Journal of Agricultural Science; Vol. 13, No. 7; 2021
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Is There a Benefit of Adding Atrazine to HPPD-Inhibiting Herbicides for Control of Multiple-Herbicide-Resistant, Including Group 5-Resistant, Waterhemp in Corn?

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Received: February 9, 2021 Accepted: May 12, 2021 Online Published: June 15, 2021

doi:10.5539/jas.v13n7p21 URL: https://doi.org/10.5539/jas.v13n7p21

Abstract

The evolution of multiple-herbicide-resistant (MHR) waterhemp (resistant to Groups 2, 5, 9, and 14) in Ontario, Canada is challenging for growers. The complementary activity of the co-application of hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides with atrazine has been well documented. The objective of this research was to determine if the addition of atrazine to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides applied postemergence improves their consistency of MHR waterhemp (including Group 5 resistance) in corn. Five field trials were conducted over a two-year period (2018, 2019) in Ontario, Canada. Five HPPD-inhibiting herbicides [isoxaflutole (105 g ha^{-1}), mesotrione (100 g ha^{-1}), topramezone (12.5 g ha^{-1}), tembotrione (90 g ha^{-1}), and tolpyralate (30 g ha^{-1})] were applied postemergence with and without atrazine to 10-cm-tall waterhemp. Corn injury (≤ 10%) was observed at specific sites where the application of tembotrione, isoxaflutole and isoxaflutole + atrazine resulted in characteristic white bleaching of corn foliage; however, yield was not affected. Averaged across field sites, the addition of atrazine to isoxaflutole, mesotrione, topramezone, or tembotrione improved MHR waterhemp control 15%, 11%, 7%, and 7%, respectively at 4 weeks after application (WAA). Averaged across herbicide treatments and sites, the addition of atrazine reduced the standard error of MHR waterhemp control by 13% to 100%. This study concludes that the co-application of atrazine with HPPD-inhibitors applied postemergence reduced the risk of herbicide failure and resulted in greater and more consistent control of MHR waterhemp.

Keywords: atrazine, multiple-herbicide resistant, postemergence herbicides, weed management

1. Introduction

Waterhemp (*Amaranthus tuberculatus*) is ranked as one of the most troublesome weeds in corn production in the United States (Nordby & Hartzler, 2004). Waterhemp is a late-emerging, summer-annual broadleaf weed species with several biological characteristics that have enabled it to expand its geographic range and thrive in agricultural cropping systems (Hartzler et al., 1999; Olsen & Waselkov, 2014). The distribution of herbicide-resistant (HR) waterhemp currently includes 18 states in the United States and three Canadian provinces, including Ontario (Heap, 2020). Over the past 18 years, waterhemp has spread across Ontario and is now present in 14 counties (Benoit et al., 2019a; Costea et al., 2005). Glyphosate-resistant (GR) waterhemp was first confirmed on Walpole Island, Ontario in 2014 (Schryver et al., 2017). Multiple-herbicide-resistant (MHR) waterhemp populations have since been identified in Ontario with four-way resistance to acetolactate synthase (ALS) (Group 2), photosystem II (PS II) (Group 5), 5-enolpyruyl shikimate-3-phosphate synthase (EPSPS) (Group 9) and protoporphyrinogen oxidase (PPO) (Group 14) inhibiting herbicides (Benoit et al., 2019a). A waterhemp population resistant to six herbicide modes-of-action (MOA) was reported in Missouri in 2015 with resistance to Group 2, synthetic auxins (Group 4), 5, 9, 14, and 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Group 27) (Shergill et al., 2018). Waterhemp is the first weed species to evolve resistance to HPPD-inhibiting herbicides (Heap, 2020; McMullan & Green, 2011).

The evolution of MHR waterhemp populations and the concomitant decrease in effective herbicide options warrants the need for Ontario corn producers to implement waterhemp management practices that provide near-perfect, full-season control to reduce waterhemp seed return to zero. Complete waterhemp control, and the subsequent prevention of seed production, can deplete the soil seed bank by more than 99% in four years and reduce the selection intensity for MHR biotypes (Steckel et al., 2007). Waterhemp is a prolific seed producer and typically produces approximately 300,000 seeds plant^{-1}; however, seed production of up to 4.8 million seeds plant^{-1} has been documented in the absence of competition (Hartzler et al., 2004). As the growing season progresses and waterhemp emergence is delayed relative to crop emergence, seed production declines; however, late-emerging plants can still contribute to the soil seed bank given MHR waterhemp's prolific nature.

Weeds that emerge simultaneously with corn have a greater detrimental impact on yield (Kropff & Spitters, 1991). Corn is sensitive to early season weed interference and must be kept weed-free from emergence up to the V4 corn growth stage to prevent yield loss (Hall et al., 1992; Page et al., 2012; Swanton et al., 1999). Waterhemp left uncontrolled can reduce grain corn yield by up to 74% (Steckel & Sprague, 2004). In Ontario, corn yield losses of up to 48% have been reported when MHR waterhemp population resistant to Group 2 and 5 herbicides were left uncontrolled (Soltani et al., 2009).

