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Isoxaflutole and metribuzin interactions in isoxaflutole-resistant soybean

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Abstract

Herbicide-resistant weeds are a growing concern globally; in response, new herbicide resistance traits are being inserted into crops. Isoxaflutole-resistant soybean [*Glycine max* (L.) Merr.] will provide a new mode of action for use in this crop. Ten experiments were conducted over a 2-yr period (2017, 2018) to determine herbicide interactions between isoxaflutole and metribuzin on soybean injury, weed control efficacy, and soybean yield on a range of soil types. Soybean leaf-bleaching injury caused by isoxaflutole was most severe at sites with higher levels of rainfall after application. Control of weed species with isoxaflutole (52.5, 79, and 105 g ai ha^{-1}) and metribuzin (210, 315, and 420 g ai ha^{-1}) differed by site based on amount of rainfall after application. At sites where there was sufficient rainfall for herbicide activation, isoxaflutole at all rates controlled common lambsquarters (*Chenopodium album* L.), *Amaranthus* spp., common ragweed (*Ambrosia artemisiifolia* L.), and velvetleaf (*Abutilon theophrasti* Medik.) >90%; metribuzin at all rates controlled *Amaranthus* spp. and witchgrass (*Panicum capillare* L.) >80%. Control of every weed species evaluated was reduced when there was limited rainfall after herbicide application. The co-application of isoxaflutole + metribuzin resulted in additive or synergistic interactions for the control of *C. album*, *Amaranthus* spp., *A. artemisiifolia*, *A. theophrasti*, *Setaria* spp., barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv], and *P. capillare*. Isoxaflutole and metribuzin can be an effective management strategy for common annual broadleaf and grass weeds in Ontario if timely rainfall events occur after herbicide application.

Introduction

Weeds must be controlled during the critical weed-free period (CWFP) to avoid yield loss in soybean [*Glycine max* (L.) Merr.]. Weed interference would reduce soybean yield an average of 52% or US$16.5 billion annually if North American soybean producers did not implement any weed management strategies (Soltani et al. 2017). The soybean yield component most prominently impacted by weed interference is pod number per square meter, which can be reduced by up to 64% under season-long weed interference (Van Acker et al. 1993b). A yield loss of ≤5% occurs if soybean is maintained weed-free between the V2 and V3 growth stages in Ontario, based on the CWFP for soybean in Ontario developed by Van Acker et al. (1993a). Employing integrated weed management strategies such as cultural, biological, mechanical, and chemical practices to eliminate weed interference during the CWFP helps mitigate soybean yield loss.

Glyphosate-resistant (GR) soybean has been rapidly adopted since its introduction in 1996; by 2006, 95% of soybean in the United States was seeded to GR cultivars (Young 2006). The broad spectrum and reliable weed control with glyphosate led to it being the sole herbicide applied on many hectares across North America; glyphosate was frequently the only weed control measure implemented. This dependence on glyphosate contributed to the evolution of more than 40 GR weed species worldwide, including six species in Canada: giant ragweed (*Ambrosia trifida* L.), horseweed (*Erigeron canadensis* L.), *A. artemisifolia*, waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], kochia [*Bassia scoparia* (L.) A. J. Scott], and birdsrape mustard (*Brassica rapa* L.) (Heap 2018). GR weeds cost US farmers US$2 billion annually from crop yield loss and additional weed management costs (Davis 2014). To address herbicide-resistant (HR) weeds, innovative strategies must be implemented to reduce the selection intensity for additional HR weeds and slow down the geographic spread of resistant biotypes.

New transgenic soybean cultivars with traits that confer resistance to isoxaflutole are currently under development. Combinations of glyphosate, glufosinate, and mesotrione resistance will also be incorporated into soybean cultivars with isoxaflutole resistance. Preliminary studies have shown potential for isoxaflutole-resistant (IR) soybean to be used as a tool to manage GR

Table 1. Soil characteristics, planting date, application dates, and rainfall of 10 field experiments in Ontario, Canada, in 2017 and 2018.[a]

No.	Location	Year	Soil type	Sand	Silt	Clay	OM	pH	CEC	Planting date	Application date	Rainfall 0–7 DAA	Rainfall 0–14 DAA	Rainfall 0–28 DAA
				%					meq/100 g soil			mm		
1	Ridgetown	2017	Clay loam	33	30	37	4.0	7.0	24	June 9	June 13	17.1	34.8	58.4
2	Ridgetown	2018	Clay loam	35	30	35	4.2	6.7	19	May 25	May 29	5	4.3	35.2
3	Ridgetown	2017	Clay loam	41	28	31	4.0	7.1	14	June 2	June 7	2.7	24.8	46.4
4	Ridgetown	2018	Clay loam	43	26	31	3.6	6.8	16	May 31	June 1	4.9	7.2	42.6
5	Exeter	2018	Loam	41	35	24	2.9	7.7	27	May 18	May 22	5.2	14.7	28.3
6	Exeter	2017	Loam	35	43	22	3.9	7.8	30	June 3	June 5	0.8	12.5	84.8
7	Ennotville	2017	Silt loam	41	52	7	3.8	7.8	18	May 31	June 2	9.8	22.7	114
8	Ennotville	2018	Silt loam	41	52	7	3.8	7.8	18	May 25	May 28	14.9	15.8	48.2
9	Cambridge	2017	Sandy loam	68	26	6	2.2	7.2	9	May 31	June 2	5.9	7.3	70.5
10	Cambridge	2018	Sandy loam	68	26	6	2.2	7.2	9	May 25	May 28	10.8	14	58

[a] Abbreviations: DAA, days after application: OM, organic matter.

weeds. A mixture of isoxaflutole (105 g ai ha^{-1}), metribuzin (420 or 630 g ai ha^{-1}), and *S*-metolachlor (1,068 g ai ha^{-1}) controlled GR Palmer amaranth (*Amaranthus palmeri* S. Watson) 95% and 85%, respectively, at 4 and 7 wk after application (WAA) (Meyer et al. 2015). Isoxaflutole (105 g ha^{-1}) plus metribuzin (420 g ha^{-1}) controlled GR *A. tuberculatus* 75% and GR *E. canadensis* 78% at 8 WAA (Ditschun et al. 2016; Schryver et al. 2017). Herbicide efficacy in these studies may be underestimated due to the lack of soybean competition because IR seed was not available.

Isoxaflutole inhibits the 4-hydroxyphenyl pyruvate dioxygenase (HPPD) enzyme, which is essential for tocopherol and plastoquinone biosynthesis (Pallett et al. 1998). Typically, isoxaflutole is applied PRE or early POST (ePOST) in corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) production; however, the development of IR soybean will expand the use of isoxaflutole in soybean, applied PRE. According to the herbicide label, isoxaflutole (105 g ha^{-1}) provides full-season residual control of a broad spectrum of annual grass and broadleaf weed species (Anonymous 2017). Isoxaflutole applied at lower rates of 52.5 and 79 g ha^{-1} controls a narrower spectrum of weeds and provides shorter residual weed control. In corn, isoxaflutole is commonly applied in combination with atrazine, which increases the spectrum of weeds controlled and improves control of annual grass and broadleaf weeds. However, soybean is not tolerant to atrazine; therefore, metribuzin is proposed for use with isoxaflutole in IR soybean (ACK, personal observation). Metribuzin is a photosystem II (PSII) inhibitor that binds to the Q_B binding niche on the D1 protein of PSII, displacing the electron transporter, plastoquinone, causing a buildup of high-energy electrons that leads to lipid peroxidation of organelle and cellular membranes (Shaner 2014; Trebst 2008).

Herbicides applied as a mixture may interact within the tank before application or afterward within the plant, resulting in an antagonistic, additive, or synergistic response. The interaction is classified as antagonistic, additive, or synergistic by comparing the actual response of the herbicides applied together to the expected response when applied alone determined by Colby's equation (Colby 1967). Antagonism occurs when the actual response of the herbicides applied together is lower than the expected response, additive responses occur when the expected and actual responses are similar, and synergy occurs when the actual response is greater than the expected response. Synergism has been documented with combinations of HPPD- and PSII-inhibiting herbicides in certain weed species. Ditschun et al. (2016) observed synergistic control of *E. canadensis* with isoxaflutole + metribuzin at 79 + 316 g ha^{-1} and 105 + 410 g ha^{-1}, respectively.

Limited research has been conducted using isoxaflutole and metribuzin in IR soybean, with few studies addressing the interaction of the two herbicides on annual weeds. The objectives of this research were to (1) determine the spectrum of weeds controlled and (2) tolerance of IR soybean to isoxaflutole and metribuzin applied alone and in a mixture and (3) quantify interactions between isoxaflutole and metribuzin for the control of annual grass and broadleaf weeds.

Materials and Methods

Ten field experiments were conducted over a 2-yr period (2017, 2018) at four sites near Ridgetown (two trials per year), Exeter, Ennotville, and Cambridge, Ontario. The sites were chosen to represent some common soil types found in Ontario. Soil characteristics, planting dates, and application dates are presented in Table 1. The land was conventionally tilled before planting. Regionally appropriate IR soybean cultivars were planted approximately 5-cm deep at approximately 372,500 seeds ha^{-1}. Plots were 3.0-m wide (4 rows spaced 76 cm apart) and 8- or 10-m long.

