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Managing Palmer Amaranth (*Amaranthus palmeri*) with Sequential Applications of Glufosinate and 2,4-D

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Determine the influence of sequential spray order and role of glufosinate when used in a system with 2,4-D to control Palmer amaranth at two different growth stages.
Study Design: Randomized complete block design with three replications.
Place and Duration of Study: A fallow, non-crop field at the Texas A&M AgriLife Research and Extension Center, Lubbock, Texas, during the 2018 and 2019 growing seasons.
Methodology: Herbicides were applied to 7 to 15 cm and 25 to 30 cm Palmer amaranth with a handheld 1.93m CO_2-pressurized backpack calibrated to deliver 140 L ha^{-1} at 207 kPa. Palmer amaranth control was visually estimated on a scale of 0 (no control) to 100% (complete control) relative to the nontreated control. Palmer amaranth biomass and density were collected 43 and 36 days after the last sequential application for 7 to 15 cm and 25 to 30 cm Palmer amaranth in 2019. Palmer amaranth control, biomass, and density were subjected to analysis of variance and means were separated using Fisher's Protected LSD at *P*=.05.
Results: 2,4-D choline + glyphosate followed by glufosinate provided the greatest level of Palmer amaranth control for both sizes of Palmer amaranth. Overall, Palmer amaranth control was not

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influenced by sequential application timing. Biomass and density were not significantly different among treatments.

Conclusion: Sequential application order of glufosinate and 2,4-D was not an important factor for Palmer amaranth control. However, due to 2,4-D label restrictions, applying 2,4-D choline + glyphosate followed by glufosinate may be the best option for maximum efficacy.

Keywords: 2,4-D choline; glufosinate; glyphosate; Palmer amaranth; postemergence; pyrithiobac; sequential applications.

ABBREVIATIONS

ALS *: acetolactate synthase*
DAIA *: days after initial application*
DASA *: days after sequential application*
EPSPS *: 5-enolpyruvylshikimate-3-phosphate synthase*

1. INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Watson*)* is one of the most common and troublesome weeds in cotton [1]. New herbicide-resistant populations and limited in-season options make it difficult to manage this weed. While 2,4-D has been used to effectively control Palmer amaranth in other cropping systems, it was not an option for cotton producers until the release of 2,4-D tolerant cotton varieties in 2016. 2,4-D-based weed management systems will require additional herbicide modes of action to slow the development of herbicide resistance and create the robust level of control required for Palmer amaranth [2]. One option for Palmer amaranth management is the addition of glufosinate as a second postemergence herbicide applied in sequence with 2,4-D choline. This sequential application system is a promising option for in-season Palmer amaranth control, but it is currently unknown if sequential order of these two herbicides impacts overall herbicide efficacy.

The Texas High Plains planted over 1,700,000 hectares of cotton *(Gossypium hirsutum* L.) in 2019 [3]. In this area producers manage limited water and a harsh climate to produce nearly 40% of the nation’s cotton. Weed management is challenging in this region where drought and high winds challenge the success of many weed control options. Palmer amaranth can thrive under these harsh conditions and can produce over half a million seeds and use twice as much water as cotton [4]. Palmer amaranth can reduce cotton yield by 54% at densities of 10 Palmer amaranth per 9 m of row [5].

Over-reliance on glyphosate created a favorable environment for selection of glyphosate resistant Palmer amaranth in Roundup Ready® crops [2]. In West Texas, this weed is reported to be resistant to two herbicide modes of action, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase; Group 9) and ALS (acetolactate synthase; Group 2) inhibitors and up to eight herbicide modes of action globally [6].

