8) | 20.9 (148.9) | 36.7 (66.5) | 46.0 (175.0) | 8.9|
| SE                   | 11.3                 | 9.8      | 5.6        | 7.0      | 8.6         |    |

aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.

bAppropriate adjuvants were used with each treatment: 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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹⁻.<sup>1</sup>

<sup>1</sup>Means followed by the same lower case letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

<sup>2</sup>Values in parentheses are expected values calculated from Colby's equation (Equation 1). Asterisks indicate significant differences between observed and expected values based on a two-sided t-test: *P < 0.05; **P < 0.01.
Setaria spp.

Results for Setaria spp. are the pooled results of four experiments. The interaction between HPPD inhibitors and ROS generators was significant for Setaria spp. control at 2, 4, and 8 WAA (P < 0.0001). Control of Setaria spp. followed similar trends at 2, 4, and 8 WAA (Table 10). The PSII inhibitors did not control Setaria spp., whereas glufosinate provided 78% to 84% control of Setaria spp. at 2, 4, and 8 WAA (Table 10). Similar to this study, Bethke et al. (2013) reported that glufosinate provided 73% control of S. faberi. The lack of Setaria spp. control with the PSII inhibitors is consistent with previous research (Armel et al. 2007; Corbett et al. 2004; Jordan et al. 1993). The addition of mesotrione or tolpyralate to glufosinate did not improve Setaria spp. control. Mesotrione and tolpyralate controlled Setaria spp. 5% to 11% and 71% to 75%, respectively, over the course of the study. The results from this study are similar to those of other studies in which mesotrione provided <25% control of S. faberi (Armel et al. 2003, 2007, 2008a; Whaley et al. 2006). The addition of atrazine, bromoxynil, or bentazon to mesotrione did not improve Setaria spp. control. Atrazine, bromoxynil, or bentazon to mesotrione improved control of Setaria spp. control at 2 WAA; all other interactions with mesotrione were additive. The level of Setaria spp. control with mesotrione and its tank mixes with PSII inhibitors was not acceptable at any assessment timing despite some reported synergistic responses. Similar to the current study, Armel et al. (2007) reported synergy between mesotrione and atrazine for the control of S. faberi. At 2, 4, and 8 WAA, Setaria spp. control was not enhanced with the addition of the PSII inhibitors to tolpyralate. Similarly, Metzger et al. (2018) documented that atrazine did not improve control of S. viridis with tolpyralate at 2, 4, and 8 WAA. The addition of tolpyralate to atrazine or bentazon resulted in a synergistic increase in Setaria spp. control at 2 and 4 WAA. The interaction between tolpyralate and the PSII inhibitors for the control of Setaria spp. at 8 WAA was additive. Control of Setaria spp. at 2, 4, and 8 WAA with tolpyralate was improved with the addition of glufosinate. At 2 WAA, the interaction between tolpyralate and glufosinate was additive. Control of Setaria spp. at 2 WAA was greater with tolpyralate + glufosinate than with tolpyralate + bromoxynil and tolpyralate + bentazon. Although the interaction between tolpyralate and glufosinate was antagonistic for the control of Setaria spp. at 4 and 8 WAA, glufosinate was the only ROS generator to improve the control of Setaria spp. with tolpyralate at 4 and 8 WAA. Glufosinate tank mixed with glyphosate has been reported to control S. faberi less than expected (Besançon et al. 2018; Bethke et al. 2013). The reduced efficacy of the tank mix has been attributed to reduced translocation of glyphosate (Besançon et al. 2018). It is possible that tolpyralate translocation in Setaria spp. is reduced in tolpyralate + glufosinate tank mixes; however, this remains speculative. The possibility of antagonism induced by tolpyralate on glufosinate activity should not be ignored.

There was a significant interaction between HPPD inhibitors and ROS generators for Setaria spp. density (P = 0.0197) and dry biomass (P = 0.0351); therefore, the levels of HPPD inhibitor were analyzed by each level of ROS generator and the levels of ROS

Table 10. Setaria spp. control (at 2, 4, and 8 wk after application), density, and dry biomass in corn following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.a

