Early Postemergence Herbicide Tank-Mixtures for Control of Waterhemp Resistant to Four Herbicide Modes of Action in Corn

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How to cite this paper: Willemse, C., Soltani, N., Benoit, L., Jhala, A.J., Hooker, D.C., Robinson, D.E. and Sikkema, P.H. (2021) Early Postemergence Herbicide Tank-Mixtures for Control of Waterhemp Resistant to Four Herbicide Modes of Action in Corn. *Agricultural Sciences*, **12**, 354-369.
https://doi.org/10.4236/as.2021.124023

Received: February 13, 2021
Accepted: April 6, 2021
Published: April 9, 2021

Abstract

Multiple-herbicide-resistant (MHR) waterhemp has been confirmed and is difficult to control for growers in Ontario, Canada and in the Midwestern United States. The objective of this study was to evaluate early post-emergence (EPOST) herbicides for control of MHR waterhemp in field corn. Five field trials were conducted over a two-year period (2019, 2020) at sites on Walpole Island, ON and near Cottam, ON, Canada. Thirteen herbicide tank-mixtures containing multiple modes-of-action (MOA) were applied EPOST to 5 cm MHR waterhemp in field corn. Control of MHR waterhemp varied by site due to variable plant density, plant biomass, and number of herbicide-resistant individuals across research sites and years. Control of MHR waterhemp ranged from 90% to 100% with glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone/atrazine, glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine, glyphosate + *S*-metolachlor/atrazine/mesotrione, glyphosate + mesotrione + atrazine, glyphosate/*S*-metolachlor/mesotrione + atrazine, glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone, glyphosate/2,4-D choline + rimsulfuron + mesotrione, and glyphosate + pyroxasulfone + dicamba/atrazine at 4, 8, and 12 WAA. Control of MHR waterhemp ranged from 70% to 100% with glyphosate + topramezone/dimethenamid-P + dicamba/atrazine, glyphosate + isoxaflutole + atrazine, and glyphosate + tolpyralate + atrazine at 4, 8, and 12 WAA. Control of MHR waterhemp was similar for all herbicide programs, except glyphosate + dicamba/atrazine and glyphosate + *S*-metolachlor/atrazine which resulted in the lowest control at three of five sites that ranged from 63% to 89% and 61% to 76%, respectively. Crop injury was ≤10% for herbicide programs tested, except 28% to 31% corn injury with glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine; however, without effect on corn grain yield. Corn yield was comparable with all herbicide programs evaluated in this study. It is concluded that there are herbicide programs that provide control of emerged and full-season residual control of MHR waterhemp in field corn.

Keywords

Density, Biomass, Residual Weed Control, Weed Management

1. Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] has become one of the most problematic weed species in midwestern United States crop production. Reductions in tillage, greater reliance on herbicides for weed management, and the evolution of resistance to multiple herbicide modes of action (MOA) have contributed to the rapid increase of waterhemp in agricultural cropping systems [1] [2]. Waterhemp has been reported in 19 states of the USA and three provinces in Canada where it interferes with corn and soybean production [3] [4]. The rapid movement of waterhemp and evolution of herbicide resistance among individuals and populations is facilitated by its dioecism, rapid growth rate, high reproductive rate, delayed emergence, and extended emergence pattern [5] [6]. Resistance to photosystem II (PS II)-, acetolactate synthase (ALS)-, and protoporphyrinogen oxidase (PPO)-inhibiting herbicides was identified in waterhemp in 1990, 1993, and 2001, respectively [4] [7] [8] [9]. Waterhemp resistant to 5-enolpyruyl shikimate-3-phosphate synthase (EPSPS)-inhibitors was first reported in the USA in 2005 and Ontario, Canada in 2014 [4]. More recent reports from Ontario have identified multiple-herbicide-resistant (MHR) waterhemp populations resistant to ALS-, PS II-, EPSPS-, and PPO-inhibiting herbicides. Waterhemp continues to evolve resistance to currently used MOA and is the first weed species to develop resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides [4] [7] [8] [9]. The first MHR waterhemp population with six-way resistance to synthetic auxins and ALS-, PS II-, EPSPS-, PPO-, and HPPD-inhibiting herbicides was identified in Missouri in 2015 [10]. Resistance to very-long-chain fatty-acid (VLCFA)-inhibiting herbicides has since been detected within a MHR waterhemp population resistant to 2,4-D and ALS-, PS II-, PPO-, and HPPD-inhibiting herbicides [11]. The ability of MHR waterhemp to rapidly evolve and accumulate traits that confer resistance to multiple MOA makes it difficult to manage in agricultural cropping systems [12].

