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SHORT COMMUNICATION

Efficacy of HPPD-inhibiting herbicides applied preemergence or postemergence for control of multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus* var. *rudis*)

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Abstract: Research was conducted in 2017 and 2018 to determine the relative efficacy of five HPPD-inhibitors, tank mixed with atrazine, for the control of multiple-resistant waterhemp. At 12 weeks after application (WAA), isoxaflutole + atrazine, mesotrione + atrazine and tembotrione + atrazine, applied PRE, controlled waterhemp 90, 87 and 81%, respectively. None of the HPPD-inhibiting herbicides applied PRE controlled waterhemp equivalent to the weed-free control 12 WAA. Applied POST, topramezone + atrazine, mesotrione + atrazine, tolypyralate + atrazine, and tembotrione + atrazine controlled waterhemp 87, 94, 97, and 98% 12 WAA, respectively, and were all equivalent to the weed-free control.

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Abbreviations: **GR**, glyphosate-resistant; **MR**, multiple herbicide-resistant; **POST**, postemergence; **PRE**, preemergence; **WAA**, weeks after application.

Key words: Biomass, density, glyphosate resistance, multiple herbicide-resistant, waterhemp, ALS resistance, triazine resistance, corn

Waterhemp is native to the American south-west and was first confirmed in Ontario in 2002 (Vyn et al. 2006). In 2014, waterhemp was the fourth glyphosate-resistant (GR) weed that was documented in the province, after common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.) and Canada fleabane (*Conyza canadensis* L. Cronq.) (Sikkema et al. 2009). Many biological traits make waterhemp a troublesome and difficult to control weed. In Ontario, waterhemp emerges from May to October; therefore, herbicides must provide full season residual control of both the initial population and subsequent flushes (Schryver et al. 2017). Waterhemp is dioecious, housing male and female reproductive organs on separate plants, this increases the chances of pollen mediated herbicide-resistance gene flow from one population to another. (Liu et al. 2012; Sarangi et al. 2017). Wind pollination is the predominant method of pollen dispersal; pollen grains are small, spherical (20 μm in diameter) and structured to remain airborne for long distances (Franssen et al. 2001; Sarangi et al. 2017). Once released from the male anther, pollen can remain viable for up to 5 days and travel as far as 800 m (Liu et al. 2012). On average a waterhemp plant will produce around 300 000 seed, under optimal conditions a single female waterhemp plant can produce up to 4.8 million seeds (Hartzler et al. 2004).

Waterhemp has been identified at 81 locations in southwestern Ontario. A field survey conducted by Schryver et al. (2017) reported that seeds collected from waterhemp populations

from 81 different locations were 100% resistant to the ALS-inhibitors, 82% resistant to glyphosate, and 76% resistant to atrazine; 74% of the populations were resistant to all three modes-of-action. Multiple resistances to these groups of herbicides, particularly atrazine and glyphosate, which have been cornerstones in corn weed control programs, poses a significant challenge for Ontario grain farmers. Hydroxyphenylpyruvate dioxygenase- (HPPD) inhibiting herbicides, WSSA classification Group 27, have proven to be effective for the control of waterhemp in corn in Ontario (Vyn et al. 2006). The HPPD chemistries are an attractive herbicide option for Ontario grain farmers, since they have a flexible application window and control a wide spectrum of broadleaf weeds (OMAFRA 2018). Bicyclopyrone, isoxaflutole and mesotrione are registered for use preplant (PP), preemergence (PRE) and early postemergence (POST) in corn. Tembotrione, tolpyralate and topramezone are registered as POST options in corn (OMAFRA 2018). Tank-mixing HPPD-inhibitors with atrazine can result in a synergistic effect, broadening the weed control spectrum and improving consistency of control (Woodyard et al. 2009). The objective of this study was to determine the efficacy of the currently available HPPD-inhibiting herbicides applied PRE or POST for the control of multiple-resistant waterhemp in Ontario. This study investigated each HPPD inhibitor at both PRE- and POST application timings.