Using multiple modes of action in herbicide systems may delay resistance and increase control, particularly when integrated into a system that uses tillage and cultural weed management practices such as planting weed-free seed, crop rotation, competitive seeding rates, and appropriate planting date. Cotton varieties that are tolerant to 2,4-D and use Enlist™ technology (Corteva™ Agriscience, Wilmington, Delaware, USA) offer producers an option to control glyphosate resistant Palmer amaranth by using a herbicide that was previously not available for use in-season. 2,4-D, a synthetic auxin, can cause significant crop injury when exposed to non-tolerant cotton at rates 1/200 of the standard rate [7]. Enlist™ cotton is also tolerant to glyphosate and glufosinate. 2,4-D choline, glyphosate, and glufosinate may be tank-mixed or applied sequentially. This tolerance provides the opportunity to create a postemergence herbicide system with 3 modes of action (Group 4, Group 9, Group 10). Furthermore, this system offers the option to integrate currently labeled soil-active herbicides and postemergence-directed herbicides. Pyrithiobac (Staple® LX), a soil-active herbicide, should be beneficial at controlling many weeds as they emerge. Systems with multiple modes of action, a variety of application timings, and integration of tillage and cultural practices could all be helpful tools for producers facing hard-to-manage Palmer amaranth infestations [2, 8, 9].

As glyphosate resistant populations of Palmer amaranth increase, alternative management strategies are needed to effectively manage this weed. The use of glufosinate and 2,4-D choline for Palmer amaranth control is one option, but the importance of application order for these herbicides remains unclear. To optimize a 2,4-D tolerant cotton system, research is required to evaluate the influence of sequential application order on Palmer amaranth control. Therefore, the objectives of this study were to 1.) Determine how sequential application order influences efficacy of 2,4-D or glufosinate when applied to Palmer amaranth at different growth stages (7 to 15 cm and 25 to 30 cm) and 2.) Evaluate the influence of pyrithiobac, a soil residual herbicide, on the sequential application system. To evaluate these objectives, two field studies were conducted in a non-crop field with high soil seedbank populations of Palmer amaranth during the 2018 and 2019 growing seasons. Information from this research could serve to improve Palmer amaranth control and provide information on factors that may contribute to the success of 2,4-D choline herbicide systems.

2. MATERIALS AND METHODS

2.1 Study Area

Non-crop field studies were conducted at the Texas A&M AgriLife Research and Extension Center near Lubbock, Texas (33°69'N, -101°82'W) in 2018 and 2019 to evaluate sequential applications of glufosinate, 2,4-D choline, and 2,4-D choline + glyphosate. The study location was selected because of its dense population of Palmer amaranth (approximately 70 Palmer amaranth m^2). The soil was an Acuff loam with 1% organic matter and a pH of 7.5. Plot size was 4.05 m by 9.14 m. In-furrow irrigation was used to promote weed emergence, but no supplemental irrigation was used during the duration of the trial. Rainfall during the duration of the trials was 100 mm (54% of the average summer rainfall) from June 1 to August 31 in 2018 and 302 mm (163% of the average summer rainfall) during the same period in 2019. Precipitation averages were based on rainfall for the 4 km region surrounding the study location from June 1 to August 31 over a 30-year period from 1981 to 2010 [10].

2.2 Experimental Design

Pendimethalin (Prowl H2O®) at 0.86 kg active ingredient (ai) ha^{-1} was applied preplant each year approximately 30 days before trial initiation to reduce Palmer amaranth density. Applications were made on May 3, 2018 and April 25, 2019 using a tractor mounted three-point sprayer equipped with Turbo TeeJet 11002 nozzles. Turbo TeeJet Induction 11002 nozzles were used for all 2,4-D alone and 2,4-D tank mixed treatments. Turbo TeeJet 11002 nozzles were used for all non-2,4-D treatments. Preplant and herbicide treatments were made using an application speed of 4.8 km hr^{-1} using 140 L ha^{-1} carrier volume. Glufosinate treatments included ammonium sulfate at 2.86 kg $ha^{-1.}$ Treatments were initiated when Palmer amaranth height was 7 to 15 cm or 25 to 30 cm (Table 1).

Initial applications were made to 7 to 15 cm Palmer amaranth on May 25, 2018 and June 7, 2019 with sequential applications made on June 4, 2018 and June 17, 2019. Initial applications were made to 25 to 30 cm Palmer amaranth on June 4, 2018 and June 13, 2019 with sequential applications on June 14, 2018 and June 24, 2019. The experiment was performed with treatments arranged in a randomized complete block design with three replications.