Weed interference must be prevented during the early stages of corn (*Zea mays* L.) growth and development to prevent yield loss [13]. The relative time of crop and weed emergence has a greater effect on corn yield than weed density and biomass [13]. Weeds that emerge with the crop have the greatest impact on corn yield [13]. Steckel and Sprague [2] reported MHR waterhemp emerging at

VE corn growth stage reduced grain yield 74% compared to only 2% yield loss when waterhemp emerged at V8 and was left uncontrolled for the remainder of the growing season. In contrast, Cordes *et al.* [14] reported corn yield loss was dependent on waterhemp density. When competing with corn, waterhemp can be placed at a disadvantage due to its characteristic late emergence; however, corn yield losses of up to 17% have been reported when densities of 369 to 445 plants m^{-2} emerge and compete up to V7 corn growth stage [14] [15]. Steckel and Sprague [2] reported corn yield reductions when waterhemp emerged before the V8 corn growth stage. In Ontario, corn yield losses of up to 48% have been reported when waterhemp populations are left uncontrolled [16]. The critical period of weed control in corn to prevent yield loss varies with the relative time of weed and crop emergence, weed density, species, and environment [17] [18]. It is recommended that corn remain waterhemp-free from emergence to V6 to maximize grain yield [2]. Early-season control of MHR waterhemp is imperative to reduce early-season weed interference, prevent corn yield loss, and reduce weed escapes.

Current herbicide-based MHR waterhemp management strategies include preemergence (PRE), postemergence (POST), and PRE followed-by (*fb*) POST herbicide applications that utilize multiple effective MOA [16] [19] [20] [21]. The HPPD-inhibitors isoxaflutole, mesotrione, and tolpyralate are often applied in combination with a PS II-inhibitor such as atrazine and result in excellent control of MHR waterhemp [22] [23] [24] [25] [26]. Complementary activity between HPPD-inhibitors and atrazine has been reported for the control of triazine-susceptible and triazine-resistant redroot pigweed (*Amaranthus retroflexus* L.), waterhemp, and Palmer amaranth (*Amaranthus palmeri* S. Watson) [25] [26] [27]. HPPD-inhibitors inhibit the production of carotenoids, α-tocopherols, and plastoquinone, and atrazine increases the production of reactive oxygen species [28] [29]. The enhanced weed control efficacy when a HPPD-inhibitor is co-applied with a PS II-inhibitor is due to 1) increased binding efficiency of atrazine to the D1 protein of PS II-inhibitor caused by the shortage of plastoquinone, and 2) enhanced reactive oxygen species (ROS) levels due to the lack of quenching carotenoids, tocopherols, and plastoquinone. Synthetic auxin herbicides are another effective MOA for MHR waterhemp control; however, current literature reports variable responses [16] [30] [31]. Synthetic auxin herbicides provide control of broadleaf weeds by mimicking plant growth hormones which causes unregulated plant growth and death in some plants [32]. Superior MHR waterhemp control with dicamba/atrazine compared to other POST tank-mixtures has been reported [33]. Anderson *et al.* [30], Soltani *et al.* [16] and Vyn *et al.* [31] reported dicamba/atrazine provided ≥86% control of herbicide-resistant waterhemp. Benoit *et al.* [33] and Schryver *et al.* [34] found that POST applications of dicamba are more effective than PRE applications. The application of a new glyphosate/2,4-D choline formulation registered for application to ENLIST™ (Corteva Agriscience, Wilmington, DE) corn hybrids allows for a second synthetic auxin herbicide for MHR waterhemp control in corn [34] [35] [36]. ENLIST™ corn hybrids contain transgenes that confer resistance to glyphosate and glufosinate plus the aryloxyalkanoate dioxygenase-1 (AAD-1) transgene which enables them to exhibit resistance to glyphosate, glufosinate, and greater tolerance to 2,4-D and the arlyloxyphenoxy propionates than traditional glyphosate (RoundupReady®) (Bayer CropScience Inc., 160 Quarry Park Boulevard SE, Calgary, AB) and glufosinate (LibertyLink®) (BASF Canada Inc., 100 Milverton Drive, Mississauga, ON) resistant hybrids [35] [37] [38]. Robinson *et al.* [36] reported up to 94% control of common waterhemp (*Amaranthus rudis* Sauer) with 2,4-D (1120 g ae) and 99% control with 2,4-D + glyphosate (280 + 840 g ae); however, it is important to note glyphosate (840 g ae) alone provided 100% control 4 WAA in that study. Similarly, Miller and Norsworthy [39] obtained ≥87% control of glyphosate-resistant Palmer amaranth in soybean. Numerous PRE, POST, and PRE *fb* POST herbicide applications in corn have been developed for the control of MHR waterhemp with varying resistance profiles [3] [19] [31] [33] [40] [41].

Waterhemp control is affected by herbicide application timing. Previous studies have shown that PRE applications can control MHR waterhemp better than POST applications [33] [42] [43]. Hager *et al.* [44] reported 8% to 9% greater control of ALS-resistant-waterhemp 1, 2, and 3 WAA when herbicides were applied to 5 cm waterhemp early POST (EPOST) compared to 10 cm waterhemp (POST) in soybean. Similarly, Hedges *et al.* [43] observed a 20% reduction in waterhemp control as POST applications were delayed from 5 to 25 cm tall waterhemp. These studies suggest differences between EPOST and POST can be attributed to slower herbicide activity on larger waterhemp plants and reduced interception due to shading of younger plants caused by the extended emergence pattern [33] [42] [43]. Corn producers should eliminate MHR waterhemp interference from VE to V6 corn growth stage and control it before it exceeds 10 cm in height [2] [44] [45].

