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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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Short Title: Palmer amaranth control

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

Keywords: Enlist corn; herbicide program; orthogonal contrast; Palmer amaranth biomass; seed production; weed density.

Nomenclature: 2,4-D; acetochlor; clopyralid; flufenacet; flumetsulam; glufosinate; glyphosate; isoxaflutole; mesotrione; thiencarbazone-methyl; Palmer amaranth, *Amaranthus palmeri* S. Watson; corn, *Zea mays* L.

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 that 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 in soybean (*Glycine max* L. Merrill) 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 acetolactate synthase (ALS)-inhibitor, 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS)-inhibitor, microtubule assembly-inhibitor, photosystem II-inhibitor, protoporphyrinogen oxidase (PPO)-inhibitor (Chahal et al. 2017; Garetson et al. 2019; Ward et al. 2013), 4-hydroxyphenyl pyruvate dioxygenase (HPPD)-inhibitor (Chahal et al. 2015; Jhala et al. 2014), synthetic auxins (Kumar et al. 2019), and very long chain fatty acid-inhibitor (Brabham et al. 2019). A Palmer amaranth biotype resistant to glufosinate has been confirmed in Arkansas (Priess et al. 2022) and dicamba-resistant Palmer amaranth has been reported in Tennessee in 2021 (Foster and Steckel 2022). In addition to resistance to herbicide with a single SOA, Palmer amaranth resistance to multiple herbicides with different SOA 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 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 resistant to ALS-inhibitor, atrazine, and glyphosate in Michigan. As of March 2024, Palmer amaranth has evolved resistance to ten herbicide SOA (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 herbicide applied preemergence (PRE)-only or postemergence (POST)-only (Shyam et al. 2021b; Mausbach et al. 2022). Herbicide applied PRE generally lose their residual activity 20–40 d after application depending on the herbicide used and soil type; however, most POST herbicides commonly applied in corn have minimal to no soil residual activity (Wiggins et al. 2015). The late-emerging Palmer amaranth often escapes POST herbicide and produces seed, leading to the replenishment of the soil seedbank (Bagavathiannan and Norsworthy 2012). Therefore, herbicide programs 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 foliar active POST herbicide in corn up to certain growth stages to provide overlapping residual activity to control weeds (McDonald et al. 2023; Jhala et al. 2015; Sarangi and Jhala 2019).

A new MHR corn trait 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 EPSP-synthase-inhibitor-resistant Palmer amaranth with the aid of herbicide programs that cannot be applied in conventional or glyphosate-resistant corn. The objectives of this study were to evaluate the effect of herbicide programs applied PRE, early-POST (EPOST), and PRE fb late-POST (LPOST) 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. We hypothesized that a season-long control of multiple herbicide-resistant Palmer amaranth would be achieved with reduced seed production in a PRE fb a LPOST herbicide 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 early spring to control glyphosate-resistant horseweed (*Erigeron canadensis* L. Cronq.) 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) was planted at 67,500 seed ha⁻¹ on May 12, 2020 and May 18, 2021 in 76 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.