Glufosinate rate was dependent upon the initial herbicide treatment. If glufosinate was applied in sequence, the second application rate was 0.59 kg ai ha^{-1} due to restrictions per the 2018 label [11]. Initial applications of glufosinate and those applied sequentially after 2,4-D choline + glyphosate were made at 0.88 kg ai ha^{-1}. 2,4-D choline + glyphosate (Enlist Duo™ with Colex D Technology™) was applied at 1.62 kg acid equivalent (ae) ha^{-1}, 2,4-D choline (Enlist One™ with Colex D Technology™) was applied at 0.80 kg ae ha^{-1}, and pyrithiobac-sodium (Staple® LX) was applied at 0.073 kg ai ha^{-1}. Treatments that contained pyrithiobac included concentrated crop oil at 0.5% on a volume per volume basis.

2.3 Palmer Amaranth Control

The level of Palmer amaranth control following each treatment was quantified by estimating plant death on a scale from 0 to 100%, with 0% indicating no control and 100% indicating complete weed necrosis [12]. Palmer amaranth control was evaluated by observing plant color, herbicide symptomology, new growth since application and overall vigor. Palmer amaranth control was evaluated 7 to 10 days after initial application (DAIA) and 10 to 15, 21, and 36 days after sequential application (DASA). In 2019, plants were counted and above ground fresh plant biomass was harvested from one m^2 within each plot for biomass and density 43 and 36 days after the last sequential application for 7 to

Table 1. Herbicide treatments

Treatment number	Herbicide treatments	
	Initial application	Sequential application
1	Untreated	Untreated
2	2,4-D choline + glyphosate	2,4-D choline + glyphosate
3	2,4-D choline + glyphosate	2,4-D choline
4	2,4-D choline + glyphosate	Glufosinate
5	2,4-D choline	2,4-D choline + glyphosate
6	2,4-D choline	2,4-D choline
7	2,4-D choline	Glufosinate
8	2,4-D choline + pyrithiobac	Glufosinate
9	Glufosinate	2,4-D choline + glyphosate
10	Glufosinate	2,4-D choline
11	Glufosinate	Glufosinate
12	Glufosinate + pyrithiobac	Glufosinate

15 cm and 25 to 30 cm Palmer amaranth, respectively. Palmer amaranth were dried at 35°C for one to two weeks using a plant drier and biomass weights recorded.

2.4 Statistical Analysis

Palmer amaranth percent control, density, and biomass were subject to ANOVA using the PROC GLIMMIX procedure of SAS 9.1 (SAS Institute Inc., SAS Campus Drive, Cary, NC, 27513). Random variables were year, weed size, and replication. A year by treatment interaction was observed; therefore, years were separated for analysis and discussion.

3. RESULTS AND DISCUSSION

3.1 Control of 7 to 15 cm Palmer Amaranth

In 2018, glufosinate alone controlled 7 to 15 cm Palmer amaranth 95% 10 days after initial application (DAIA), which was greater than all treatments containing 2,4-D choline (Table 2). Ten days after the sequential application (DASA), all treatments except 2,4-D choline + glyphosate followed by (fb) 2,4-D choline and 2,4-D choline fb 2,4-D choline controlled Palmer amaranth >92%. Palmer amaranth was controlled 100% by 2,4-D choline + glyphosate fb glufosinate 10 DASA and 97% 21 DASA in 2018. Pyrithiobac provided increased Palmer amaranth control 21 DASA when used with 2,4-D choline fb glufosinate (96%) compared to 2,4-D choline alone fb glufosinate (86%). Sequential application order influenced treatments of 2,4-D choline fb glufosinate, with 2,4-D choline in the initial application providing greater Palmer amaranth control 21 DASA.

In 2019, 2,4-D choline + glyphosate controlled 7 to 15 cm Palmer amaranth 92% 7 DAIA, which was greater than all other treatments (Table 3). Treatments that included 2,4-D choline + glyphosate in the initial application controlled Palmer amaranth 90 to 93% 10 DASA, while treatments with 2,4-D choline alone in the initial application controlled Palmer amaranth 62 to 75%. Two applications of 2,4-D choline + glyphosate controlled Palmer amaranth 80% 21 DASA, which was similar to 2,4-D choline + glyphosate fb glufosinate (79%).. 2,4-D choline fb 2,4-D choline + glyphosate controlled Palmer amaranth greater than 2,4-D choline + glyphosate fb 2,4-D choline 21 DASA in both years. Sequential order did not influence Palmer amaranth control when using 2,4-D choline + glyphosate and glufosinate in either year. This data supports the research by Culpepper et al. [13] who reported sequential 2,4-D choline herbicide systems controlled up to 7 cm Palmer amaranth 84%. Trends in this study support the research by Stephenson et al. [14] who reported up to 10 cm Palmer amaranth control was greatest following 2,4-D choline + glyphosate when compared to treatments of 2,4-D choline alone or co-applied with glufosinate or glyphosate.

