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Effect of hybrid varieties, application timing, and herbicide rate on field corn tolerance to tolpyralate plus atrazine

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Abstract

A wide margin of crop safety is a desirable trait of POST herbicides, and investigation of crop tolerance is a key step in evaluation of new herbicides. Six field experiments were conducted in Ontario, Canada, from 2017 to 2018 to examine the influence of corn (*Zea mays* L.) hybrid (DKC42-60RIB, DKC43-47RIB, P0094AM, and P9840AM), application rate (1X and 2X), and application timing (PRE, V1, V3, and V5) on the tolerance of field corn to tolpyralate, a new 4-hydroxyphenyl pyruvate dioxygenase inhibitor, co-applied with atrazine. Two corn hybrids (DKC42-60RIB and DKC43-47RIB) exhibited slightly greater visible injury from tolpyralate + atrazine, applied POST, than P0094AM and P9840AM at 1 to 2 wk after application (WAA); hybrids responded similarly with respect to height, grain moisture, and yield. Applications of tolpyralate + atrazine at a 2X rate (80 + 2,000 g ai ha^{-1}) induced greater injury (≤31.6%) than the field rate (40 + 1,000 g ha^{-1}) (≤11.6%); the 2X rate applied at V1 or V3 decreased corn height and slightly increased grain moisture at harvest. On average, field rates resulted in marginally higher grain yields than 2X rates. Based on mixed-model multiple stepwise regression analysis, the air temperature at application, time of day, temperature range in the 24 h before application, and precipitation following application were useful predictor variables in estimating crop injury with tolpyralate + atrazine; however, additional environmental variables also affected crop injury. These results demonstrate the margin of corn tolerance with tolpyralate + atrazine, which provides a basis for optimization of application timing, rate, and corn hybrid selection to mitigate the risk of crop injury with this herbicide tank mixture.

Introduction

Selective herbicides with a wide margin of crop safety have a fundamental role in current corn (*Zea mays* L.) weed management programs. Since their initial release in the 1990s, several selective herbicides that inhibit the hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, including triketones, isoxazoles, and pyrazolones, have been commercially applied PRE and POST in corn (Edmunds and Morris 2012; Mitchell et al. 2001). Inhibition of HPPD impedes production of homogentisic acid, an essential intermediate involved in carotenoid and tocopherol biosynthesis, leaving chlorophyll and the photosynthetic complex susceptible to photo-oxidation and causing extensive bleaching of developing plant foliage (Grossmann and Ehrhardt 2007; Hawkes 2012; Kakidani and Hirai 2003). To improve efficacy, broaden weed control spectrums, and reduce selection pressure on a single herbicide mechanism of action (MOA), HPPD inhibitors are commonly applied to corn in tank mixtures with herbicides exhibiting a different MOA, including photosystem II (PSII) inhibitors (Abendroth et al. 2006; Vollmer et al. 2017). Through exclusion of plastoquinone from the D1 binding niche, PSII inhibitors such as atrazine disrupt the passage of electrons through the photosynthetic electron transport chain, causing lipid peroxidation; this MOA is considered complementary to HPPD inhibitors (Abendroth et al. 2006; Hess 2000).

Crop selectivity is the primary factor impacting the usability of all POST herbicides for in-crop application. Previous research has confirmed varying levels of sensitivity to POST acetolactate synthase–inhibiting herbicides among various field corn hybrids (Bunting et al. 2004). Similarly, several instances of hybrid-specific sensitivities have been reported with HPPD inhibitors, including mesotrione and topramezone applied POST to sweet corn (Bollman et al. 2008; O'Sullivan et al. 2002), due to mutations of cytochrome P450 alleles in certain hybrids (Williams and Pataky 2010). In contrast, field corn typically exhibits excellent tolerance to mesotrione (Mitchell et al. 2001). Johnson et al. (2002) reported <15% injury at 1 wk after application (WAA) with mesotrione or mesotrione + atrazine applied POST, and injury declined to <8%

by 4 WAA. This tolerance has been attributed to reduced uptake and more rapid metabolism of mesotrione in field corn relative to susceptible plant species (Mitchell et al. 2001), although differential sensitivity of the target HPPD enzyme across species has also been reported (Hawkes 2012). Similarly, field corn has been observed to exhibit excellent tolerance to topramezone (Grossmann and Ehrhardt 2007; Rahman et al. 2013). Topramezone selectivity has been attributed to differential sensitivity of the target enzyme in corn compared with susceptible species (Grossmann and Ehrhardt 2007). Other HPPD inhibitors, however, including isoxaflutole, have required the addition of crop safeners (compounds that enhance crop metabolism of the herbicide) to achieve acceptable crop tolerance, particularly at POST application timings (Ahrens et al. 2013; Sprague et al. 1999).

Tolpyralate is the latest pyrazolone HPPD-inhibiting herbicide commercialized for use in all types of corn. Labeled for application POST, at 30 to 40 g ha^{-1}, tolpyralate is recommended to be applied in a tank mixture with atrazine at ≥560 g ha^{-1} (Anonymous 2017). Based on company trials, tolpyralate is reported to exhibit a wide margin of crop safety (Tonks et al. 2015). Previous work in field corn observed <10% crop injury with tolpyralate + atrazine applied at rates up to 120 + 4,000 g ai ha^{-1}, representing 3X the maximum labeled rate of tolpyralate (Metzger et al. 2018a). However, no studies have specifically examined field corn tolerance to tolpyralate. Furthermore, a limited number of hybrids were used in previous experiments, and the specific effects and interactions of tolpyralate + atrazine rate, application timing, and individual corn hybrid with respect to crop safety have not been reported in the literature. Previous experiments with other corn herbicides have linked application timing, application rate, and corn hybrid to crop tolerance (Ahrens et al. 2013; Bunting et al. 2004; Johnson et al. 2002), warranting investigation into the effects of these factors with tolpyralate as well. Therefore, the goal of this study was to examine corn tolerance to tolpyralate + atrazine by examining the effects and interactions of herbicide rate, herbicide application timing, and corn hybrid selection.