Delayed POST herbicide applications can result in reduced control due to larger weed size at application and decreased corn yield due to early-season waterhemp interference. To achieve season-long control of MHR waterhemp, it is imperative that herbicide applications include effective MOA, provide soil residual and target small weed size (≤10 cm). We hypothesized that EPOST herbicide tank mixtures made to 5 cm MHR waterhemp will provide season-long control of MHR waterhemp in corn. The objective of this research was to identify effective EPOST herbicide tank-mixtures that provide control of emerged MHR waterhemp and season-long residual control in corn while stewarding currently available herbicide MOA.

2. Materials and Methods

2.1. Experimental Methods

Five field trials were conducted over a two-year period (2019, 2020) at sites on

Walpole Island, ON (42.561492˚N, −82.501487˚W) and near Cottam, ON, Canada (42.149076˚N, −82.683687˚W) with MHR waterhemp resistant to ALS-, PS II-, EPSPS- and PPO-inhibiting herbicides (Table 1). Sites were disked or cultivated in the spring to prepare the seedbed for planting. Glyphosate- and glufosinate-resistant corn hybrid DKC45-65RIB (Monsanto, St. Louis, MO) was seeded in rows spaced 0.75 m apart at approximately 83,000 seeds ha^{-1} to a depth of 4 cm. Plots were 8 m long and 2.25 m (3 corn rows) wide. Fifteen herbicide treatments (Table 2) were arranged in a randomized complete block design with four replications. Replications included nontreated and weed-free controls and were separated by a 2 m alley. The weed-free control was maintained weed-free with a pre-emergence (PRE) application of atrazine/bicyclopyrone/mesotrione/*S*-metolachlor (2022 $g·ha^{-1}$) followed by either atrazine/dicamba (1800 $g·ha^{-1}$) applied postemergence (POST) up to V3-stage (5-leaf stage) of corn development, or glufosinate (500 $g·ha^{-1}$) between V3 and V6; hand-weeding was performed throughout the remainder of the growing season as needed. Glyphosate (450 g ae ha^{-1}) was applied POST to the entire experimental area, including the nontreated control, to remove susceptible waterhemp biotypes and other weed species.

Herbicide treatments were applied EPOST 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. All herbicide treatments were applied when MHR waterhemp reached an average 5 cm in height. Site 1 (S1) and S3 was separated temporally by applying herbicide treatments 5 days apart.

Data were collected on MHR waterhemp control estimates, density, biomass, visible corn injury, grain corn moisture content, and grain corn yield. Waterhemp control was evaluated visually on a 0% to 100% scale compared to the nontreated control at 4, 8, and 12 WAA. 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 of the plants within each quadrat was determined by cutting the MHR waterhemp at

Table 1. Soil characteristics and resistance profile of each field site where herbicide tank-mixtures were applied EPOST for control of multiple-herbicide resistant (MHR) waterhemp in Ontario, Canada in 2019 and 2020.

			Soil characteristics						Resistance profile[a]			
Site	Year	Location	Classification	Sand (%)	Silt (%)	Clay (%)	pH	OM (%)	ALS (%)	PS II (%)	EPSPS (%)	PPO (%)
S1	2019	Cottam	Sandy Loam	70	21	9	6.0	2.6	97	34	N/A	N/A
S2	2019	Walpole	Sandy Loam	70	21	9	7.6	2.3	23	6	79	N/A
S3	2019	Cottam	Sandy Loam	70	21	9	6.0	2.6	97	34	N/A	N/A
S4	2020	Cottam	Sandy Loam	70	19	11	5.9	2.6	68	54	64	43
S5	2020	Walpole	Sandy Loam	76	15	9	7.8	2.5	54	30	96	17

Abbreviations: ALS, acetolactate synthase; OM, organic matter; PS II, photosystem II; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; PPO, protoporphyrinogen oxidase. [a]Mean number of surviving waterhemp plants three weeks after application divided by the number of plants sprayed within quadrats per mode of action per site.

Table 2. Herbicide treatments, products, rates, and manufacturers for the study of herbicide tank-mixtures applied EPOST for the control of multiple-herbicide resistant (MHR) waterhemp in Ontario, Canada in 2019 and 2020.

