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Effect of herbicide programs on control and seed production of multiple herbicide–resistant Palmer amaranth (*Amaranthus palmeri*) in corn resistant to 2,4-D/glufosinate/glyphosate

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Abstract

Multiple herbicide–resistant (MHR) Palmer amaranth is among the most problematic summer annual broadleaf weeds in Nebraska and several other states. A new MHR corn cultivar (resistant to 2,4-D/glufosinate/glyphosate, also known as Enlist corn) has been commercially available in the United States since 2018. Growers are searching for herbicide programs for control and reduce seed production of MHR Palmer amaranth among Enlist corn crops. The objectives of this study were to evaluate herbicides applied preemergence, early postemergence, or preemergence followed by (fb) late postemergence for the management of MHR Palmer amaranth in Enlist corn fields and to assess their effect on Palmer amaranth biomass, density, seed production, and corn yield. Field experiments were conducted near Carleton, NE, in 2020 and 2021, in a grower's field of Enlist corn infested with acetolactate synthase–inhibitor/atrazine/glyphosate–resistant Palmer amaranth. Herbicides applied preemergence, such as flufenacet/isoxaflutole/thiencarbazone-methyl, acetochlor/clopyralid/flumetsulam, or acetochlor/clopyralid/mesotrione, provided 75% to 99% control of Palmer amaranth 30 d after preemergence. Preemergence fb late postemergence herbicides resulted in 94% Palmer amaranth control 90 d after late postemergence, reduced weed density to 0 to 8 plants m^{-2} 30 d after late postemergence, and reduced biomass to 2 to 14 g m^{-2} 15 d after late postemergence compared to preemergence-only (59% control, 0 to 15 plants m^{-2}, and 4 to 123 g m^{-2}) and early postemergence–only herbicides (78% control, 6 to 30 plants m^{-2}, and 8 to 25 g m^{-2}). Based on contrast analysis, Palmer amaranth seed production was reduced to 14,050 seeds m^{-2} in preemergence fb late postemergence herbicide programs compared with 325,490 seed m^{-2} in preemergence-only and 376,750 seed m^{-2} in early postemergence–only programs. Based on orthogonal contrast, higher corn yield of 12,340 and 11,730 kg ha^{-1} was obtained with preemergence fb late postemergence herbicide programs compared with preemergence-only (10,840 and 11,510 kg ha^{-1}) and early postemergence–only programs (10,850 and 10,030 kg ha^{-1}) in 2020 and 2021, respectively.

Introduction

Palmer amaranth is among the most problematic summer annual broadleaf weeds across the mid-south, southeastern, mid-Atlantic, and north central United States (Oliveira et al. 2022; Vencill et al. 2008). In a survey conducted by the Weed Science Society of America, Palmer amaranth was ranked as the most troublesome weed in agronomic cropping systems in the United States (Van Wychen 2022). A widespread occurrence of Palmer amaranth is due to its unique biological attributes that include an extended period of emergence, aggressive growth rate, high photosynthetic rate, high water-use efficiency, considerable biomass accumulation, prolific seed production (up to 0.6 million seed per female plant) (Chahal et al. 2018b; Jha and Norsworthy 2009; Ward et al. 2013), and dioecious reproductive biology, which increases the pollen-mediated gene flow and spread of herbicide resistance alleles (Jhala et al. 2021). If not controlled, Palmer amaranth can cause a significant crop yield reduction. For example, a Palmer amaranth density of 3 plants m^{-2} caused 60% yield loss of soybean (*Glycine max* L. Merill) in a study conducted in Arkansas (Klingaman and Oliver 1994). Bensch et al. (2003) reported 78%

soybean yield loss at a density of 8 plants m^{-2} in Kansas. Massinga et al. (2001) reported that Palmer amaranth at 0.5 to 8 plants m^{-1} row reduced corn yield from 11% to 91%.

In addition to its biological characteristics, the evolution of herbicide-resistant Palmer amaranth in agronomic cropping systems has become a challenge for growers for effective management (Chahal et al. 2018a; Mausbach et al. 2021). Palmer amaranth has evolved resistance to herbicides from several site-of-action (SOA) groups, including those that inhibit acetolactate synthase (ALS), 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), microtubule assembly, photosystem II, protoporphyrinogen oxidase (PPO) (Chahal et al. 2017; Garetson et al. 2019; Ward et al. 2013), 4-hydroxyphenyl pyruvate dioxygenase (HPPD) (Chahal et al. 2015; Jhala et al. 2014), synthetic auxins (Kumar et al. 2019), and very long chain fatty acids (Brabham et al. 2019). A Palmer amaranth biotype that is resistant to glufosinate has been confirmed in Arkansas (Priess et al. 2022) and dicamba-resistant Palmer amaranth was reported in Tennessee in 2021 (Foster and Steckel 2022). In addition to resistance to herbicides with a single SOA, Palmer amaranth resistance to multiple herbicides with different SOAs has been reported. One of the most prevalent forms of multiple herbicide resistance in Palmer amaranth is resistance to glyphosate and ALS-inhibiting herbicides, which has been confirmed in eight states (Chahal et al. 2017; Heap 2024; Jhala et al. 2014). In addition, Palmer amaranth that is resistant to atrazine, chlorsulfuron, 2,4-D, glyphosate, and mesotrione has been reported in Kansas (Kumar et al. 2019; 2020). Kohrt et al. (2016) confirmed Palmer amaranth resistance to ALS inhibitor, atrazine, and glyphosate in Michigan. As of March 2024, Palmer amaranth has evolved resistance to 10 herbicide SOAs (Heap 2024).