Materials and Methods

Experimental Methods

Three field experiments were conducted in 2017 and in 2018 at one location near Ridgetown (42.458N, 81.882W) and one location near Exeter (43.316N, 81.512W), ON, Canada, for a total of 6 site-year combinations. Experiments were spatially or temporally separated to capture a broader range of random environmental conditions. Trial sites were moldboard plowed each fall and cultivated twice each spring following application of fertilizer as required according to soil test results. Experimental sites were maintained weed-free for the duration of the study by applying *S*-metolachlor/atrazine (2,880 g ai ha^{-1}) PRE followed by glyphosate (900 g ae ha^{-1}) POST and hand hoeing as required. In 2017, dimethenamid-*P*/saflufenacil (735 g ai ha^{-1}) was applied to the experiment near Exeter instead of *S*-metolachlor/atrazine. No crop injury was observed as a result of any of the PRE broadcast treatments.

Four corn hybrids were planted 4- to 5-cm deep in plots that were 1.5-m wide (two corn rows 0.76 m apart and 8- or 10-m long), organized in a split-split-block design, with corn hybrid designated as the main plot and tolpyralate + atrazine rate and application timing designated as subplots. Each combination of subplots was randomized within the main plots across four replications (blocks) at each site. Due to equipment and spatial limitations, corn hybrids were seeded in a continuous pattern across the four blocks at each trial site and were not randomized. The specific seeding arrangement of corn hybrids was selected arbitrarily during trial initiation and held constant across all 6 site-years. Corn hybrids were selected to represent current hybrids in Ontario at the time of trial initiation: ‘Pioneer P0094AM’ and ‘Pioneer P9840AM’ (Pioneer Hi-Bred International, Johnston, IA) and ‘Dekalb DKC42-60RIB’ and ‘Dekalb DKC43-47RIB’ (Monsanto, St Louis, MO). Herbicide treatments consisted of tolpyralate + atrazine applied at a label rate of 40 + 1,000 g ha^{-1}, and a 2X label rate of tolpyralate + atrazine at 80 + 2,000 g ha^{-1}, respectively. Methylated seed oil (MSO; Concentrate®, Loveland Products, Loveland, CO) at 0.50% v/v and urea ammonium nitrate (UAN; 2.50% v/v) were included with each treatment in accordance with tolpyralate label recommendations (Anonymous 2017). Plots that received the 2X herbicide rate were sprayed twice in immediate succession with a 1X rate, so as to simulate a spray overlap scenario. Each herbicide treatment was applied PRE and at the V1, V3, and V5 corn stages as determined with the leaf collar method. Nontreated control (NTC) plots for each hybrid were included within each block. Treatments were applied using a CO_2-powered backpack sprayer calibrated to deliver 187 L ha^{-1} application volume at 255-kPa spray pressure. At Ridgetown, a 1-m-wide spray boom equipped with three ULD12002 nozzles (Pentair, New Brighton, MN) spaced 50 cm apart was used. At Exeter, a 2.5-m boom fit with identical nozzles at 50-cm spacing was used. Soil characteristics, application information, and planting dates are presented in Table 1.

Crop injury was assessed visually at 1 and 2 wk after each application and 4 and 8 wk after the V5 applications. At each of these timings, total plot phytotoxicity was evaluated on a percent scale, with 0 indicating no visible injury and 100 indicating complete death of the treated plants. Plant height was assessed at 2 wk after the V5 applications by measuring and recording the height of 10 randomly selected corn plants per plot and calculating an average height for each treatment plot. To minimize confounding effects introduced through uncontrolled environmental variables across sites, plant height was subsequently expressed as a percent, relative to the average height of the corresponding hybrid in the NTC plot within blocks. Potential corn stand loss was determined at 2 wk after the V5 application by indiscriminately placing a 2-m measuring stick between the two corn rows, counting and recording the number of corn plants on either side of the stick, and calculating the average number of plants per meter of row for each plot. At maturity, the complete plot was harvested using a small-plot combine that recorded grain weight and moisture. For data analysis, grain yields were corrected to 15.5% moisture. As with height, grain moisture and corrected grain yields were expressed as percent relative to the NTC by dividing moisture and yield of each treatment plot by moisture and yield of the corresponding hybrid in NTC plots within blocks.

Statistical Analysis

Variance Analysis

A mixed-model variance analysis was performed on each response parameter using PROC GLIMMIX in SAS software v. 9.4 (SAS Institute, Cary, NC). The main plot factor (corn hybrid), subplot factors (herbicide rate, application timing), and all two- and three-way factorial interactions were designated as fixed effects in the model. Significance of these fixed effects and their interactions was determined using an *F*-test. Environment (comprising year and location), interaction of environment with each fixed effect,

Table 1. Planting dates, harvest dates, soil characteristics, and application information for experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[a]