Herbicide treatment	Herbicide trade name	Rate (g ae or ai ha^{-1})	Herbicide manufacturer[b]
Glyphosate + *S*-metolachlor/atrazine	Roundup WeatherMAX® + Primextra® II Magnum®	900 + 1600/1280	Bayer CropScience/Syngenta Canada
Glyphosate + dicamba/atrazine	Roundup WeatherMAX® + Marksman® Herbicide	900 + 504/996	Bayer CropScience/BASF Canada.
Glyphosate + tolpyralate + atrazine	Roundup WeatherMAX® + Shieldex™ 400 SC Herbicide + Aatrex®	900 + 40 + 1120	Bayer CropScience/ISK Biosciences
Glyphosate + isoxaflutole + atrazine	Roundup WeatherMAX® + Converge Flexx Herbicide + Aatrex® Liquid 480	900 + 105 + 1063	Bayer CropScience/Bayer CropScience/Syngenta Canada
Glyphosate + topramezone/ dimethenamid-P + dicamba/atrazine	Roundup WeatherMAX® + Armezon® PRO + Marksman® Herbicide	900 + 12.5/630 + 488/966	Bayer CropScience/BASF Canada/BASF Canada
Glyphosate + pyroxasulfone + dicamba/atrazine	Roundup WeatherMAX® + Zidua™ SC + Marksman® Herbicide	900 + 150 + 488/966	Bayer CropScience/BASF Canada/BASF Canada
Glyphosate/2,4-D choline + rimsulfuron + mesotrione	Roundup WeatherMAX® + Enlist Duo™ Herbicide + Matrix® SG + Callisto® 480SC Herbicide	563/591 + 15 + 144	Bayer CropScience/Corteva Agriscience/Corteva Agriscience/Syngenta Canada
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone	Roundup WeatherMAX® + Acuron® Flexi	900 + 1268/141/35	Bayer CropScience/Syngenta Canada
Glyphosate/*S*-metolachlor/mesotrione + atrazine	Roundup WeatherMAX® + Halex® GT Herbicide	1050/1050/105 + 280	Bayer CropScience/Syngenta Canada
Glyphosate + mesotrione + atrazine	Roundup WeatherMAX® + Callisto® 480SC Herbicide + Aatrex® Liquid 480	900 + 100 +280	Bayer CropScience/Syngenta Canada/Syngenta Canada
Glyphosate + *S*-metolachlor/atrazine/mesotrione	Roundup WeatherMAX® + Lumax® EZ Herbicide	900 + 1393/524/139	Bayer CropScience/Syngenta Canada
Glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine	Roundup WeatherMAX® + Enlist Duo™ Herbicide + Matrix® SG + Callisto® 480SC Herbicide + Aatrex® Liquid 480	563/591 + 15 + 144 + 1008	Bayer CropScience/Corteva Agriscience/Corteva Agriscience/Syngenta Canada/Syngenta Canada
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone/atrazine	Roundup WeatherMAX® + Acuron® Herbicide	900 + 588/35/140/1259	Bayer CropScience/Syngenta Canada

Note: Herbicide treatments glyphosate + mesotrione + atrazine and glyphosate/*S*-metolachlor/mesotrione + atrazine included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.) (0.2% v/v); and glyphosate + tolpyralate + atrazine included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Ave., Loveland, CO) (0.5% v/v), and urea ammonium nitrate (UAN 28-0-0) (Sylvite, 3221 North Service Road, Burlington, ON) (2.5% v/v). [a]DMA, dimethylamine. [b]BASF Canada Inc., 100 Milverton Drive, Mississauga, ON; Bayer CropScience Inc., 160 Quarry Park Boulevard SE, Calgary, AB; Corteva Agriscience, 735 Chestnut Run Plaza, Wilmington, DE; ISK Biosciences Corporation, 7470 Auburn Road, Concord, OH; Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON).

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 to calculate MHR waterhemp biomass per unit area (g·m^{-2}). Visible corn injury was assessed on a 0% to 100% scale at 1 and 4 weeks after herbicide application (WAA); 0% represented no visible injury and 100% represented complete plant death. Grain corn yield (t·ha^{-1}) and moisture (%) were collected by harvesting two rows of each plot at maturity using a small-plot combine. Grain yields were adjusted to 15.5% moisture prior to statistical analysis.

2.2. Statistical Analysis

Data were subjected to variance analysis using the PROC GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Car, NC). An initial mixed model analysis was conducted to evaluate site-by-treatment interactions. Site, site-by-treatment, and replication within site were considered the random effect and the fixed effect was treatment. Site-by-treatment interaction was significant for all parameters with no difference between S1 and S3, and S2 and S5; therefore, data were combined for S1 and S3, and S2 and S5, and are presented separately for S4. A second mixed model analysis was conducted to analyze herbicide treatment effects on visible MHR waterhemp control, density, biomass, visible corn injury, and grain corn yield. The fixed effect was herbicide treatment and the random effect was replication. Normality and homogeneity of variance were tested using the Shapiro-Wilk test via the PROC UNIVARIATE procedure. Normality assumptions (residuals are independent, homogeneous and normally distributed) were confirmed by plotting the residuals for treatment, replication, and site. A normal distribution was used to analyze visible MHR waterhemp control, visible crop injury, and corn yield data. MHR waterhemp density and biomass data were analyzed using a lognormal distribution to satisfy assumptions of variance analysis. MHR waterhemp density and biomass least-square means were back-transformed from the log-scale using the omega method (M. Edwards, Ontario Agricultural College Statistician, University of Guelph, personal communication). Means were separated using the Tukey-Kramer grouping for Least Square Means. A significance level of $\alpha = 0.05$ was used for data analysis.

