



Management of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in 2,4-D-, glufosinate-, and glyphosate-resistant soybean⁴

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Research Article

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2,4-D chlorimuron-ethyl; cloransulam-methyl; flumioxazin; glufosinate; glyphosate; imazethapyr; metribuzin; pyroxasulfone; saflufenacil; sulfentrazone; Palmer amaranth; *Amaranthus palmeri* S. Watson; soybean; *Glycine max* (L.) Merr.

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Abstract

Glyphosate-resistant (GR) Palmer amaranth is a problematic, annual broadleaf weed in soybean production fields in Nebraska and many other states in the United States. Soybean resistant to 2,4-D, glyphosate, and glufosinate (Enlist E3™) has been developed and was first grown commercially in 2019. The objectives of this research were to evaluate the effect of herbicide programs applied PRE, PRE followed by (fb) late-POST (LPOST), and early-POST (EPOST) fb LPOST on GR Palmer amaranth control, density, and biomass reduction, soybean injury, and yield. Field experiments were conducted near Carleton, NE, in 2018, and 2019 in a grower's field infested with GR Palmer amaranth in 2,4-D-, glyphosate-, and glufosinate-resistant soybean. Sulfentrazone + cloransulam-methyl, imazethapyr + saflufenacil + pyroxasulfone, and chlorimuron ethyl + flumioxazin + metribuzin applied PRE provided 84% to 97% control of GR Palmer amaranth compared with the nontreated control 14 d after PRE. Averaged across herbicide programs, PRE fb 2,4-D and/or glufosinate, and sequential application of 2,4-D or glufosinate applied EPOST fb LPOST resulted in 92% and 88% control of GR Palmer amaranth, respectively, compared with 62% control with PRE-only programs 14 d after LPOST. Reductions in Palmer amaranth biomass followed the same trend; however, Palmer amaranth density was reduced 98% in EPOST fb LPOST programs compared with 91% reduction in PRE fb LPOST and 76% reduction in PRE-only programs. PRE fb LPOST and EPOST fb LPOST programs resulted in an average soybean yield of 4,478 and 4,706 kg ha⁻¹, respectively, compared with 3,043 kg ha⁻¹ in PRE-only programs. Herbicide programs evaluated in this study resulted in no soybean injury. The results of this research illustrate that herbicide programs are available for the management of GR Palmer amaranth in 2,4-D-, glyphosate-, and glufosinate-resistant soybean.

Introduction

Commercialization of herbicide-resistant (HR) crop technology in the late 1990s led to a turning point in the history of weed management (Reddy and Nandula 2012). Glyphosate-resistant (GR) soybean and corn (*Zea mays* L.) were rapidly adopted by growers in the United States because of its ease of use, cost-effectiveness, and broad-spectrum weed control (Green and Owen 2011). Currently, HR soybean constitutes 94% of the total soybean planted in the United States (USDA 2019). HR soybean including a single trait such as glyphosate resistance or glufosinate resistance, or multiple HR traits such as glyphosate and dicamba resistance are grown in the United States. The cultivation and widespread adoption of GR corn and soybean after their commercialization reduced the use of residual herbicides because of flexibility in application timing, excellent weed control, and wide margin of crop safety with glyphosate (Green 2012); however, repeated application of glyphosate resulted in the evolution of GR weeds (Heap and Duke 2018). Currently, 48 weed species have been reported to have evolved resistance to glyphosate globally, including 17 species in the United States (Heap 2020).

Native to the southwestern United States, Palmer amaranth has been ranked as the most troublesome weed in agronomic cropping systems in the United States in a survey conducted by the Weed Science Society of America (Van Wychen 2019). It is also one of the most economically important weeds in agronomic crops in the United States (Beckie 2011; Chahal et al. 2018). Palmer amaranth is a prolific seed producer (Keeley et al. 1987) and can survive and produce a high amount of seeds even under moisture-stressed situations (Chahal et al. 2018). It has a high photosynthetic rate, resulting in a fast growth rate and considerable biomass accumulation compared with other *Amaranthus* species (Ehleringer 1983; Jha and Norsworthy 2009), along with continuous emergence throughout the growing season, leading to season-long crop interference (Jha and Norsworthy 2009). In addition to the aforementioned weedy

characteristics, the evolution of herbicide resistance in Palmer amaranth has made control of this weed especially challenging in cotton (*Gossypium* sp. L.) and soybean. GR Palmer amaranth was first reported in Georgia (Culpepper et al. 2006), and since then, 27 other U.S. states have documented the presence of GR Palmer amaranth (Heap 2020).