				Soil characteristics[b]						Application information				
Year	Trial[c]	Planting date	Harvest date	Sand	Silt	Clay	OM[a]	CEC	pH	Timing	Date	TOD	Temp	RH
				%				meq 100 g^{-1}				hours	C	%
2017	E1	May 12	October 17	52	29	19	4.6	12.6	6.0	PRE	May 15	1030	17.4	53
										V1	May 30	0615	16.8	80
										V3	June 7	1715	20.2	47
										V5	June 13	2000	23.5	67
	E2	May 19	October 18	56	28	17	4.0	11.0	6.2	PRE	May 22	0715	13.3	76
										V1	June 5	0630	14.0	96
										V3	June 12	0715	25.1	70
										V5	June 17	0730	24.2	79
	E3	May 18	October 19	41	40	19	3.6	28	7.9	PRE	May 23	0930	14.0	72
										V1	June 5	1530	17.0	76
										V3	June 13	1100	28.8	47
										V5	June 19	0845	24.9	62
2018	E4	May 8	October 29	51	32	17	4.7	13.9	6.4	PRE	May 9	0700	13.5	67
										V1	May 25	0730	25.4	57
										V3	June 2	0830	16.7	69
										V5	June 8	0700	18.3	80
	E5	May 24	November 8	57	31	12	4.2	12.4	7.1	PRE	May 25	0750	25.3	57
										V1	June 1	0900	21.5	49
										V3	June 8	0800	21.6	66
										V5	June 18	0715	26.7	84
	E6	May 11	October 20	39	35	26	3.4	28.3	8.0	PRE	May 16	1450	27.9	29
										V1	May 26	0800	22.4	62
										V3	June 1	0900	23.5	74
										V5	June 12	0950	23.5	65

[a] Abbreviations: OM, organic matter; RH, relative humidity; Temp, temperature; TOD, time of day.

[b] Represents average within-trial sites ($n = 10$ to 16 soil cores per trial; 15-cm depth). Soil characteristics were measured within replications in 2018 for multiple regression analysis.

[c] E1, E2, E4, and E5 denote experiments located at Ridgetown; E3 and E6 denote experiments located near Exeter.

block (nested within environment), and the block by hybrid interaction were designated as random effects; their significance was determined using a restricted log-likelihood test. Significance was set to $\alpha = 0.05$ for all statistical analysis. Residuals were examined to confirm assumptions that they were homogeneous, had mean equal to zero, and were normally distributed, using scatter plots of studentized residual values paired with a Shapiro-Wilk test. A distribution and link function were subsequently selected for each assessment parameter that best met these assumptions. Crop injury at 1, 2, and 4 WAA, as well as relative plant height, relative grain moisture, and relative grain yield were each determined to be normally distributed, while injury at 8 WAA required lognormal transformation before analysis. In this case, data were back-transformed for presentation using the omega procedure (M Edwards, Ontario Agricultural College Statistician, University of Guelph, personal communication). Least-square means of main plot and subplot effects as well as their interactions were computed and separated using the Tukey-Kramer test. Significant differences in main effect or interaction least-square means were illustrated in tables using letter codes, assigned using the *pdmix800* macro (Bowley 2015) and *slicediff* commands. When two and three-way factorial interactions were insignificant, only the main effect least-square means are presented for a given parameter, averaged across all levels of the other two factors (Table 2).

Multiple Stepwise Regression

A secondary analysis was conducted on visible injury parameters at 1, 2, 4, and 8 WAA to identify contributing factors that may have accentuated crop injury at these timings. A mixed-model multiple stepwise regression analysis was performed with 12 distinct candidate predictor variables using PROC GLIMMIX (SAS Institute). Environment (comprising location and year) was considered a random effect in the model; its statistical significance was tested using the log-likelihood ratio test ($\alpha = 0.05$). Candidate predictor variables were selected from 20 initial variables that were unique to particular trials (environments) or applications within trials; 8 of the variables were immediately eliminated from the initial list because of probable collinearity. Candidate predictor variables were further thinned based on scatter plots (generated using PROC SGPLOT) of each variable plotted against each response parameter, which were visually inspected for potential correlation. Subsequently, a mixed model was optimized (based on the corrected Akaike information criterion fit statistic) for visible injury at 1, 2, 4, and 8 WAA, averaged across hybrids, rates, and POST application timings through repeated, stepwise elimination of insignificant ($P > 0.05$ using the *F*-test) predictor variables from the analysis. Data from PRE applications were excluded from regression analysis, as no injury was observed with PRE applications (Tables 2–15). A variance inflation (VIF) analysis was conducted on all candidate predictor variables for each assessment parameter using PROC REG; variables were eliminated with evidence of collinearity (VIF values ≥ 4). Residual assumptions were confirmed using PROC UNIVARIATE, consistent with the procedure outlined for the initial variance analysis. Results of the mixed-model multiple regression analyses are outlined in Tables 16–20 for each visible injury assessment parameter.

Results and Discussion

Factorial Analysis of Fixed Effects

Crop injury varied with herbicide rate, application timing, and corn hybrid. There was no evidence of injury with PRE applications; however, injury (>30%) was observed with certain POST

Table 2. Least-square means and P-values for main effects and interactions of hybrid, tolpyralate + atrazine rate, and application timing on visible corn injury at 1, 2, 4 and 8 WAE/WAA,[a] and on corn height, grain moisture, and yield, relative to nontreated control plots within replications, from field experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[b]