Two field studies were completed in 2017 and 2018 at three locations in Ontario for a total of six site-years per study. One of the locations was near Cottam, Ontario and two locations were on Walpole Island, Ontario. The trials were conducted on commercial fields and no additional waterhemp seed was added. The soil at Cottam was sandy-loam with 66% sand, 24% silt, 10% clay, 2.2% organic matter and a PH of 6.4. The soil at Walpole 1 was loamy-sand with 78% sand, 14% silt, 8% clay, 2.3% organic matter and a PH of 8.3. The soil at Walpole 2 was loamy-

sand with 76% sand, 18% silt, 6% clay, 2.4% organic matter and a PH of 8.0. Waterhemp populations at all three locations are multiple-resistant to three herbicide modes-of-action Groups: 2, 5 and 9 (Schryver et al. 2017). The first study investigated the efficacy of HPPD-inhibitors applied PRE, and the second study investigated their efficacy applied POST. The studies were established as a randomized complete block design with four replications. Each replication included a weedy - and a weed-free control. Herbicide treatments are based on the manufacturer's recommended rates in Ontario and are presented in Tables 1 and 2.

Recommended adjuvants were added to the POST herbicides and are presented in Table 2.

The soil at each site was cultivated in the fall and disked twice in the spring to prepare the seedbed prior to planting, the previous crop at all location was soybean. Glyphosate- and glufosinate- resistant corn ('DKC 46-82RIB') was planted at a rate of 83,000 seeds ha⁻¹ in mid-May to early-June, depending on the field location. Each plot was 2.25m wide (3 rows spaced 76 cm apart) and 8-m long. Weed-free controls were maintained with S-metolachlor/mesotrione/atrazine/bicyclopyrone (2022 g ai ha⁻¹) applied PRE, followed by dicamba/atrazine (1800 g ai ha⁻¹) applied POST and subsequent hand weeding throughout the growing season as needed. Glyphosate (450 g ae ha⁻¹) was applied early POST to the entire experimental area to control glyphosate-susceptible biotypes and all other weed species.

Herbicide treatments were applied using a CO₂ pressurized backpack sprayer calibrated to deliver 200 L ha⁻¹ at 250 kPa. The sprayer was equipped with a 1.5-m handheld boom and four ULD 12002 nozzles spaced 50 cm apart (Pentair, New Brighton, MN USA) producing a spray width of 2.0 m. The PRE herbicides were applied 1-5 days after corn planting and POST treatments were applied when waterhemp plants reached approximately 10 cm in height and corn was up to V6 growth stage.

In the PRE study, corn injury was assessed 2 and 4 weeks after emergence (WAE) and in the POST study corn injury was assessed 1 and 4 weeks after herbicide application (WAA). Crop injury was assessed visually on a scale of 0 to 100; 0% corresponded to no injury and 100% corresponded to complete plant death. Weed control was assessed visually 4, 8 and 12 WAA in both studies on a scale of 0 to 100% reduction in perceived biomass compared to the weedy control. Waterhemp density and biomass were determined 8 WAA by counting plants in two 0.25 m² quadrats per plot, the same plants were cut at the soil surface, placed in paper bags, dried at 50°C for three weeks to consistent moisture, and weighed. Corn was harvested from two rows in the fall with a small-plot combine, yield and moisture were recorded. Yield data was converted to 15.5% moisture before statistical analysis. Due to excessive rainfall, yield data was only collected from two locations in 2018.

Data from the PRE- and POST- studies were analyzed separately in PROC GLIMMIX in SAS v. 9.4 (SAS Institute Inc., Cary, NC). Random effects were environment and replication within environment. Herbicide treatment was the only fixed effect. The objective of these studies is to determine the most consistent herbicide option across varying Ontario environments, for this reason data from all sites for each study were pooled. The Shapiro-Wilk test was applied to test for normality using SAS PROC UNIVARIATE. Residuals were plotted for, predicted, treatment, block and trial in order to confirm assumptions.