3. Results and Discussion

Most EPOST herbicide tank-mixtures provided greater than 90% control of MHR waterhemp. The density, biomass, and population resistance profile are reflected in the differences in control between sites. At 4, 8, and 12 WAA, control of MHR waterhemp ranged from 61% to 100% across sites and was lower at S1, S3, and S4 due to greater density and biomass compared to S2 and S5 (**Table 1**, **Tables 3-5**). Density and biomass of MHR waterhemp in the nontreated control at S1, S3, and S4 averaged 263 to 962 plants m^{-2} and 70.2 to 259.4 $\text{g}\cdot\text{m}^{-2}$, respectively, compared to 60 plants m^{-2} and 72.2 $\text{g}\cdot\text{m}^{-2}$ at S2 and S5. Vyn *et al.* [31] reported similar site differences in POST MHR waterhemp control which they attributed to plant density and site-specific MHR waterhemp resistance profiles. In that study, one waterhemp population exhibited resistance to ALS-inhibiting herbicides and the other to both ALS- and PS II-inhibiting herbicides [31]. All sites contained waterhemp resistant to ALS-, PS II-, EPSPS- and PPO-inhibitors; however, the proportion of individuals resistant to each MOA varied by site. The MHR waterhemp population at S1, S3, and S4 contained a greater number of individuals exhibiting resistance to ALS-, PS II-, and PPO-inhibitors than that of S2 and S5.

Table 3. Means for multiple-herbicide-resistant (MHR) waterhemp control [4, 8 and 12 weeks after EPOST application (WAA)] in corn treated with herbicide tank-mixtures applied EPOST from five field trials conducted in Ontario, Canada in 2019 and 2020.

Treatment[†]	Rate (g ae or ai ha^{-1})	Visible control (%)								
		4 WAA			8 WAA			12 WAA		
		S4	S1, S3	S2, S5	S4	S1, S3	S2, S5	S4	S1, S3	S2, S5
Weed-free control	-	100	100	100	100	100	100	100	100	100
Nontreated control	-	0	0	0	0	0	0	0	0	0
Glyphosate + *S*-metolachlor/atrazine	900 + 1600/1280	75 b	73 c	100 ab	67 bc	73 c	100 a	61 c	76 c	100 a
Glyphosate + dicamba/atrazine	900 + 504/996	76 b	83 bc	99 b	65 c	86 b	99 b	63 c	89 b	99 b
Glyphosate + tolpyralate + atrazine	900 + 40 + 1120	95 a	99 a	100 ab	83 ab	99 a	100 a	79 bc	99 a	100 a
Glyphosate + isoxaflutole + atrazine	900 + 105 + 1063	92 a	94 ab	100 a	87 a	95 ab	100 a	84 ab	96 ab	100 a
Glyphosate + topramezone/dimethenamid-P + dicamba/atrazine	900 + 12.5/630 + 488/966	98 a	99 a	100 ab	90 a	99 a	100 a	86 ab	99 ab	100 a
Glyphosate + pyroxasulfone + dicamba/atrazine	900 + 150 + 488/966	95 a	93 ab	100 ab	92 a	95 ab	100 a	90 ab	97 ab	100 a
Glyphosate/2,4-D choline + rimsulfuron + mesotrione	563/591 + 15 + 144	98 a	99 a	100 a	92 a	99 a	100 a	90 ab	99 a	100 a
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone	900 + 1268/141/35	95 a	97 a	100 a	95 a	97 a	100 a	92 ab	98 ab	100 a
Glyphosate/*S*-metolachlor/mesotrione + atrazine	1050/1050/105 + 280	97 a	97 ab	100 a	94 a	97 ab	100 a	92 ab	98 ab	100 a
Glyphosate + mesotrione + atrazine	900 + 100 + 280	98 a	98 a	100 ab	95 a	99 a	100 a	95 ab	99 ab	100 a
Glyphosate + *S*-metolachlor/atrazine/ mesotrione	900 + 1393/524/139	99 a	99 a	100 a	98 a	99 a	100 a	97 ab	99 a	100 a
Glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine	563/591 + 15 + 144 + 1008	99 a	100 a	100 a	96 a	99 a	100 a	96 ab	100 a	100 a
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone/atrazine	900 + 1259/140/35/588	99 a	99 a	100 ab	99 a	99 a	100 a	99 a	100 a	100 a

Note: Herbicide treatments glyphosate + mesotrione + atrazine and glyphosate/*S*-metolachlor/mesotrione + atrazine included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.) (0.2% v/v); and glyphosate + tolpyralate + atrazine included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Ave., Loveland, CO.) (0.5% v/v), and urea ammonium nitrate (UAN 28-0-0) (Sylvite, 3221 North Service Road, Burlington, ON.) (2.5% v/v). a-c: Means followed by the same letter are not significantly different ($P < 0.05$).

Table 4. Density and biomass of multiple-herbicide-resistant (MHR) waterhemp 4 weeks after EPOST application (WAA) in corn treated with herbicide tank-mixtures applied EPOST from five field trials conducted in Ontario, Canada in 2019 and 2020.