HPPD-inhibiting herbicides are widely used in corn production due to their crop safety, flexible application timing, broad spectrum weed control and activity on many HR weed species. Mesotrione was the most widely used HPPD-inhibitor and was applied to 42% of planted corn hectares in the USA in 2018 (NASS, 2019). Other HPPD-inhibitors used in corn production include bicyclopyrone, isoxaflutole, tembotrione, topramezone, and tolpyralate (Nurse et al., 2010; Osipitan et al., 2018; US-EPA, 2015). Common tank-mixes used in Ontario corn production consist of an HPPD-inhibiting herbicide with atrazine. Atrazine is a triazine herbicide that was released commercially in 1958 as a preemergence and postemergence herbicide for broadleaf weed control in corn (Ulrich et al., 2012). Atrazine is the most widely used PS II-inhibitor and the second most widely used herbicide in corn following glyphosate (NASS, 2019). In 2018, atrazine was applied to 65% of planted corn hectares at an average rate of 1,161 g ha^{-1}. In susceptible plant species, atrazine occupies the Q_B binding site on the D1 protein of PS II, increasing the production of reactive oxygen species (ROS); singlet oxygen, and triplet chlorophyll (Hess, 2000; Shaner et al., 2014). Reactive oxygen species cause lipid peroxidation and cell membrane destruction, resulting in plant death (Bartosz, 1997; Hankamer et al., 1997). HPPD-inhibiting herbicides prevent the conversion of hydroxyphenyl pyruvate to homogentisate (Lee et al., 1997). Homogentisate is the precursor to antioxidant compounds plastoquinone, *α*-tocopherols and carotenoids. Antioxidant compounds quench ROS, reducing or eliminating the impact of oxidative stress on the plant (Lee et al., 1997). In the absence of plastoquinone, *α* tocopherols, and carotenoids the plant is unable to quench ROS and succumbs to oxidative stress resulting in plant death (Trebst et al., 2002). When applied in a mixture, atrazine + HPPD-inhibiting herbicides cause a simultaneous increase in ROS and decrease in antioxidants, resulting in more effective weed control (Abendroth et al., 2006).

Synergism between atrazine and HPPD-inhibiting herbicides has been reported for the control of several broadleaf weed species including waterhemp, Palmer amaranth (*Amaranthus palmeri* S. Watson), redroot pigweed (*Amaranthus retroflexus* L.), Canada fleabane [*Conyza canadensis* (L.) Cronq.], and common lambsquarters (*Chenopodium album* L.) (Abendroth et al., 2006; Armel et al., 2009; Benoit et al., 2019b; Khort & Sprague, 2017; Osipitan et al., 2018; Williams et al., 2011; Woodyard et al., 2009). Complementary activity between HPPD- and PS II-inhibitors can also be observed with weed species that exhibit resistance to one or more applied active ingredients (Hugie et al., 2008; Walsh et al., 2012; Woodyard et al., 2009). Hugie (2008) documented complementary activity between atrazine + mesotrione and bromoxynil + mesotrione applied to triazine-resistant redroot pigweed. The two primary mechanisms of resistance associated with triazine resistance are target site resistance, conferred by an amino acid substitution on the D1 protein of PS II, and enhanced metabolism of atrazine by glutathione s-transferase (GST) (Holt et al., 1993; Oettmeier, 1999). Synergism between atrazine and HPPD-inhibiting herbicides was also observed in wild radish (*Raphanus raphanistrum*) and velvetleaf (*Abutilon theophrasti*), where triazine-resistance was conferred by a target-site mutation and enhanced metabolism, respectively (Walsh et al., 2012; Woodyard et al., 2009). Previous studies have found that a mixture of atrazine with HPPD-inhibitors and other corn herbicides provides a greater and more consistent level of weed control while improving grain yields and profitability (Swanton et al., 2007; Woodyard et al., 2009). Swanton (2007) estimated the benefit of atrazine to Ontario corn production was $26.1 million CAD in 2004. Atrazine is one of the most widely used herbicides for weed management in corn production and its stewardship is imperative to ensure its future use to control MHR waterhemp and other HR weeds.

Postemergence applications of HPPD-inhibitors in the absence of atrazine often provide unacceptable control of MHR waterhemp in corn (Shergill et al., 2018). It is hypothesized that herbicide applications consisting of an HPPD-inhibitor plus atrazine will provide a greater and more consistent level of MHR waterhemp control than an HPPD-inhibitor applied alone. The objective of this research was to determine if the co-application of atrazine with HPPD-inhibiting herbicides improves the level and consistency of MHR waterhemp control, including Group 5 resistance, in Ontario corn.

2. Materials and Methods

This field study consisted of five site-years of trials, which included two sites (S2, S3) on Walpole Island, ON in 2018, one site (S4) on Walpole Island, ON in 2019 and one site near Cottam, ON, Canada in 2018 (S1) and 2019 (S5). MHR waterhemp resistant to ALS-, PS II-, EPSPS, and PPO-inhibiting herbicides was present at all field sites (Schryver et al., 2017). The classification and description of the soil types are presented in Table 1. S2, S3 and S5 were disked in the fall and cultivated twice in the spring and S1 and S4 were disked and cultivated in the spring prior to planting. Field corn (cv. "DKC46-82RIB", Bayer CropScience, Guelph, ON) was planted at approximately 83,000 seeds ha^{-1} to a depth of 4 cm. Each trial consisted of 13 treatments arranged in a randomized complete block design with four replications. Replications included a non-treated (weedy) and weed-free control and were separated by a 2 m alley. Plots were 8 m long and 2.25 m (3 corn rows spaced 0.75 m apart) wide. The weed-free control was maintained weed-free with a preemergence application of atrazine/bicyclopyrone/mesotrione/*S*-metolachlor (2,022 g ha^{-1}) followed by atrazine/dicamba (1,800 g ha^{-1}) applied postemergence at the V3-stage (5-leaf stage) of corn development; hand-weeding was performed throughout the remainder of the growing season as needed. A postemergence application of glyphosate was applied at 450 g ha^{-1} to the entire trial area, including the nontreated control, to eliminate interference of glyphosate-susceptible waterhemp biotypes and other weed species.