Glyphosate is a systemic, nonselective POST herbicide that targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the chloroplast of sensitive plants, causing inhibition of aromatic amino acid production (Bentley 1990). Current mechanisms of target-site resistance to glyphosate in Palmer amaranth have been due to either mutation in the EPSPS gene, the molecular target of glyphosate (Dominguez-Valenzuela et al. 2017), or overamplification and expression of the EPSPS gene (Chahal et al. 2017; Gaines et al. 2010; Koo et al. 2018). Reduced glyphosate absorption and translocation have also been reported to impart nontarget-site resistance to glyphosate in Palmer amaranth (Dominguez-Valenzuela et al. 2017; Nandula et al. 2012). Considering the extent of GR Palmer amaranth populations, effective management programs in soybean should focus on methods that reduce survival, seed production, and transfer of herbicide-resistance alleles.

Palmer amaranth interference can cause substantial yield losses in agronomic crops. For example, a Palmer amaranth density of 3 plants m^{-2} caused 60% yield loss in soybean in Arkansas (Klingaman and Oliver 1994), and Bensch et al. (2003) reported 79% soybean yield loss at a density of 8 plants m^{-2} in Kansas. Early emergence (0–1 wk after crop emergence) and establishment of Palmer amaranth reduce soybean yield compared with late emergence (2–8 wk after crop emergence) (Korres et al. 2019). Van Acker et al. (1993) reported the critical period of weed control in soybean ranged from the second soybean-node growth stage (V2) to the beginning pod growth stage (R3). However, in a recent multilocation and multiyear study conducted in Nebraska, the critical period of weed removal in soybean was delayed from V1–V2 to V4–R5 using PRE herbicides such as saflufenacil + imazethapyr + pyroxasulfone and saflufenacil + imazethapyr (Knezevic et al. 2019).

Herbicide applied PRE is a foundation for management of GR weeds such as Palmer amaranth and waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] followed by (fb) POST herbicides to control late-emerged weeds (Jhala et al. 2017). Sarangi and Jhala (2019) reported 97% and 86% control of Palmer amaranth with the application of PRE fb POST herbicide with and without layered residual activity, respectively. This finding suggests that POST herbicide mixed with an additional foliar, active herbicide such as glufosinate + 2,4-D or a residual herbicide such as acetochlor can lead to better weed control compared with POST herbicide applied alone for control of Palmer amaranth (Aulakh and Jhala 2015).

A GR Palmer amaranth population was confirmed in a grower's field in a continuous GR corn–soybean rotation located in Thayer County, NE (Chahal et al. 2017). A subsequent greenhouse dose-response bioassay confirmed that Palmer amaranth was 37- to 40-fold resistant to glyphosate compared with a glyphosate-susceptible population. Moreover, the mechanism of resistance was found to be amplification of EPSPS gene (32- to 105-fold) compared with a glyphosate-susceptible biotype (Chahal et al. 2017). Interestingly, the GR Palmer amaranth population was less sensitive to POST herbicides such as atrazine, mesotrione, and halosulfuron-methyl, but was effectively controlled ($\geq 95\%$) with glufosinate at 593 g ai ha^{-1} (Chahal et al. 2017).

A new, multiple HR-soybean trait that exhibits resistance to 2,4-D, glufosinate, and glyphosate has been developed by

Corteva™ Agriscience and was commercialized in 2019 in the United States. However, little scientific literature exists pertaining to the most effective herbicide programs for managing GR Palmer amaranth in this new soybean production system. Therefore, the objectives of this research were to investigate and compare the effect of three preformulated herbicide mixtures applied PRE alone or fb a late-POST (LPOST) application of either 2,4-D or glufosinate or both and sequential applications (early-POST [EPOST] fb LPOST) of 2,4-D or glufosinate on Palmer amaranth density, biomass, crop injury, and yield in a multiyear field study in Nebraska.

Materials and Methods

Site Description

Field experiments were conducted in 2018 and 2019 in a grower's field infested with GR Palmer amaranth in Thayer County, Carleton, NE (40.30°N, 97.67°E) (Chahal et al. 2017). The field was rain fed without supplementary irrigation. Palmer amaranth was the predominant weed species at the research site. The soil at the experimental site was silt loam with 63% silt, 19% sand, 18% clay, 2.63% organic matter, and 4.8 pH. The previous crop at the site was no-till soybean. The experiment was arranged in a randomized complete block design with four replications. Individual plots were 3 m wide (four soybean rows spaced 0.76 m apart) and 9 m long. Glyphosate-, 2,4-D-, and glufosinate-resistant soybean with 2.5 maturity group was no-till planted at a rate of 322,000 seeds ha^{-1} at a depth of 3 cm on May 10, 2018, and May 6, 2019.