3.2 Control of 25 to 30 cm Palmer Amaranth

In 2018, 25 to 30 cm Palmer amaranth control ranged from 54 to 85% 10 DAIA (Table 2). 2,4-D choline + glyphosate, glufosinate, glufosinate + pyrithiobac, and glufosinate alone controlled Palmer amaranth better than 2,4-D choline alone or with pyrithiobac. 2,4-D choline + glyphosate applied twice, 2,4-D choline + glyphosate fb glufosinate, and glufosinate fb 2,4-D choline + glyphosate controlled Palmer amaranth 95 to

98% 11 DASA, which was greater than all other treatments. Glufosinate + pyrithiobac fb glufosinate controlled Palmer amaranth 55% 11 DASA and 35% 21 DASA. Treatments containing an application of 2,4-D choline alone controlled Palmer amaranth ≤ 70% 21 DASA, except for 2,4-D choline + glyphosate fb 2,4-D choline (91%).

In 2019, 2,4-D choline + glyphosate controlled Palmer amaranth 80% 7 DAIA, while other treatments controlled Palmer amaranth 58 to 66% (Table 3). Palmer amaranth control ranged from 25 to 65% 15 DASA. 2,4-D choline + glyphosate fb 2,4-D choline or glufosinate controlled Palmer amaranth > 60% 15 DASA. Palmer amaranth control was ≤ 30% for 9 out of 11 treatments 36 DASA in 2019. Treatments with 2,4-D choline + glyphosate and glufosinate applied in either order were among the treatments with the greatest Palmer amaranth control 21 DASA in 2018 and 15 DASA in 2019. 2,4-D choline fb glufosinate provided greater weed control than glufosinate fb 2,4-D choline in both years. Merchant et al. [15] reported 93 to 94% control of 20 cm Palmer amaranth following 2,4-D choline fb glufosinate applications made 10 days apart.

In general, herbicide efficacy decreased as Palmer amaranth size at initial application increased, making timely applications to smaller weeds important for success of this system. This trend was expected as most 2,4-D studies have focused on controlling smaller Palmer amaranth. 2,4-D choline alone is generally considered a weak choice as a "rescue" option for all mature weeds [16, 17].

There were no differences among treatments for measurements of Palmer amaranth density or biomass for either size of Palmer amaranth (data not shown). In many cases, Palmer amaranth density in treated plots was greater than untreated controls. This may be due to lack of soil residual control provided by 2,4-D choline, 2,4-D choline + glyphosate, or glufosinate and subsequent emergence of Palmer amaranth after the sequential application.

Table 2. Palmer amaranth control following initial and sequential applications of glufosinate and 2,4-D choline in Lubbock, Texas in 2018

Initial application	Sequential application	Palmer amaranth size at initial application					
		7 to 15 cm[a]			25 to 30 cm		
		10 DAIA[bc]	10 DASA	21 DASA	10 DAIA	11 DASA	21 DASA
		---------------------------------------%---------------------------------------					
2,4-D choline + glyphosate		91 b			85 a		
	2,4-D choline + glyphosate		97 bc	91 abc		95 a	96 a
	2,4-D choline		87 e	78 de		88 b	91 ab
	glufosinate		100 a	97 a		98 a	98 a
2,4-D choline		69 c			54 d		
	2,4-D choline + glyphosate		92 d	90 abc		77 d	53 de
	2,4-D choline		73 f	63 f		63 f	62 cd
	glufosinate		95 c	86 bcd		78 d	70 bcd
2,4-D choline + pyrithiobac		67 c			65 c		
	glufosinate		98 ab	96 a		84 c	77 abc
Glufosinate		95 a			79 b		
	2,4-D choline + glyphosate		99 ab	92 abc		95 a	86 ab
	2,4-D choline		92 d	73 ef		68 e	48 de
	glufosinate		98 ab	83 cd		89 b	84 abc
Glufosinate + pyrithiobac		92 ab			82 ab		
	glufosinate		98 bc	93 ab		55 g	35 e