Table 1. Soil characteristics and multiple-herbicide-resistant (MHR) waterhemp resistance profile of each field site where atrazine, HPPD-inhibitors and HPPD-inhibitors plus atrazine were applied postemergence in corn in Ontario, Canada in 2018 and 2019 [a]

Site	Year	Location	Soil characteristics						Resistance profile			
			Classification	Sand (%)	Silt (%)	Clay (%)	pH	OM (%) [b]	ALS- (%) [c]	PS II- (%) [d]	EPSPS- (%) [e]	PPO- (%) [f]
S1	2018	Cottam	Sandy Loam	66	24	10	6.4	2.2	84	24	88	-[a]
S2	2018	Walpole I	Loamy Sand	76	18	6	8.0	2.4	57	26	60	-
S3	2018	Walpole II	Loamy Sand	78	14	8	8.3	2.3	59	5	53	-
S4	2019	Cottam	Sandy Loam	70	21	9	6.0	2.6	68	54	64	43
S5	2019	Walpole I	Sandy Loam	70	21	9	7.6	2.3	54	30	96	17

Note. [a] Populations were not screened for resistance to PPO-inhibitors in 2018;

[b] OM: organic matter;

[c] ALS-: acetolactate synthase;

[d] PS II-: photosystem II;

[e] EPSPS-: 5-enolpyruvylshikimate-3-phosphate synthase;

[f] PPO-: protoporphyrinogen oxidase.

Herbicide treatments were applied postemergence using a CO_2-powered backpack sprayer equipped with four, 120-02 ultra low drift (ULD) nozzles (Pentair, New Brighton, MN) spaced 50 cm apart and calibrated to deliver 200 L ha^{-1} at 240 kPa. Herbicides were applied when MHR waterhemp reached an average 10 cm height or before the V6 corn growth stage. Herbicide trade names, herbicide manufacturers, recommended adjuvants, and adjuvant manufacturers are listed in Table 2. The rates of atrazine used are variable and represent the current registered (or proposed registered) rates in Canada when co-applied with the respective Group 27 herbicides.

Table 2. Herbicide active ingredient, adjuvants and manufacturers used to study atrazine, HPPD-inhibitors and HPPD-inhibitors plus atrazine applied postemergence for the control of multiple-herbicide resistant (MHR) waterhemp in Ontario, Canada in 2018 and 2019

Herbicide Active Ingredient [a]	Tradename	Adjuvant(s)	Adjuvant Rate	Herbicide Manufacturer	Adjuvant Manufacturer
Atrazine	Aatrex® Liquid 480	Assist oil concentrate	1.0 (% v/v)	Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.	BASF Canada Inc., 100 Milverton Dr., Mississauga, ON.
Isoxaflutole	Converge Flexx	-	-	Bayer CropScience Inc., 160 Quarry Park Blvd S. E., Calgary, AB.	-
Mesotrione	Callisto® 480SC	Agral 90®	0.2 (% v/v)	Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.	Syngenta Canada Inc., 140 Research Ln, Guelph, ON.
Tembotrione	Laudis™	A) Hasten™ B) 28% UAN [b]	1.75 (L ha^{-1}) 3.5 (L ha^{-1})	Bayer CropScience Inc., 160 Quarry Park Blvd S. E., Calgary, AB.	A) Victorian Chemical Company Pty. Ltd. 83 Maffra St. Coolaroo, Victoria, AUS. B) Sylvite, 3221 North Service Rd., Burlington, ON.
Tolpyralate	Shieldex™ 400SC Herbicide	A) MSO™ concentrate B) 28% UAN [b]	0.5 (% v/v) 2.5 (% v/v)	ISK Biosciences Corporation., 740 Auburn Road, Concord, OH.	A) Loveland Products, 3005 Rocky Mountain Ave., Loveland, CO. B) Sylvite, 3221 North Service Rd., Burlington, ON.
Topramezone	Armezon® Herbicide	Merge®	0.5 (% v/v)	BASF Canada Inc., 100 Milverton Drive, Mississauga, ON.	BASF Canada Inc., 100 Milverton Dr., Mississauga, ON.

Note. [a] The rates of atrazine represent the current registered rates in Canada when co-applied with the respective Group 27 herbicides;

[b] Urea Amonium Nitrate.

Data were collected on MHR waterhemp control, density and biomass, corn injury, moisture content, and grain yield. MHR waterhemp control was evaluated visually on a 0% to 100% scale at 4, 8, and 12 weeks after application (WAA); 0% represented no control and 100% represented complete plant death. MHR waterhemp density and biomass were determined at 4 WAA by counting and harvesting the plants within two randomly placed 0.25 m^2 quadrats in each plot. The aboveground biomass (g m^{-2}) of the plants within each quadrat was obtained by cutting the MHR waterhemp at the soil surface, the plants placed inside a paper bag, kiln-dried for three weeks to a consistent moisture, then weighed using an analytical balance. Corn injury was assessed visually on a scale of 0% to 100% at 1 and 4 WAA; 0% represented no injury and 100% represented complete plant death. Grain corn yield (kg ha^{-1}) and moisture (%) were collected by harvesting two middle rows of each plot at maturity using a small-plot combine. Grain yields were adjusted to 15.5% moisture prior to statistical analysis.