Herbicide Treatments

Herbicide programs included PRE, PRE fb LPOST, and EPOST fb LPOST applications with a total of 15 treatments, including a non-treated control (Table 1). Herbicides were applied using a CO₂-pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 210 kPa equipped with a 2-m wide spray boom equipped with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., Wheaton, IL) spaced 50 cm apart for PRE herbicides and AIXR11004 for 2,4-D and XR11005 nozzles for glufosinate application. The PRE herbicides were applied the same day after planting soybean; EPOST herbicides were applied at 39 d after planting (DAP) on June 18, 2018, and 35 DAP on June 10, 2019; and LPOST herbicides were applied at 18 d after EPOST (DA-EPOST) on July 6, 2018, and 14 DA-EPOST herbicides on June 24, 2019. Soybean stages corresponding to application timings were V2–V3 soybean growth stage for EPOST and V4–V5 for LPOST application. Palmer amaranth height corresponding to the EPOST applications was 8 to 12 cm and for LPOST applications was 10 to 15 cm.

Data Collection

Palmer amaranth control and soybean injury were visually assessed at 14 d after PRE (DA-PRE), 14 DA-EPOST, and 14 d after late POST (DA-LPOST) on a scale of 0% to 100%, where 0% was equivalent to no Palmer amaranth control or soybean injury and 100% was equivalent to complete control or soybean-plant death. Palmer amaranth density was recorded from two randomly placed, 0.5 m^2 quadrats $plot^{-1}$ at 14 DA-EPOST and 14 DA-LPOST. Likewise, aboveground Palmer amaranth biomass was collected from two randomly placed 0.5- m^2 quadrats $plot^{-1}$ at 14 DA-EPOST and 14 DA-LPOST. At each of these intervals, Palmer amaranth plants were placed in paper bags, oven-dried, and weighed.

Table 1. Details of herbicide programs, application timing, and rates used for control of glyphosate-resistant Palmer amaranth in 2,4-D-, glyphosate-, and glufosinate-resistant soybean in field experiments conducted at Carleton, NE, during the 2018 and 2019 growing seasons.

Treatment No.	Herbicide program (rate) ^a	Timing	Trade name		
			PRE/EPOST	LPOST ^b	Manufacturer
1	Nontreated control	–	–	–	–
2	Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹)	PRE	Sonic [®]	–	Corteva Agriscience, Wilmington, DE 19880
3	Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	Sonic [®]	Enlist One [®]	Corteva Agriscience
4	Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	Sonic [®]	Liberty [®] 280 SL	Corteva Agriscience; BASF Corp., Research Triangle Park, NC 27709
5	Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	Sonic [®]	Enlist One [®] + Liberty [®] 280 SL	Corteva Agriscience; Corteva Agriscience + BASF Corp.
6	Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹)	PRE	Zidua [®] Pro	–	BASF Corp.
7	Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	Zidua [®] Pro	Enlist One [®]	BASF Corp.; Corteva Agriscience
8	Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	Zidua [®] Pro	Liberty [®] 280 SL	BASF Corp.
9	Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	Zidua [®] Pro	Enlist One [®] + Liberty [®] 280 SL	BASF Corp.; Corteva Agriscience + BASF Corp.
10	Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹)	PRE	Trivence [®]	–	Corteva Agriscience
11	Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	Trivence [®]	Enlist One [®]	Corteva Agriscience
12	Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	Trivence [®]	Liberty [®] 280 SL	Corteva Agriscience; BASF Corp.
13	Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	Trivence [®]	Enlist One [®] + Liberty [®] 280 SL	Corteva Agriscience; Corteva Agriscience + BASF Corp.
14	Glufosinate (656 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	EPOST fb LPOST	Liberty [®] 280 SL	Liberty [®] 280 SL	BASF Corp.
15	2,4-D (2,080 g ae ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	EPOST fb LPOST	Enlist One [®]	Enlist One [®]	Corteva Agriscience

^aAbbreviations: –, not applicable; EPOST, early POST; fb, followed by; LPOST, late POST.

^bAmmonium sulphate (N-Pak AMS Liquid; Winfield Solutions, St Paul, MN 55164) at 2.5% vol/vol was mixed with glufosinate treatments.

At 14 DA-PRE, data were collected from the plots treated with PRE herbicides and the nontreated control; at 14 DA-EPOST and 14 DA-LPOST, Palmer amaranth control, density, and biomass data were collected from all plots. Palmer amaranth density and aboveground biomass data were converted into percent density and biomass reduction compared with the nontreated control, using Equation 1:

$$\text{Percent density or biomass reduction} = [(C - B)/C] \times 100 \quad [1]$$

where *C* is Palmer amaranth density or aboveground biomass of the nontreated control plot and *B* is the Palmer amaranth density or aboveground biomass collected from an individual experimental plot. At maturity, soybean was harvested from the middle two rows using a plot combine, weighed, and soybean yield was adjusted to 13.5% moisture content. However, due to pending export approval of 2,4-D-, glyphosate-, and glufosinate-resistant soybean in few countries, the field experiment was destroyed in 2018, so yield data are available only for 2019.