Palmer amaranth has an extended emergence pattern from early May through August in the Midwest (Chahal et al. 2021), and from late April to early September in the southern United States (Liu et al. 2022), making it difficult to control with herbicides applied preemergence only or postemergence only (Mausbach et al. 2021; Shyam et al. 2021b). Herbicides applied preemergence generally lose their residual activity 20 to 40 d after application depending on the herbicide used and soil type; however, most postemergence herbicides commonly applied to corn have minimal to no soil residual activity (Wiggins et al. 2015). The late-emerging Palmer amaranth often escapes a postemergence herbicide and produces seed, leading to the replenishment of the soil seedbank (Bagavathiannan and Norsworthy 2012). Therefore, herbicide practices should be focused on season-long control of Palmer amaranth to reduce seed production and infestation during subsequent crop seasons (Striegel and Jhala 2022). In addition, soil residual herbicides such as acetochlor, dimethenamid-*P*, fluthiacet-methyl, or pyroxasulfone can be applied with a foliar-active postemergence herbicide to corn up to certain growth stages to provide overlapping residual activity to control weeds (Jhala et al. 2015; McDonald et al. 2023; Sarangi and Jhala 2019).

A new multiple herbicide–resistant (MHR) corn trait that is resistant to 2,4-D, glufosinate, and glyphosate, also known as Enlist corn, has been commercially available in the United States since 2018. It provides an opportunity for management of ALS-, PS II-, and EPSPS-inhibitor-resistant Palmer amaranth with the aid of herbicide practices that cannot be applied to conventional or glyphosate-resistant corn. The objectives of this study were to evaluate the effect of herbicides applied preemergence, early postemergence, and preemergence fb late postemergence for control of ALS-inhibitor/atrazine/glyphosate-resistant Palmer amaranth, and their effect on Palmer amaranth density, biomass, seed production, crop injury, and yield in Enlist corn crops. We hypothesized that a season-long control of MHR Palmer amaranth would be achieved with reduced seed production in a preemergence fb a late postemergence herbicide application program.

Materials and Methods

Field Experiments

Field experiments were conducted in 2020 and 2021 in a grower's field infested with ALS-inhibitor/atrazine/glyphosate-resistant Palmer amaranth near Carleton, NE (40.30°N, 97.67°W). The experiments were established under no-till conditions. The previous crops at the site were no-till soybean in 2019 and no-till corn in 2020. Palmer amaranth was the dominant summer weed at the experimental site and was confirmed to be resistant to ALS-inhibitor/atrazine/glyphosate (Chahal et al. 2017). The soil at the experimental site was a silt loam (montmorillonitic, mesic, Pachic Argiustolls), with 19% sand, 63% silt, 18% clay, pH 6.0, and 2.5% organic matter content. The herbicide 2,4-D (Enlist ONE; Corteva Agriscience, Indianapolis, IN) was applied in early spring to control glyphosate-resistant horseweed (*Erigeron canadensis* L. Cronq.) that was present at the experimental site. The treatments were laid out in a randomized complete block design with four replications. The dimensions of individual experimental plots were 3 m wide and 9 m long. Enlist E3 corn (8097 SXE Enlist Corn SmartStax; Corteva AgriScience, Indianapolis, IN) was planted at 67,500 seed ha^{-1} on May 12, 2020, and May 18, 2021, in 78-cm-row spacing. The experimental site setup was without supplemental irrigation. Precipitation received during the crop growing season for both years is listed in Table 1.

Herbicides to control Palmer amaranth were applied preemergence only, early postemergence only, and preemergence fb late postemergence with a total of 15 treatments, including a nontreated control and a weed-free control for comparison purpose (Table 2). Herbicides were applied using a handheld CO_2-pressurized backpack sprayer equipped with TeeJet AIXR 110015 flat-fan nozzles (Spraying Systems Co., Glendale, IL) calibrated to deliver a flow rate of 140 L ha^{-1} at 276 kPa at a constant speed of 4.8 km h^{-1}. Glufosinate was mixed with liquid ammonium sulfate at 3% vol/vol (Anonymous 2017) and was applied with TeeJet XR 11005 flat-fan nozzles (Spraying Systems Co.). The preemergence herbicides were applied 2 d after corn planting on May 14, 2020, and on the day of corn planting on May 18, 2021. Early postemergence herbicides were applied 36 d after corn planting on June 18, 2020, and 28 d after corn planting on June 16, 2021; and late postemergence herbicides were applied on June 23, 2020, and on June 25, 2021. Early postemergence and late postemergence herbicides were applied when Palmer amaranth was 10 to 15 cm and 20 to 30 cm tall, respectively. The height of Palmer amaranth was variable because of its extended emergence pattern.

Data Collection

Visible estimates of Palmer amaranth control were recorded 15 and 30 d after preemergence and after early postemergence, and 15, 30, and 90 d after late postemergence using a 0% to 100% scale, with 0% meaning no Palmer amaranth control and 100% meaning complete control. Corn injury was assessed on a 0% to 100% scale 15 and 30 d after each application with 0% meaning no corn injury and 100% meaning plant death. Palmer amaranth density was recorded by counting the number of Palmer amaranth plants in

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Table 1. Monthly mean air temperature and total precipitation during the 2020 and 2021 growing seasons along with the 30-yr average at the experiment site near Carleton, NE.[a]

Month	Mean air temperature			Total precipitation		
	2020	2021	30-yr average	2020	2021	30-yr average
	C			mm		
March	6.1	7.5	4.6	147.8	147.1	45.2
April	9.2	10.0	10.6	37.8	73.7	66.3
May	15.0	15.8	16.4	80.3	81.5	135.4
June	24.7	23.9	22.3	147.6	13.5	115.1
July	24.7	24.2	24.9	424.2	45.5	105.2
August	23.6	24.7	23.7	42.9	105.1	94.0
September	17.8	21.4	19.1	87.63	46.7	66.0

[a]Data were obtained from the National Oceanic and Atmospheric Administration.