	Assessment parameter						
	Visible injury[c]						
Main effects	1 WAA	2 WAA	4 WAA	8 WAA	Relative height[d]	Relative grain moisture	Relative yield
Corn hybrid	%						
P0094AM	10.5	5.5 a	1.2	0.0	100	99	101
P9840AM	10.8	5.7 a	1.2	0.0	100	101	103
DKC42-60RIB	11.4	6.4 b	1.3	0.0	99	100	100
DKC43-47RIB	11.8	6.7 b	1.5	0.0	98	101	99
Hybrid P-value	0.0245	0.0001	0.2656	0.4838	0.4458	0.1230	0.4753
Rate g ai ha^{-1}							
40 + 1,000	5.3	2.6	0.3	0.0 a	100	100	102 a
80 + 2,000	17.0	9.5	2.3	0.1 b	98	100	100 b
Rate P-value	0.0004	0.0034	0.0360	0.0403	0.0577	0.8800	0.0258
Timing							
PRE	0.0	0.0	0.0	0.1	101	100	100
V1	21.6	10.7	0.7	0.1	97	101	101
V3	19.6	11.2	2.6	0.1	99	101	100
V5	3.3	2.4	1.9	0.1	100	100	102
Timing P-value	<0.0001	0.0004	0.0248	0.1038	0.0124	0.4285	0.5863
Two-way interactions							
Hybrid*timing	0.0346	0.1821	0.5255	0.4353	0.8561	0.8359	0.8927
Rate*hybrid	0.8699	0.3622	0.8729	0.9313	0.8319	0.8483	0.1366
Rate*timing	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	0.0167	0.4456
Three-way interaction							
Rate*hybrid*timing	0.9989	0.9930	0.7436	0.5098	0.9516	0.9975	0.1972

[a] Abbreviations: V1, 3, 5, corn vegetative growth stages 1, 3 and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.

[b] Means within main effect columns followed by different letters denote significant differences according to Tukey-Kramer multiple range test (α = 0.05).

[c] Visual assessments were conducted 1 and 2 WAE for PRE applications; 4 and 8 WAA assessments were conducted 4 and 8 wk after V5 applications, respectively.

[d] Plant height, grain moisture, and yield were each expressed as a percent, relative to the corresponding nontreated control plots within replications.

Table 3. Influence of corn hybrid on visible injury at 1 WAE/WAA[a] of tolpyralate + atrazine at four application timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing			
Hybrid	PRE	V1	V3	V5
	Visible injury at 1 WAA			
	%			
P0094AM	0.0 a	20.6 a	18.5 a	2.9 a
P9840AM	0.0 a	21.5 a	18.5 a	3.1 a
DKC42-60RIB	0.0 a	22.5 a	19.8 ab	3.4 a
DKC43-47RIB	0.0 a	21.9 a	21.6 b	3.8 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.

[b] Means followed by the same letter within columns (a, b) for a given application timing are not significantly different according to Tukey's multiple means comparison test (α = 0.05).

Table 4. Influence of application timing on visible injury to four corn hybrids at 1 WAE/WAA[a] of tolpyralate + atrazine in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Hybrid			
Application timing	P0094AM	P9840AM	DKC42-60RIB	DKC43-47RIB
	Visible injury at 1 WAA			
	%			
PRE	0.0 a	0.0 a	0.0 a	0.0 a
V1	20.6 b	21.5 b	22.5 b	21.9 b
V3	18.5 b	18.5 b	19.8 b	21.6 b
V5	2.9 a	3.1 a	3.4 a	3.8 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.

[b] Means followed by the same letter within columns (a, b) for a given hybrid are not significantly different according to Tukey's multiple means comparison test (α = 0.05).

timings, particularly with 2X rates (Tables 5–15), producing a rate by application timing interaction for visible injury at 1, 2, 4, and 8 WAA ($P < 0.0001$), relative plant height ($P = 0.0009$), and relative grain moisture ($P = 0.0167$) (Table 2). Crop injury symptoms varied across application timings, but generally manifested as white bleaching or yellow chlorosis in the youngest corn leaves, which progressed to leaf necrosis in severe cases. Similar symptomology in certain sweet corn hybrids was reported by O'Sullivan et al. (2002) and Bollman et al. (2008), following application of mesotrione alone or tank mixed with atrazine. Injury was most severe in our study at 1 WAA and gradually diminished by 8 WAA as injured plants developed new, unaffected leaves and shed necrotic leaf tissue. Corn stand loss was not observed with any treatment (data not presented). Because visible injury was highest at 1 to 2

Table 5. Influence of herbicide rate on visible injury at 1 WAE/WAA[a] of tolpyralate + atrazine at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing			
Rate	PRE	V1	V3	V5
	Visible injury 1 WAA[c]			
g ai ha^{-1}	%			
40 + 1,000	0.0 a	11.6 a	8.6 a	0.9 a
80 + 2,000	0.0 a	31.6 b	30.6 b	5.7 b

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted at 1 WAE for PRE applications.

Table 6. Influence of application timing on visible injury at 1 WAE/WAA[a] of tolpyralate + atrazine at two rates in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Rate	
Application timing	40 + 1,000 (g ai ha^{-1})	80 + 2,000 (g ai ha^{-1})
	Visible injury 1 WAA[c]	
	%	
PRE	0.0 a	0.0 a
V1	11.6 b	31.6 b
V3	8.6 b	30.6 b
V5	0.9 a	5.7 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted at 1 WAE for PRE applications.

Table 7. Influence of herbicide rate on visible injury at 2 WAE/WAA[a] of tolpyralate + atrazine at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing			
Rate	PRE	V1	V3	V5
	Visible injury at 2 WAA[c]			
g ai ha^{-1}	%			
40 + 1,000	0.0 a	5.3 a	4.3 a	0.8 a
80 + 2,000	0.0 a	16.1 b	18.1 b	4.0 b

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted at 2 WAE for PRE applications.

