

Efficacy of 2,4-D Choline as Influenced by Weed Size in the Texas High Plains

10 **ABSTRACT**

11
12 **Aims.** Postemergence timing trials based on weed size were conducted near Lubbock, TX to assess the effectiveness of 2,4-D choline + glyphosate on control of Palmer amaranth (*Amaranthus palmeri* S. Wats.), Russian-thistle (*Salsola tragus* L.), and kochia (*Kochia scoparia* L.) at three growth stages (3 to 5 cm, 10 to 15 cm, and 20 to 30 cm).

16 **Study design.** All trials were arranged in a randomized complete block design with four replications.

17 **Place and duration of study.** Field experiments were conducted in 2013, 2014, and 2015 in Lubbock, TX at the Texas A&M AgriLife Research and Extension Center near Lubbock, TX.

19 **Methodology.** Herbicide treatments consisted of a single postemergence application of 2,4-D choline + glyphosate at two rates, 2,4-D choline + glyphosate at two rates + glufosinate, 2,4-D choline + glyphosate + S-metolachlor, 2,4-D choline + glyphosate + acetochlor, 2,4-D choline + glufosinate, glyphosate, or glufosinate.

22 **Results.** The greatest level of weed control for all three weed species was achieved at the 3 to 5 cm timing; however, weed size was most critical for Palmer amaranth and Russian-thistle compared to kochia. Averaged over all three years, Palmer amaranth control decreased from 93 to 74% when evaluated 21 days after treatment following applications that included 2,4-D choline when applied to plants 3 to 5 and 10 to 30 cm, respectively. For Russian-thistle, control decreased from 98 to 78% when evaluated 21 days after treatment following treatments that included 2,4-D choline when applied to plants 3 to 5 and 10 to 30 cm, respectively. For kochia, control decreased from 98 to 84% when evaluated 21 days after treatment following treatments that included 2,4-D choline when applied to plant 3 to 5 and 10 to 30 cm, respectively.

31 *Keywords: Application timing, growth stage, tank mixtures*

32 **1. INTRODUCTION**

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34 Effective, economical, and sustainable weed management is crucial to a profitable cotton production system. Weeds decrease cotton lint yield and quality by competing for nutrients, water, and light [1]. Palmer amaranth (*Amaranthus palmeri* S. Wats.), Russian-thistle (*Salsola tragus* L.), and kochia (*Kochia scoparia* L.) are among the most difficult-to-control weeds in Texas High Plains cotton. Palmer amaranth was ranked as the most troublesome cotton weed in the southern United States in 2009, occurring in nine of 10 states surveyed [2]. It also has become one of the most economically damaging glyphosate-resistant weed species in the United States [3]. Russian-thistle, a C4 summer annual broadleaf weed that is prevalent in the western United States, is extremely competitive due in part to its aggressive root system [4,5]. Early seedling emergence, tolerance to drought, heat, and salinity, hermaphroditic flowers that are out-crossed and self-fertile, and wind-mediated pollen dispersal also contribute to its competitiveness [6-8]. The competitiveness of kochia, also a troublesome C4 summer annual broadleaf weed in croplands and noncroplands over the Great Plains of North America, is attributed to its early seedling emergence, rapid growth rate, heat and salt tolerance, prolific seed production, and long-distance seed dispersal by tumbling [9-15].

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46 Additionally, all three of these weeds have developed resistance to critical herbicides modes of action. In the United States, Palmer
 47 amaranth has even evolved resistance to multiple herbicide modes of action such as EPSP synthase inhibitors (Group 9), ALS
 48 inhibitors (Group 2), HPPD inhibitors (Group 27), PPO inhibitors (Group 14), microtubule assembly inhibitors (Group 3), and
 49 photosystem II inhibitors (Groups 5-7) [16]. Russian-thistle and kochia populations resistant to ALS and/or EPSP synthase
 50 inhibitors have been documented and kochia populations resistant to synthetic auxins and photosystem II inhibitors also have
 51 developed [17-18]. Therefore, the list of available modes of action to control these weed species in cotton is limited;
 52 however, an additional option became available with the release of Enlist™ technology in cotton in 2016. Enlist™
 53 technology utilizes cotton tolerance to 2,4-D choline, glyphosate, and glufosinate. Cotton tolerant to 2,4-D choline was
 54 conferred by the insertion of a gene (AAD-12) that codes for an aryloxyalkanoate dioxygenase enzyme [19]. Plants
 55 transformed to include this gene can metabolize certain auxin herbicides, including 2,4-D, to a nonlethal form [20]. Enlist™
 56 cotton provides growers with a new tool to effectively manage Palmer amaranth, Russian-thistle, kochia, and other difficult-to-
 57 control weeds in Texas High Plains cotton.