Table 2. Herbicides, application timings, and rates used for control of acetolactate synthase inhibitor/atrazine/glyphosate–resistant Palmer amaranth in a 2,4-D/glufosinate/glyphosate–resistant corn in field experiments conducted near Carleton, NE, in 2020 and 2021.

Herbicide program[a]	Trade name	Application timing[b]	Rate	Manufacturer
			g ae or ai ha^{-1}	
Acetochlor/clopyralid/mesotrione	Resicore	PRE	2,300	Corteva Agriscience
Acetochlor/clopyralid/flumetsulam	Surestart II	PRE	1,190	Corteva Agriscience
Flufenacet/isoxaflutole/thiencarbazone-methyl	TriVolt	PRE	536	Bayer CropScience
Glyphosate/2,4-D	Enlist DUO	EPOST	1,630	Corteva Agriscience
2,4-D	Enlist ONE	EPOST	1,060	Corteva Agriscience
Glufosinate	Liberty	EPOST	656	BASF Corp.
2,4-D + glufosinate	Enlist ONE + Liberty	EPOST	800 + 656	Corteva Agriscience + BASF Corp.
Acetochlor/clopyralid/mesotrione fb 2,4-D	Resicore fb Enlist ONE	PRE fb LPOST	2,300 fb 800	Corteva Agriscience
Acetochlor/clopyralid/flumetsulam fb 2,4-D	Surestart II fb Enlist ONE	PRE fb LPOST	1,190 fb 800	Corteva Agriscience
Flufenacet/isoxaflutole/thiencarbazone-methyl fb 2,4-D	TriVolt fb Enlist ONE	PRE fb LPOST	536 fb 800	Bayer CropScience, Corteva Agriscience
Acetochlor/ clopyralid/mesotrione fb glufosinate	Resicore fb Liberty	PRE fb LPOST	2,300 fb 656	Corteva Agriscience, BASF Corp.
Acetochlor/clopyralid/flumetsulam fb glufosinate	Surestart II fb Liberty	PRE fb LPOST	1,190 fb 656	Corteva Agriscience, BASF Corp.
Flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate	TriVolt fb Liberty	PRE fb LPOST	536 fb 656	Bayer CropScience, BASF Corp.

[a]Glufosinate treatments were mixed with liquid ammonium sulfate (N PAK AMS, Winfield United, Arden Hills, MN) at 3% vol/vol.
[b]Abbreviations: EPOST, early postemergence; fb, followed by; LPOST, late postemergence; POST, postemergence; PRE, preemergence.

0.5-m^2 quadrats from each plot 15 and 30 d after preemergence, 30 d after early postemergence, and 30 d after late postemergence. Aboveground biomass was collected from 0.5-m^2 quadrats plot^{-1} 30 d after early postemergence and 15 d after late postemergence. Palmer amaranth plants were clipped at the soil surface, kept in paper bags, dried at 65 C in an oven for a week, and weighed. Palmer amaranth seed production was recorded by placing a 1.0-m^2 quadrat in the center two rows of corn and collecting the inflorescences of female plants from each quadrat. Palmer amaranth inflorescences were stripped from the stems and separated by passing them through a series of USA standard testing sieves (Gilson Company, Worthington, OH) with mesh size ranging from 0.50 to 3.35 mm. Material collected from the 0.50-mm sieve was processed with a seed cleaner (Hoffman Manufacturing, Albany, OR) that used air to remove the lighter floral chaff from the Palmer amaranth seed (Sosnoskie et al. 2014). The seeds were thoroughly cleaned, weighted, and number of seeds per square meter was determined. At maturity, corn was harvested from the center two rows of each plot using a plot combine, weighed, and the moisture content was recorded. The grain yield was adjusted to 15.5% moisture content and converted into kilograms per hectare (kg ha^{-1}).

Statistical Analysis

Palmer amaranth control, density, aboveground biomass, and Palmer amaranth seed production, as well as corn yield data, were subjected to ANOVA using the GLIMMIX procedure with SAS software (version 9.4; SAS Institute Inc, Cary, NC). Before analysis, data were subjected to the UNIVARIATE procedure for testing normality and homogeneity of variance with normal Q-Q plots and Levene's test, respectively. Type III tests were used to assess fixed effects, and treatment comparisons were made based on Tukey Kramer's pairwise comparison test and Sidak adjustments. Palmer amaranth control data were log transformed and fit to generalized linear mixed-effect models using the GLIMMIX procedure with beta distribution. Palmer amaranth density and biomass data were square root–transformed, and back-transformed values are presented. Palmer amaranth seed production and corn yield data were analyzed with the GLIMMIX procedure using gaussian (link = "identity") error distributions selected for response variables based on the restricted maximum likelihood technique. Year and herbicide treatments were considered fixed effects in the model, while replications were considered a random effect. Orthogonal contrasts were considered to compare herbicide programs (preemergence vs. early postemergence, preemergence

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vs. preemergence fb late postemergence, and early postemergence vs. preemergence fb late postemergence) at $P \leq 0.05$ for Palmer amaranth control at 15 and 30 d after early postemergence; 15, 30, and 90 d after late postemergence; Palmer amaranth seed production; and corn yield.