Preemergence

There was no visual evidence that the HPPD + atrazine herbicide tankmixes applied PRE caused any corn injury in this study (data not presented). At 4 WAA, waterhemp control by

HPPD-inhibiting herbicides + atrazine varied between 69 and 93% depending on the herbicide (Table 1). The herbicide treatments isoxaflutole + atrazine and mesotrione + atrazine had the highest waterhemp control of as much as 93 and 91%, respectively. This result is consistent with an earlier study that reported 97% control of triazine-resistant waterhemp with isoxaflutole + atrazine (105 + 1063 g ai ha⁻¹) 10 WAA (Vyn et al. 2006). Previous field research from Ontario reported 82% waterhemp control 8 WAA for both isoxaflutole + atrazine (105 + 1063 g ai ha⁻¹) and mesotrione + atrazine (140 + 1500 g ai ha⁻¹) applied preemergence. Of the treatments tested, topramezone + atrazine had the lowest control of 69%; other HPPD + atrazine treatments provided greater than 83% control. Waterhemp control with herbicides evaluated decreased 3 to 10 percentage points from 4 WAA to 8 WAA. Similar downward trend in waterhemp control was not observed between 8 and 12 WAA. The 12 WAA waterhemp control rating for atrazine, topramezone + atrazine, tembotrione + atrazine and tolpyralate + atrazine increased as much as 9 percentage points compared to the 8 WAA rating. It is noteworthy that these four treatments provided the least control 8 WAA. The authors suggest the perceived increase in control could be due to the natural thinning of waterhemp populations as the season progresses, a phenomenon previously reported by Heneghan and Johnson (2017). Additionally, corn was bigger and may have shaded waterhemp. At 12 WAA no herbicide treatment provided waterhemp control that was equivalent to the weed-free control. Waterhemp density was reduced 73-97% compared to the weedy control with PRE-herbicides evaluated (Table 1). Similarly, waterhemp biomass was reduced 69-96% with PRE-herbicides evaluated (Table 1). Waterhemp interference in the weedy control reduced corn yield 12% compared to the weed-free control in this study. Although the minimal, late-season, waterhemp competition had no impact on final yield in any treatment, it is

still necessary that farmers target perfect, full-season weed control to reduce weed-seed return to the soil. This was not achieved with the soil-applied herbicides evaluated in this study.

Postemergence

At 2 and 4 WAA, the HPPD + atrazine herbicide tankmixes, applied POST, caused up to 3 and 1% corn injury, primarily bleaching, respectively. At 4 WAA, all HPPD + atrazine treatments provided 86-98% control (Table 2). At 12 WAA, tembotrione + atrazine, tolpyralate + atrazine, and mesotrione + atrazine all provided greater than 94% waterhemp control. Atrazine provided 64% control; reduced control can be attributed to triazine resistance at all three trial locations. The trend was similar throughout the remainder of the season. Mesotrione + atrazine provided 91 and 93 % control 4 and 8 WAA, respectively. Results are similar to Soltani et al. (2009) who reported 91 and 86% control of a triazine-resistant Ontario waterhemp population with mesotrione + atrazine ($100 + 280 \text{ g ha}^{-1}$) 4 and 10 WAA, respectively. Waterhemp control varied 86 to 87% with topramezone + atrazine at 4, 8 and 12 WAA. Variable control of *Amaranthus* spp. with topramezone has been previously reported. Topramezone + atrazine ($18 + 560 \text{ g ha}^{-1}$) applied to 15 cm Palmer Amaranth (*Amaranthus palmeri*) resistant to Groups 2, 5 and 9, provided 70% control 3 WAA (Kohrt and Sprague 2017). Waterhemp density was reduced as much as 99% compared to the weedy control with POST-herbicides evaluated (Table 2). Similarly, waterhemp biomass was reduced 81-100% with POST-herbicides evaluated (Table 2). There was a 10% decrease in corn yield due to waterhemp interference in this study when comparing tembotrione + atrazine and the weedy control. There was no other effect on corn yield.

This study concludes that isoxaflutole + atrazine, mesotrione + atrazine and tolfenpyrat + atrazine are the most effective HPPD-inhibiting herbicides applied PRE, and mesotrione + atrazine, tolfenpyrat + atrazine and tolfenpyrat + atrazine are the most effective HPPD-inhibiting herbicides applied POST. Effective weed control in field crops is influenced by a large variety of factors including: herbicide choice, application timing, rainfall and crop stage; however, relying on a single in-crop application for weed control introduces the risk of weather delays and weeds growing larger than the recommended application size, which can reduce control using any POST treatments (Eure et al. 2013). Although HPPD-inhibitors plus atrazine, applied PRE, provide up to 90% control of waterhemp and up to 98% control when applied POST, in practise it is important for farmers to apply multiple effective modes of action to reduce the likelihood of evolving resistances to these currently effective chemistries (Janseniuk et al. 1996).