Data were subjected to variance analysis using the PROC GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Car, NC). Replication was considered the random effect and herbicide treatment within site was considered the fixed effect. Covariance analysis determined significant treatment by site interactions; therefore, data were partitioned by site, and an analysis of simple effects was conducted for each parameter. Normality and homogeneity of variance were tested using the Shapiro-Wilk test via the PROC UNIVARIATE procedure. Normality assumptions were confirmed by plotting the residuals for treatment, replication, and site. A normal distribution with the identity link function was used to analyze MHR waterhemp control and corn yield data. MHR waterhemp density and biomass data were analyzed using a lognormal distribution with the identity link to satisfy assumptions of variance analysis. MHR waterhemp density and biomass least-square means and standard errors were back-transformed from the log-scale using the delta method (Colby, 1967). Non-orthogonal contrasts were constructed to determine if there was a benefit of adding atrazine to each individual HPPD-inhibitor averaged across all field sites. All statistical comparisons were based on a significance level of 0.05.

3. Results and Discussion

3.1 Control of MHR Waterhemp

Control of MHR waterhemp with atrazine was variable within and across sites ranging from 28% to 96% while the standard error ranged from 1.4% to 10.3% 4, 8, and 12 WAA (Table 3). In general, atrazine provided the lowest level of MHR waterhemp control which can be attributed to confirmed Group 5 (triazine resistance) in populations across the sites (data not shown). Similarly, McMullan and Green (2011) reported 0% control of an HPPD- and PS II-inhibitor resistant waterhemp biotype with atrazine 4 WAA. In the absence of atrazine, HPPD-inhibitors provided inconsistent control of MHR waterhemp across sites that ranged from 40% to 99%

with standard error ranging from 0% to 12.3% 4, 8, and 12 WAA. The co-application of atrazine with each HPPD-inhibitor was evaluated using the simple effects ($P < 0.05$) and the herbicide treatment means for each parameter are presented in Table 3. The addition of atrazine to isoxaflutole increased control of MHR waterhemp at S1, S2, and S4 from 74% to 99%, 68% to 87%, and 40% to 51%, respectively 4 WAA. Compared to isoxaflutole applied alone, the addition of atrazine reduced variability of MHR waterhemp control at all sites, except S2 at 4 WAA, and improved control of MHR waterhemp in three of five sites at 4 and 8 WAA.

Table 3. Visible control of multiple-herbicide resistant (MHR) waterhemp 4, 8, and 12 weeks after postemergence application (WAA) with atrazine (ATR), HPPD-inhibitors and HPPD-inhibitors plus atrazine applied postemergence across five field sites in Ontario, Canada in 2018 and 2019. Treatment means of HPPD-inhibitor applied without (-) and with (+) atrazine are significantly different using Tukey's LSD ($P < 0.05$). Values in parentheses are the standard errors of herbicide treatment means. Different letters (a-d) within columns means significant differences according to Tukey's LSD ($P > 0.05$)

Tankmix partner with atrazine [a, b]	Control (%)									
	S1		S2		S3		S4		S5	
	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR
4 WAA										
None	-	75(6.5)b	-	53(6.3)b	-	70(6.8)b	-	30(6.8)d	-	86(9.0)a
Isoxaflutole	74(5.5)a	99*(0)a	68(4.3)a	87*(5.8)a	79(8.5)a	92(4.5)ab	40(7.1)b	51*(5.5)cd	95(1.9)a	99(0.5)a
Mesotrione	93(3.5)a	99(0)ab	79(6.6)a	92(5.7)a	90(4.4)a	98(1.0)ab	51(2.4)b	70*(5.4)b	92(7.3)a	99(0.3)a
Topramezone	77(8.3)a	92(7.3)ab	86(5.5)a	97(1.2)a	84(3.1)a	81(10.7)ab	65(4.6)ab	61(3.8)bc	81(9.2)a	97(0.9)a
Tembotrione	93(6.0)a	99(0)a	89(5.4)a	99*(0)a	91(4.1)a	99*(0)a	76(3.1)a	88*(4.8)a	99(0.4)a	99(1.3)a
Tolpyralate	92(7.3)a	97(2.3)ab	91(5.4)a	98*(1.0)a	95(1.8)a	97(1.2)ab	73(4.3)a	66(3.1)b	98(1.3)a	100(0)a
8 WAA										
None	0	80(6.1)b	0	64(6.3)b	0	80(5.4)b	0	29(2.4)d	0	95(3.4)a
Isoxaflutole	68(6.6)b	99*(0)a	85(2.0)a	98*(1.0)a	90(5.9)a	97*(1.2)a	40(7.4)b	53(4.3)c	99(1.2)a	100(0)a
Mesotrione	97(2.3)a	97(2.3)ab	96(1.0)a	97(2.3)a	97(1.2)a	99(0)a	54(2.4)b	81*(3.8)b	98(1.1)a	99(0.3)a
Topramezone	65(11.7)ab	95*(3.3)ab	94(2.2)a	98(1.0)a	95(1.8)a	88(9.5)ab	65(8.7)ab	66(5.5)bc	93(3.5)a	99(0.3)a
Tembotrione	92(7.3)ab	99(0)a	98(1.0)a	99(0)a	95(1.8)a	99*(0)a	91(2.4)a	96(2.1)a	99(0.3)a	99(0.3)a
Tolpyralate	77(10.9)ab	97*(2.3)ab	97(1.2)a	99(0)a	97(1.2)a	98(1.0)a	86(3.1)a	73(6.3)b	99(1.3)a	100(0)a
12 WAA										
None	0	79(9.0)a	0	64(10.3)a	0	74(3.8)a	0	28(1.4)d	0	96(2.3)a
Isoxaflutole	68(6.0)ab	99*(0)a	90(2.9)a	99(0)a	92(5.7)a	97(1.2)a	44(9.7)b	58(3.2)c	99(1.3)a	99(0.3)a
Mesotrione	92(5.7)a	92(4.5)a	97(2.3)a	97(1.2)a	94(2.2)a	98(1.0)a	56(3.1)b	85*(4.6)ab	99(0.3)a	99(0.3)a
Topramezone	58(8.5)b	89*(6.3)a	96(2.1)a	98(1.0)a	94(2.2)a	87(9.1)a	69(8.0)ab	70(5.4)bc	95(2.6)a	99(0.3)a
Tembotrione	87(12.3)ab	98(1.0)a	97(2.3)a	98(1.0)a	98(1.0)a	99(0)a	93(1.4)a	98(1.0)a	100(0)a	100(0)a
Tolpyralate	71(12.3)ab	93*(4.5)a	99(0)a	99(0)a	98(1.0)a	99(0)a	90(3.1)a	75(5.4)b	99(0.3)a	100(0)a