Treatments were arranged in a randomized complete block design, with four replications at each location. Herbicides were applied PRE using a compressed-CO_2 backpack sprayer calibrated to deliver 200 L ha^{-1} at 240 kPa and equipped with a 1.5-m boom with four 120-02 ultra–low drift nozzles (Hypro® ULD 120-02, New Brighton, MN) spaced 50 cm apart producing a spray width of 2.0 m. One untreated and one weed-free control were included in each replicate; the weed-free control was treated with imazethapyr (100 g ai ha^{-1}) and metribuzin (400 g ha^{-1}) applied PRE, followed by glyphosate (900 g ae ha^{-1}) applied POST, followed by hand weeding as needed for the remainder of the season. Herbicide treatments included isoxaflutole at 52.5, 79, and 105 g ha^{-1}, hereafter referred to as the low, medium, and high rates of isoxaflutole, respectively; metribuzin at 210, 316, and 420 g ha^{-1}, hereafter referred to as the low, medium, and high rates of metribuzin, respectively; and a mixture of the low, medium, and high rates of isoxaflutole and metribuzin.

Soybean injury was visually evaluated at 1, 2, and 4 wk after emergence (WAE) on a scale of 0 to 100 based on percent affected leaf area, where 0 represented no visible soybean injury and 100 represented soybean death. Weed control of naturally occurring weed species was visually estimated at 4, 8, and 12 WAA on a scale of 0 to 100, where 0 was no decrease in weed biomass relative to the weedy control and 100 was complete control (data only presented from 8 WAA). At 8 WAA, weed density and biomass were

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Table 2. Soybean leaf-bleaching injury symptoms at 1, 2, and 4 wk after emergence (WAE) from 10 field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

		Visible bleaching injury						
		1 WAE	2 WAE		4 WAE			
Treatment	Rate	All sites	Sites 1, 2, 3, 4, 5, 7, 8, 9, 10	Site 6	Sites 5, 8, 10	Sites 1, 2	Sites 3, 4, 7, 9	Site 6
Observed	g ai ha^{-1}	%						
Isoxaflutole	52.5	0	0	0a	0	0abc	1b	17b
Isoxaflutole	79	0	0	2b	0	0abc	4cd	26cd
Isoxaflutole	105	0	0	0ab	0	4bc	8ef	27de
Metribuzin	210	0	0	0a	0	0a	0a	0a
Metribuzin	315	0	0	0a	0	0a	0a	0a
Metribuzin	420	0	0	0a	0	0a	0a	0a
Isoxaflutole + metribuzin	52.5 + 210	0	0	0ab	0	0abc	3bc	20bc
Isoxaflutole + metribuzin	79 + 315	0	0	1ab	0	3abc	5ef	27de
Isoxaflutole + metribuzin	105 + 420	0	0	2b	0	5c	10f	34e
Expected ϵ[b]								
Isoxaflutole + metribuzin	52.5 + 210	0	0	0	0	0	1*	17*
Isoxaflutole + metribuzin	79 + 315	0	0	2	0	0*	4*	26*
Isoxaflutole + metribuzin	105 + 420	0	0	0	0	4*	8*	27*

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value (P < 0.05) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where E is the expected injury with isoxaflutole + metribuzin; and X and Y are the observed percent injury of isoxaflutole + metribuzin, respectively.

measured by counting the number of weeds, by species, from two 0.5-m^2 quadrats per plot. The weeds were cut at the soil surface, placed in a paper bag by species, dried at 60 C to constant moisture, and then weighed. The center two rows of soybean were harvested with a small-plot research combine. The seed weight and moisture were recorded, and weights were adjusted to 13% moisture before analysis.

Data were analyzed in SAS software (v. 9.4; SAS Institute, Cary, NC) using PROC GLIMMIX. An initial mixed-model analysis was conducted when analyzing injury, weed control at 4 WAA, and yield to determine whether there was a significant site by treatment interaction. The fixed effects were treatment, site, and the site by treatment interaction, and the random effect was replication within site. If there was a significant site by treatment interaction, a Tukey-Kramer multiple means comparison test was performed to determine how the sites grouped. The same site groupings were used for control at 8 and 12 WAA, density, and biomass analyses. Sites were pooled for the remaining analyses if there was not a significant site by treatment interaction. A second mixed-model analysis was conducted on each group to determine treatment effects on soybean injury, visible weed control at 4, 8, and 12 WAA, weed density, biomass, and soybean yield once sites were organized into groups that responded similarly. In this second mixed-model analysis, the fixed effect was treatment, and the random effects were site, site by treatment interactions, and replication within site. An *F*-test was performed to test the significance of fixed effects, and a Wald test was used to test the significance of random effects. Residual plots were used to test that variances were randomly distributed, independent, and homogenous across treatments. A Shapiro-Wilk test was conducted to test the assumption that residuals were normally distributed. Natural log and arcsine square-root transformations were used when necessary to normalize data; transformed means were transformed back to the original scale for presentation of results. A Tukey-Kramer test was conducted to compare means at a confidence level of 0.05. To determine the interactions of isoxaflutole and metribuzin, Colby's equation was used to calculate the expected injury, control, density, and biomass. The expected values were then compared with the observed values using a *t*-test. If the values did not differ, the interaction was considered additive; however, if there was a significant difference in the expected and observed values, the interaction was classified as antagonistic or synergistic.

Results and Discussion

Weed control was visually assessed at 4, 8, and 12 WAA; however, only the 8 WAA assessments are presented to minimize data within the article.

Soybean Injury

No soybean injury was observed at any sites in this study at 1 WAE (Table 2). Soybean leaf bleaching occurred at Site 6 at 2 WAE (Table 2). Isoxaflutole at the medium rate caused 2% bleaching injury. Isoxaflutole + metribuzin at the low, medium, and high rates caused 0%, 1%, and 2% soybean bleaching, respectively. Based on Colby's equation, the interaction was additive. Soybean bleaching symptoms occurred at seven sites at 4 WAE (Table 2). There was a significant site by treatment interaction (data not presented); therefore, Sites 1 and 2 were combined; Sites 3, 4, 7, and 9 were combined; and Site 6 was analyzed separately. At Sites 1 and 2, isoxaflutole at the high rate caused 4% soybean injury. Isoxaflutole + metribuzin at the medium and high rates caused 3% and 5% injury, respectively. The addition of metribuzin to isoxaflutole at the medium and high rates caused a synergistic increase in soybean injury. At Sites 3, 4, 7, and 9, isoxaflutole at the low, medium, and high rates caused 1%, 4%, and 8% injury, respectively. The addition of metribuzin to isoxaflutole at the low, medium, and high rates caused a synergistic increase in soybean injury to 3%, 5%, and 10%, respectively. At Site 6, isoxaflutole at the low, medium, and high rates caused 17%, 26%, and 27% soybean bleaching, respectively. Isoxaflutole + metribuzin at the low, medium, and high rates caused a synergistic increase in soybean injury to 20%, 27%, and 34%, respectively. The higher injury at Site 6 was probably due to a large rainfall

Table 3. *Chenopodium album* control, density, and biomass at 8 wk after application from seven field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

		Visible control			Density			Biomass		
Treatment	Rate	Site 3	Sites 5, 9, 10	Sites 1, 4, 6	Site 3	Sites 5, 9, 10	Sites 1, 4, 6	Site 3	Sites 5, 9, 10	Sites 1, 4, 6
Observed	g ai ha^{-1}		—%—			—no. m^{-2}—			—g m^{-2}—	
Untreated					51.7b	27.5a	97.6c	29.8a	38.3a	20.1c
Isoxaflutole	52.5	26abc	72bcd	88a	16.9ab	35.5a	31.5bc	15.8a	39.5a	8.9abc
Isoxaflutole	79	49abc	84abc	96a	11.0ab	28.6a	13.9abc	9.9a	31.4a	5.4abc
Isoxaflutole	105	76ab	95ab	98a	10.6ab	18.0a	9.8ab	9.1a	23.6a	2.2ab
Metribuzin	210	1c	41cd	19b	24.7ab	27.0a	47.0bc	6.3a	33.6a	22.1c
Metribuzin	315	4bc	32d	84a	13.1ab	29.7a	31.6bc	12.9a	57.5a	13.6bc
Metribuzin	420	26abc	58cd	84a	26.7ab	16.5a	18.abc	17.7a	29.2a	6.9abc
Isoxaflutole + metribuzin	52.5 + 210	71abc	80abc	99a	3.7a	42.1a	6.7ab	3.5a	51.7a	5.5abc
Isoxaflutole + metribuzin	79 + 315	62abc	97ab	95a	4.8ab	15.6a	2.8a	2.7a	19.9a	1.8ab
Isoxaflutole + metribuzin	105 + 420	98a	100a	99a	5.9ab	14.2a	2.0a	14.5a	18.7a	1.3a
Expected ϵ[b]										
Isoxaflutole + metribuzin	52.5 + 210	27	84	92*	1.1	8.1	0.1	3.4	33.8	0.3
Isoxaflutole + metribuzin	79 + 315	53	90*	99	1.7	7.4	0.1	3.2	14.0	0.4
Isoxaflutole + metribuzin	105 + 420	82*	98*	100	1.0	1.0	0.04	0.7	1.8	0.2

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value (P < 0.05) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.