Statistical Analysis

ANOVA was performed on Palmer amaranth control, density, and aboveground biomass, and soybean yield data using PROC GLIMMIX in SAS, version 9.4 (SAS Institute, Cary, NC). Before analysis, data were subjected to PROC UNIVARIATE analysis for testing normality and homogeneity of variance. Visual estimates of Palmer amaranth control and percent density and biomass reduction data were arc-sine square-root transformed and were back-transformed for presentation. For Palmer amaranth

control, percent density, and biomass reduction at 14 DA-PRE, 14 DA-EPOST, and 14 DA-LPOST, treatment and years were considered fixed effects; blocks were considered a random effect. For soybean yield, treatment was considered as a fixed effect (because there was only 1 yr of data) and blocks were considered as a random effect. Post hoc comparison of treatments was accomplished using Fisher LSD test at $\alpha = 0.05$. Orthogonal contrasts were performed to compare herbicide programs (PRE-only vs. PRE fb LPOST; PRE fb LPOST vs. EPOST fb LPOST) at 14 DA-LPOST for Palmer amaranth control, density, and aboveground biomass at $\alpha = 0.05$.

Results and Discussion

Year-by-treatment interactions for Palmer amaranth control, density, and aboveground biomass at 14 DA-PRE, 14 DA-EPOST, and 14 DA-LPOST were not significant ($P \geq 0.05$); therefore, data from both years were pooled. The average monthly temperature during May 2018 was higher than May 2019 and the 30-yr average (Table 2). Apart from that, monthly temperatures during the crop season in both years were similar to the 30-yr average. More precipitation fell in 2019 compared with 2018 and the 30-yr average (Table 2). There was no soybean injury from the herbicides evaluated (data not presented).

Palmer Amaranth Control

Herbicides applied PRE in this study provided 84% to 97% control of GR Palmer amaranth at 14 DA-PRE, and no differences in control were observed among the PRE herbicides (Table 3).

Table 2. Average air temperature and total precipitation during the 2018 and 2019 growing seasons and the 30-yr average at the Hebron, NE, weather station near Carleton, NE.^a

Timing	Average temperature			Average precipitation		
	2018	2019	30-yr average	2018	2019	30-yr average
	°C			mm		
May	20.6	14.6	16.5	78	172.7	122
June	25	21.8	22.2	96	153.2	121.4
July	24.7	25.1	25.1	95.5	137.2	105
August	23.3	23.1	24	92.2	155	94.7
September	20.6	22.6	18.8	153.4	120.4	74.2
October	10.6	9.6	11.8	99.8	118.1	56.1
Annual	10.6	10.3	10.9	614.9	856.5	797.3

^aAir temperature and precipitation data were obtained from High Plains Regional Climate Center (HPRCC 2020).

Although statistically similar, sulfentrazone + cloransulam-methyl provided 84% to 87% control, whereas imazethapyr + saflufenacil + pyroxasulfone, and chlorimuron ethyl + flumioxazin + metribuzin provided 87% to 97% control at 14 DA-PRE (Table 3). Similar to our findings, Sarangi and Jhala (2019) reported 97% to 100% control of Palmer amaranth with chlorimuron ethyl + flumioxazin + metribuzin, saflufenacil + imazethapyr + dimethenamid-P, and sulfentrazone + metribuzin at 14 DA-PRE in soybean. Aulakh and Jhala (2015) reported 95% control of waterhemp with sulfentrazone + cloransulam-methyl at 15 DA-PRE in glufosinate-resistant soybean in Nebraska.

The residual activity of herbicides applied PRE declined as the season progressed. For example, sulfentrazone + cloransulam-methyl provided 27% control of Palmer amaranth 14 DA-LPOST compared with 75% control with imazethapyr + saflufenacil + pyroxasulfone and chlorimuron ethyl + flumioxazin + metribuzin. In 2018, greater Palmer amaranth control (86%) was achieved at 14 DA-EPOST compared with 2019 (74%). Less Palmer amaranth control obtained in 2019 may be attributed to much greater seedling emergence due to increased soil moisture availability (Table 2).