Results and Discussion

Year-by-treatment interaction for Palmer amaranth control, aboveground biomass, and seed production was not significant ($P \geq 0.05$); therefore, data from both years were combined. Year-by-treatment interaction for Palmer amaranth density and corn yield was significant; therefore, data are presented separately for both years. No corn injury was observed from any herbicide program (data not shown), indicating that the herbicides evaluated in this study are safe to use in Enlist corn when applied according to label instructions.

Temperature and Precipitation

The average monthly temperature during the 2021 growing season was higher than 2020, except June and July (Table 1). Below-average precipitation of 13.5 mm fell in June and 45.5 mm in July 2021, while above-average precipitation of 147.6 mm in occurred June and 424.2 mm in July occurred in 2020 compared to the 30-yr average of 115.1 mm and 105.2 mm for June and July, respectively.

Palmer Amaranth Control

Herbicides applied preemergence in this study provided ≥96% control of Palmer amaranth 15 d after preemergence, and 75% to 99% control 30 d after preemergence, without difference among treatments (Table 3). The residual activity of most herbicides applied preemergence declined as the season progressed. For example, acetochlor/clopyralid/flumetsulam, and flufenacet/isoxaflutole/thiencarbazone-methyl controlled Palmer amaranth by 44% at 90 d after late postemergence compared with 87% control with acetochlor/clopyralid/mesotrione (Table 3). Rain fell within 10 d of applying preemergence herbicides in both years with an average of 80.3 mm in 2020 and 81.5 mm in 2021, which was comparatively less than the 30-yr average of 135.4 mm for May (Table 1).

Among the early postemergence herbicides, 2,4-D + glufosinate controlled Palmer amaranth by 90%; and glufosinate provided 83% control compared with 57% control with glyphosate/2,4-D; and 62% control with 2,4-D 15 d after early postemergence (Table 3). Glufosinate + 2,4-D and glyphosate/2,4-D provided similar Palmer amaranth control, ranging from 71% to 78% at 30 d after early postemergence and 82% to 84% at 30 d after late postemergence, respectively. As the season progressed, Palmer amaranth control with glufosinate alone decreased to 66% compared to 85% with 2,4-D + glufosinate, 82% with glyphosate/2,4-D, and 80% with 2,4-D alone 90 d after late postemergence (Table 3).

Herbicides applied preemergence without a follow-up postemergence herbicide could not provide economically acceptable Palmer amaranth control compared with preemergence fb late postemergence herbicide programs later in the season, except for acetochlor/clopyralid/mesotrione. This is because Palmer amaranth at the study site was resistant to the ALS inhibitor. Thus, lower Palmer amaranth control was obtained with acetochlor/clopyralid/flumetsulam, and flufenacet/isoxaflutole/thiencarbazone-methyl applied preemergence because both premixes contain an ALS inhibitor. Palmer amaranth was not resistant to acetochlor/clopyralid/mesotrione. A similar decline in residual activity of soil-applied preemergence herbicides has been reported with soybean in multiyear field studies in Nebraska, where preemergence herbicides resulted in 66% control of Palmer amaranth compared with 86% control by preemergence fb late postemergence herbicide programs 28 d after late postemergence (Sarangi and Jhala 2019). Liu et al. (2021) concluded that a preemergence fb late postemergence herbicide routine resulted in 83% Palmer amaranth control 7 wk after late postemergence compared to 67% control with a preemergence-only application to glufosinate/glyphosate-resistant corn.

The preemergence fb late postemergence herbicide routine provided ≥94% control of Palmer amaranth 15 d after late postemergence, and 87% to 97% control 90 d after late postemergence without difference among treatments (Table 3). This was attributed to an early-season control of Palmer amaranth by the residual activity of preemergence herbicides, whereas the late-emerged flushes of Palmer amaranth were controlled by a follow-up application of late postemergence herbicides. The preemergence fb late postemergence herbicide programs provided similar Palmer amaranth control (87% to 97%) 90 d after late postemergence. While Palmer amaranth is known for its extended emergence pattern, emergence is reported to be higher from early May to mid-July (Chahal et al. 2021). Meyer et al. (2015) showed that auxin-based late postemergence herbicides can control glyphosate-resistant Palmer amaranth in soybean fields.

Contrast analysis showed that preemergence fb late postemergence herbicide programs resulted in 94% Palmer amaranth control compared with 59% and 78% control with preemergence-only and early postemergence–only programs, respectively (Table 3). Similarly, Sarangi et al. (2017) reported 90% control of herbicide-resistant *Amaranthus* species in soybean fields with a preemergence fb late postemergence herbicide regimen. Several other studies have found greater control of *Amaranthus* species with preemergence fb late postemergence herbicide applications compared with preemergence-only or early postemergence–only applications (Aulakh and Jhala 2015; Johnson et al. 2012; Liu et al. 2021; Striegel and Jhala 2022).

Palmer Amaranth Density and Biomass

Year-by-treatment interaction for Palmer amaranth density was significant, thus Palmer amaranth density data were presented separately by year. Year-by-treatment interaction for Palmer amaranth biomass data were nonsignificant, so data were combined across both years. Palmer amaranth density and biomass were affected by the herbicide programs compared with the nontreated control (Table 4). Palmer amaranth emergence was greater in 2020 than in 2021. For example, Palmer amaranth density in the nontreated control ranged from 61 to 149 plants m^{-2} in 2020 compared with 43 to 72 plants m^{-2} in 2021. This was most likely due to more precipitation and low temperature in 2020 compared with 2021, particularly in June 2020, when 147.6 mm of rainfall provided plenty of moisture for Palmer amaranth emergence and growth (Table 1).