Note. [a] Herbicide treatment application rates were based on current Ontario herbicide labels and were atrazine (1063); isoxaflutole (105); atrazine + isoxaflutole (1063 + 105); mesotrione (100); atrazine + mesotrione (280 + 100); topramezone (12.5); atrazine + topramezone (500 + 12.5); tembotrione (90); atrazine + tembotrione (1000 + 90); tolpyralate (30); and atrazine + tolpyralate (560 + 30 g ai ha^{-1});

[b] Herbicide treatments with mesotrione included Agral® 90 (0.2% v/v); with topramezone included MERGE® (0.5% v/v); with tembotrione included Hasten™ (1.75 L ha^{-1}) and urea ammonium nitrate (UAN 28-0-0) (3.5 L ha^{-1}); and with tolpyralate included MSO™ concentrate (0.5% v/v) and UAN (2.5% v/v).

Mesotrione controlled MHR waterhemp 51% to 99%; the co-application with atrazine improved control to 70% to 99% across all sites 4, 8, and 12 WAA (Table 3). Tank mixing atrazine with mesotrione improved control 29% at S4 despite a 3.0% increase in the standard error. Woodyard et al. (2009) reported similar variability with mesotrione alone providing 48% to 62% waterhemp control 30 days after application which increased to 95% to 99% with the addition of atrazine. Vyn (2006) reported the addition of atrazine to mesotrione increased control of MHR waterhemp from 90% to 99% and 93% to 99% at 4 and 7 WAA, respectively. In this study, the addition of atrazine to mesotrione increased MHR waterhemp control at 4, 8, and 12 WAA at S4 (Tables 3). Mesotrione exhibits excellent activity on pigweed species which explains why MHR waterhemp control increased numerically from the addition atrazine at the remaining four sites in this study; however, these increases were not statistically significant (Hugie et al., 2008; Vyn et al., 2006; Woodyard et al., 2009). Control of MHR waterhemp with topramezone varied considerably across sites, and spatially within each site, with control ranging from 58% to 96% with standard errors between 1.8% and 11.7% at 4, 8, and 12 WAA. The addition of atrazine improved MHR waterhemp control with topramezone by 30% and 31% and reduced standard error by 8.4% and 2.2% at 8 and 12 WAA, respectively at S1 (Tables 3). This is consistent with Khort and Sprague (2017) who reported 20% greater control of 8 cm Palmer amaranth 3 WAA with atrazine + topramezone (560 + 18 g ha^{-1}) compared to topramezone alone.

Across all sites, tembotrione alone provided 76% to 99% control with standard errors ranging from 0% to 12.3% at 4, 8, and 12 WAA (Table 3). Except for S4 at 4 WAA, atrazine + tembotrione provided ≥ 96% control of MHR waterhemp 4, 8, and 12 WAA. The addition of atrazine to tembotrione provided a greater and more consistent level of MHR waterhemp control at various sites 4 and 8 WAA; however, at 12 WAA, there was no benefit of adding atrazine to tembotrione. These results are consistent with Benoit (2019b) who reported atrazine + tembotrione (1000 + 90 g ha^{-1}) applied postemergence provided greater than 97% control of MHR waterhemp 4, 8, and 12 WAA. Similarly, Control of Palmer amaranth biotypes from Louisiana and Mississippi increased from 92% to 97% with the addition of atrazine (2,240 g ha^{-1}) to tembotrione (92 g ha^{-1}) 4 WAA (Stephenson et al., 2015). Williams (2011) also reported a 15% increase in redroot pigweed control, and 17% decrease in the standard error, 2 WAA with the addition of atrazine (370 g ha^{-1}) to tembotrione (31 g ha^{-1}) applied postemergence.