[a]Abbreviations: DAIA, days after initial application; DASA, days after sequential application.
[b]Initial application applied May 25 for 7 to 15 cm, June 4 for 25 to 30 cm; rating dates are given as days after initial application (DAIA). Sequential applications were applied June 4 for 7 to 15 cm, June 14 for 25 to 30 cm; rating dates are given as days after sequential application (DASA).
[c]Means within a column followed by a common letter were similar according to Fishers Protected LSD in SAS at P = 0.05

Table 3. Palmer amaranth control following initial and sequential applications of glufosinate and 2,4-D choline in Lubbock, Texas in 2019

Initial application	Sequential application	Palmer amaranth size at initial application[a]					
		7 to 15 cm[a]			25 to 30 cm		
		7 DAIA[bc]	10 DASA	21 DASA	7 DAIA	15 DASA	36 DASA
		---------------%					
2,4-D choline + glyphosate		92 a			80 a		
	2,4-D choline + glyphosate		91 ab	80 a		59 ab	35 b
	2,4-D choline		90 abc	51 bc		65 a	30 bc
	glufosinate		93 a	79 a		64 a	22 cde
2,4-D choline		40 d			58 b		
	2,4-D choline + glyphosate		75 de	66 ab		58 ab	50 a
	2,4-D choline		62 f	32 d		33 cd	20 def
	glufosinate		73 de	35 cd		46 bc	22 cde
2,4-D choline + pyrithiobac		55 c			65 b		
	glufosinate		82 bcd	52 bc		50 b	27 bcd
Glufosinate		79 b			66 b		
	2,4-D choline + glyphosate		89 abc	67 ab		54 ab	22 cde
	2,4-D choline		67 ef	32 d		25 d	7 gh
	glufosinate		78 d	40 cd		27 d	12 fg
Glufosinate + pyrithiobac		83 b			66 b		
	glufosinate		80 bcd	42 cd		32 d	13 efg

[a]Abbreviations: DAIA, days after initial application; DASA, days after sequential application.
[b]Initial Application applied May 25 for 7 to 15 cm, June 4 for 25 to 30 cm; rating dates are given as days after initial application (DAIA). Sequential applications were applied June 4 for 7 to 15 cm, June 14 for 25 to 30 cm; rating dates are given as days after sequential application (DASA).
[c]Means within a column followed by a common letter were similar according to Fishers Protected LSD in SAS at P= 0.05

Differences observed in soil-active herbicide efficacy may be due to canopy cover at the time of application, with the canopy intercepting herbicide and preventing it from reaching the soil. Lack of rainfall in-season in 2018 also may have influenced incorporation of the soil-active herbicide. However, above average rainfall in 2019 may have led to an increase in Palmer amaranth germination.

4. CONCLUSION

With increasing occurrence of glyphosate resistant Palmer amaranth, herbicide management strategies that use multiple modes of action are essential. The results of this study indicate that sequential application order was not an important factor for Palmer amaranth control. However, due to 2,4-D choline label restrictions [18], applying 2,4-D choline + glyphosate followed by glufosinate may be the best option for maximum efficacy. This sequence of application provides 3 herbicide modes of action, but when considering that glyphosate resistant populations are common across Texas [19], only 2 of the 3 modes of action may be effective in some situations. Thus, although this treatment was successful in the study location, it may be less successful in an area with widespread glyphosate resistance.

When Palmer amaranth was < 30 cm in height at the time of initial application, 2,4-D choline + glyphosate followed by glufosinate provided the greatest level of control. Initial applications of 2,4-D choline were more effective when followed by glufosinate sequentially, but 2,4-D choline alone applied sequentially failed to control Palmer amaranth and is not recommended. When paired with other weed management strategies, such as tillage and cultural control practices(plant weed-free seed, appropriate planting date, crop rotation, etc.), sequential applications of 2,4- D choline + glyphosate followed by glufosinate may be an effective tactic to control glyphosate resistant Palmer amaranth.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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