| Herbicide treatmentb | No tank-mix partnerc | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|----------------------|----------------------|----------|------------|----------|-------------|----|
| **Control at 2 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 c Y                | 0 c Y    | 0 c Y      | 1 c Y    | 84 a Z      | 3.7|
| Mesotrione           | 11 b Y               | 15 b Y (11) | 15 b Y (12) | 16 b Y (12) | 88 a Z (86) | 3.3|
| Tolpyralate          | 71 a Y               | 81 a Y (71)** | 78 a Y (72) | 79 a Y (72)** | 92 a Z (96) | 1.1|
| SE                   | 4.6                  | 5.1      | 4.9        | 4.9      | 1.1         |    |
| **Control at 4 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 c Y                | 0 c Y    | 0 c Y      | 0 c Y    | 79 a Z      | 3.6|
| Mesotrione           | 8 b Y                | 14 b Y (8) | 12 b Y (9) | 15 b Y (8)* | 85 a Z (81) | 3.3|
| Tolpyralate          | 72 a Y               | 80 a Y (72)* | 75 a Y (72) | 79 a Y (72)* | 89 a Z (94)* | 1.5|
| SE                   | 4.7                  | 5.1      | 4.8        | 5.0      | 1.2         |    |
| **Control at 8 WAA** |                      |          |            |          |             |    |
| No tank-mix partner  | 0 c Y                | 0 c Y    | 0 c Y      | 0 c Y    | 78 a Z      | 3.5|
| Mesotrione           | 5 b Y                | 13 b Y (5)** | 8 b Y (5) | 11 b Y (5)* | 83 a Z (80) | 3.4|
| Tolpyralate          | 75 a Y               | 80 a Y (75) | 76 a Y (75) | 79 a Y (75) | 89 a Z (95)* | 1.6|
| SE                   | 5.0                  | 5.1      | 5.0        | 5.1      | 1.4         |    |
| **Density**          |                      |          |            |          |             |    |
| No tank-mix partner  | 71 b Y               | 53 ab Y  | 65 a Y     | 64 a Y  | 21 a Z      | 7.9|
| Mesotrione           | 77 b YZ              | 78 b Y (86) | 86 b Y (93) | 81 a Y (101) | 44 a Z (31) | 10.9|
| Tolpyralate          | 30 a Z               | 30 a Y (36) | 50 a Y (41) | 38 a Y (40) | 23 a Z (10)** | 4.8|
| SE                   | 11.8                 | 8.9      | 11.0       | 10.8     | 11.7        |    |
| **Dry biomass**      |                      |          |            |          |             |    |
| No tank-mix partner  | 105.0 b Y            | 86.5 ab Y | 163.3 ab Y | 110.2 ab Y | 10.0 a Z | 17.3|
| Mesotrione           | 184.6 b Y            | 225.7 b Y (424.0) | 219.8 b Y (593.3) | 219.0 b Y (486.0) | 33.8 a Z (27.1) | 19.8|
| Tolpyralate          | 15.8 a Y             | 17.7 a Y (40.1) | 36.9 a Y (52.7) | 27.5 a Y (39.3) | 7.9 a Z (3.5)* | 5.2|
| SE                   | 20.8                 | 22.5     | 27.0       | 23.3     | 3.5         |    |

aAbbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.

bAppropriate adjuvants were used with each treatment: mesotrione included Agral (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. Atrazine applied with no tank-mix partner included Assist® Oil Concentrate (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada) at 2 L ha⁻¹.

cMeans followed by the same lowercase letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

dValues in parentheses are expected values calculated from Colby’s equation (Equation 1). Asterisks indicate significant differences between observed and expected values based on a two-sided t-test: *P < 0.05; **P < 0.01.
Cides applied alone did not cause corn injury (Table 11). The dissipation of each herbicide treatment was complete by 4 WAA. Previous research has also reported synergism with tolpyralate was co-applied with atrazine, bromoxynil, or bentazon. There was a synergistic increase in corn injury when mesotrione was co-applied with bromoxynil or bentazon and when glufosinate was added to an HPPD-inhibiting herbicide. There was no increase in corn injury when atrazine or glufosinate was added to an HPPD-inhibiting herbicide. There was a synergistic increase in corn injury when mesotrione was co-applied with bromoxynil or bentazon and when tolpyralate was co-applied with atrazine, bromoxynil, or bentazon at 1 WAA. Previous research has also reported synergism with tolpyralate + bromoxynil and tolpyralate + bentazone for corn injury at 1 WAA (Willems et al. 2021). At 1 and 2 WAA, tolpyralate caused greater corn injury than mesotrione when tank mixed with bromoxynil. Willems et al. (2021) also documented greater corn injury with tolpyralate + bromoxynil than with mesotrione + bromoxynil at 1 WAA. At 2 WAA, mesotrione and tolpyralate only increased the corn injury with bromoxynil but did not increase corn injury of the other ROS generators.

The interaction between HPPD-inhibiting and ROS-generating herbicides for corn yield was not significant, so the main effects are presented (P = 0.0774). When averaged across the ROS-generating herbicides, mesotrione and tolpyralate increased corn yield 35% and 49%, respectively, although the corn yield was not different between the two herbicides (data not presented).