At 14 DA-EPOST, glufosinate or 2,4-D provided 88% and 65% control of Palmer amaranth, respectively (Table 3). Less control by 2,4-D could be attributed to variable Palmer amaranth height at the time of application. For instance, Craigmyle et al. (2013) and Everitt and Keling (2007) reported that weed height at the time of 2,4-D application can affect the level of broadleaf weed control achieved. At 14 DA-LPOST, herbicides applied PRE without a follow-up POST herbicide did not maintain Palmer amaranth control compared with PRE fb POST or EPOST fb LPOST herbicide programs (Table 3). A similar decline in residual activity of soil-applied PRE herbicides has been reported in no-till soybean in Nebraska where PRE-only herbicides resulted in 66% control of Palmer amaranth compared with 86% control by PRE fb POST herbicide programs at 28 d after POST (Sarangi and Jhala 2019).

Among the PRE fb LPOST programs, sulfentrazone + cloransulam-methyl fb 2,4-D provided the lowest (70%) Palmer amaranth control (Table 3). GR Palmer amaranth control provided by the remaining PRE fb LPOST programs ranged from 88% to 100% (Table 3). Interestingly, chlorimuron ethyl + flumioxazin + metribuzin applied PRE alone provided statistically similar control (85%) as sequential application of 2,4-D (93%) or glufosinate (92%) (Table 3). This might be attributed to a high level of GR Palmer amaranth control by the residual activity of this premix. Palmer amaranth is known for its extended emergence pattern (Jha and Norsworthy 2009); however, emergence is

reported to be higher from early May to mid-July, which is before soybean canopy closure (Jha and Norsworthy 2009). Thus, PRE herbicide would not only provide emerging soybean seedlings a weed-free start but also result in reduced reliance on POST herbicides (Norsworthy et al. 2012).

The sequential application of 2,4-D or glufosinate provided 85% to 92% control of Palmer amaranth 14 DA-LPOST (Table 3). Similarly, Chahal and Jhala (2015) reported 86% to 98% waterhemp control 75 DA-LPOST with single as well as sequential application of glufosinate in glufosinate-resistant soybean. Meyer et al. (2015) showed that synthetic auxin herbicide-based LPOST programs can be options to control GR Palmer amaranth and waterhemp in soybean traits resistant to 2,4-D or dicamba. Contrast analysis showed that PRE fb LPOST programs resulted in 92% Palmer amaranth control compared with 62% control with PRE-only programs (Table 3). Similarly, Sarangi et al. (2017) reported 90% control of GR waterhemp in soybean with PRE fb LPOST herbicide programs. There was no difference in Palmer amaranth control provided by PRE fb LPOST (92%) and EPOST fb LPOST programs (88%) at 14 DA-LPOST. Contrary to our findings, authors of several studies have found greater control of GR waterhemp with PRE fb POST herbicide programs compared with POST-only programs in soybean (Aulakh and Jhala 2015; Johnson et al. 2012; Sarangi et al. 2017).

Palmer Amaranth Density and Biomass Reduction

Palmer amaranth emergence was greater in 2019 compared with 2018, leading to greater Palmer amaranth density. For example, Palmer amaranth density in the nontreated control ranged from 100 to 200 plants m⁻² in 2018 compared with 300 to 500 plants m⁻² in 2019 (data not shown). This was most likely due to adequate rainfall in 2019 compared with 2018 (Table 2), and a substantial Palmer amaranth seed bank at this location. Adequate soil moisture favors the germination of Palmer amaranth seeds (Hartzler et al. 1999). At 14 DA-EPOST, greater density reduction was obtained in 2018 (89%) compared with 71% in 2019, which can be attributed to greater emergence in 2019. At 14 DA-EPOST, glufosinate provided 86% Palmer amaranth density reduction compared with a 60% reduction with 2,4-D (Table 4). Palmer amaranth was at a variable height when EPOST herbicides were applied and it is well known that the efficacy of auxinic herbicides, as well as glufosinate, can vary with weed height and density (Barnett et al. 2013; Craigmyle et al. 2013; Everitt and Keeling 2007; Jhala et al. 2017; Steckel et al. 1997). At 14 DA-LPOST, all PRE fb LPOST herbicide programs except sulfentrazone + cloransulam-methyl fb 2,4-D provided 89% to 100% reduction in Palmer amaranth density compared with the nontreated control (Table 4). Sulfentrazone + cloransulam-methyl fb 2,4-D resulted in 42% Palmer amaranth density reduction, most likely due to declining residual activity as well as uneven Palmer amaranth size when LPOST herbicides were applied.