[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where E is the expected injury with isoxaflutole + metribuzin; and X and Y are the observed percent injury of isoxaflutole + metribuzin, respectively.

event 1 wk before the 4 WAE evaluation timing, resulting in increased herbicide absorption. No soybean injury was observed at Sites 5, 8, or 10.

Chenopodium album

Chenopodium album was present at seven sites in this study. Due to a significant site by treatment interaction (data not presented), Site 3 was analyzed separately; Sites 5, 9, and 10 were combined; and Sites 1, 4, and 6 were combined and analyzed separately.

Isoxaflutole and metribuzin controlled *C. album* 26% to 76% and 1% to 26%, respectively, at Site 3 (Table 3). Isoxaflutole + metribuzin at the low, medium, and high rates controlled *C. album* 71% to 98%. Additive control occurred with the application of the low and medium rates of isoxaflutole + metribuzin, and a synergistic increase in *C. album* control occurred with the high rate of isoxaflutole + metribuzin. At Sites 5, 9, and 10, isoxaflutole and metribuzin at the three rates controlled *C. album* 72% to 95% and 32% to 58%, respectively. Isoxaflutole + metribuzin controlled *C. album* 80% to 100%. The combination of isoxaflutole + metribuzin at the medium and high rates caused a synergistic increase for *C. album* control. At Sites 1, 4, and 6, all treatments provided 84% to 99% control, with the exception of the low rate of metribuzin, which controlled *C. album* 19%. The combination of isoxaflutole + metribuzin at the low rate provided a synergistic increase in *C. album* control, whereas the medium and high rates had an additive interaction.

Isoxaflutole + metribuzin at the low rate reduced *C. album* density 93% at Site 3 (Table 3). No other treatment reduced *C. album* density compared with the untreated control or differed from the low rate of isoxaflutole + metribuzin. At Sites 5, 9, and 10, no treatment reduced *C. album* density compared with the untreated control. At Sites 1, 4, and 6, isoxaflutole at the low and medium rates did not reduce *C. album* density; the high rate reduced density 90%. Metribuzin at the low, medium, or high rate did not reduce *C. album* density. Isoxaflutole + metribuzin at the low, medium, and high rates reduced *C. album* density 93% to 98%. The coapplication of isoxaflutole + metribuzin at all sites caused an additive reduction in *C. album* density.

Herbicide treatments did not reduce *C. album* biomass at Sites 3, 5, 9, and 10 (Table 3). The high rate of isoxaflutole reduced *C. album* biomass 89% compared with the untreated control at Sites 1, 4, and 6. Metribuzin at the low, medium, or high rate did not reduce *C. album* biomass. Isoxaflutole + metribuzin at the low rate did not reduce *C. album* biomass; however, the medium and high rates reduced biomass 91% to 94% compared with the untreated control. Observed *C. album* biomass values did not differ from expected values with the combination of isoxaflutole + metribuzin; therefore, the interactions were additive.

In summary, *C. album* control was lowest at Site 3. This site had one of the lowest levels of rainfall of 2.7 mm 0 to 7 d after application (DAA) (Table 1). This probably reduced the amount of herbicide dissolved in the soil water solution so that it could be absorbed by *C. album* seedlings. The other sites where *C. album* was evaluated had higher levels of control. Most of these sites received >5 mm of rainfall 0 to 7 d DAA, with the exception of Site 6, which received only 0.8 mm of rainfall and had delayed weed emergence, likely because of lack of moisture. This site received 12.5 mm of rainfall within 0 to 14 DAA, allowing for the herbicides to be dissolved into the soil water solution and absorbed by the emerging weed seedlings. Similar results were reported by Sprague et al. (1999): limited rainfall after application in 1 yr reduced control of *C. album* with isoxaflutole (79 and 105 g ai ha^{-1}) by up to 63%. Increasing rates of isoxaflutole, metribuzin, or the mixture of isoxaflutole + metribuzin rarely resulted in a significant increase in *C. album* control. Bhowmik et al. (1999) determined the effective dose of isoxaflutole to reduce *C. album* biomass 80% (ED_{80}) was 13 g ai ha^{-1}, which suggests that this species is very sensitive to isoxaflutole and would be controlled at the rates evaluated in this study. In contrast, Knezevic et al. (1998) determined the ED_{80} of isoxaflutole for *C. album* to be 60 to 130 g ha^{-1}. Results in our study suggest that at sites with the highest level of *C. album* control (Sites 1, 4, and 6), the ED_{80} would be between 79 and 105 g ha^{-1}. Generally, isoxaflutole provided greater numerical control of *C. album* compared with metribuzin for all site groups. The combination of isoxaflutole + metribuzin had additive or synergistic interactions for *C. album* control and reduction in density or biomass. The combination of

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Table 4. *Amaranthus* spp. control, density, and biomass at 8 wk after application from nine field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

Treatment	Rate	Visible control				Density				Biomass			
		Site 3	Site 5	Sites 1, 4, 6, 8, 9, 10	Site 7	Site 3	Site 5	Sites 1, 4, 6, 8, 9, 10	Site 7	Site 3	Site 5	Sites 1, 4, 6, 8, 9, 10	Site 7
Observed	g ai ha^{-1}	——% ——				——no. m^{-2}——				——g m^{-2}——			
Untreated						71.1b	3.5a	34.0e	29.2c	142.6a	22.7a	28.7c	172.7c
Isoxaflutole	52.5	34abcd	44cde	62cd	87bc	38.0ab	3.5a	4.1bcd	3.6b	68.7a	16.3a	6.6b	20.8bc
Isoxaflutole	79	56abc	73abcd	83abc	98ab	17.8ab	3.3a	3.5bcd	1.5ab	32.1a	17.4a	6.2b	1.5ab
Isoxaflutole	105	69a	80abc	88abc	99ab	16.7ab	1.7a	1.9abcd	0.03a	34.5a	4.4a	3.8ab	0.2a
Metribuzin	210	8cd	22e	40d	75c	23.0ab	1.5a	6.7d	2.5ab	58.2a	19.0a	9.4bc	12.6abc
Metribuzin	315	5d	35de	67bcd	96abc	8.8ab	0.6a	5.7cd	0.5ab	62.1a	1.6a	7.1bc	1.6ab
Metribuzin	420	12bcd	61bcde	79abcd	87bc	35.5ab	0.8a	3.7bcd	2.4ab	61.4a	6.9a	6.4b	18.7bc
Isoxaflutole + metribuzin	52.5 + 210	68a	40de	93abc	100a	9.1ab	2.1a	1.7abc	0.03a	40.5a	21.6a	4.8b	0.2a
Isoxaflutole + metribuzin	79 + 315	63ab	88ab	98ab	99ab	19.9ab	0.8a	1.0ab	0.03a	34.2a	0.9a	2.2ab	0.2a
Isoxaflutole + metribuzin	105 + 420	85a	98a	99a	100a	7.0a	0.5a	0.3a	0.03a	29.6a	1.1a	0.4a	0.2a
Expected ϵ[b]													
Isoxaflutole + metribuzin	52.5 + 210	46	57	76	98*	14.9	3.0	1.5	0.4	27.9	15.8	4.1	2.1
Isoxaflutole + metribuzin	79 + 315	62	83	96	100	3.6	1.3	1.0	0.03	13.8	1.7	2.0	0.1
Isoxaflutole + metribuzin	105 + 420	73	92	98	100	11.1	0.2	0.4	0.003	14.8	2.8	2.0*	0.1

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value (P < 0.05) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, Expected value determined by Colby's equation: $E = X + Y - (XY100)$, where E is the expected control, density, or biomass with isoxaflutole + metribuzin; and X and Y are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

isoxaflutole + metribuzin rarely provided a higher level of *C. album* control compared with isoxaflutole or metribuzin alone at any rate. However, 100% control was only obtained at Sites 5, 9, and 10 at 8 WAA with isoxaflutole + metribuzin at the high rate. This suggests that, at most sites, *C. album* seeds would be returned to the soil seedbank and contribute to weed management challenges in subsequent years if weeds were not controlled by other strategies during the same season. *Chenopodium album* is controlled >95% after application of isoxaflutole + metribuzin at the low, medium, or high rate when the herbicide is sufficiently activated.

Amaranthus spp.

Powell amaranth (*Amaranthus powellii* S. Watson) and redroot pigweed (*Amaranthus retroflexus* L.) were combined during weed control ratings at nine sites in this study. There was a significant treatment by site interaction (data not presented), so Sites 3, 5, and 7 were analyzed independently; and Sites 1, 4, 6, 8, 9, and 10 were combined.