Control of MHR waterhemp with tolpyralate and atrazine + tolpyralate ranged from 73% to 98% and 66% to 100% across all sites 4 WAA, respectively. The addition of atrazine to tolpyralate increased MHR waterhemp control at S1 by 22% and reduced standard error 7.8% at 12 WAA. These results are consistent with Benoit (2019b) who reported greater than 94% control of MHR waterhemp with atrazine + tolpyralate (1000 + 30 g ha^{-1}) 4, 8, and 12 WAA. Similarly, Osipitan (2018) reported a reduction in the calculated effective dose for 90% waterhemp control 4 WAA from 28 to 12 g ha^{-1} from the addition of atrazine (560 g ha^{-1}) to tolpyralate (30 g ha^{-1}). In contrast, the addition of atrazine numerically decreased control of MHR waterhemp with tolpyralate at 4, 8, and 12 WAA at S4; however, this decrease was not statistically significant. We attribute these decreases to experimental variability and do not consider them true treatment effects.

Averaged across herbicide treatments and sites, the addition of atrazine improved control of MHR waterhemp with HPPD-inhibitors by 6% to 8% at 4, 8 or 12 WAA, respectively ($P < 0.0001$; contrasts not shown). When averaged across sites, the addition of atrazine to isoxaflutole, mesotrione, topramezone and tembotrione improved MHR waterhemp control from 71% to 86% ($P < 0.0001$), 81% to 92% ($P = 0.0014$), 79% to 86% ($P = 0.0339$), and 90% to 97% ($P = 0.0339$), respectively, at 4 WAA (contrasts not shown).

3.2 Density and Biomass of MHR Waterhemp

MHR waterhemp density and biomass varied across sites ranging from 52 to 760 plants m^{-2} and 19.2 to 203.9 g m^{-2}, respectively, in nontreated control plots (Table 4). Compared to the nontreated control, atrazine reduced MHR waterhemp density and biomass by 44% to 98% and 68% to 99%, with standard errors ranging from 1.7 to 251.4 plants m^{-2} and 1.11 to 31.33 g m^{-2}, respectively (Table 4). In general, the addition of atrazine to the HPPD-inhibitors resulted in MHR waterhemp density and biomass reductions ≥ 94% and reduced standard error ≤ 2.5% in all sites except S4. At S4, the addition of atrazine to topramezone and tolpyralate resulted in numerical increases in MHR waterhemp density and biomass; we attribute these increases to spatial field variability, by chance, and are not considered true herbicide treatment effects. Averaged across herbicide treatments and sites, the addition of atrazine to HPPD-inhibitors reduced MHR waterhemp density and biomass by 4 plants m^{-2} ($P = 0.0123$) and 1.2 g m^{-2} ($P < 0.0001$), respectively (contrasts not shown). When averaged across sites, the addition of atrazine to topramezone, mesotrione, and tolpyralate reduced MHR waterhemp density by 12 ($P < 0.0001$), 5 ($P = 0.0677$) and 1 ($P = 0.0349$) plant m^{-2} (contrast not shown), respectively. Similarly, MHR biomass was reduced by 2.5 g m^{-2} with the addition of atrazine to topramezone ($P < 0.0001$).

Table 4. Density and biomass of multiple-herbicide resistant (MHR) waterhemp 4 weeks after postemergence application (WAA) and corn grain yield with atrazine (ATR), HPPD-inhibitors and HPPD-inhibitors plus atrazine applied postemergence across five field sites in Ontario, Canada in 2018 and 2019. Treatment means of HPPD-inhibitor applied without (-) and with (+) atrazine are significantly different using Tukey's LSD (P < 0.05). Values in parentheses are the standard errors of herbicide treatment means. Different letters (a-c) within columns means significant differences according to Tukey's LSD (P > 0.05)