When averaged across herbicide programs, PRE fb LPOST programs (91%) resulted in a statistically higher density reduction of GR Palmer amaranth compared with PRE-only programs (76%). Thus, the application of a LPOST herbicide caused a 20% increase in density reduction compared with PRE-only herbicide programs alone (Table 4). Overall, Palmer amaranth density reduction ranged from 89% to 100% without a difference in the most PRE fb POST herbicide programs at 14 DA-LPOST (Table 4). EPOST fb LPOST programs (sequential application of glufosinate or 2,4-D) resulted in 98% density reduction of Palmer amaranth

Table 3. Glyphosate-resistant Palmer amaranth control as affected by herbicide programs in 2,4-D-, glyphosate-, and glufosinate-resistant soybean in field experiments conducted at Carleton, NE, during the 2018 and 2019 growing seasons.^a

Herbicide program	Timing ^b	Palmer amaranth control ^c		
		14 DA-PRE	14 DA-EPOST	14 DA-LPOST
Nontreated control	–	0	0	0
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹)	PRE	85 a	49 f	27 e
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	87 a	71 de	70 d
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb Glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	85 a	81 b–d	88 bc
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	84 a	75 c–e	96 ab
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹)	PRE	97 a	81 b–d	75 d
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	95 a	89 ab	95 ab
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	95 a	92 ab	99 a
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	87 a	94 a	96 ab
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹)	PRE	94 a	75 c–e	85 c
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	93 a	90 ab	93 a–c
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	89 a	87 a–c	92 a–c
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	97 a	92 ab	100 a
Glufosinate (656 g ai ha ⁻¹) fb Glufosinate (656 g ai ha ⁻¹)	EPOST fb LPOST	–	88 ab	85 c
2,4-D (2,080 g ae ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	EPOST fb LPOST	–	65 e	92 a–c
P-value		0.2076	<0.0001	<0.0001
Contrast analysis ^d				
PRE vs. PRE fb LPOST		–	–	62 vs. 92 ^e
PRE fb LPOST vs. EPOST fb LPOST		–	–	92 vs. 88 ^{NS}

^aData presented in this table were pooled across both years (2018 and 2019).

^bAbbreviations: –, not applicable; DA-EPOST, d after early POST application; DA-LPOST, d after late POST application; EPOST, early POST; fb, followed by; LPOST, late POST; NS, not significant.

^cMeans presented within each column with no common letter(s) are significantly different based on Fisher protected LSD, where $\alpha = 0.05$.

^dA priori orthogonal contrasts.

^eP < 0.0001.

compared with the nontreated control at 14 DA-LPOST (Table 4). Contrast analysis revealed that EPOST fb LPOST programs resulted in 98% reduction in Palmer amaranth density compared with 76% reduction from PRE fb LPOST programs (Table 4). However, this statistical difference was attributed to 42% density reduction by sulfentrazone + cloransulam-methyl fb 2,4-D, which influenced the estimated mean of the PRE fb LPOST programs. Except for sulfentrazone + cloransulam-methyl fb 2,4-D, all other PRE fb LPOST programs were comparable to EPOST fb LPOST programs.

Norsworthy et al. (2016) and Aulakh and Jhala (2015) have stressed the importance of PRE fb POST with residual herbicide programs as important in-season measures to reduce GR Palmer amaranth density and seed production in soybean. Moreover, PRE fb LPOST programs will be more sustainable than the sequential application of 2,4-D or glufosinate in EPOST fb LPOST programs, due to integration of herbicides with multiple sites of action. Miller and Norsworthy (2016) reported a lower density of GR Palmer amaranth plants with herbicide programs involving multiple sites of action compared with a single EPOST application of 2,4-D + glyphosate in 2,4-D, glyphosate, and glufosinate-resistant soybean. More importantly, repeated use of herbicides with the same site of action (e.g., 2,4-D or glufosinate) would select for HR-weed biotypes. It is important to note that 2,4-D resistance has been already confirmed in waterhemp populations from

Nebraska, Illinois, and Missouri (Bernards et al. 2012; Evans et al. 2019; Figueiredo et al. 2018; Shergill et al. 2018; Shyam et al. 2019) and Palmer amaranth population from Kansas (Kumar et al. 2019).

Aboveground biomass reduction of Palmer amaranth followed the same trend as density reduction (Table 4). In 2019, lower biomass reduction (60%) was obtained compared with 2018 (78%) at 14 DA-EPOST. Glufosinate or 2,4-D resulted in 69% to 71% reduction in GR Palmer amaranth biomass 14 DA-EPOST. Such a low reduction of biomass can be attributed to taller Palmer amaranth plants, which reduced the efficacy of glufosinate or 2,4-D. The PRE fb LPOST programs resulted in 91% to 100% biomass reduction, except sulfentrazone + cloransulam-methyl fb 2,4-D (32%) and were comparable to EPOST fb LPOST herbicide programs (99%) at 14 DA-LPOST (Table 4). Such low control with sulfentrazone + cloransulam-methyl fb 2,4-D might be attributed to a reduction in the efficacy of sulfentrazone + cloransulam-methyl in controlling GR Palmer amaranth, resulting in Palmer amaranth taller than 15 cm at the time of 2,4-D application, which ultimately resulted in reduced control. Averaged across herbicide programs, PRE fb LPOST programs resulted in 89% reduction of Palmer amaranth biomass compared with 58% with PRE-only programs 14 DA-LPOST (Table 4). EPOST fb LPOST programs (99%) provided a comparable reduction in biomass compared with PRE fb LPOST programs (89%) (Table 4). This was due to poor efficacy of