Isoxaflutole and metribuzin at the three rates controlled *Amaranthus* spp. 34% to 69% and 5% to 12%, respectively, at Site 3 (Table 4). The combination of isoxaflutole + metribuzin had an additive effect at each rate and controlled *Amaranthus* spp. 63% to 85%. Isoxaflutole + metribuzin at the varying rates provided 55% to 80% greater *Amaranthus* spp. control than metribuzin alone at the low or medium rate. Isoxaflutole and metribuzin at the three rates controlled *Amaranthus* spp. 44% to 80% and 22% to 61%, respectively, at Site 5. The co-application of isoxaflutole + metribuzin at the low, medium, and high rates exhibited an additive interaction controlling *Amaranthus* spp. 40%, 88%, and 98%, respectively. Isoxaflutole and metribuzin at the three rates controlled *Amaranthus* spp. 62% to 88% and 40% to 79%, respectively, at Sites 1, 4, 6, 8, 9, and 10. The combination of isoxaflutole + metribuzin at the three rates provided additive interactions and controlled *Amaranthus* spp. 93% to 99%. Isoxaflutole at the low, medium, and high rates controlled *Amaranthus* spp. 87% to 99% at Site 7. Metribuzin at the three rates controlled *Amaranthus* spp. 75% to 96%. Isoxaflutole + metribuzin at the low rate caused a synergistic increase in *Amaranthus* spp. control, whereas the medium and high rates had additive interactions, all providing 99% to 100% *Amaranthus* spp. control.

Isoxaflutole + metribuzin at the high rate reduced *Amaranthus* spp. density 90% compared with the untreated control at Site 3 (Table 4). Each rate of isoxaflutole + metribuzin displayed an additive interaction. At Site 5, no treatment reduced *Amaranthus* spp. density compared with the untreated control. The combination of isoxaflutole + metribuzin interacted additively at each rate. All herbicide treatments reduced *Amaranthus* spp. density compared with the untreated control at Sites 1, 4, 6, 8, 9, and 10. Isoxaflutole and metribuzin at the three rates reduced density 88% to 94% and 80% to 89%, respectively. There was an additive interaction with isoxaflutole + metribuzin at each rate, and *Amaranthus* spp. density was reduced 95% to 99% compared with the untreated control. Isoxaflutole + metribuzin at the low rate provided an additional 15% reduction in *Amaranthus* spp. density compared with metribuzin at the low rate. At Site 7, every herbicide treatment reduced *Amaranthus* spp. density compared with the untreated control. Isoxaflutole at the low, medium, and high rates reduced *Amaranthus* spp. density 88%, 95%, and 99%, respectively, compared with the untreated control. Metribuzin at the three rates reduced density 91% to 98% compared with the untreated control. The combination of isoxaflutole + metribuzin at the three rates had an additive interaction and reduced density 99% compared with the untreated control.

No herbicide treatment reduced *Amaranthus* spp. biomass compared with the untreated control at Sites 3 and 5 (Table 4). At both sites, the combination of isoxaflutole + metribuzin produced additive interactions. At Sites 1, 4, 6, 8, 9, and 10, isoxaflutole at the three rates reduced *Amaranthus* spp. biomass 77% to 87%. Metribuzin at the high rate reduced *Amaranthus* spp. biomass 78%. The combination of isoxaflutole + metribuzin at the low and medium rates had additive interactions, and the high rate had a synergistic interaction. Isoxaflutole + metribuzin at the low, medium, and high rates reduced *Amaranthus* spp. biomass 83%, 92%, and 99%, respectively, compared with the untreated

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Table 5. *Ambrosia artemisiifolia* control, density, and biomass at 8 wk after application from four field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

		Visible control			Density			Biomass		
Treatment	Rate	Site 2	Sites 1,4	Site 6	Site 2	Sites 1,4	Site 6	Site 2	Sites 1,4	Site 6
Observed	g ai ha^{-1}		%			no. m^{-2}			g m^{-2}	
Untreated					17.2d	48.5b	45.8b	2.4ab	15.4b	35.2b
Isoxaflutole	52.5	77abc	84a	97a	0.9ab	2.0a	0.7a	0.8ab	1.8a	0.3a
Isoxaflutole	79	82abc	90a	100a	0.3a	0.7a	0.5a	0.1a	0.4a	0.1a
Isoxaflutole	105	93abc	98a	100a	0.05a	0.6a	0.3a	0.1a	0.4a	0.1a
Metribuzin	210	4d	4b	0c	7.6bcd	50.3b	2.7a	1.5ab	18.7b	19.6b
Metribuzin	315	31cd	15b	60b	9.0cd	36.7b	2.6a	2.1ab	19.6b	6.2ab
Metribuzin	420	47bcd	29b	97a	3.2abcd	27.3b	0.5a	1.7ab	14.1b	0.4a
Isoxaflutole + metribuzin	52.5 + 210	90abc	95a	100a	2.0abc	0.7a	0.4a	10.5b	0.4a	0.1a
Isoxaflutole + metribuzin	79 + 315	98ab	98a	100a	0.3a	0.7a	0.06a	1.5ab	0.4a	0.1a
Isoxaflutole + metribuzin	105 + 420	100a	100a	100a	0.5a	0.3a	0.06a	0.5ab	0.4a	0.1a
Expected ϵ[b]										
Isoxaflutole + metribuzin	52.5 + 210	81	86	97	0.3	2.1	0.2	2.4	2.2	0.3
Isoxaflutole + metribuzin	79 + 315	89	93	100	0.1	0.4	0.1	0.2	0.5	0.01
Isoxaflutole + metribuzin	105 + 420	97	99	100	0.01	0.4	0.02	0.2	0.5	0.01

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05.
[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where E is the expected control, density, or biomass with isoxaflutole + metribuzin; and X and Y are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

control. At Site 7, isoxaflutole at the low rate and metribuzin at the low and high rates did not reduce *Amaranthus* spp. biomass compared with the untreated control. Isoxaflutole at the medium and high rates, metribuzin at the medium rate, and isoxaflutole + metribuzin at all three rates reduced biomass 99% compared with the untreated control. The combination of isoxaflutole + metribuzin resulted in additive interactions.

In summary, there was the lowest level of *Amaranthus* spp. control at Site 3, followed by Site 5, Sites 1, 4, 6, 8, 9, and 10, and Site 7. We think variable control may be attributed to varying rainfall amounts after herbicide application across sites. Site 3 only received 2.7 mm of rain 0 to 7 DAA, which was lower than the other sites, except for Site 6 (Table 1). Adequate rainfall is needed for activation of many soil-applied herbicides, including isoxaflutole and metribuzin. The rainfall at Site 3 was probably insufficient to dissolve the herbicide into soil water solution so that it could be taken up by the *Amaranthus* spp. seedlings, resulting in reduced control. Site 6 received only 0.6 mm of rain 0 to 7 DAA; however, this was likely not enough rainfall for weed seed germination; therefore, when more rain occurred after 7 DAA, the herbicides were likely activated and absorbed by the emerging weed seedlings. Differences in levels of *A. retroflexus* control with isoxaflutole across sites and years have been described by Sprague et al. (1999), who reported that isoxaflutole (79 g ai ha^{-1}) controlled *A. retroflexus* 8% at a site with limited rainfall; in contrast, *Amaranthus* spp. control was 88% higher at sites that received an activating rainfall. Isoxaflutole and metribuzin applied alone at the respective rates did not differ; however, numerically, isoxaflutole usually provided better control than metribuzin. An application of isoxaflutole or metribuzin at the medium and high rates rarely increased *Amaranthus* spp. control. Knezevic et al. (1998) reported the ED_{90} of isoxaflutole to reduce *A. retroflexus* biomass was 100 g ha^{-1}; in our study, only Site 7 had >90% reduction in biomass with isoxaflutole at the high rate (105 g ha^{-1}). Results at Site 7 in this study are also consistent with Zhao et al. (2017), who reported that *A. retroflexus* was controlled 92% to 95% with isoxaflutole at 100 g ha^{-1} at 30 DAA when 8.9 and 97.7 mm of rainfall was received 0 to 7 DAA in the 2 yr of their study. Sweat et al. (1998) reported 8% greater *A. retroflexus* control with metribuzin at 420 g ha^{-1} at 28 DAA than the same treatment at Site 7 at 4 WAA in this study. At 8 WAA, mixtures of isoxaflutole + metribuzin resulted in additive interactions at Sites 3 and 5. The combination of isoxaflutole + metribuzin at the low rate usually did not provide any benefit in *Amaranthus* spp. control compared with isoxaflutole or metribuzin applied alone. However, in many cases, the medium and high rates of isoxaflutole + metribuzin provided enhanced *Amaranthus* spp. control compared with isoxaflutole or metribuzin applied alone. Isoxaflutole + metribuzin at the low, medium, and high rates provided >99% control when sufficiently activated by rainfall.