Table 8. Influence of application timing on visible injury at 2 WAE/WAA[a] of tolpyralate + atrazine at two rates in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Rate	
Application timing	40 + 1,000 (g ai ha^{-1})	80 + 2,000 (g ai ha^{-1})
	Visible injury 2 WAA[c]	
	%	
PRE	0.0 a	0.0 a
V1	5.3 a	16.1 b
V3	4.3 a	18.1 b
V5	0.8 a	4.0 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application; WAE, weeks after emergence.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted 2 WAE for PRE applications.

Table 9. Influence of herbicide rate on visible injury at 4 WAA[a] of tolpyralate + atrazine at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing			
Rate	PRE	V1	V3	V5
	Visible injury at 4 WAA[c]			
g ai ha^{-1}	%			
40 + 1,000	0.0 a	0.3 a	0.6 a	0.5 a
80 + 2,000	0.0 a	1.1 a	4.6 b	3.3 b

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted 4 wk after V5 applications.

Table 10. Influence of application timing on visible injury at 4 WAA[a] of tolpyralate + atrazine at two rates in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Rate	
Application timing	40 + 1,000 (g ai ha^{-1})	80 + 2,000 (g ai ha^{-1})
	Visible injury at 4 WAA[c]	
	%	
PRE	0.0 a	0.0 a
V1	0.3 a	1.1 a
V3	0.6 a	4.6 b
V5	0.5 a	3.3 b

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted 4 wk after V5 applications.

WAA, differences in corn hybrid response were more evident at these timings. Corn injury across application timings depended on the hybrid, which produced a timing by hybrid interaction at 1 WAA ($P = 0.0346$; Table 2), while hybrid alone was statistically significant as a main effect at 2 WAA ($P = 0.0001$; Table 2). Overall, no three-way interactions between fixed effects were statistically significant for any assessment parameter ($P \geq 0.1972$).

Visible corn injury across all four hybrids was most severe at 1 WAA with V1 or V3 applications when averaged across rates (Table 4). No injury was observed with PRE applications, while injury from V5 applications was $\leq$3.8% regardless of hybrid. O'Sullivan et al. (2002) also reported no injury to sweet corn hybrids with mesotrione applied PRE; whereas POST applications caused severe injury. Hybrids responded similarly to tolpyralate + atrazine applications made at the V1 stage (Table 3); injury ranged from 20.6% to 22.5% and consisted mainly of white

Table 11. Influence of herbicide rate on visible injury at 8 WAA[a] of tolpyralate + atrazine at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing			
Rate	PRE	V1	V3	V5
	Visible injury at 8 WAA[c]			
g ai ha^{-1}	%			
40 + 1,000	0.0 a	0.1 a	0.1 a	0.1 a
80 + 2,000	0.0 a	0.1 a	0.1 b	0.1 b

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method; WAA, weeks after application.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Visual assessments were conducted 8 wk after V5 applications.

Table 13. Influence of application timing of tolpyralate + atrazine on corn height when applied at two rates in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Rate	
Application timing[a]	40 + 1,000 (g ai ha^{-1})	80 + 2,000 (g ai ha^{-1})
	Relative height[c]	
	%	
PRE	101 a	101 a
V1	99 a	95 c
V3	101 a	97 bc
V5	100 a	99 ab

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method;.
[b] Means followed by the same letter within columns (a, b, c) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Plant height was expressed as a percent relative to the corresponding nontreated control plots within replications.

Table 15. Influence of application timing of tolpyralate + atrazine on corn grain moisture when applied at two rates in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Rate	
Application timing[a]	40 + 1,000 (g ai ha^{-1})	80 + 2,000 (g ai ha^{-1})
	Relative grain moisture[c]	
	%	
PRE	100 a	99 a
V1	100 a	101 ab
V3	100 a	101 b
V5	100 a	100 ab

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method;.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Grain moisture was expressed as a percent relative to the corresponding nontreated control plots within replications.

Table 12. Influence of tolpyralate + atrazine rate on corn height when applied at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing[a]			
Rate	PRE	V1	V3	V5
	Relative height[c]			
g ai ha^{-1}	%			
40 + 1,000	101 a	99 a	101 a	100 a
80 + 2,000	101 a	95 b	97 b	99 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method;.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Plant height was expressed as a percent relative to the corresponding nontreated control plots within replications.

Table 14. Influence of tolpyralate + atrazine rate on corn grain moisture when applied at four timings in field experiments conducted in Ontario, Canada, in 2017 and 2018.[b]

	Application timing[a]			
Rate	PRE	V1	V3	V5
	Relative grain moisture[c]			
g ai ha^{-1}	%			
40 + 1,000	100 b	100 a	100 a	100 a
80 + 2,000	99 a	101 a	101 b	100 a

[a] Abbreviations: V1, 3, and 5, corn vegetative growth stages 1, 3, and 5, respectively, as determined with the leaf collar method;.
[b] Means followed by the same letter within columns (a, b) are not significantly different according to Tukey's multiple means comparison test ($\alpha = 0.05$).
[c] Grain moisture was expressed as a percent relative to the corresponding nontreated control plots within replications.