This study provides novel and comprehensive findings regarding the interaction of HPPD-inhibiting and ROS-generating herbicides in four broadleaf and two grass weed species. To our knowledge, this is the first study investigating the interaction of mesotrione or tolpyralate with glufosinate. It is important to note that at 8 WAA, synergy was documented for the control of A. threophrasti, A. artenmisifolia, and C. album with mesotrione + glufosinate; however, the interaction was additive for the control of S. arvensis, S. herba, and E. cras-galli. In contrast, the interaction between tolpyralate and glufosinate was antagonistic for the control of A. artenmisifolia, S. herba, and E. cras-galli. The interaction between tolpyralate and glufosinate was additive for the control of A. threophrasti, C. album, and S. arvensis. Therefore, the interaction of HPPD inhibitors with glufosinate is HPPD inhibitor and weed species. Research should be conducted to determine the mechanism of antagonism between tolpyralate and glufosinate; the mechanism or mechanisms of antagonism may be different from the reduced translocation and absorption mechanisms associated with the reduced weed control with glufosinate + glyphosate tank mixes. Additionally, research should investigate whether increasing or decreasing tolpyralate or glufosinate rate, applying tolpyralate and glufosinate sequentially, or using a different adjuvant system can alleviate or eliminate antagonism between tolpyralate and glufosinate, as these approaches have

### Table 11. Corn injury (at 1 and 2 wk after application) and corn grain yield following the application of HPPD-inhibiting, ROS-generating, and HPPD-inhibiting plus ROS-generating herbicides from field trials in Ontario, Canada, in 2020 and 2021.

| Herbicide treatment | No tank-mix partner | Atrazine | Bromoxynil | Bentazon | Glufosinate | SE |
|---------------------|---------------------|----------|------------|----------|-------------|----|
| Corn injury at 1 WAA |                     |          |            |          |             |    |
| No tank-mix partner | 0.0 a Z             | 0.0 a Z  | 2.4 a Y    | 0.3 a Z  | 0.0 a Z     | 0.2|
| Mesotrione          | 0.0 a Z             | 0.0 a Z (0.0) | 3.8 a X (2.4)** | 1.2 b Y (0.3)** | 0.0 a Z (0.0) | 0.2|
| Tolpyralate         | 0.0 a Z             | 0.3 b VZ (0.0)** | 5.8 b X (2.5)** | 1.0 b Y (0.3)** | 0.0 a Z (0.0) | 0.3|
| SE                  | 0.0                 | 0.1      | 0.3        | 0.2      | 0.0         |    |
| Corn injury at 2 WAA |                     |          |            |          |             |    |
| No tank-mix partner | 0.0 a Z             | 0.0 a Z  | 1.2 a Y    | 0.1 a Z  | 0.0 a Z     | 0.1|
| Mesotrione          | 0.0 a Z             | 0.0 a Z (0.0) | 2.0 b Y (1.2)** | 0.2 a Z (0.1)** | 0.0 a Z (0.0) | 0.1|
| Tolpyralate         | 0.0 a Z             | 0.1 a Z (0.0)* | 2.8 c Y (1.3)** | 0.2 a Z (0.1) | 0.0 a Z (0.0) | 0.1|
| SE                  | 0.1                 | 0.0      | 0.1        | 0.0      | 0.0         |    |
| Corn yield          |                     |          |            |          |             |    |
| No tank-mix partner | 5,000               | 5,200    | 6,400      | 6,400    | 8,300       | 500|
| Mesotrione          | 7,500               | 7,600    | 8,400      | 9,000    | 9,800       | 440|
| Tolpyralate         | 8,500               | 9,700    | 10,400     | 9,900    | 8,700       | 380|
| SE                  | 670                 | 550      | 610        | 630      | 510         |    |

*Abbreviations: HPPD, 4-hydroxyphenylpyruvate dioxygenase; ROS, reactive oxygen species; WAA, weeks after application.

1. Appropriate adjuvants were used with each treatment: 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; Atrazine applied with no tank-mix partner included Assist®-test: *P < 0.05; **P < 0.01.

2. Means followed by the same lowercase letter within a column and response parameter or means followed by the same uppercase letter within a row are not significantly different according to the Tukey-Kramer multiple range test (P < 0.05).

3. Values in parentheses are expected values calculated from Colby’s equation (Equation 1). Asterisks indicate significant differences between observed and expected values based on a two-sided t-test: *P < 0.05; **P < 0.01.
been effective with other antagonistic herbicide combinations (Burke et al. 2005; Culpepper et al. 1998; Jordan and York 1989; Mueller et al. 1989; Myers and Coble 1992; Rhodes and Coble 1984). Results from this study demonstrated that mesotrione tank mixed with all ROS generators evaluated provided >90% control of all broadleaf weed species at 8 WAA; however, glufosinate was the best ROS-generating herbicide to tank mix with mesotrione for the control of the annual grass weed species. In contrast, atrazine, bromoxynil, bentazox, or glufosinate tank mixed with tolpyralate controlled all grass weed species equivalently. At 8 WAA, tolpyralate tank mixed with atrazine, bromoxynil, or bentazox controlled all broadleaf weed species >90%; glufosinate was an inferior tank-mix partner with tolpyralate for the control of A. artemisiifolia and C. album. Future research should investigate the interaction of isoxaflutole, tembotrione, and topopramezone with glufosinate for the control of several weed species. In addition, a herbicide interaction investigation should be conducted with different rate combinations of glufosinate and HPPD inhibitors to improve the understanding of the interaction between glufosinate and HPPD inhibitors.

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