At 30 d after preemergence, acetochlor/clopyralid/mesotrione, acetochlor/clopyralid/flumetsulam, and flufenacet/isoxaflutole/thiencarbazone-methyl resulted in Palmer amaranth densities of 0 to 5, 10 to 66, and 2 to 47 plants m^{-2}, respectively, during both years (Table 4). As the season progressed, the efficacy of preemergence herbicides was reduced, except acetochlor/

Table 3. Control of multiple herbicide–resistant Palmer amaranth affected by herbicide programs in a 2,4-D/glufosinate/glyphosate–resistant corn in field experiments conducted near Carleton, NE, in 2020 and 2021.[a]

		Palmer amaranth control[b,c]						
Herbicide program	Timing	15 DA-PRE	30 DA-PRE	15 DA-EPOST	30 DA-EPOST	15 DA-LPOST	30 DA-LPOST	90 DA-LPOST
		%						
Nontreated control	–	0	0	0	0	0	0	0
Weed free	–	99	99	99	99	99	99	99
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1})	PRE	96 a	97 a	90 a	90 a	99 a	89 ab	87 ab ab
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1})	PRE	97 a	79 a	41 f	49 d	91 c	58 e	44 d d
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1})	PRE	97 a	75 a	43 f	40 d	87 b	40 f	45 d d
Glyphosate/2,4-D (1,630 g ae ha^{-1})	EPOST	–	–	57 e	71 b	89 b	82 b	82 b b
2,4-D (1,060 g ae ha^{-1})	EPOST	–	–	62 d	60 c	77 c	68 d	80 b b
Glufosinate (656 g ai ha^{-1})	EPOST	–	–	83 b	57 c	88 b	73 c	66 c c
2,4-D (800 g ae ha^{-1}) + glufosinate (656 g ai ha^{-1})	EPOST	–	–	90 a	78 b	95 a	84 b	85 b b
Acetochlor/ clopyralid/mesotrione (2,300 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	98 a	99 a	93 a	97 a	99 a	89 b	97 a a
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	98 a	86 a	78 b	73 b	95 a	92 a	95 a a
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	97 a	83 a	72 c	69 c	96 a	87 b	94 a a
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	99 a	99 a	99 a	98 a	94 a	89 b	87 ab ab
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	98 a	83 a	92 a	92 a	99 a	95 a	95 a a
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	99 a	79 a	93 a	93 a	99 a	93 b	93 a a
P-value		0.725	0.157	0.0004	0.0001	0.8633	0.005	0.0004
Contrast analysis[d]								
PRE vs. EPOST				58 vs 73 [e]	60 vs 67 [NS]	92 vs 87 [NS]	62 vs 77 [NS]	59 vs 78 [NS]
PRE vs. PRE fb LPOST				58 vs 88 [e]	60 vs 87 [e]	92 vs 97 [NS]	62 vs 91 [e]	59 vs 94 [e]
EPOST vs. PRE fb LPOST				73vs 88 [NS]	67 vs 87 [e]	87 vs 97 [e]	77 vs 91 [e]	78 vs 94 [e]

[a]Abbreviations: DA-PRE, days after preemergence application; DA-EPOST, days after early postemergence application; DA-LPOST, days after late postemergence application; EPOST, early postemergence; fb, followed by; LPOST, late postemergence; NS, not significant.
[b]Year-by-treatment interaction for Palmer amaranth control was nonsignificant; therefore, data were pooled across both years (2020 and 2021).
[c]Means presented within each column with no common letters are significantly different as according to the Tukey Kramer pairwise comparison test.
[d]A priori orthogonal contrasts.
[e]$P < 0.0001$.

Table 4. Multiple herbicide–resistant Palmer amaranth density and above-ground biomass as affected by the herbicide programs in a 2,4-D/glyphosate/glufosinate–resistant corn in field experiments conducted near Carleton, NE, in 2020 and 2021.[a,b]

Herbicide program	Timing	Palmer amaranth density[b,c]							Palmer amaranth biomass[c,d]	
		15 DA-PRE		30 DA-PRE		30 DA-EPOST		30 DA-LPOST[e]	30 DA-EPOST	15 DA-LPOST
		2020	2021	2020	2021	2020	2021	2021		
		number m^{-2}							g m^{-2}	
Nontreated control		149 a	43 a	108 a	55 a	61 a	53 a	72 a	94 a	143 a
Weed free		0	0	0	0	0	0	0	0	0
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1})	PRE	6 c	0 b	5 b	0 d	2 c	0 e	0 e	5 d	4 e
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1})	PRE	3 c	0 b	66 a	14 c	14 a	9 bc	12 b	40 b	123 ab
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1})	PRE	2 c	0 b	47 a	9 c	22 a	11 bc	15 b	26 b	72 b
Glyphosate/2,4-D (1,630 g ae ha^{-1})	EPOST	47 b	46 a	33 a	30 b	25 a	10 bc	18 b	36 b	25 c
2,4-D (1,060 g ae ha^{-1})	EPOST	59 b	45 a	30 a	33 b	9 b	17 b	22 b	55 b	21 cd
Glufosinate (656 g ai ha^{-1})	EPOST	69 b	45 a	34 a	33 b	41 a	9 bc	30 ab	22 c	8 cd
2,4-D (800 g ae ha^{-1}) + glufosinate (656 g ai ha^{-1})	EPOST	45 b	40 a	36 a	18 b	42 a	6 c	6 c	13 c	12 d
Acetochlor/ clopyralid/mesotrione (2,300 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	2 c	0 b	3 b b	0 d	2 c	0 e	0 e	5 d	3 e
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	3 c	0 b	24 a	10 bc	7 b	7 c	8 c	17 c	14 d
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	4 c	0 b	15 b	10 bc	10 ab	2 d	2 d	22 c	13 d
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	0 d	0 b	2 b	2 cd	3 c	5 c	1 d	48 b	2 e
Acetochlor/ clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	3 c	0 b	19 ab	11 bc	8 b	0 e	0 e	19 c	2 e
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	0 d	0 b	23 a	2 cd	12 ab	0 e	0 e	17 c	2 e
P-value							< 0.0001			