Tankmix partner with atrazine [a, b]	S1		S2		S3		S4		S5	
	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR	-ATR	+ATR
Density (plants ha^{-1})										
Non-treated control[c]	287 (175.0)a	287 (182.9)a	52 (16.1)a	52 (16.1)a	147 (45.2)a	147 (45.1)a	760 (462.6)a	760 (483.5)a	66 (20.3)a	66 (20.2)a
None	-	5(3.1)b	-	20(8.0)a	-	9(3.6)b	-	424 (251.4)a	-	4(1.7)b
Isoxaflutole	21(7.1)b	0*(0)b	13(7.4)ab	1*(0.5)b	3(1.6)b	1(0.2)bc	175 (60.1)ab	240 (47.0)a	1(0.5)b	0(0)b
Mesotrione	1(0.3)c	0(0)b	8(4.5)ab	3(2.5)ab	2(1.3)b	0(0)bc	596 (231.2)a	42* (34.4)ab	0(0)b	1(1.0)b
Topramezone	57(38.5)ab	1*(0.3)b	3(2.2)ab	0(0)b	5(3.8)b	3(2.0)bc	111 (74.8)ab	258 (117.8)a	9(6.9)ab	1(0.7)b
Tembotrione	0(0)c	0(0)b	1(0.4)b	0(0)b	1(0.5)b	0(0)c	55(5.5)b	27(9.7)b	1(0.3)b	1(0.1)b
Tolpyralate	2(1.3)bc	0(0)b	1(0.3)b	0(0)b	1(0.4)b	2(0.9)bc	103 (76.3)ab	177 (43.6)a	1(0.4)b	0(0)b
Biomass (g ha^{-1})										
Non-treated control[c]	70.3 (23.36)a	70.3 (28.61)a	62.6 (15.56)a	62.6 (15.56)a	142.2 (35.36)a	142.2 (35.36)a	203.9 (67.80)a	203.9 (83.03)a	19.2 (4.77)a	19.2 (4.77)a
None	-	3.0(2.20)b	-	20.3 (11.01)a	-	2.1 (1.11)b		43.1 (31.33)abc	-	2.3 (1.23)ab
Isoxaflutole	4.0(1.38)b	0*(0)b	6.6 (1.73)b	0.9* (0.22)b	1.2 (0.32)b	0.1 (0.03)bc	44.4 (15.12)abc	39.4 (10.29)b	0.2 (0.05)b	0(0)c
Mesotrione	0(0)d	0(0)b	3.5 (1.06)bc	0.6* (0.23)bc	1.1 (0.33)b	0(0)c	60.4 (5.98)b	8.4* (4.13)bc	0(0)b	0.6 (0.21)bc
Topramezone	6.4 (3.28)bc	1.0(0.56)b	2.6 (1.38)bc	0.3 (0.13)bc	0.4 (0.22)b	1.2 (0.50)bc	19.5 (9.9)bcd	28.8 (15.92)ab	4.7 (2.46)ab	0.3* (0.12)bc
Tembotrione	0.2(0.03)cd	0(0)b	0.5(0.12)c	0(0)c	0.2(0.04)b	0(0)c	4.3(0.66)d	2.1*(0.51)c	0(0.01)b	0()c
Tolpyralate	0.5 (0.16)bcd	0(0)b	0.4(0.06)c	0(0)c	0(0.01)b	0(0)c	9.5 (3.18)cd	18.8 (4.84)b	0(0.01)b	0(0)c
Corn yield (kg ha^{-1})										
Weed-free control§	10,500 (260)a	10,500 (260)a	11,200 (1,140)a	11,200 (1,140)a	11,800 (670)a	11,800 (670)a	10,400 (310)a	10,400 (310)a	7,000 (390)a	7,000 (390)a
Nontreated control[c]	9,400 (1,790)ab	9,400 (1,790)a	8,800 (2,170)a	8,800 (2,170)a	11,000 (620)a	11,000 (620)a	7,100 (380)ab	7,100 (380)ab	6,100 (610)a	6,100 (610)a
None	-	8,900 (590)a	-	10,300 (880)a	-	12,600 (440)a	-	7,800 (250)b	-	6,300 (430)a
Isoxaflutole	7,100 (1,180)b	10,600* (350)a	10,200 (1,660)a	12,500* (600)a	12,300 (920)a	11,700 (1,140)a	7,800 (460)ab	8,900 (230)b	6,300 (410)a	7,000 (90)a
Mesotrione	9,600 (610)ab	9,800 (380)a	10,300 (1,700)a	11,200 (920)a	12,600 (950)a	12,700 (350)a	8,200 (170)b	9,200 (310)ab	6,300 (200)a	6,600 (620)a
Topramezone	7,900 (1,070)ab	9,500 (790)a	11,000 (1,510)a	10,500 (1,680)a	12,900 (300)a	13,200 (320)a	8,300 (260)ab	8,700 (570)ab	6,800 (360)a	6,400 (410)a
Tembotrione	10,800 (670)ab	9,400 (670)a	12,400 (660)a	10,400 (1,320)a	11,600 (540)a	13,100 (280)a	8,800 (390)ab	9,200 (340)ab	5,800 (350)a	6,600 (300)a
Tolpyralate	8,800 (1,100)ab	10,600 (300)a	11,400 (1,380)a	8,500* (1,380)a	12,200 (640)a	12,300 (680)a	9,100 (630)ab	8,800 (270)b	6,000 (90)a	6,300 (130)a

Note. [a] Herbicide treatment application rates were based on current Ontario herbicide labels and were atrazine (1063); isoxaflutole (105); atrazine + isoxaflutole (1063 + 105); mesotrione (100); atrazine + mesotrione (280 + 100); topramezone (12.5); atrazine + topramezone (500 + 12.5); tembotrione (90); atrazine + tembotrione (1000 + 90); tolpyralate (30); and atrazine + tolpyralate (560 + 30 g ha^{-1});

[b] Herbicide treatments with mesotrione included Agral® 90 (0.2% v/v); with topramezone included Merge® (0.5% v/v); with tembotrione included Hasten™ (1.75 L ha^{-1}) and urea ammonium nitrate (UAN 28-0-0) (3.5 L ha^{-1}); and with tolpyralate included MSO™ concentrate (0.5% v/v) and UAN (2.5% v/v);

[c] Non-treated control did not receive herbicide treatment and was not subject to – and + ATR treatments.