Table 16. Summary of candidate predictor variables entered in mixed-model multiple stepwise regression analysis for visible crop injury at 1, 2, 4, and 8 wk following application of tolpyralate + atrazine at two rates and three application timings to four corn hybrids in experiments conducted in Ontario, Canada, in 2017 and 2018.[a]

Predictor variable	Abbreviation
Soil parameters	
Sand, %	%Sa
Silt, %	%Si
Clay, %	%Cl
Organic matter, %	OM
pH	pH
CEC, meq 100 g^{-1}	CEC
Air temperature parameters, C[a]	
Temperature differential 24 h before application	ΔTPRE
Temperature differential 24 h after application	ΔTPO
Air temperature at application[b]	T
Application parameters[b]	
Relative humidity at application, %	RH
Time of application, 24-h clock	TOD
Precipitation parameter[b]	
Cumulative precipitation 7 d after application, mm	PRECIP

[a] Temperature differentials (daily max – daily min) and cumulative precipitation were obtained from permanent weather stations situated <1 km from trial sites.
[b] Application variables measured using a portable digital weather meter (Kestrel Meters, Boothwyn, PA).

bleaching, symptoms characteristic of HPPD-inhibitor injury ((Abendroth et al. 2006; Grossmann and Ehrhardt 2007). In accordance with label recommendations (Anonymous 2017a) tolpyralate was co-applied with atrazine in this study. Previously, Choe et al. (2014) reported an increase in sweet corn bleaching injury when atrazine was added to mesotrione, topramezone, and tembotrione relative to each HPPD-inhibitor applied alone; however, this

Table 17. Summary of mixed-model multiple stepwise regression analysis of significant ($P < 0.05$) predictor variables for visible crop injury at 1 wk after application (WAA) of tolpyralate + atrazine at two rates and three timings to four corn hybrids, from experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[a]

	Visible injury at 1 WAA		
Predictor variable	Estimate (±SE)	*F*-value	Pr > *F*
Temperature differential 24 h prior (ΔTPRE)	0.68 (0.218)	9.67	0.0020
Air temperature at application (T)	0.51 (0.175)	8.51	0.0037
Precipitation 7 d post (PRECIP)	−0.65 (0.057)	130.49	<0.0001
Time of day (TOD)	−0.01 (0.002)	38.10	<0.0001
Random effect	Estimate (±SE)	χ^2	Pr > χ^2
Environment	16.30 (11.932)	25.31	<0.0001
Equation			
Injury at 1 WAA = 12.37 + 0.68(ΔTPRE) + 0.51 (T) − 0.65(PRECIP) − 0.01(TOD) + 16.3			

[a]Predictor variables deemed insignificant ($P > 0.05$) were eliminated from the model.

Table 19. Summary of mixed-model multiple stepwise regression analysis of significant ($P < 0.05$) predictor variables for visible crop injury at 4 wk after application (WAA) of tolpyralate + atrazine at two rates and three timings to four corn hybrids, from experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[a]

	Visible injury at 4 WAA		
Predictor variable	Estimate (±SE)	*F*-value	Pr > *F*
Air temperature at application (T)	0.26 (0.037)	48.29	<0.0001
Precipitation 7 d post (PRECIP)	−0.03 (0.012)	7.50	0.0064
Random effect	Estimate (±SE)	χ^2	Pr > χ^2
Environment	2.43 (1.579)	135.76	<0.0001
Equation			
Injury at 4 WAA = −3.52 + 0.26(T) − 0.03(PRECIP) + 2.43			

[a]Predictor variables deemed insignificant ($P > 0.05$) were eliminated from the model.

Table 18. Summary of mixed-model multiple stepwise regression analysis of significant ($P < 0.05$) predictor variables for visible crop injury at 2 wk after application (WAA) of tolpyralate + atrazine at two rates and three timings to four corn hybrids, from experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[a]

	Visible injury at 2 WAA		
Predictor variable	Estimate (±SE)	*F*-value	Pr > *F*
Temperature differential 24 h prior (ΔTPRE)	0.80 (0.141)	32.28	<0.0001
Precipitation 7 d post (PRECIP)	−0.26 (0.031)	67.32	<0.0001
Random effect	Estimate (±SE)	χ^2	Pr > χ^2
Environment	5.72 (4.030)	31.22	<0.0001
Equation			
Injury at 2 WAA = 0.88 + 0.8(ΔTPRE) − 0.26(PRECIP) + 5.72			

[a]Predictor variables deemed insignificant ($P > 0.05$) were eliminated from the model.

Table 20. Summary of mixed-model multiple stepwise regression analysis of significant ($P < 0.05$) predictor variables for visible crop injury at 8 wk after application (WAA) of tolpyralate + atrazine at two rates and three timings to four corn hybrids, from experiments conducted near Ridgetown and Exeter, ON, Canada, in 2017 and 2018.[a]

	Visible injury at 8 WAA		
Predictor variable	Estimate (±SE)	*F*-value	Pr > *F*
Air temperature at application (T)	0.07 (0.011)	37.19	<0.0001
Precipitation 7 d post (PRECIP)	−0.01 (0.004)	7.83	0.0053
Random effect	Estimate (±SE)	χ^2	Pr > χ^2
Environment	0.05 (0.038)	27.66	<0.0001
Equation			
Injury at 8 WAA = −1.16 + 0.07(T) − 0.01(PRECIP) + 0.05			

[a]Predictor variables deemed insignificant ($P > 0.05$) were eliminated from the model.

difference in injury did not translate to final ear yield. At one site in 2018 (E4; Table 1), substantial leaf burn was observed with V1 applications; affected foliage exhibited gray, water-soaked lesions within 4 h of application (data not presented). These symptoms were inconsistent with typical HPPD-inhibitor bleaching injury (Abendroth et al. 2006) and were perhaps a result of the adjuvants used. When applications were made at V3, DKC43-47RIB was slightly more sensitive to tolpyralate + atrazine compared with P0094AM and P9840AM at 1 WAA (Table 4). By 2 WAA, both DKC43-47RIB and DKC42-60RIB were comparatively more sensitive to tolpyralate + atrazine than either P0094AM or P9840AM, irrespective of application rate or timing (Table 2). Interestingly, these hybrids are not reported to be sensitive to HPPD inhibitors (Monsanto 2018); however, previous research has identified Ontario sweet corn hybrids that are sensitive to other HPPD inhibitors, including mesotrione, due to the presence of mutant recessive *CYP* alleles (Bollman et al. 2008; Meyer et al. 2010; O'Sullivan et al. 2002). As visible injury diminished with time, no differences in hybrid sensitivity were observed at later assessment timings or in quantitative assessment parameters ($P \geq 0.1366$; Table 2), suggesting that the observed differences in hybrid sensitivity in this study have negligible biological significance but may warrant more thorough tolerance screening of additional corn hybrids.