[a]Abbreviations: DA-PRE, days after preemergence application; DA-EPOST, days after early postemergence application; DA-LPOST, days after late postemergence application; EPOST, early postemergence; fb, followed by; LPOST, late postemergence.
[b]Year by treatment interaction for Palmer amaranth density was significant; therefore, data are presented separately for both years (2020 and 2021).
[c]Means presented within each column with no common letters are significantly different according to the Tukey Kramer pairwise comparison test. Year-by-treatment for Palmer amaranth biomass was nonsignificant; therefore, data were combined across both years.
[d]Year-by-treatment interaction for Palmer amaranth biomass was nonsignificant; therefore, data of both years were combined.
[e]Palmer amaranth density data were not collected at 30 d after late postemergence herbicide application in 2020; therefore, data from only 2021 are presented.

clopyralid/mesotrione, which reduced Palmer amaranth density to 0 to 2 plants m^{-2} at 30 d after early postemergence. Among early postemergence herbicides, 2,4-D resulted in a Palmer amaranth density of 9 and 17 plants m^{-2} in 2020 and 2021, respectively, whereas 2,4-D + glufosinate and glufosinate applied alone resulted in a Palmer amaranth density of 6 and 9 plants m^{-2} in 2021, respectively. Adequate soil moisture at the beginning of the season favors the germination of Palmer amaranth and, due to the lack of preemergence herbicide, provides an opportunity for Palmer amaranth to emerge and compete with corn. Palmer amaranth was at a variable height when early postemergence herbicides were applied, and it is known that the efficacy of auxinic herbicides, as well as glufosinate, can vary with weed height and density (Barnett et al. 2013; Jhala et al. 2017; Steckel et al. 1997).

Among preemergence fb late postemergence herbicide programs, acetochlor/clopyralid/mesotrione fb 2,4-D; acetochlor/clopyralid/flumetsulam fb glufosinate; or flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate recorded no Palmer amaranth plants 30 d after late postemergence. Chahal and Jhala (2015) observed just one *Amaranthus* plant per square meter with glufosinate applied early postemergence fb late postemergence 45 d after late postemergence compared with 6 plants m^{-2} in the nontreated control in glufosinate-resistant soybean in Nebraska. Among the preemergence fb late postemergence herbicide programs, acetochlor/clopyralid/flumetsulam fb 2,4-D resulted in higher Palmer amaranth density (8 plants m^{-2}) 30 d after late postemergence, most likely due to declining residual activity of the preemergence herbicide and uneven Palmer amaranth height when 2,4-D was applied. The preemergence fb late postemergence herbicide applications recorded 0 to 8 Palmer amaranth plants m^{-2} compared with 6 to 30 and 0 to 15 plants m^{-2} with early postemergence-only and preemergence-only herbicides, respectively, 30 d after late postemergence (Table 4). Thus, the late postemergence herbicide caused a 50% density reduction compared with the preemergence-only herbicides. Norsworthy et al. (2016) and Aulakh and Jhala (2015) have explained that preemergence fb late postemergence herbicide applications were more effective than early postemergence–only or preemergence-only herbicides due to multiple herbicide application timings and the integration of herbicides with diversified SOAs. Miller and Norsworthy (2016) reported a lower density of Palmer amaranth with herbicide applications that involve multiple SOAs compared with a single herbicide SOA. Furthermore, repeated use of herbicides with the same SOA (e.g., 2,4-D or glufosinate) would select for the herbicide-resistant weed biotype. Resistance to 2,4-D has already been confirmed by Palmer amaranth in Kansas (Kumar et al. 2019) and by a waterhemp biotype in Nebraska (Bernards et al. 2012). Therefore, a sequential and repeated application of 2,4-D to Enlist corn and soybean should be avoided.

The aboveground biomass of Palmer amaranth followed a similar trend to that of density (Table 4). The lowest (≤5 g m^{-2}) Palmer amaranth biomass was recorded after acetochlor/clopyralid/mesotrione was applied compared with other preemergence-only and early postemergence–only herbicides at 30 d after early postemergence and 15 d after late postemergence. Palmer amaranth biomass at 30 d after early postemergence was greater after application of preemergence-only and early postemergence–only herbicides (i.e., acetochlor/clopyralid/flumetsulam, flufenacet/isoxaflutole/thiencarbazone-methyl, glyphosate/2,4-D, and 2,4-D). This might be due to the reduced efficacy of the applied residual herbicide and some Palmer amaranth plants being taller than 15 cm at the time early postemergence herbicides were applied.