Herbicide programs included PRE-only, EPOST-only, and PRE fb LPOST 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 CO₂-pressurized backpack sprayer equipped with AIXR 110015 flat-fan nozzles (TeeJet® Technologies, Wheaton, IL) calibrated to deliver a 140 L ha⁻¹ flow rate 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 XR 11005 flat-fan nozzles (TeeJet® Technologies). The PRE herbicides were applied 2 d after corn planting on May 14 in 2020 and on the day of corn planting on May 18 in 2021. Early POST herbicides were applied 36 d after corn planting on June 18, 2020, and 28 d after corn planting on June 16, 2021; and LPOST herbicides were applied on June 23, 2020, and on June 25, 2021. EPOST and LPOST herbicides were applied when Palmer amaranth was 10–15 cm and 20–30 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 PRE (DA-PRE) and EPOST (DA-EPOST), and 15, 30, and 90 d after LPOST (DA-LPOST) 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 0.5 m² quadrats from each plot 15 and 30 DA-PRE, 30 DA-EPOST, and 30 DA-LPOST. Aboveground biomass was collected from 0.5-m² quadrats plot⁻¹ 30 DA-EPOST and 15 DA-LPOST. 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 1.0 m² 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, INC, 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, INC. Albany, OR) that used air to remove the lighter floral chaff from the Palmer amaranth seed (Sosnoskie et al. 2014). The seed were thoroughly cleaned, weighted and number of seed per m² were 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 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 PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc, Cary, NC). Before analysis, data were subjected to UNIVARIATE procedure for testing normality and homogeneity of variance with normal Q-Q plots and levene 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 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 GLIMMIX 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 (PRE vs EPOST, PRE vs PRE fb LPOST, and EPOST vs PRE fb LPOST) at $P \leq 0.05$ for Palmer amaranth control at 15 and 30 DA-EPOST, 15, 30, and 90 DA-LPOST, 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 in June and 45.5 mm in July was observed in 2021, while above-average precipitation of 147.6 mm in June and 424.2 mm in July was observed in 2020 compared to 30-yr average (115.1 mm and 105.2 mm).

Palmer amaranth Control

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

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

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

The PRE fb LPOST herbicide programs provided $\geq 94\%$ control of Palmer amaranth 15 DA-LPOST, and 87% to 97% control 90 DA-LPOST without difference among treatments (Table 3). This was attributed to an early season control of Palmer amaranth by the residual activity of PRE herbicides, whereas the late-emerged flushes of Palmer amaranth were controlled by follow up application of LPOST herbicides. The PRE fb LPOST herbicide programs provided similar Palmer amaranth control (87% to 97%) 90 DA-LPOST. 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 LPOST herbicides can control glyphosate-resistant Palmer amaranth in soybean.

Contrast analysis showed that PRE fb LPOST herbicide programs resulted in 94% Palmer amaranth control compared with 59% and 78% control with PRE-only and EPOST-only programs, respectively (Table 3). Similarly, Sarangi et al. (2017) reported 90% control of herbicide-resistant *Amaranthus* species in soybean with PRE fb LPOST herbicide programs. Several other studies have found greater control of *Amaranthus* species with PRE fb LPOST herbicide programs compared with PRE-only or EPOST-only programs (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 non-significant, 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 compared with 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, 147.6 mm of rainfall provided plenty of moisture for Palmer amaranth emergence and growth (Table 1).

At 30 DA-PRE, acetochlor/clopyralid/mesotrione, acetochlor/clopyralid/flumetsulam, and flufenacet/isoxaflutole/thiencarbazone-methyl resulted in Palmer amaranth density 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 PRE herbicides was reduced, except acetochlor/clopyralid/mesotrione, which reduced Palmer amaranth density to 0 to 2 plants m^{-2} 30 DA-EPOST. Among EPOST herbicide programs, 2,4-D had 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 recorded 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 PRE herbicide, provides an opportunity for Palmer amaranth to emerge and compete with corn. Palmer amaranth was at a variable height when EPOST 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 PRE fb LPOST herbicide programs, acetochlor/clopyralid/mesotrione fb 2,4-D; acetochlor/clopyralid/flumetsulam fb glufosinate; or flufenacet/isoxaflutole/ thien carbazone-methyl fb glufosinate recorded no Palmer amaranth (0 plant m^{-2}) 30 DA-LPOST. Chahal and Jhala (2015) observed 1 *Amaranthus* species m^{-2} with glufosinate applied EPOST fb LPOST 45 DA-LPOST compared with 6 plants m^{-2} in nontreated control in glufosinate-resistant soybean in Nebraska. Among the PRE fb LPOST herbicide programs, acetochlor/clopyralid/flumetsulam fb 2,4-D resulted in higher Palmer amaranth density (8 plants m^{-2}) 30 DA-LPOST, most likely due to declining residual activity of the PRE herbicide and uneven Palmer amaranth height when 2,4-D was applied. The PRE fb LPOST herbicide programs recorded 0 to 8 Palmer amaranth plants m^{-2} compared with 6 to 30 and 0 to 15 plants m^{-2} with EPOST-only and PRE-only herbicides, respectively, 30 DA-LPOST (Table 4). Thus, the LPOST herbicide caused a 50% density reduction compared with the most PRE-only herbicides. Norsworthy et al. (2016) and Aulakh and Jhala (2015) have explained that PRE fb LPOST herbicide programs were more effective compared with EPOST-only or PRE-only herbicides due to multiple herbicide application timings and the integration of herbicides with diversified SOA. Miller and Norsworthy (2016) reported a lower density of Palmer amaranth with herbicide programs involving multiple SOA 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. It is important to note that 2,4-D resistance has already been confirmed in Palmer amaranth from Kansas (Kumar et al. 2019) and in a waterhemp biotype in Nebraska (Bernards et al. 2012). Therefore, a sequential and repeated application of 2,4-D in 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 ($\leq 5 \text{ g m}^{-2}$) Palmer amaranth biomass was obtained with acetochlor/clopyralid/mesotrione compared to other PRE-only and EPOST-only herbicides at 30 DA-EPOST and 15 DALPOST. Some PRE-only and EPOST-only herbicides (acetochlor/clopyralid/flumetsulam, flufenacet/isoxaflutole/thien carbazone-methyl, glyphosate/2,4-D, and 2,4-D) showed higher Palmer amaranth biomass at 30 DA-EPOST. This