Table 4. Glyphosate-resistant Palmer amaranth density and above-ground biomass as affected by the herbicide programs in 2,4-D/glyphosate/glufosinate resistant soybean in field experiments conducted in Carleton, NE, during the 2018 and 2019 growing seasons.^a

Herbicide program	Timing ^b	Reduction in Palmer amaranth density ^c		Reduction in Palmer amaranth biomass ^c	
		14 DA-EPOST	14 DA-LPOST	14 DA-EPOST	14 DA-LPOST
Nontreated control		–	–	–	–
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹)	PRE	67 bc	47 b	30 d	29 d
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	69 bc	42 b	56 bc	32 d
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	80 ab	93 a	54 cd	93 ab
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	69 bc	99 a	64 a–c	98 ab
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹)	PRE	75 a–c	90 a	72 a–c	81 b
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	88 a	96 a	81 ab	91 ab
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	92 a	100 a	76 a–c	100 a
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	93 a	100 a	82 a	100 a
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹)	PRE	81 ab	93 a	64 a–c	63 c
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	92 a	96 a	71 a–c	92 ab
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	86 ab	89 a	79 a–c	94 ab
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	92 a	100 a	88 a	100 a
Glufosinate (656 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	EPOST fb LPOST	86b ab	98 a	69 a–c	99 a
2,4-D (2,080 g ae ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	EPOST fb LPOST	60 c	98 a	71 a–c	99 a
P-value		<0.0001	<0.0001	<0.0001	<0.0001
Contrast analysis ^d					
PRE vs. PRE fb LPOST		–	76 vs. 91 ^e	–	58 vs. 99 ^e
PRE fb LPOST vs. EPOST fb LPOST		–	91 vs. 98 ^f	–	89 vs. 99 ^f

^aData presented in this table were pooled across both years (2018 and 2019).

^bAbbreviations: –, not applicable; DA-EPOST, d after early POST application; DA-LPOST, d after late POST application; EPOST, early POST; fb, followed by; LPOST, late POST.

^cMeans presented within each column with no common letter(s) are significantly different based on Fisher protected LSD, where $\alpha = 0.05$.

^dA priori orthogonal contrasts.

^eP < 0.0001.

^fP < 0.05.

sulfentrazone + cloransulam-methyl fb 2,4-D, which failed to reduce Palmer amaranth biomass.

Chahal and Jhala (2015) reported a higher reduction in GR-volunteer corn biomass with sequential application of glufosinate in glufosinate-resistant soybean. Sarangi and Jhala (2019) showed high biomass reduction of Palmer amaranth in soybean with PRE fb POST herbicides with residual activity ranging from 96% to 100%. PRE herbicides in this study were fb a mixture of 2,4-D + glufosinate; however, Palmer amaranth biomass reduction was similar to PRE fb 2,4-D or glufosinate. Tank mixing herbicides such as glufosinate + 2,4-D can further improve control of GR Palmer amaranth if applied with PRE herbicide application at planting. For example, Ganie and Jhala (2017) reported that tank mixing 2,4-D with glufosinate provided 80% to 92% control of GR giant ragweed.

In this study, preformulated mixtures of imazethapyr + saflufenacil + pyroxasulfone, and chlorimuron ethyl + flumioxazin + metribuzin applied PRE fb 2,4-D or glufosinate, controlled GR Palmer amaranth 92% to 99% and reduced density and biomass by 89% to 100% and 91% to 100%, respectively; therefore, a tank mixture of 2,4-D + glufosinate is not needed to achieve optimum Palmer amaranth control if PRE herbicide is applied; however, if grass weeds are present in the field, tank mixing 2,4-D with glyphosate or glufosinate will be needed. To maintain

the effectiveness of this herbicide program, however, it will be crucial to follow labeled application timings with appropriate soybean and Palmer amaranth growth stages, because 2,4-D can be applied up to the R2 soybean growth stage whereas glufosinate cannot be applied after soybean starts flowering.