Treatment[†]	Rate (g ae or ai ha^{-1})	Density (plants m^{-2}) and site			Biomass (g·m^{-2}) and site		
		S4	S1, S3	S2, S5	S4	S1, S3	S2, S5
Weed-free control	-	0	0	0	0	0	0
Nontreated control	-	962 a	263 a	60 a	259.4 a	70.2 a	72.2 a
Glyphosate + *S*-metolachlor/atrazine	900 + 1600/1280	177 abc	86 ab	0 b	60.0 b	15.7 b	0 b
Glyphosate + dicamba/atrazine	900 + 504/996	246 ab	45 bc	0 b	28.0 b	5.3 bc	0 b
Glyphosate + tolpyralate + atrazine	900 + 40 + 1120	79 abcd	1 e	0 b	0.9 c	0.1 d	0 b
Glyphosate + isoxaflutole + atrazine	900 + 105 + 1063	38 bcde	7 cde	0 b	5.7 bc	0.5 d	0 b

Continued

Glyphosate + topramezone/dimethenamid-P + dicamba/atrazine	900 + 12.5/630 + 488/966	7 cde	1 e	0 b	0.8 c	0 d	0 b
Glyphosate + pyroxasulfone + dicamba/atrazine	900 + 150 + 488/966	27 bcde	19 bcd	0 b	6.6 bc	1.4 cd	0 b
Glyphosate/2,4-D choline + rimsulfuron + mesotrione	563/591 + 15 + 144	9 cde	1 de	0 b	0.7 c	0 d	0 b
Glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone	900 + 1268/141/35	37 bcde	10 cde	0 b	2.0 c	0.6 d	0 b
Glyphosate/*S*-metolachlor/mesotrione + atrazine	1050/1050/105 + 280	10 cde	5 cde	0 b	2.1 c	0.4 d	0 b
Glyphosate + mesotrione + atrazine	900 + 100 +280	10 cde	7 cde	0 b	0.1 c	0.3 d	0 b
Glyphosate + *S*-metolachlor/atrazine/mesotrione	900 + 1393/524/139	2 de	1 de	0 b	0.1 c	0.1 d	0 b
Glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine	563/591 + 15 + 144 + 1008	3 de	0 e	0 b	0.5 c	0 d	0 b
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone/atrazine	900 + 1259/140/35/588	1 e	1 de	0 b	0.2 c	0 d	0 b

Note: Herbicide treatments glyphosate + mesotrione + atrazine and glyphosate/*S*-metolachlor/mesotrione + atrazine included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.) (0.2% v/v); and glyphosate + tolpyralate + atrazine included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Ave., Loveland, CO.) (0.5% v/v), and urea ammonium nitrate (UAN 28-0-0) (Sylvite, 3221 North Service Road, Burlington, ON.) (2.5% v/v). a-d: Means followed by the same letter are not significantly different ($P < 0.05$).

Table 5. Effect of herbicide tank-mixtures applied EPOST on corn injury [1, 2 and 4 weeks after the EPOST application (WAA)] and yield from five field trials conducted in Ontario, Canada in 2019 and 2020.

		Injury (%)					
Treatment†	Rate (g ae or ai ha^{-1})	1 WAA	2 WAA	4 WAA	Yield (t·ha^{-1}) and site		
		S2	S2	S2	S4	S1, S3	S2, S5
Weed-free control	-	-	-	-	8.0 a	8.4 a	8.7 ab
Nontreated control	-	-	-	-	4.9 b	7.3 a	8.3 ab
Glyphosate + *S*-metolachlor/atrazine	900 + 1600/1280	1 a	0 a	0 a	7.9 a	8.0 a	9.4 a
Glyphosate + dicamba/atrazine	900 + 504/996	0 a	0 a	0 a	8.5 a	8.6 a	8.7 ab
Glyphosate + tolpyralate + atrazine	900 + 40 + 1120	8 ab	4 a	1 a	8.8 a	8.2 a	8.1 ab
Glyphosate + isoxaflutole + atrazine	900 + 105 + 1063	0 a	0 a	0 a	8.4 a	8.0 a	9.2 ab
Glyphosate + topramezone/dimethenamid-P + dicamba/atrazine	900 + 12.5/630 + 488/966	1 a	1 a	1 a	8.2 a	7.6 a	8.5 ab
Glyphosate + pyroxasulfone + dicamba/atrazine	900 + 150 + 488/966	3 a	3 a	3 a	7.9 a	8.0 a	8.5 ab
Glyphosate/2,4-D choline + rimsulfuron + mesotrione	563/591 + 15 + 144	3 a	6 a	5 a	8.6 a	8.5 a	8.3 ab
Glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone	900 + 1268/141/35	0 a	0 a	0 a	8.2 a	8.0 a	8.7 ab
Glyphosate/*S*-metolachlor/mesotrione + atrazine	1050/1050/105 + 280	0 a	0 a	0 a	9.2 a	8.1 a	8.5 ab
Glyphosate + mesotrione + atrazine	900 + 100 +280	0 a	0 a	0 a	9.8 a	8.9 a	9.0 ab
Glyphosate + *S*-metolachlor/atrazine/mesotrione	900 + 1393/524/139	0 a	0 a	0 a	8.3 a	8.6 a	8.5 ab
Glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine	563/591 + 15 + 144 + 1008	28 b	31 b	31 b	8.2 a	8.5 a	7.3 b
Glyphosate + *S*-metolachlor/mesotrione/ bicyclopyrone/atrazine	900 + 1259/140/35/588	0 a	0 a	0 a	8.8 a	8.5 a	8.4 ab