might be due to the reduced efficacy of the applied residual herbicide and some Palmer amaranth plants more than 15 cm tall at the time EPOST herbicides were applied.

At 15 DA-LPOST, 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 2 to 4 g m⁻² compared to 143 g m⁻² in the nontreated control accounting for ≥ 97% Palmer amaranth biomass reduction (Table 4). Shyam et al. (2021b) reported 99% reduction in Palmer amaranth biomass with PRE fb LPOST herbicides in Enlist soybean. Sarangi and Jhala (2019) reported ≥ 96% Palmer amaranth biomass reduction in soybean with PRE fb LPOST herbicide programs. Thus, acetochlor/clopyralid/mesotrione fb 2,4-D, acetochlor/clopyralid/flumetsulam fb glufosinate, flufenacet/isoxaflutole/thiencarbazone-methyl fb glufosinate, and acetochlor/clopyralid/mesotrione had provided 100% Palmer amaranth density reduction and ≥ 97% biomass reduction. Therefore, no seed production was observed in these treatments at the end of season (Table 5). To maintain the effectiveness of any herbicide program, however, it will be crucial to follow application timings with appropriate crop and weed growth stages as described on the product label. For example, the 2,4-D label suggests applying when broadleaf weeds are less 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 higher precipitation that provided sufficient moisture for better corn growth and development as it was a dryland field. Herbicide programs 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 in untreated control. The lowest corn yield was obtained in the nontreated control, which was comparable to flufenacet/isoxaflutole/thiencarbazone-methyl, glyphosate/2,4-D, and 2,4-D (9,391 and 9,107 kg ha⁻¹ in 2020 and 2021, respectively). Orthogonal contrast analysis suggested that herbicides applied E-POST-only resulted in 10,850 kg ha⁻¹ grain yield compared with 12,340 kg ha⁻¹ with the PRE fb LPOST herbicide programs. Similarly, Jones et al. (2001) concluded that PRE fb LPOST herbicide programs produced 8,890

to 9,570 kg ha⁻¹ grain yield compared with glufosinate (8,300 kg ha⁻¹) and nontreated control (5,810 kg ha⁻¹) in multi-year studies in glufosinate-resistant corn in Texas. Contrast analysis showed that there was no difference in corn yield between PRE fb LPOST programs (11,730 to 12,340 kg ha⁻¹) and PRE-only herbicides (10,840 to 11,510 kg ha⁻¹). Liu et al. (2021) reported that no difference in corn yield was observed with PRE-only, PRE fb EPOST and PRE fb LPOST herbicide programs, and it ranged from 9,210 to 10,215 kg ha⁻¹.