Soybean Yield

Averaged across treatments, PRE fb LPOST herbicide programs resulted in greater yield (4,478 kg ha⁻¹) compared with PRE-only programs (3,043 kg ha⁻¹) (Table 5). Therefore, the PRE fb LPOST programs prevented a 32% soybean yield loss that would have occurred with PRE-only herbicide programs. The lowest yield (2,398 kg ha⁻¹) was obtained in the nontreated control, which was comparable to the PRE-only herbicide programs of sulfentrazone + cloransulam-methyl (2,835 kg ha⁻¹) and chlorimuron ethyl + flumioxazin (2,832 kg ha⁻¹) (Table 5), indicating if Palmer amaranth is the predominant weed in soybean production field, using PRE-only program will not provide optimum yield. Similarly, Whitaker et al. (2010) reported greater soybean yield with PRE herbicide fb fomesafen compared with PRE-only programs.

There was no difference in yield obtained from PRE fb LPOST programs (4,478 kg ha⁻¹) and sequential application of 2,4-D or glufosinate (EPOST fb LPOST) programs (4,706 kg ha⁻¹)

Table 5. Soybean yield affected by herbicide programs in 2,4-D-, glyphosate-, and glufosinate-resistant soybean in field experiment conducted at Carleton, NE, in 2019.^a

Herbicide program	Timing ^b	Soybean yield ^c
Nontreated control	–	kg ha ⁻¹ 2,398 f
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹)	PRE	2,835 f
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	4,394 abcd
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	4,068 d
Sulfentrazone + cloransulam-methyl (195 + 25 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	4,561 abcd
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹)	PRE	3,462 e
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	4,752 ab
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	4,325 bcd
Imazethapyr + saflufenacil + pyroxasulfone (70 + 25 + 120 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	4,657 abc
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹)	PRE	2,832 f
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	PRE fb LPOST	4,712 ab
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	PRE fb LPOST	4,162 cd
Chlorimuron ethyl + flumioxazin + metribuzin (22 + 72 + 231 g ai ha ⁻¹) fb 2,4-D + glufosinate (2,080 g ae ha ⁻¹ + 656 g ai ha ⁻¹)	PRE fb LPOST	4,671 ab
Glufosinate (656 g ai ha ⁻¹) fb glufosinate (656 g ai ha ⁻¹)	EPOST fb LPOST	4,574 abc
2,4-D (2,080 g ae ha ⁻¹) fb 2,4-D (2,080 g ae ha ⁻¹)	EPOST fb LPOST	4,837 a
P-value		<0.001
Contrast analysis ^d		
PRE vs. PRE fb LPOST		3,043 vs. 4,478 ^e
PRE fb LPOST vs. EPOST fb LPOST		4,478 vs. 4,706 ^{NS}

^aThe field experiment in 2018 was destructed due to pending export approval of 2,4-D, glyphosate, and glufosinate-resistant soybean in few countries, so yield data are available only for 2019.

^bAbbreviations EPOST, early POST; fb, followed by; LPOST, late POST; NS, not significant.

^cMeans presented within each column with no common letter(s) are significantly different based on Fisher protected LSD test, where $\alpha = 0.05$.

^dA priori orthogonal contrasts.

^e $P < 0.0001$.

(Table 5). However, it is advisable to use PRE fb POST herbicides because it will provide more opportunity to use an herbicide program with multiple sites of action and reduce the exposure of a substantial number of Palmer amaranth plants to POST herbicides. Sarangi and Jhala (2019) reported that PRE fb POST herbicide programs were effective for controlling Palmer amaranth and obtaining greater soybean yield, whereas Grey et al. (2013) showed that combinations of multiple herbicide sites of action applied PRE and POST would be necessary to manage multiple HR Palmer amaranth. Butts et al. (2016) stated the importance of PRE fb POST herbicide programs as a component of an integrated weed management program to effectively control *Amaranthus* species as well as increase soybean yield. Similarly, multiyear field experiments conducted in Nebraska have shown that PRE fb POST herbicides with or without residual activity can effectively control weeds such as GR waterhemp and prevent yield loss of GR and glufosinate-resistant soybean (Jhala et al. 2017; Sarangi et al. 2017).

We conclude 2,4-D-, glyphosate-, and glufosinate-resistant soybean will provide additional POST herbicide options such as 2,4-D or glufosinate to soybean growers for managing not only GR but also multiple HR Palmer amaranth. Moreover, the adoption of integrated weed management practice is necessary to maintain the effectiveness of 2,4-D-, glyphosate-, and glufosinate-resistant crop technology (Miller and Norsworthy 2016). For example, Enlist 3 corn, resistant to 2,4-D, glufosinate, glyphosate, and aryloxyphenoxypropionates is available commercially, but it should not be planted in rotation with 2,4-D-, glyphosate-, and glufosinate-resistant soybean to avoid the use of the same POST herbicides in the same field.

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