Note: Herbicide treatments glyphosate + mesotrione + atrazine and glyphosate/*S*-metolachlor/mesotrione + atrazine included Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Research Park, Guelph, ON.) (0.2% v/v); and glyphosate + tolpyralate + atrazine included methylated seed oil (MSO Concentrate®) (Loveland Products Inc., 3005 Rocky Mountain Ave., Loveland, CO) (0.5% v/v), and urea ammonium nitrate (UAN 28-0-0) (Sylvite, 3221 North Service Road, Burlington, ON.) (2.5% v/v). a-b: Means followed by the same letter are not significantly different ($P < 0.05$).

At S1, S3, and S4, glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone/atrazine, glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine, glyphosate + *S*-metolachlor/atrazine/mesotrione, and glyphosate + mesotrione + atrazine controlled MHR waterhemp 95% to 100% at 4, 8, and 12 WAA and reduced plant density and biomass by 97% to 100%. Glyphosate/*S*-metolachlor/mesotrione + atrazine, glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone, glyphosate/2,4-D choline + rimsulfuron + mesotrione, and glyphosate + pyroxasulfone + dicamba/atrazine controlled MHR waterhemp 90% to 99% at 4, 8, and 12 WAA and reduced plant density and biomass 96% to 100%. Control of MHR waterhemp ranged from 99% to 100% and plant density and biomass were reduced 100% with all herbicides at S2 and S5. These findings are consistent with Sarangi and Jhala [46] who reported excellent control of MHR waterhemp with *S*-metolachlor/mesotrione/bicyclopyrone/atrazine applied POST to 8 to 10 cm and 15 to 18 cm waterhemp. Benoit *et al.* [3] [19] [33] and Vyn *et al.* [31] reported comparable control of MHR waterhemp with mesotrione + atrazine applied POST providing ≥ 90% control 4, 8 and 12 WAA in multiple trials. Vyn *et al.* [31] reported similar control of MHR waterhemp with 2,4-D/atrazine (1404 $g \cdot ha^{-1}$) of 84% to 100% 4 and 10 WAA. Additionally, Sarangi *et al.* [47] and Chahal *et al.* [45] reported excellent control of MHR waterhemp with glyphosate + 2,4-D choline (840 + 800 $g \cdot ha^{-1}$) applied POST, however, Chahal *et al.* [45] suggested that plants should be less than 10 cm in height at application. At S1, S3, and S4, glyphosate + topramezone/dimethenamid-P + dicamba/atrazine, glyphosate + isoxaflutole + atrazine, and glyphosate + tolpyralate + atrazine controlled MHR waterhemp 79% to 99% at 4, 8 and 12 WAA and reduced plant density and biomass 92% to 100%. These results complement another Ontario study that reported ≥96% MHR waterhemp control from 4 to 12 WAA with glyphosate + tolpyralate + atrazine (900 + 30 + 560 $g \cdot ha^{-1}$) applied POST [26]. Moreover, MHR waterhemp control with isoxaflutole + atrazine applied EPOST was similar to PRE applications that provided 82% to 100% control in other studies [16] [19] [31] [48]. Control of MHR waterhemp was similar for all herbicide tank-mixtures except glyphosate + *S*-metolachlor/atrazine and glyphosate + dicamba/atrazine at S4, S1 and S3 which resulted in the lowest level of control ranging from 73% to 75% and 76% to 83%, respectively. Glyphosate + *S*-metolachlor/atrazine resulted in lower MHR waterhemp control than glyphosate + dicamba/atrazine at S1 and S3; however, control was similar at S4. This is consistent with Benoit *et al.* [19] who reported 84% and 87% MHR waterhemp control 4 and 8 WAA with dicamba/atrazine POST. In contrast, Soltani *et al.* [16] reported greater MHR waterhemp control and reductions in density and biomass with dicamba/atrazine (1800 $g \cdot ha^{-1}$) POST that were similar to mesotrione + atrazine POST and isoxaflutole + atrazine PRE in that study. Schryver *et al.* [34] reported MHR waterhemp control with dicamba (600 $g \cdot ha^{-1}$) is greatest when applied POST resulting in 91% to 100% control compared to 60% to 65% at 10 WAA when applied PRE. Lower control with glyphosate + dicamba/atrazine may be due to lower dicamba application rate (504 g·ha^{-1}). Control of MHR waterhemp from 4 to 12 WAA increased at S1 and S3 and decreased at S4 for most herbicide tank-mixtures evaluated. Increasing MHR waterhemp control over the course of the growing season could be due to natural thinning of waterhemp populations reported by Benoit *et al.* [19] and [49]; in contrast, late emerging cohorts have been reported to reduce end-of-season control as well [2].