At 15 d after late postemergence, acetochlor/clopyralid/mesotrione fb glufosinate, acetochlor/clopyralid/flumetsulam fb glufosinate, flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate, acetochlor/clopyralid/mesotrione fb 2,4-D, and acetochlor/clopyralid/mesotrione reduced Palmer amaranth biomass to 2 to 4 g m^{-2} compared with a biomass of 143 g m^{-2} in the nontreated control group, accounting for ≥97% Palmer amaranth biomass reduction (Table 4). Shyam et al. (2021b) reported 99% reduction in Palmer amaranth biomass with preemergence fb late postemergence herbicides applied to Enlist soybean. Sarangi and Jhala (2019) reported ≥96% Palmer amaranth biomass reduction in soybean with preemergence fb late postemergence herbicide applications. Thus, applications of acetochlor/clopyralid/mesotrione fb 2,4-D, acetochlor/clopyralid/flumetsulam fb glufosinate, flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate, and acetochlor/clopyralid/mesotrione resulted in 100% Palmer amaranth density reduction and ≥97% biomass reduction. Therefore, no seed production was observed after these treatments at the end of season (Table 5). To maintain the effectiveness of any herbicide program, however, it is crucial application timings be followed with appropriate crop and weed growth stages as described on the product label. For example, the 2,4-D label suggests applying the herbicide when broadleaf weeds are shorter than 15 cm (Anonymous 2022), therefore, if it is applied late, Palmer amaranth control can be compromised.

Corn Yield

Year-by-treatment interaction was significant; therefore, yield data are presented separately for both years (Table 5). Corn yield in 2020 was higher due to greater precipitation that provided sufficient moisture for better corn growth and development as it was a dryland field. Herbicide applications resulted in better grain yield in the range of 11,080 kg ha^{-1} to 12,910 kg ha^{-1} and 10,280 kg ha^{-1} to 12,420 kg ha^{-1}, respectively, in 2020 and 2021 compared with 8,750 and 5,790 kg ha^{-1} yield from the untreated control. The lowest corn yield was obtained from the nontreated control, and was comparable to yields after applications of flufenacet/isoxaflutole/thiencarbazone-methyl, glyphosate/2,4-D, and 2,4-D. Orthogonal contrast analysis suggested that herbicides applied early postemergence only resulted in 10,850 kg ha^{-1} grain yield compared with 12,340 kg ha^{-1} after application of preemergence fb late postemergence herbicides. Similarly, Jones et al. (2001) concluded that preemergence fb late postemergence herbicide applications produced 8,890 to 9,570 kg ha^{-1} grain yield compared with glufosinate alone (8,300 kg ha^{-1}) and the nontreated control (5,810 kg ha^{-1}) in multiyear studies of 0glufosinate-resistant corn in Texas. Contrast analysis showed no difference in corn yield between preemergence fb late postemergence applications (11,730 to 12,340 kg ha^{-1}) and preemergence-only applications (10,840 to 11,510 kg ha^{-1}). Liu et al. (2021) observed no difference in corn yield with preemergence-only, preemergence fb early postemergence, and preemergence fb late postemergence herbicide applications, ranging from 9,210 to 10,215 kg ha^{-1}.

Palmer Amaranth Seed Production

Year-by-treatment interaction for Palmer amaranth seed production was nonsignificant; therefore, data were pooled across both years (Table 5). The highest Palmer amaranth seed production (1,077,650 seed m^{-2}) resulted from glufosinate applied alone compared with the nontreated control (939,690 seed m^{-2}) (Table 5). Miranda et al. (2021) reported that Palmer amaranth

Table 5. Corn yield and Palmer amaranth seed production affected by herbicide programs in a 2,4-D–, glyphosate-, and glufosinate-resistant corn in field experiment conducted near Carleton, NE, in 2020 and 2021.[a]

Herbicide program	Timing	Corn yield[b,c] 2020	Corn yield[b,c] 2021	Palmer amaranth seed production[c,d,e]
		kg ha^{-1}	kg ha^{-1}	seed m^{-2}
Nontreated control		8,750 d	5,790 e	939,690 b
Weed-free		11,215 ab	10,620 abcd	0
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1})	PRE	11,080 a	12,160 a	0
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1})	PRE	11,180 abc	11,125 abcd	464,940 c
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1})	PRE	10,250 bcd	11,250 abc	511,540 c
Glyphosate/2,4-D (1,630 g ae ha^{-1})	EPOST	10,205 bcd	10,585 abcd	168,960 d
2,4-D (1,060 g ae ha^{-1})	EPOST	9,390 cd	9,110 d	138,090 d
Glufosinate (656 g ai ha^{-1})	EPOST	12,065 ab	9,555 cd	1,077,650 a
2,4-D (800 g ae ha^{-1}) + glufosinate (656 g ai ha^{-1})	EPOST	11,740 ab	10,875 abcd	122,310 d
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	12,910 a	12,415 a	0
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	12,880 a	11,860 ab	42,940 e
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb 2,4-D (800 g ae ha^{-1})	PRE fb LPOST	12,570 a	11,080 abcd	12,000 e
Acetochlor/clopyralid/mesotrione (2,300 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	11,240 abc	10,280 abcd	29,360 e
Acetochlor/clopyralid/flumetsulam (1,190 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	12,380 ab	12,350 a	0
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha^{-1}) fb glufosinate (656 g ai ha^{-1})	PRE fb LPOST	12,070 ab	12,400 a	0
P-value		<0.0001	<0.0001	<0.0001
Contrast analysis[f]				
PRE vs. EPOST		10,835 vs 10,850 [NS]	11,510 vs 10,030 [g]	325,490 vs 376,750 [g]
PRE vs. PRE fb LPOST		10,835 vs 12,340 [g]	11,510 vs 11,730 [NS]	325,490 vs 14,050 [g]
EPOST vs. PRE fb LPOST		10,850 vs 12,340 [g]	10,030 vs 11,730 [g]	376,750 vs 14,050 [g]

[a]Abbreviations: EPOST, early postemergence; fb, followed by; LPOST, late postemergence; NS, not significant; POST, postemergence.
[b]Year-by-treatment interaction for corn yield was significant; therefore, data are presented separately for both years.
[c]Means presented within each column with no common letters are significantly different according to the Tukey Kramer pairwise comparison test.
[d]Year-by-treatment interaction for Palmer amaranth seed production was nonsignificant; therefore, data were pooled across both years.
[e]Treatments with 0 Palmer amaranth seed production were excluded from the analysis.
[f]A priori orthogonal contrasts.
[g]$P < 0.0001$.