3.3 Corn Injury and Yield

Corn injury was specific to herbicide treatment and site (data not shown). White bleaching of plant foliage, characteristic HPPD-inhibitor injury (Abendroth et al., 2006), was observed with isoxaflutole and atrazine + isoxaflutole, which caused 10% and 9% corn injury at S1, respectively. Similar symptomology was observed at S5, tembotrione caused 10% corn injury at 1 WAA, injury was reduced to 5% at 4 WAA. All other herbicide treatments caused ≤ 2% injury at 1 and 4 WAA. Previous studies have shown good corn tolerance to HPPD-inhibitors (Mitchell et al., 2001; Williams et al., 2011; Woodyard et al., 2009). Corn damage was caused by wildlife at S5 where the removal of corn ears occurred following pollination; therefore, these data were not included in the corn grain yield analysis (data not shown). Corn grain yield varied between herbicide treatment and site. Between sites, corn grain yield from the weed-free control plots ranged from 7,000 to 11,800 kg ha^{-1} with standard errors ranging from 260 to 1,140 kg ha^{-1} (Table 4). When MHR waterhemp was left uncontrolled, corn grain yield ranged from 6,100 to 11,000 kg ha^{-1} between sites while standard error ranged from 380 to 2,170 kg ha^{-1}. MHR waterhemp interference with atrazine alone at S4 reduced corn grain yield 2,600 kg ha^{-1}; greater corn yield loss can be attributed to greater MHR waterhemp density at this site and poorer weed control by this treatment. The addition of atrazine to tolpyralate reduced corn grain yield by 2,900 kg ha^{-1} at S2; we attribute this decrease to field variability and is not considered a true treatment effect. Corn grain yield averaged across herbicide treatments and sites did not increase from the addition of atrazine to HPPD-inhibitors ($P > 0.05$). The inability to detect yield differences among treatments could be due to the minimal effect of later emerging MHR waterhemp at low densities on corn grain yield (Cordes et al., 2004; Steckel et al., 2003; Wu & Owen, 2014). Waterhemp seedlings germinating after the postemergence application are less able to compete with the established crop. When averaged across sites, isoxaflutole + atrazine and mesotrione + atrazine resulted in corn grain yields 1,500 and 500 kg ha^{-1} greater than isoxaflutole and mesotrione alone ($P < 0.05$), respectively. In general, HPPD-inhibitors applied with atrazine provided greater and more consistent control of MHR waterhemp and provided greater reductions in density and biomass than atrazine alone; however, this did not translate into a yield response. Steckel and Sprague (2004) showed that waterhemp emerging later in the growing season, between V8 and V12 corn, produce as few as 0 seeds $plant^{-1}$. While the addition of atrazine to HPPD-inhibitors did not directly influence corn grain yield, more consistent MHR waterhemp control with HPPD-inhibitors may reduce in-season escapes and the return of weed seed to the soil seed bank (Swanton et al., 1999). Reducing weed seed return of this prolific and competitive weed species may also warrant the co-application of atrazine HPPD-inhibitors in corn production to limit resistance evolution.

In general, the co-application of atrazine with HPPD-inhibitors applied postemergence improved the consistency and control of MHR waterhemp compared with HPPD-inhibitors applied alone. Averaged across treatments and at four of five sites, HPPD-inhibitors plus atrazine provided 81% to 99% control of MHR waterhemp and reduced standard errors by 13% to 100% at 4, 8, and 12 WAA. At S4, MHR waterhemp control varied from 51% to 98% because of extremely high waterhemp density up to 760 plants m^{-2} and biomass up to 204 g m^{-2} compared to other sites. Averaged across sites, the co-application of atrazine with isoxaflutole, mesotrione, topramezone, or tembotrione improved MHR waterhemp control by 15%, 11%, 7% and 7%, respectively, at 4 WAA. Across treatments, there were few statistical differences between MHR waterhemp control, density, biomass, and corn grain yield. Despite the increase in MHR waterhemp control with the addition of atrazine, there was no effect on corn grain yield. In this study the benefit of the addition of the co-application of atrazine with HPPD-inhibitors is best realized through greater and more consistent MHR waterhemp control. The use of atrazine also broadens the spectrum of weed control and reduces weed seed return (Swanton et al., 2007). The use of HPPD-inhibitors in combination with other PS II-inhibitors, such as bromoxynil and metribuzin, has been reported (Abendroth et al., 2006; Hugie et al., 2008). Moreover, the combination of HPPD-inhibitors with other MOA increases the spectrum of weed control and delays the evolution of herbicide resistance, which will allow future use of currently effective MOA. The occurrence of MHR waterhemp resistant to both HPPD-inhibitors and atrazine (Jacobs et al., 2020; McMullan & Green, 2011) as well as Palmer amaranth Jhala et al. (2014) suggests these herbicides should be used strategically, and growers should strive for 100% control. The ability of MHR waterhemp to escape herbicide applications, germinate late in the growing season, produce a plethora of viable offspring and evolve resistance to multiple herbicide MOA necessitates full-season control programs. The use of HPPD-inhibitors plus atrazine may provide greater and more consistent control of MHR waterhemp in Ontario; however, cultural practices such as a diverse crop rotation, optimizing crop density, reducing crop row spacing, herbicide rotation, tank-mixing multiple, effective MOA, and planting cover crops would also help delay the evolution of herbicide resistance in weeds.

4. Conclusion

Results from this study indicate that the co-application of atrazine with HPPD-inhibitors applied postemergence are effective herbicide tank mixtures that reduced the risk of herbicide failure and resulted in greater and more consistent control of MHR waterhemp, including Group 5 resistance, control in Ontario corn.

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