Palmer amaranth Seed Production

Year by treatment interaction for Palmer amaranth seed production was non-significant; 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 seed production per plant decreased as Palmer amaranth density increased, and concluded that the highest seed production (376,000 seeds plant⁻¹) was found 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 PRE-only, EPOST-only, and PRE fb LPOST herbicide programs, respectively (Table 4). Therefore, lower seed production in the nontreated control compared with the glufosinate applied EPOST may have been caused by higher inter-plant competition in the nontreated control. Acetochlor/clopyralid/mesotrione applied PRE without a follow-up LPOST herbicide resulted in no Palmer amaranth seed production (Table 5) compared with flufenacet/isoxaflutole/thiencarbazone-methyl applied PRE-only and acetochlor/clopyralid/flumetsulam applied PRE-only, which produced about 0.5 million seed m⁻². This might be due to acetochlor/clopyralid/mesotrione was effective reducing Palmer amaranth density and biomass compared with flufenacet/isoxaflutole/thiencarbazone-methyl and acetochlor/clopyralid/flumetsulam (Table 4) that resulted in no Palmer amaranth seed production.

Among the PRE fb LPOST 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 PRE fb LPOST herbicide programs had Palmer amaranth seed production of 14,050 seed m⁻² compared with PRE-only (325,490 seed m⁻²) and EPOST-only (376,750 seed m⁻²) programs. Striegel and Jhala (2022) reported that Palmer amaranth seed production was 1,634 seed plant⁻¹ with PRE fb POST herbicide programs compared with 7,544 seed plant⁻¹ with a POST-only herbicide. Similarly, Norsworthy et al. (2016) concluded that the inclusion of a PRE 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 PRE fb LPOST and PRE-only herbicide programs 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 in PRE fb LPOST herbicide programs compared with 325,490 seed m⁻² and grain yield of 10,840 and 11,510 kg ha⁻¹ in PRE-only and 376,750 seed m⁻² and 10,850 and 10,030 kg ha⁻¹ in EPOST-only programs, respectively, in 2020 and 2021. Enlist technology provides an option for growers with a long window/flexibility for POST application of 2,4-D (Enlist ONE) for management of herbicide-resistant Palmer amaranth till V8 growth stage or 76 cm height or even more than this stage of Enlist corn with precautionary measures. For instance, if corn is taller than 76 cm, 2,4-D 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 in 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, resistance 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 SOA, there is a need to integrate best management practices with cultural and non-chemical 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 PRE herbicide was important to manage MHR *Amaranthus* species.

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Competing Interests

The authors declare none.

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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, Nebraska.

Month	Mean air temperature ^a			Total precipitation ^a		
	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, Nebraska in 2020 and 2021.

Herbicide program ^a	Trade name	Application timing ^b	Rate g ae or ai ha ⁻¹	Manufacturer
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, WI) at 3% vol/vol.

^b Abbreviations: PRE, preemergence; EPOST, early POST; fb, followed by; LPOST, late POST; POST, postemergence.

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 at Carleton, Nebraska in 2020 and 2021.

Herbicide program	Timing ^a	Palmer amaranth control ^{a,b,c}													
		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	a b
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 LPOST fb	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 LPOST fb	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 LPOST fb	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 LPOST fb	99	a	99	a	99	a	98	a	94	a	89	b	87 ab	a b
Acetochlor/ clopyralid/flumetsulam (1,190 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE LPOST fb	98	a	83	a	92	a	92	a	99	a	95	a	95 a	a

ha ⁻¹)																
Flufenacet/isoxaflutole/thiencarbazone-methyl (536 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE	fb	99	a	79	a	93	a	93	a	99	a	93	b	93 a	a
	LPOST															
P-value			0.72		0.15		0.0004		0.0001		0.8633		0.005		0.0004	
			5		7											
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							73 vs 88 NS	67 vs 87 e	87 vs 97 e	77 vs 91 e	78 vs 94 ^e					

^aAbbreviations: -, not applicable, DA-PRE, days after PRE application; DA-EPOST, days after early-POST application; DA-LPOST, days after late-POST application; EPOST, early POST; fb, followed by; LPOST, late POST; NS, not significant.