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Table 1. Means for visual waterhemp control (4, 8 and 12 WAA), waterhemp density and dry biomass (8 WAA), corn yield with pre-emergence herbicide treatments. Data were averaged across all trials in 2017 and 2018.

Treatments	Rate (g ai ha ⁻¹)	Waterhemp control (%)			Waterhemp		Corn Yield ^a (t ha ⁻¹)
		4 WAA	8 WAA	12 WAA	Density (No. m ⁻²)	Dry Weight (g m ⁻²)	
Weedy Control		0	0	0	486 a	248 a	10.0 b
Weed-Free Control		100 a	100 a	100 a	0 b	0 b	11.3 a
Atrazine	1063	75 cd	65 c	71 d	97 b	52 b	11.4 a
Isoxaflutole + atrazine	105 + 1063	93 ab	90 ab	90 b	13 b	11 b	11.0 ab
Mesotrione + atrazine	140 + 1490	91 ab	88 ab	87 b	28 b	13 b	11.7 a
Topramezone + atrazine	12.5 + 500	69 d	65 c	74 cd	133 b	78 b	11.3 a
Tembotrione + atrazine	90 + 1000	83 bc	76 bc	81 bcd	53 b	36 b	11.6 a
Tolpyralate + atrazine	30 + 1000	84 bc	79 ab	84 cb	48 b	54 b	11.8 a
	SE	5.0	7.8	6.1	65.5	39.4	0.6

^aStandardized to 15.5% moisture

^{a-d} Means followed by the same letter within a column are not significantly different using Tukeys LSD (P>0.05).

Table 2. Means for visual waterhemp control (4, 8 and 12 WAA), waterhemp density and dry biomass (4 WAA), corn yield with POST-applied herbicide treatments. Data were averaged across all trials in 2017 and 2018.

Treatments ^a	Rate (g ai ha ⁻¹)	Crop Injury (%)		Waterhemp control (%)			Waterhemp		Corn Yield ^b (t ha ⁻¹)
		2 WAA	4 WAA	4 WAA	8 WAA	12 WAA	Density (No. m ⁻²)	Dry Weight (g m ⁻²)	
Weedy Control		0 c	0 b	0 c	0	0	757 a	189 a	9.6 b
Weed-Free Control		0 c	0 b	100 a	100	100	0 b	0 b	10.4 ab
Atrazine	1063	0 c	0 b	64 c	67 c	65 c	330 ab	35 b	10.3 ab
Isoxaflutole + atrazine	105 + 1063	1 bc	0.3 ab	88 b	90 ab	86 b	67 b	7 b	10.4 ab
Mesotrione + atrazine	100 + 280	0 c	0 b	91 ab	93 ab	94 ab	35 b	1 b	10.3 ab
Topramezone + atrazine	12.5 + 500	0 c	0 b	86 b	88 b	87 ab	279 b	9 b	10.3 ab
Tembotrione + atrazine	90 + 1000	2 ab	0 b	98 a	97 a	98 a	4 b	0 b	10.6 a
Tolpyralate + atrazine	30 + 1000	3 a	1 a	94 ab	96 ab	97 ab	25 b	0 b	10.5 ab
	SE	0.6	0.2	6.0	4.5	6.2	165.6	17.8	0.3

^aAll treatments applied with glyphosate (900 g ai ha⁻¹). Atrazine, mesotrione + atrazine, topramezone + atrazine, tembotrione + atrazine and tolpyralate + atrazine treatments included Assist oil concentrate (1.0% v/v), Agral 90 (0.2% v/v), Merge (0.5% v/v), Hasten 28% UAN (1.75 L ha⁻¹), 3.5 (L ha⁻¹), MSO concentrate (0.5% v/v), and 28% UAN (2.5% v/v), respectively.

^bStandardized to 15.5% moisture

^{a-c} Means followed by the same letter within a column are not significantly different using Tukeys LSD (P>0.05).