When averaged across the four hybrids at 1 and 2 WAA, the 2X rate of tolpyralate + atrazine resulted in greater crop injury than the 1X rate, regardless of POST application timing (Tables 5–8). It is possible that the 2X rate of adjuvants (MSO + UAN) used in this study accentuated crop injury in addition to the effect of increasing herbicide rate. Grossmann and Ehrhardt (2007) reported an increase in weed uptake of topramezone when a nonionic surfactant (Dash HC®, BASF SE 2018) was added to topramezone. Similarly, Zhang et al. (2013) reported an increase in weed control efficacy and risk of crop phytotoxicity when MSO was applied with topramezone. Therefore, the 2X adjuvant rate used in this study to simulate a spray overlap may have increased corn uptake of tolpyralate + atrazine, contributing to greater phytotoxicity. At 1 WAA, injury was 20 and 22 percentage points higher with the 2X rate than with the 1X rate, applied at V1 and V3, respectively (Tables 5 and 6). In contrast, the V5 application resulted in only 5.7% injury when the 2X rate was applied. Regardless of rate, corn was most susceptible to injury when tolpyralate + atrazine was applied at V1 or V3. By 2 WAA however, only the 2X rate applied at V1 or V3 caused injury greater than that observed with either PRE (0%) or V5 applications (0.8% to 4%) (Tables 7 and 8). Johnson et al. (2002) observed a similar trend; crop injury with mesotrione + atrazine was higher when applied to V3 corn compared with V4 or V5 corn. However, these results were attributed to environmental conditions at the time of application rather than physiological characteristics of the corn plants.

Because 4 and 8 WAA assessments were conducted in relation to the V5 application, injury was generally more evident in plots treated at V3 and V5 timings compared with V1 timings. Injury with 2X rates applied at V1 decreased to 1.1% by 4 WAA, which was statistically similar to the 0% injury observed in plots treated PRE

(Table 10). At both 4 and 8 WAA assessment timings, the rate by timing interaction was statistically significant ($P < 0.0001$; Table 2). When applied at the field rate (1X), application timing had no effect on crop injury at 4 or 8 WAA, although applications at V3 or V5 resulted in greater crop injury at 4 WAA than applications PRE or at V1 (Tables 9–11). This difference is likely due to the relatively shorter time period from the V3 and V5 applications to the 4 WAA assessments compared with the time from the V1 application. Similarly, an increase in injury was observed at 4 and 8 WAA when the 2X rate was applied at V3 and V5, but not at earlier application timings. Regardless, crop injury was ≤4.6% at 4 WAA; by 8 WAA application timings were similar (data not presented), and no treatment resulted in >0.1% injury (Table 11), which would be within commercially acceptable tolerance levels.

Consistent with early-season crop injury assessments, 2X rates of tolpyralate + atrazine applied at either V1 or V3 resulted in corn stunting relative to the NTC, while no difference in height was observed with either rate applied PRE or at V5 (Tables 12 and 13). This effect led to a statistically significant rate by timing interaction for relative plant height ($P = 0.0009$; Table 2). Presumably due to the more severe injury observed with the 2X rate applied at V1 and V3 (Tables 4–8), a 4-percentage point decrease in plant height was observed when a 2X rate was applied compared with a 1X rate (Table 12). Similarly, 6- and 4-percentage point decreases in height were observed when the 2X rate was applied at V1 and V3, respectively, compared with when it was applied PRE. Therefore, despite plants showing <1% injury at 8 WAA, plants that were most severely injured were also stunted as a result of early-season herbicide injury. Furthermore, stunted plants reached anthesis later (≤7 d) than the nontreated control (data not presented). Despite a delay in development, negligible trends were observed in final grain yield or harvest moisture, although the rate by timing interaction was statistically significant for relative grain moisture at harvest ($P = 0.0167$; Table 2). A 1-percentage point increase in grain moisture was observed when a 2X rate of tolpyralate + atrazine was applied to V3 corn compared with a 1X rate; a 2-percentage point increase in moisture was observed when the 2X rate was applied at V3 compared with PRE (Tables 14 and 15). The Ontario Ministry of Agriculture, Food and Rural Affairs estimates corn drying costs to be Can$2.33 per 1,000 kg for each percentage point in grain moisture ([OMAFRA] Ontario Ministry of Agriculture, Food and Rural Affairs 2018). Therefore, despite its statistical significance, the observed difference in grain moisture is unlikely to be economically significant. Contrary to all other assessment parameters, differences in grain yield were only detected across herbicide rates ($P = 0.0258$; Table 2). A 2X rate of tolpyralate + atrazine resulted in a 2-percentage point lower grain yield compared with the 1X rate. However, yield when a 2X rate was applied was still 100% of that obtained in the NTC when averaged across application timings and hybrids; no decrease in yield was observed as a result of the herbicide treatments. Similar results have been reported with sweet corn, in which visible crop injury caused by mesotrione, topramezone, and tembotrione did not translate to yield loss (Bollman et al. 2008; Soltani et al. 2007). Similarly, Gitsopoulos et al. (2010) reported no effect of topramezone rate or application timing on corn grain yield. In contrast, O'Sullivan et al. (2002) reported a yield decrease in a sensitive sweet corn hybrid ('Del Monte 2038') with mesotrione applied POST.