Corn injury was ≤10% for all herbicide treatments 1, 2, and 4 WAA at all sites except S2 (**Table 5**). Glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine caused 28%, 31%, and 31% corn injury 1, 2, and 4 WAA , respectively at S2; symptoms included brace root malformation and lodging which resulted in reduced corn stand. Applications of glyphosate/2,4-D choline to non-ENLIST™ hybrids can cause stalk brittleness, leaning, malformed brace roots, and leaf rolling in the whorl [50] [51]. Ruen *et al.* [38] reported similar leaf necrosis and leaning of ENLIST™ corn hybrids treated with single applications of glyphosate/2,4-D choline at V4 and V7 corn growth stages and sequential applications at V4 *fb* V7. Interestingly, the addition of atrazine to glyphosate/2,4-D choline + rimsulfuron + mesotrione increased corn injury 25%, 25%, and 26% at 1, 2, and 4 WAA, respectively. We do not have an explanation for this observation; the response should be evaluated in future studies to determine if this is a real response. Tolerance of conventional corn hybrids to 2,4-D varies with hybrid, corn growth stage at application, soil characteristics, and weather conditions [38]. It is recommended that glyphosate/2,4-D choline (ENLIST DUO) only be applied to ENLIST™ field corn hybrids that contain the AAD-1 transgene [35]. Glyphosate/2,4-D choline applications can also be made up to the V8 corn growth stage; in this study, herbicides were applied to V4 corn (data not shown). Corn injury caused by glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine resulted in lower corn yield than glyphosate + *S*-metolachlor/atrazine; however, yield was similar to the weed-free control. When waterhemp was left uncontrolled, corn yield was reduced 39% at S4 and was similar to another Ontario study that reported a corn yield reduction of 48% [16]. Relative to the weed-free control, corn yield was not reduced at S1, S2, S3, and S5 which could again be the result of comparatively lower MHR waterhemp density and biomass. Cordes *et al.* [14] reported corn yield reductions due to the late removal of waterhemp when plants reached 15 cm. This result supports previous research that suggests EPOST herbicide applications reduce early season weed interference and minimize corn yield loss [13].

4. Conclusion

Most of the EPOST herbicide tank-mixtures evaluated in this study resulted in greater than 90% control of MHR waterhemp. The top four herbicides treatments were glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone/atrazine, glyphosate/2,4-D choline + rimsulfuron + mesotrione + atrazine, glyphosate +

S-metolachlor/atrazine/mesotrione, and glyphosate + mesotrione + atrazine which controlled MHR waterhemp 95% to 100% throughout the growing season at all sites. Glyphosate/*S*-metolachlor/mesotrione + atrazine, glyphosate + *S*-metolachlor/mesotrione/bicyclopyrone, glyphosate/2,4-D choline + rimsulfuron + mesotrione, and glyphosate + pyroxasulfone + dicamba/atrazine provided 90% to 100% MHR waterhemp control 4, 8, and 12 WAA. Control of MHR waterhemp was similar amongst all herbicide tank-mixtures, except glyphosate + *S*-metolachlor/atrazine and glyphosate + dicamba/atrazine which resulted in lower control than all other treatments, control ranged from 61% to 100% and 63% to 100%, respectively. Reductions in MHR waterhemp control with these herbicides resulted in greater MHR waterhemp density and biomass 4 WAA at three of five sites. Differences in MHR waterhemp control were the result of variation in population resistance profiles, competitiveness of individual populations, and comparatively lower MHR waterhemp density and biomass at two of five sites. Furthermore, herbicide tank-mixtures reduced MHR waterhemp density and biomass > 91% and 97%, respectively, except glyphosate + *S*-metolachlor/atrazine and glyphosate + dicamba/atrazine (67% to 82% and 74% to 92%). Weed interference caused by MHR waterhemp did not reduce corn yield in this study at four out of five sites. This study identifies EPOST herbicide tank-mixtures that expand POST MHR waterhemp management and have a wide margin of crop safety. Corn producers should incorporate the use of strategic tillage, cover crops, crop row spacing, crop density, PRE herbicides, EPOST herbicides, POST herbicides, and PRE *fb* POST herbicide programs to steward the use of currently effective MOA and reduce the evolution of herbicide resistance. Strategies to manage MHR waterhemp should be implemented early in the growing season, herbicide applications should be made before MHR waterhemp reaches 10 cm in height to prevent corn yield loss.

Acknowledgements

The authors acknowledge Chris Kramer for his technical support, Dr. Michelle Edwards for her statistical support, the University of Guelph, Ridgetown Campus summer staff for their field support and the Grain Farmers of Ontario and herbicide manufacturers for providing funding for this research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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