Ambrosia artemisiifolia

Ambrosia artemisiifolia was evaluated at four sites in this study. There was a significant treatment by site interaction (unpublished data); therefore, Sites 2 and 6 were analyzed separately; and Sites 1 and 4 were combined for analysis.

Isoxaflutole and metribuzin controlled *A. artemisiifolia* 77% to 93% and 4% to 47%, respectively, at site 2 (Table 5). An additive interaction occurred with isoxaflutole + metribuzin at all three rates and controlled *A. artemisiifolia* 90% to 100%. At Sites 1 and 4, isoxaflutole and metribuzin at the three rates controlled *A. artemisiifolia* 84% to 98% and 4% to 29%, respectively. The combination of isoxaflutole + metribuzin resulted in additive activity and controlled *A. artemisiifolia* 95% to 100%. Isoxaflutole + metribuzin at the low, medium, and high rates provided 66% to 96% greater control of *A. artemisiifolia* than metribuzin; however, this combination did not provide higher control than any rate of isoxaflutole. At site 6, isoxaflutole controlled *A. artemisiifolia* 97% to 100%. There was increased *A. artemisiifolia* control with increasing rates of metribuzin; the low, medium, and high rates controlled *A. artemisiifolia* 0%, 60%, and 97%, respectively. The combination of isoxaflutole + metribuzin at each rate had an additive interaction and controlled *A. artemisiifolia* 100%.

Ambrosia artemisiifolia density was reduced 95% to 99% and 88% to 98% with all rates of isoxaflutole and isoxaflutole + metribuzin, respectively, at site 2 (Table 5). Metribuzin did not reduce *A. artemisiifolia* density. Similarly, at Sites 1 and 4, all rates of isoxaflutole and isoxaflutole + metribuzin reduced density compared with the untreated control; however, metribuzin was not

Table 6. *Abutilon theophrasti* control, density, and biomass at 8 wk after application from three field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

		Visible control		Density		Biomass	
Treatment	Rate	Sites 2, 3	Site 1	Sites 2, 3	Site 1	Sites 2, 3	Site 1
Observed	g ai ha^{-1}	———%	———	———no. m^{-2}	———	———g m^{-2}	———
Untreated				6.2a	31.7d	4.3a	27.8d
Isoxaflutole	52.5	82a	95a	1.3a	3.9bc	1.3a	3.1bc
Isoxaflutole	79	96a	98a	0.6a	0.9ab	1.1a	0.8ab
Isoxaflutole	105	93a	100a	0.4a	0.04a	0.5a	0.05a
Metribuzin	210	16b	2c	6.2a	26.2d	4.9a	15.1cd
Metribuzin	315	0b	2c	4.3a	18.6d	5.9a	25.0d
Metribuzin	420	15b	32b	4.2a	12.9cd	2.7a	12.6cd
Isoxaflutole + metribuzin	52.5 + 210	98a	97a	0.3a	0.7ab	1.0a	0.3ab
Isoxaflutole + metribuzin	79 + 315	97a	97a	1.2a	0.9ab	2.3a	0.3ab
Isoxaflutole + metribuzin	105 + 420	99a	100a	0.2a	0.2a	0.6a	0.2ab
Expected ϵ[b]							
Isoxaflutole + metribuzin	52.5 + 210	93	96	2.5	3.2	3.3	1.8
Isoxaflutole + metribuzin	79 + 315	96	98	0.6	0.7	2.5	1.1
Isoxaflutole + metribuzin	105 + 420	96*	100	0.4	0.04	0.6	0.1

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value ($P < 0.05$) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where *E* is the expected control, density, or biomass with isoxaflutole + metribuzin; and *X* and *Y* are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

effective. Isoxaflutole and isoxaflutole + metribuzin reduced *A. artemisiifolia* density 96% to 99% and 99%, respectively. At site 6, all herbicide treatments reduced *A. artemisiifolia* density compared with the untreated control. There were no treatment differences among the herbicides; all herbicides reduced density 94% to 99%.

The herbicide treatments evaluated did not decrease *A. artemisiifolia* biomass compared with the untreated control at Site 2 (Table 5). At Sites 1 and 4, all rates of isoxaflutole and isoxaflutole + metribuzin reduced *A. artemisiifolia* biomass 88% to 97% compared with the untreated control. In contrast, metribuzin did not reduce biomass. At Site 6, isoxaflutole at all three rates, metribuzin at the high rate, and isoxaflutole + metribuzin at all three rates reduced biomass 99% compared with the untreated control.

In summary, herbicides at site 2 provided the lowest *A. artemisiifolia* control; greater control was achieved at Sites 1 and 4; and the highest level of control occurred at Site 6. Sites 1, 2, 4, and 6 received 35.2, 58.4, 42.6, and 84.8 mm of rain 0 to 28 DAA, respectively (Table 1). Improved *A. artemisiifolia* control can likely be attributed to higher levels of rainfall, especially considering the theorized "reactivation" or "recharge" activity of isoxaflutole. Isoxaflutole is stable and unavailable for uptake by plants under relatively dry conditions, because it is readily adsorbed to soil colloids. When rainfall moves isoxaflutole into soil water solution, isoxaflutole is converted into diketonitrile, a more phytotoxic metabolite, which is not as highly adsorbed by soil colloids and is more available for uptake by plants. This results in control of susceptible species after each rain event while isoxaflutole remains in the seed germination zone (Taylor-Lovell et al. 2000). Due to this phenomenon, rainfall received up to 28 DAA may be important for the control of late weed flushes and small seedling weeds. *Ambrosia artemisiifolia* was more susceptible to isoxaflutole at the medium and high rates than metribuzin at the low and medium rates at many of the groups of sites and evaluation timings. This finding is not surprising, as Byker et al. (2018) reported the ED_{90} for metribuzin to control GR *A. artemisiifolia* at 4 WAA was 824 g ha^{-1}, which is much higher than any rate used in this study. Greater than 90% control was obtained at Site 6 with metribuzin at 420 g ha^{-1}; the increased rainfall may have contributed to the improved control at this site. *A. artemisiifolia* was effectively controlled with isoxaflutole at the rates used in this study, which is corroborated by Sprague et al. (1999), who reported that isoxaflutole at 79 and 105 g ha^{-1} controlled *A. artemisiifolia* >95%. The interaction for the co-application of isoxaflutole + metribuzin was mostly additive for *A. artemisiifolia* control, although in a few instances the interaction was synergistic. At 8 WAA, isoxaflutole + metribuzin at the high rate controlled *A. artemisiifolia* 100% at each group of sites, preventing any weed seed to return to the soil seedbank. However, if sufficiently activated, the low, medium, or high rate of isoxaflutole + metribuzin can provide 100% control.

Abutilon theophrasti

Abutilon theophrasti was assessed at three sites in this study. There was a significant treatment by site interaction (unpublished data); therefore, Sites 2 and 3 were combined; and Site 1 was analyzed independently.

At the two site groupings, control did not differ between isoxaflutole applied alone and with the addition of metribuzin at any of the rates (Table 6). At Sites 2 and 3, isoxaflutole and isoxaflutole + metribuzin controlled *A. theophrasti* 82% to 99%; at Site 1, *A. theophrasti* control was 95% to 100%. At Sites 2 and 3, metribuzin controlled *A. theophrasti* 0% to 16% and did not provide control equivalent to isoxaflutole or isoxaflutole + metribuzin. At Site 1, metribuzin at the low and medium rates controlled *A. theophrasti* 2%; increasing the rate provided 30% greater control. At Sites 2 and 3, the high rate of isoxaflutole + metribuzin controlled *A. theophrasti* synergistically. All other combinations of isoxaflutole + metribuzin at either of the site groups had additive interactions.

There was no decrease in *A. theophrasti* density with the herbicide treatments evaluated at Sites 2 and 3 (Table 6). Isoxaflutole at the low, medium, and high rates reduced *A. theophrasti* density 88%, 97%, and 99%, respectively, at Site 1. Metribuzin at the three rates did not decrease *A. theophrasti* density compared with the untreated control. Isoxaflutole + metribuzin at the low, medium, and high rates reduced *A. theophrasti* density 97% to 99% compared with the untreated control.