Multiple Stepwise Regression of Predictor Variables

Based on multiple regression analysis, all soil parameters collected (sand, silt, clay, organic matter, pH, and CEC) were insignificant factors in modeling crop injury at 1, 2, 4, and 8 WAA and were therefore eliminated from the models. Conversely, several environmental variables related to temperature, precipitation, and time of day were determined to be statistically significant predictors of crop injury ($P \leq 0.0064$; Tables 17–20). At 1 WAA, when injury symptoms were most pronounced, four predictor variables were significant ($P \leq 0.0037$; Table 17). Injury at 1 WAA was accentuated with higher temperatures at the time of application and with a larger temperature differential (daily high − daily low) in the 24 h before application (Table 17); this predictor variable was also significant for injury at 2 WAA (Table 18). In agreement with the model, temperature differentials 24 h before POST applications in this study ranged from 6.5 to 17.5 C; wider differentials were generally associated with increased injury (data not presented). Similarly, previous controlled-environment studies have determined that corn development is delayed by increasing the daily ambient temperature differential from 8.6 to 17.2 C (Coligado and Brown 1975), suggesting that wider daily temperature differentials may induce physiological stress in corn. Higher relative humidity (RH) has been suggested to increase herbicide diffusion through the leaf cuticle (Müller and Appleby 2012) and has been previously demonstrated to increase uptake and activity of several herbicides, including fluroxypyr, glufosinate, acifluorfen, and mesotrione (Johnson and Young 2002; Lubbers et al. 2007; Ramsey et al. 2002; Ritter and Coble 1981). However, RH was not determined to have a significant effect on crop injury in this study, despite a range from 47% to 96% RH across POST timings (Table 1). At 1 WAA, crop injury was generally higher with applications made earlier in the day compared with those made in the evening (Table 17), although time of application in this study was arbitrary. In studies with applications made at discrete time intervals, time of day effects on herbicide efficacy have been widely reported; herbicide efficacy generally peaks during midday, possibly due to temperature, RH, leaf orientation, or presence/absence of dew (Budd et al. 2017; Skuterud et al. 1998; Stewart et al. 2009; Stopps et al. 2013). Precipitation was also determined to be a potential factor in crop injury at each of the four assessment timings in this study (Tables 17–20). Generally, crop injury was reduced with higher cumulative precipitation in the 7 d following application; alleviation of any preexisting moisture stress likely diminished the effects of herbicide injury. Similar to injury at 1 WAA, visible injury at 4 and 8 WAA was associated with higher temperatures at the time of application according to the regression model (Tables 19 and 20). Johnson and Young (2002) previously reported increased foliar activity of mesotrione at higher temperatures; it is possible that a similar relationship exists with tolpyralate. Additionally, the cuticular wax on plant foliage becomes less viscous at higher temperatures, potentially allowing for greater diffusion of herbicide across the leaf membrane (Sargent 1965). While several environmental variables were identified as possible predictors of crop injury in this study, the random effect of environment was also significant in each regression model (Tables 17–20). Therefore, although the multiple regression models provide insight into factors that may have influenced crop injury with tolpyralate + atrazine, factors other than those outlined here undoubtedly contributed to the injury observed in this study.

This research provides insight into the variable tolerance levels exhibited by four corn hybrids to tolpyralate + atrazine, as affected by application timing and rate. Although none of the hybrids used in this study were previously known to exhibit sensitivity to HPPD-inhibiting herbicides, DKC43-47RIB and DKC42-60RIB appear to be marginally more susceptible to short-term injury following application of tolpyralate + atrazine compared with P0094AM and P9840AM (Tables 2 and 3). As injury diminished with time, hybrids recovered similarly; no hybrid effects were observed at 4 or 8 WAA or in quantitative assessment parameters (Table 2). Generally, applications of tolpyralate + atrazine at either the V1 or V3 timing induced greater crop injury than applications made at V5; injury was accentuated when a 2X rate was applied (Tables 5–8). Conversely, PRE applications caused no injury regardless of application rate or hybrid. Based on mixed-model multiple stepwise regression analysis, crop injury with POST applications was generally associated with applications that occurred under warmer temperatures and with wider daily temperature differentials recorded in the 24 h before application (Tables 17–20). In contrast, injury was reduced with applications made later in the day and when greater cumulative precipitation occurred in the 7 d following application. Soil parameters were determined to have no effect on corn injury in this study; however, the significance of the random effect of environment suggests that factors other than those described here influenced the level of injury. The increased corn injury observed with 2X rates of tolpyralate + atrazine applied at V1 and V3 translated to a decrease in plant height relative to the NTC and a slight increase in grain moisture at harvest (Tables 12–15). Similarly, final relative corn grain yield was marginally higher with 1X rates compared with 2X rates when averaged across hybrids and timings, although yield with each rate was similar to that of the NTC plots (Table 2). Therefore, the application of tolpyralate + atrazine within the parameters of this study would be unlikely to have economic consequences, despite substantial visible injury and a degree of stunting. Future investigation into additional corn hybrids and environmental and application variables that may affect corn tolerance to tolpyralate and tolpyralate + atrazine in field and controlled environments could aid in minimizing the risk of crop injury with this herbicide.

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