^bYear by treatment interaction for Palmer amaranth control was non-significant; therefore, data were pooled across both years (2020 and 2021).

^cMeans presented within each column with no common letter (s) are significantly different as per Tukey Kramer's pairwise comparison test.

^dA priori orthogonal contrasts.

^eP < 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 in Carleton, Nebraska in 2020 and 2021.^{a,b}

Herbicide program	Timin g ^a	Palmer amaranth density ^{a,b,c}												Palmer amaranth biomass ^{a,c,d}					
		15 DA- PRE				30 DA- PRE				30 DA- EPOST				30 DA- LPOST ^c		30 DA- EPOST		15 DA- LPOST	
		number m ⁻²												g m ⁻²					
		2020		2021		2020		2021		2020		2021		2021					
Nontreated control		14 9	a	4 3	a	10 8	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	b c	12	b	40	b	123	ab
Flufenacet/isoxaflutole/thiencarbazon e-methyl (536 g ai ha ⁻¹)	PRE	2	c	0	b	47	a	9	c	22	a	11	b c	15	b	26	b	72	b
Glyphosate/2,4-D (1,630 g ae ha ⁻¹)	EPOST	47	b	4 6	a	33	a	30	b	25	a	10	b c	18	b	36	b	25	c
2,4-D (1,060 g ae ha ⁻¹)	EPOST	59	b	4 5	a	30	a	33	b	9	b	17	b	22	b	55	b	21	cd
Glufosinate (656 g ai ha ⁻¹)	EPOST	69	b	4 5	a	34	a	33	b	41	a	9	b c	30	a b	22	c	8	cd
2,4-D (800 g ae ha ⁻¹) + glufosinate (656 g ai ha ⁻¹)	EPOST	45	b	4 0	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 T	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	PRE fb	3	c	0	b	24	a	10	bc	7	b	7	c	8	c	17	c	14	d

ha ⁻¹)	LPOST																		
Flufenacet/isoxaflutole/thiencarbazon e-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/thiencarbazon e-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																	

^aAbbreviations: DA-PRE, days after PRE application; DA-EPOST, days after early-POST application; DA-LPOST, days after late-POST application; EPOST, early POST; fb, followed by; LPOST, late POST.

^bYear by treatment interaction for Palmer amaranth density was significant; therefore, data are presented separately for both years (2020 and 2021).

^cMeans presented within each column with no common letter(s) are significantly different as per Tukey Kramer's pairwise comparison test. Year by treatment for Palmer amaranth biomass was non-significant; therefore, data were combined across both years.

^dYear by treatment interaction for Palmer amaranth biomass was non-significant; therefore, data of both years were combined.

^ePalmer amaranth density data were not collected at 30 d after late-POST herbicide application in 2020; therefore, data of only 2021 are presented.

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 at Carleton, Nebraska in 2020 and 2021.^a

Herbicide program	Timing ^a	Corn yield ^{b,c}				Palmer amaranth seed production ^{c,d,e}	
		kg ha ⁻¹				seed m ⁻²	
		2020		2021			
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	11,510 vs 10,030 ^g		325,490 vs 376,750	

	10,850 ^{NS}		^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

^aAbbreviations: EPOST, early POST; fb, followed by; LPOST, late POST; NS, not significant; POST, postemergence.

^bYear by treatment interaction for corn yield was significant; therefore, data are presented separately for both years.

^cMeans presented within each column with no common letter(s) are significantly different as per Tukey Kramer's pairwise comparison test.

^dYear by treatment interaction for Palmer amaranth seed production was non-significant; therefore, data were pooled across both years.

^eTreatments with 0 Palmer amaranth seed production were excluded from the analysis.

^fA priori orthogonal contrasts.

^gP < 0.0001