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Table 7. *Setaria* spp. control, density, and biomass at 8 wk after application from eight field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

Treatment	Rate	Visible control			Density			Biomass		
		Sites 2, 3, 5, 8	Sites 1, 4, 10	Site 6	Sites 2, 3, 5, 8	Sites 1, 4, 10	Site 6	Sites 2, 3, 5, 8	Sites 1, 4, 10	Site 6
Observed	g ai ha^{-1}	——————%——————			——————no. m^{-2}——————			——————g m^{-2}——————		
Untreated					101.4b	48.6b	116.7e	51.1d	15.8ab	16.7c
Isoxaflutole	52.5	24cde	58ab	33cd	40.0ab	35.5ab	23.4d	22.0abcd	13.8ab	7.8bc
Isoxaflutole	79	41bcd	75ab	76abc	46.2ab	20.0ab	11.7cd	17.7abcd	13.8ab	1.1ab
Isoxaflutole	105	52abc	79ab	83ab	25.4a	15.4ab	6.6cd	10.6ab	9.1ab	0.8a
Metribuzin	210	5e	36b	25d	44.4ab	49.6b	14.8cd	22.8abcd	23.2b	7.6bc
Metribuzin	315	20de	38b	55bcd	47.1ab	60.1b	3.2bc	42.7cd	29.4b	2.2ab
Metribuzin	420	25cde	50ab	96a	47.6ab	26.8ab	4.5bc	35.3bcd	18.2ab	0.2a
Isoxaflutole + metribuzin	52.5 + 210	53abc	78ab	96a	27.1ab	23.3ab	0.6ab	16.0abcd	21.0ab	0.6a
Isoxaflutole + metribuzin	79 + 315	67ab	91ab	100a	18.8a	8.5ab	0.04a	5.9a	5.5ab	0.06a
Isoxaflutole + metribuzin	105 + 420	76a	96a	100a	23.7a	4.4a	0.04a	13.0abc	3.1a	0.06a
Expected ϵ[b]										
Isoxaflutole + metribuzin	52.5 + 210	35*	78	56*	17.3	40.8	3.0	12.0	32.0	3.3
Isoxaflutole + metribuzin	79 + 315	58	87	91*	19.3	30.1	0.4	18.1*	34.8*	0.2
Isoxaflutole + metribuzin	105 + 420	66	92*	99	14.5	12.3	0.3	9.6	14.3	0.03

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value (P < 0.05) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where *E* is the expected control, density, or biomass with isoxaflutole + metribuzin; and *X* and *Y* are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

There was no decrease in *A. theophrasti* biomass with the evaluated herbicide treatments compared with the untreated control, consistent with density data at Sites 2 and 3 (Table 6). Isoxaflutole at the low, medium, and high rates reduced *A. theophrasti* biomass 89%, 97%, and 99%, respectively, at Site 1. Metribuzin at each rate did not reduce *A. theophrasti* biomass compared with the untreated control. Isoxaflutole + metribuzin at the three rates reduced *A. theophrasti* biomass 99%.

In summary, there was similar *A. theophrasti* control at Sites 2 and 3 compared with Site 1. At 4 WAA (unpublished data), greater differences were seen between the two groups of sites, probably due to the rainfall received at each site from 0 to 7 DAA (Table 1). Site 1 had received 6 and 3 times more rainfall than Sites 2 and 3, respectively; however, by 8 WAA, differences in *A. theophrasti* control between the two groups of sites diminished, which can partially be explained by similar rainfall 0 to 28 DAA of 35.2, 46.4, and 58.4 mm at Sites 1, 2 and 3, respectively, contributing to herbicide activation and *A. theophrasti* control. The results from this study are consistent with Sprague et al. (1999), who reported that isoxaflutole at 79 and 105 g ha^{-1} controlled *A. theophrasti* 15% and 35%, respectively, in a year that received 17 mm of rainfall 0 to 28 DAA, in comparison to 95% to 99% control with isoxaflutole at 79 and 105 g ha^{-1} in years with 53 to 93 mm of rainfall 0 to 28 DAA. *Abutilon theophrasti* is more sensitive to isoxaflutole than metribuzin. Metribuzin across all sites and assessment timings controlled *A. theophrasti* 0% to 32%, while isoxaflutole controlled *A. theophrasti* 28% to 100%. In contrast, Oliveira et al. (2017) reported that metribuzin at 280 g ha^{-1} controlled *A. theophrasti* 97% and 96% at 40 and 60 DAA, respectively, appreciably higher control than in this study. Knezevic et al. (1998) determined the ED_{90} for isoxaflutole to reduce *A. theophrasti* biomass was 90 g ai ha^{-1}. Bhowmik et al. (1999) found the ED_{80} for isoxaflutole to reduce *A. theophrasti* biomass was only 6.1 g ha^{-1}. Results from this study are not in agreement with results from either Knezevic et al. (1998) or Bhowmik et al. (1999). At Sites 2 and 3 there was an 89% reduction in biomass with isoxaflutole at the high rate (105 g ha^{-1}); however, these sites had relatively low pressure of *A. theophrasti* in comparison to Site 1, where there were larger *A. theophrasti* populations and the low, medium, and high rates of isoxaflutole (52.5, 79, and 105 g ha^{-1}) reduced biomass 89%, 97%, and 99%, respectively. The combination of isoxaflutole + metribuzin resulted in mostly additive interactions; however, synergistic increases in *A. theophrasti* control occurred in a few instances. At Sites 2 and 3, isoxaflutole + metribuzin at the high rate consistently had synergistic responses across all evaluation timings. Mixtures of isoxaflutole + metribuzin at each rate typically provided better control than metribuzin alone at the three rates; however, control was equivalent to isoxaflutole at all three rates. Although *A. theophrasti* can be effectively controlled with isoxaflutole alone, it is not recommended, as other weed species are likely present in the field, and the use of two modes of action will help to delay the evolution of HR weed biotypes. *Abutilon theophrasti* was controlled >97% with any rate of isoxaflutole + metribuzin.

Setaria spp

Green foxtail [*Setaria viridis* (L.) P. Beauv.] and giant foxtail (*Setaria faberi* Herrm.) were combined during field evaluations at eight sites in this study. There was a significant treatment by site interaction; therefore, Sites 2, 3, 5, and 8 were combined; Sites 1, 4, and 10 were combined; and Site 6 was analyzed independently.

Isoxaflutole and metribuzin at the three rates controlled *Setaria* spp. 24% to 52% and 5% to 25%, respectively, at Sites 2, 3, 5, and 8 (Table 7). The combination of isoxaflutole + metribuzin at the low rate resulted in a synergistic increase in *Setaria* spp. control, while the medium and high rates had an additive interaction. Isoxaflutole + metribuzin at the low, medium, and high rates controlled *Setaria* spp. 53% to 76%. Isoxaflutole and metribuzin controlled *Setaria* spp. 58% to 79% and 36% to 50%, respectively, at Sites 1, 4, and 10. Combinations of isoxaflutole + metribuzin at the low and medium rates had additive control of *Setaria* spp., and the high rate had a synergistic interaction. Isoxaflutole + metribuzin at the three rates controlled *Setaria* spp. 78% to 96%. At Site 6, isoxaflutole at the low, medium, and high rates controlled *Setaria* spp. 33%, 76%, and 83%, respectively. Metribuzin at the low, medium, and high rates controlled *Setaria* spp. 25%, 55%, and 96%, respectively. The co-application of isoxaflutole + metribuzin at the low and medium

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Table 8. *Echinochloa crus-galli* control, density, and biomass at 8 wk after application from seven field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

Treatment	Rate	Visible control		Density		Biomass	
		Sites 1, 2, 5, 8	Sites 4, 6, 10	Sites 1, 2, 5, 8	Sites 4, 6, 10	Sites 1, 2, 5, 8	Sites 4, 6, 10
Observed	g ai ha^{-1}	%		no. m^{-2}		g m^{-2}	
Untreated				6.1a	5.3a	4.2a	3.0a
Isoxaflutole	52.5	37cd	53ab	4.8a	3.7a	4.0a	5.7a
Isoxaflutole	79	71abc	81ab	2.2a	2.2a	2.1a	2.5a
Isoxaflutole	105	89ab	87ab	1.6a	1.4a	1.4a	2.0a
Metribuzin	210	14d	32b	4.0a	3.4a	4.4a	3.9a
Metribuzin	315	37cd	33b	5.8a	6.9a	5.7a	9.1a
Metribuzin	420	48bcd	62ab	2.3a	2.5a	2.1a	2.9a
Isoxaflutole + metribuzin	52.5 + 210	83abc	83ab	1.2a	3.8a	1.4a	3.6a
Isoxaflutole + metribuzin	79 + 315	89ab	95a	3.5a	1.1a	2.7a	1.8a
Isoxaflutole + metribuzin	105 + 420	95a	98a	1.3a	0.5a	1.1a	0.5a
Expected ϵ[b]							
Isoxaflutole + metribuzin	52.5 + 210	47*	73	3.1	2.0	6.4	6.8
Isoxaflutole + metribuzin	79 + 315	80	90	1.5	4.9	2.6	13.4
Isoxaflutole + metribuzin	105 + 420	93	95	1.1	2.1	1.8	5.3

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value ($P < 0.05$) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, expected value determined by Colby's equation $E = X + Y - (XY/100)$, where E is the expected control, density, or biomass with isoxaflutole + metribuzin; and X and Y are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

rates resulted in a synergistic increase in *Setaria* spp. control; there was an additive interaction at the high rate. The three rates of isoxaflutole + metribuzin controlled *Setaria* spp. 96% to 100%.