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seed production per plant decreased as Palmer amaranth density increased, and concluded that the highest seed production (376,000 seed plant^{-1}) occurred at the lowest density of 0.2 plants m^{-1} row, and that it declined by 12%, 28%, 55%, and 75% when density increased to 0.3, 0.5, 1, and 2 plants m^{-1} row, respectively. Palmer amaranth density in this study was 43 to 149 plants m^{-2} in the nontreated control compared with 0 to 15, 6 to 30, and 0 to 8 plants m^{-2} in preemergence-only, early postemergence–only, and preemergence fb late postemergence herbicide programs, respectively (Table 4). Therefore, lower seed production in the nontreated control compared with glufosinate applied early postemergence may have been caused by greater interplant competition in the nontreated control. Acetochlor/clopyralid/mesotrione applied preemergence without a follow-up late postemergence herbicide resulted in no Palmer amaranth seed production (Table 5) compared with flufenacet/isoxaflutole/thiencarbazone-methyl applied preemergence only and acetochlor/clopyralid/flumetsulam applied preemergence only, which produced about 0.5 million seed m^{-2}. This might be due to acetochlor/clopyralid/mesotrione effectively reducing Palmer amaranth density and biomass compared with that of flufenacet/isoxaflutole/thiencarbazone-methyl and acetochlor/clopyralid/flumetsulam (Table 4), which resulted in no Palmer amaranth seed production.

Among the preemergence fb late postemergence herbicide programs, acetochlor/clopyralid/mesotrione fb 2,4-D, acetochlor/clopyralid/flumetsulam fb glufosinate, and flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate resulted in no Palmer amaranth seed production (Table 5). Flufenacet/isoxaflutole/thiencarbazone-methyl fb 2,4-D, acetochlor/clopyralid/mesotrione fb glufosinate, and acetochlor/clopyralid/flumetsulam fb 2,4-D resulted in Palmer amaranth seed production of 12,000 to 42,940 seed m^{-2} without difference among them. The contrast analysis showed that preemergence fb late postemergence herbicide programs had Palmer amaranth produced 14,050 seed m^{-2} compared with preemergence-only (325,490 seed m^{-2}) and early postemergence–only (376,750 seed m^{-2}) applications. Striegel and Jhala (2022) reported that Palmer amaranth seed production was 1,634 seed plant^{-1} with preemergence fb POST herbicide applications compared with 7,544 seed plant^{-1} with a postemergence-only herbicide. Similarly, Norsworthy et al. (2016) concluded that the inclusion of a preemergence herbicide with diversified SOA fb glufosinate/glyphosate resulted in ≥97% reduction in Palmer amaranth seed production compared to a glyphosate-only treatment.

Practical Implications

Results of this study indicated that preemergence fb late postemergence and preemergence-only herbicide regimens are available for season-long Palmer amaranth control and reduce seed production in Enlist corn. Based on contrast analysis, Palmer amaranth seed production was reduced to 14,050 seed m^{-2}, and corn yield of 12,340 and 11,730 kg ha^{-1} was obtained after preemergence fb late postemergence herbicide applications compared with 325,490 seed m^{-2} and grain yield of 10,840 and 11,510 kg ha^{-1} in preemergence-only and 376,750 seed m^{-2} and 10,850 and 10,030 kg ha^{-1} in early postemergence–only herbicide programs, respectively, in 2020 and 2021. Enlist technology provides an option for growers with a long window and the flexibility for postemergence application of 2,4-D choline (Enlist ONE) for management of herbicide-resistant Palmer amaranth until the V8 growth stage or a height of 76 cm, or even more than this stage of Enlist corn with precautionary measures. For instance, if corn is taller than 76 cm, 2,4-D choline should be applied using drop nozzles aligned so that spraying does not reach into the whorl of Enlist corn plants (Anonymous 2022). Enlist corn adoption will likely be higher in the future due to resistance to aryloxyphenoxypropionates, which allow the use of quizalofop-p-ethyl on Enlist corn for controlling glyphosate/glufosinate-resistant volunteer corn (Striegel et al. 2020). This is particularly important in states such as Nebraska, where continuous corn production is common. Metabolic resistance in the Palmer amaranth biotype from Kansas, which is resistant to six commonly used corn herbicides, is challenging for corn growers (Shyam et al. 2021a). Therefore, apart from using Enlist corn technology and herbicides with diversified SOAs, there is a need to integrate best management practices with cultural and nonchemical approaches such as scouting of fields before and after herbicide application, row width manipulation, cover cropping, diverse crop rotations, weed seed destruction for persistent control of MHR Palmer amaranth, and reducing seedbank additions. For instance, Price et al. (2012) reported that a high-residue cereal cover crop in combination with broadcast preemergence herbicide was important for managing MHR *Amaranthus* species.

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