Isoxaflutole at the low and medium rates did not reduce *Setaria* spp. density relative to the untreated control; isoxaflutole at the high rate reduced density 75% at Sites 2, 3, 5, and 8 (Table 7). Metribuzin did not reduce *Setaria* spp. density. Isoxaflutole + metribuzin at the low rate did not reduce *Setaria* spp. density; however, the medium and high rates reduced density 77% to 81%. Isoxaflutole + metribuzin at the high rate was the only treatment that reduced *Setaria* spp. density compared with the untreated control; this treatment reduced density 91% at Sites 1, 4, and 10. Isoxaflutole and metribuzin at the low, medium, and high rates reduced foxtail density 80% to 94% and 87% to 97%, respectively, at Site 6. Isoxaflutole + metribuzin at the three rates reduced *Setaria* spp. density 99%, and control was greater than at any rate of isoxaflutole and metribuzin.

Isoxaflutole at the low and medium rates did not reduce *Setaria* spp. biomass; the high rate reduced biomass 79% compared with the untreated control at Sites 2, 3, 5, and 8 (Table 7). Metribuzin at the three rates did not reduce biomass compared with the untreated control. Isoxaflutole + metribuzin at the low rate did not reduce *Setaria* spp. biomass; however, the medium and high rates reduced *Setaria* spp. biomass 75% to 88%. The co-application of isoxaflutole + metribuzin at the low and high rates resulted in an additive reduction in biomass; the medium rate had a synergistic interaction. No herbicide treatment reduced *Setaria* spp. biomass compared with the untreated control at Sites 1, 4, and 10. The combination of isoxaflutole + metribuzin at the medium rate caused a synergistic interaction, whereas the low and high rates had an additive interaction. At Site 6, isoxaflutole at the low rate did not reduce biomass compared with the untreated control. Isoxaflutole at the medium and high rates decreased *Setaria* spp. biomass 93% to 95% compared with the untreated control. Similarly, metribuzin at the low rate did not reduce biomass compared with the untreated control, although the medium and high rates reduced biomass 87% to 98%. Isoxaflutole + metribuzin at the three rates reduced biomass 96% to 99% compared with the untreated control.

In summary, herbicides at Sites 2, 3, 5, and 8 provided the lowest *Setaria* spp. control; control increased at Sites 1, 4, and 10; and *Setaria* spp. control was highest at Site 6. The combined action of isoxaflutole + metribuzin resulted in additive and synergistic interactions. At Site 6, the medium and low rates effectively controlled *Setaria* spp. 100% for the entire season. The rainfall amount 0 to 28 DAA (Table 1) probably contributed to the variable *Setaria* spp. control. Sites 2, 3, 5, and 8 received an average of 39.5 mm of rainfall 0 to 28 DAA; Sites 1, 4; and 10 received an average of 53 mm; and Site 6 received 84.8 mm. Similar results were reported by Sprague et al. (1999), who reported that *S. faberi* was controlled 23% and 48% with isoxaflutole at 79 and 105 g ha^{-1}, respectively, in a year when only 17 mm of rainfall occurred 0 to 28 DAA compared with 77% to 89% and 84% to 87% control with isoxaflutole at 79 and 105 g ha^{-1}, respectively, in years when 53 to 93 mm of rainfall occurred 0 to 28 DAA. Interestingly, Johnson et al. (2012) reported up to 53% reduction in *S. faberi* control when conditions were wet after planting, which may have been due to herbicide dilution from surface runoff. In addition to rainfall, *Setaria* spp. density may have had an impact on *Setaria* spp. control. Sites 1, 4, and 10, which on average had the lowest *Setaria* spp. density, had higher levels of control with isoxaflutole at the low rate compared with the other groups of sites with the same treatment. *Setaria* spp. populations of more than 100 plants m^{-2} may have been too dense for isoxaflutole at the low rate to control effectively. When sufficiently activated by rainfall, isoxaflutole + metribuzin at the medium and high rates can control *Setaria* spp. >91%.

Echinochloa crus-galli

Echinochloa crus-galli was evaluated at seven sites in this study. There was a significant treatment by site interaction (unpublished data); therefore Sites 1, 2, 5, and 8 were combined; and Sites 4, 6, and 10 were combined for analysis.

Isoxaflutole at the low, medium, and high rates controlled *E. crus-galli* 37%, 71%, and 89%, respectively, at Sites 1, 2, 5, and 8 (Table 8). Metribuzin at the three rates controlled *E. crus-galli* 14% to 48%. The co-application of isoxaflutole + metribuzin at the low rate resulted in a synergistic increase in *E. crus-galli* control; the medium and high rates had an additive interaction. Isoxaflutole + metribuzin at the three rates controlled *E. crus-galli* 83% to 95%.

Table 9. *Panicum capillare* control, density, and biomass at 8 wk after application from four field experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

Treatment	Rate	Visible control		Density		Biomass	
		Site 8	Sites 7, 9, 10	Site 8	Sites 7, 9, 10	Site 8	Sites 7, 9, 10
Observed	g ai ha^{-1}	——%——		——no. m^{-2}——		——g m^{-2}——	
Untreated				6.8a	7.9b	1.6a	6.6b
Isoxaflutole	52.5	61ab	76c	4.6a	3.8ab	1.4a	1.3ab
Isoxaflutole	79	89ab	88abc	5.6a	2.1ab	1.7a	1.0ab
Isoxaflutole	105	91ab	98abc	8.3a	1.4ab	2.1a	0.5ab
Metribuzin	210	47b	81bc	6.4a	3.7ab	3.6a	2.0ab
Metribuzin	315	82ab	99ab	12.8a	1.1ab	5.1a	0.4a
Metribuzin	420	88ab	99ab	4.1a	0.3a	1.5a	0.1a
Isoxaflutole + metribuzin	52.5 + 210	98ab	98abc	3.6a	0.4a	1.0a	0.5ab
Isoxaflutole + metribuzin	79 + 315	99a	100a	1.3a	0.1a	2.3a	0.06a
Isoxaflutole + metribuzin	105 + 420	99a	100a	0.3a	0.06a	0.2a	0.06a
Expected ϵ^b							
Isoxaflutole + metribuzin	52.5 + 210	78	96	2.1	2.0*	1.3	0.8
Isoxaflutole + metribuzin	79 + 315	97	100	9.3	0.4	7.4	0.2
Isoxaflutole + metribuzin	105 + 420	99	100	7.0	0.03	5.0	0.02

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05. An asterisk (*) indicates expected values significantly lower than observed value (P < 0.05) as determined by a *t*-test, indicating synergistic interactions of isoxaflutole + metribuzin.
[b] ϵ, expected value determined by Colby's equation: $E = X + Y - (XY100)$, where E is the expected control, density, or biomass with isoxaflutole + metribuzin; and X and Y are the observed percent control, density, or biomass of isoxaflutole and metribuzin, respectively.

At Sites 4, 6, and 10, isoxaflutole and metribuzin at the three rates controlled *E. crus-galli* 53% to 87% and 32% to 62%, respectively. The co-application of isoxaflutole + metribuzin at the three rates had additive interactions and controlled *E. crus-galli* 83% to 98%.

In summary, Sites 1, 2, 5, and 8 had lower *E. crus-galli* control than Sites 4, 6, and 10. On average Sites 1, 2, 5, and 8 received 42.5 mm of rainfall 0 to 28 DAA, whereas Sites 4, 6, and 10 received 61.8 mm on average. The higher level of control at Sites 4, 6, and 10 was probably a result of more rainfall. Metribuzin alone at the three rates suppressed *E. crus-galli*, providing up to 71% control across all rates and evaluation timings. Oliveira et al. (2017) reported metribuzin (280 g ha^{-1}) controlled *E. crus-galli* 73%, which is much higher than the maximum control of 39% with metribuzin at 315 g ha^{-1} in this study. Isoxaflutole at the low, medium, and high rates controlled *E. crus-galli* up to 70%, 83%, and 89%, respectively. In contrast, isoxaflutole at 72 g ha^{-1} controlled 99% of *E. crus-galli* (Bhowmik et al. 1999), which is higher than the control obtained in this study. Different results were also reported by Meyer et al. (2016), who noted that isoxaflutole (100 g ha^{-1}) controlled *E. crus-galli* 99% at 4 WAA; however, at 7 WAA, *E. crus-galli* control had decreased to 75% with the same treatment. The same study also found a 9% reduction in control over the same time period with application of isoxaflutole + metribuzin (100 + 414 g ai ha^{-1}). There was no appreciable decrease in *E. crus-galli* control over time in this study. Additive and synergistic interactions occurred with the application of isoxaflutole + metribuzin. The mixture at the high rate provided the best control and had improved control compared with metribuzin at the low and medium rates. In contrast, isoxaflutole and metribuzin applied alone at the high rates generally provided control equivalent to that of the mixtures. *Echinochloa crus-galli* can be controlled >95% with isoxaflutole + metribuzin at the medium and high rates when sufficiently activated by rainfall.

Panicum capillare

Panicum capillare was assessed at four sites in this study. A significant treatment by site interaction occurred (unpublished data); therefore, Site 8 was analyzed independently; and Sites 7, 9, and 10 were combined.

Isoxaflutole and metribuzin at the various rates controlled *P. capillare* 61% to 91% and 47% to 88%, respectively, at Site 8 (Table 9). The mixtures of isoxaflutole + metribuzin resulted in additive control of *P. capillare* at each rate and provided 98% to 99% control. At Sites 7, 9, and 10, isoxaflutole and metribuzin at the three rates controlled *P. capillare* 76% to 98% and 81% to 99%, respectively. The mixtures of isoxaflutole + metribuzin at the three rates resulted in an additive interaction and controlled *P. capillare* 98% to 100%.

Herbicide treatments did not reduce *P. capillare* density relative to the untreated control at Site 8 (Table 9). At Sites 7, 9, and 10, the three rates of isoxaflutole did not reduce *P. capillare* density compared with the untreated control. Metribuzin at the low and medium rates did not reduce *P. capillare* density; however, the high rate reduced *P. capillare* density 96%. The combination of isoxaflutole + metribuzin at the low rate had a synergistic reduction in *P. capillare* density, whereas the medium and high rates had an additive response. Isoxaflutole + metribuzin at the low, medium, and high rates reduced *P. capillare* density 94% to 99% compared with the untreated control.

There were no differences in *P. capillare* biomass among the treatments at Site 8 (Table 9). Isoxaflutole at the low, medium, and high rates did not reduce *P. capillare* biomass compared with the untreated control at Sites 7, 9, and 10. Metribuzin at the low rate did not affect *P. capillare* biomass; however, the medium and high rates reduced *P. capillare* biomass 94% to 98%. Similarly, isoxaflutole + metribuzin at the low rate did not reduce *P. capillare* biomass; isoxaflutole + metribuzin at the medium and high rates reduced *P. capillare* biomass 99%.

In summary, at Site 8, isoxaflutole and metribuzin controlled *P. capillare* less than at Sites 7, 9, and 10. Site 8 received 48.2 mm of rainfall 0 to 28 DAA, which was lower than the rainfall at Sites 7, 9, and 10, which received 114, 70.5, and 58 mm of rain, respectively. The lower rainfall at site 7 probably limited the control of *P. capillare* by isoxaflutole and metribuzin. At Site 8, there was a large increase in control from 4 WAA to 8 WAA; this was probably due to reactivation of isoxaflutole during the 21 to 28 DAA time frame, as injury symptoms would not have shown up at the 4 WAA evaluations. Additionally, the increase in control

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Table 10. Soybean yield from 10 field experiments in Ontario, Canada, conducted in 2017 and 2018.[a]

Treatment	Rate	Site 10	Site 9	Sites 1, 3, 6	Sites 2, 4, 5, 7	Site 8
	g ai ha^{-1}	1000 kg ha^{-1}				
Untreated control	—	0.9d	2.3b	3.0c	3.4d	4.4d
Weed-free control	—	3.8a	3.4a	4.2a	5.1a	6.5a
Isoxaflutole	52.5	1.5cd	2.8ab	3.6abc	4.2bc	4.9bcd
Isoxaflutole	79	1.7c	2.7b	3.7ab	4.4abc	5.5bc
Isoxaflutole	105	2.2bc	2.8ab	3.7ab	4.4ab	5.3bc
Metribuzin	210	1.6c	2.7b	3.2bc	3.7cd	4.7cd
Metribuzin	315	1.6c	2.4b	3.5bc	3.9bcd	4.8cd
Metribuzin	420	1.6c	2.6b	3.6abc	4.1bcd	5.2bc
Isoxaflutole + metribuzin	52.5 + 210	1.7c	2.8b	3.8ab	4.3bc	5.2bc
Isoxaflutole + metribuzin	79 + 315	2.9ab	2.9ab	3.8ab	4.5ab	5.6b
Isoxaflutole + metribuzin	105 + 420	3.2a	2.7b	3.8ab	4.5ab	5.3bc

[a] Means followed by the same letter within a column are not significantly different according to the Tukey-Kramer multiple range test at P = 0.05.

was probably due to competition with other weed species and soybean, as control with metribuzin also increased during this time period. At Sites 7, 9, and 10, *P. capillare* control ranged from 62% to 98% across the three rates and assessment timings. A study by DeCauwer et al. (2014) determined the ED_{90} of isoxaflutole applied POST was 231.2 ± 84.68 g ha^{-1}. In this study, >90% control was achieved with isoxaflutole at 105 g ha^{-1}, suggesting the ED_{90} is lower than reported in DeCauwer et al. (2014). The combination of isoxaflutole + metribuzin resulted in mostly additive interactions, with the exception of a synergistic response at the 4 WAA evaluation timing at both groups of sites. *Panicum capillare* can be controlled >98% with isoxaflutole + metribuzin at any rate.

Soybean Yield

There was a significant site by treatment interaction (data not presented) for yield; therefore, yield at Sites 9 and 10 were analyzed independently; Sites 1, 3, and 6 were combined; Sites 2, 4, 5, and 7 were combined; and Site 8 was analyzed independently.

Weed interference reduced soybean yield 76% at Site 10. Weed interference with isoxaflutole at the low, medium, and high rates reduced soybean yield 60%, 55%, and 42%, respectively; the low rate results did not differ from those of the untreated control (Table 10). At Site 9, soybean yield in the weed-free control was 1100 kg ha^{-1} higher than in the untreated control. Treatments did not differ from untreated control, with soybeans yields reduced 18% to 24%. At Sites 1, 3, and 6, weed interference reduced soybean yield 29%. Reduced weed interference with isoxaflutole and isoxaflutole + metribuzin at all three rates resulted in soybean yield that was similar to that of the weed-free control. At Sites 2, 4, 5, and 7, weed interference reduced soybean yield 33%. Weed interference with metribuzin at the low, medium, and high rates reduced soybean yield 27%, 23%, and 19%, respectively. Weed interference with isoxaflutole + metribuzin and isoxaflutole at the medium and high rates had reduced weed interference, which resulted in soybean yield that was similar to that of the weed-free control. At Site 8, weed interference reduced soybean yield 32%. Weed interference with isoxaflutole at the low, medium, and high rates reduced soybean yield 25%, 15%, and 18%, respectively. Weed interference with metribuzin at the low, medium, and high rates reduced soybean yield 28%, 26%, and 20%, respectively. Weed interference with isoxaflutole + metribuzin at the low, medium, and high rates reduced soybean yield 20%, 14%, and 18%, respectively. No treatment provided control similar to that of the weed-free control.

In conclusion, isoxaflutole applied PRE at the low, medium, and high rates provided >85% control of *C. album*, *Amaranthus* spp., *A. artemisiifolia*, and *A. theophrasti* and >70% control of *E. crus-galli* and *P. capillare* at 12 WAA at sites where sufficient rainfall for activation occurred after application. Isoxaflutole at the low rate controlled *Setaria* spp. <66%; however, the medium and high rates provided >72% control at 12 WAA when sufficient rainfall for activation occurred. Metribuzin at the low, medium, and high rates had the potential to control *Amaranthus* spp. and *P. capillare* >81% at 12 WAA at sites that received an activating rainfall. The medium and high rates were required to control *C. album* and *Setaria* spp. >75% at 12 WAA at sites where an activating rainfall had occurred after application. Metribuzin at the high rate was required for >71% control of *A. artemisiifolia* and *E. crus-galli* at 12 WAA at sites where an activating rainfall had occurred after application. Metribuzin at the low, medium, and high rates did not control *A. theophrasti*. When adequate rainfall was received after application, isoxaflutole + metribuzin co-applied at the low, medium, and high rates provided >91% control of *C. album*, *Amaranthus* spp., *A. artemisiifolia*, *A. theophrasti*, *Setaria* spp., and *P. capillare* at 12 WAA. Isoxaflutole + metribuzin at the low rate controlled *E. crus-galli* up to 81%; however, the medium and high rates provided up to 96% and 99% control, respectively, at 12 WAA when the herbicides had been activated by rainfall after application. Control of every species was reduced at sites where there was a lack of adequate rainfall for herbicide activation, which included Sites 1, 2, 3, 5, and 8.

The mixture of isoxaflutole + metribuzin generally provided additive control of each weed species evaluated. A synergistic interaction for weed control occurred several times in this study, when the observed control was greater than the expected. Synergism can increase the spectrum of weeds controlled and improve the level of control. In this study, a synergistic increase in weed control was observed when isoxaflutole + metribuzin provided an increase in grass control, especially *Setaria* spp. For example, at Site 6, isoxaflutole and metribuzin at the low rate controlled *Setaria* spp. 8% and 0%, respectively, at 4 WAA; however, the combination of the two herbicides provided 93% control. Herbicide synergism benefits crop producers by increasing the spectrum of weeds controlled, increasing the level of weed control, decreasing weed interference, and increasing crop yield and net returns. In addition, the co-application of synergistic herbicides may be a cost-saving measure, as fewer herbicides passes in the field are required when herbicides are mixed, thus reducing time, labor, and equipment costs. Caution should be used when applying low herbicide rates,

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as sublethal doses have been shown to increase the evolution of HR weeds (Powles and Busi 2009). Alternatively, application of synergistic herbicides with multiple effective modes of action can be used as a tool to help prevent the selection of resistant biotypes.

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