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 59 Weed size at the time of application [21-24] and tank-mix combinations [25-27] are two factors that often impact the success of
 60 a herbicide. The importance of weed size at the time of 2,4-D application has been well-documented (Everitt and Keeling 2007;
 61 Siebert et al. 2004). Therefore, weed size should be considered when making 2,4-D choline applications. The objective of this
 62 research was to evaluate the effectiveness of mixtures of 2,4-D choline with glyphosate, glufosinate, S-metolachlor, and/or
 63 acetochlor on control of Palmer amaranth, Russian-thistle, and kochia at various growth stages.

64 2. MATERIAL AND METHODS

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 67 **2.1 EXPERIMENTAL SITE.** Field experiments were conducted in 2013, 2014, and 2015 in Lubbock, TX at the Texas
 68 A&M AgriLife Research and Extension Center (33.415319°N, -101.483274°W, elevation 1,001 m). The soil type was an
 69 Amarillo fine sandy loam (fine-loamy, mixed, superactive, thermic Aridic Paleustalfs) with less than 1% organic matter and
 70 pH of 7.5. All studies were arranged in a randomized complete block design with four replications. Individual plots were
 71 3.0 m wide by 6.1 m in length. Annual rainfall was 292 mm in 2013, 460 mm in 2014, and 354 mm in 2015. No
 72 supplemental irrigation was provided. On average over all three years, there were approximately 1,200 Palmer amaranth,
 73 30 Russian-thistle, and 10 kochia plants per plot.

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 75 **2.2 EXPERIMENTAL DESIGN AND DATA COLLECTION.** In 2013, postemergence applications were made to 3 to 5, 10
 76 to 15, and 20 to 30 cm Palmer amaranth, Russian-thistle, and kochia (Table 1). Weeds were susceptible to all herbicides
 77 (no resistance had developed); however, the Palmer amaranth population was in the initial stages of developing
 78 glyphosate resistance. In 2014, applications were made to 10 to 15 and 20 to 30 cm Palmer amaranth and 3 to 5 and 20
 79 to 30 cm Russian-thistle and kochia. In 2015, applications were made to 3 to 5, 10 to 15, and 20 to 30 cm Palmer
 80 amaranth and 10 to 15 and 20 to 30 cm Russian-thistle. Kochia was not evaluated in 2015 as a late freeze eliminated
 81 most of the populations at this location. The nontreated control did not receive a herbicide application. All applications
 82 were made at 4.8 km per hour with a CO₂-pressurized backpack sprayer equipped with AIXR11002 spray tips (TeeJet®
 83 Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha⁻¹ at 205 kPa. No adjuvants were included with any
 application.

Table 1. Dates of Palmer amaranth, Russian-thistle, and kochia applications at several weed sizes near Lubbock, TX.

Weed size (cm)	Palmer amaranth			Russian-thistle/kochia		
	2013	2014	2015	2013	2014	2015
3 to 5	June 14	-	June 3	April 13	May 15	-
10 to 15	June 27	July 23	June 18	May 14	-	April 21
20 to 30	July 8	August 19	June 24	June 13	June 3	May 4

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 85 Treatments consisted of a single postemergence application of 2,4-D choline + glyphosate at two rates, 2,4-D choline +
 86 glyphosate at two rates + glufosinate, 2,4-D choline + glyphosate + S-metolachlor, 2,4-D choline + glyphosate +
 87 acetochlor, 2,4-D choline + glufosinate, glyphosate alone, or glufosinate alone. Herbicides and application rates are listed
 88 in Table 2. Visual control estimates were recorded 14, 21, and 28 days after treatment (DAT) using a scale of 0 to 100

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percent, where 0 was no weed control and 100 was complete control. Foliar chlorosis, necrosis, tissue distortion, and plant stunting were considered when making visual control estimates.

Table 2. Herbicide treatments and application rates for 2013, 2014, and 2015 application timing trials near Lubbock, TX.

Herbicide common names	Brand names or designations	Application rates	Manufacturer
2,4-D choline + glyphosate	Enlist Duo™	1.64 or 2.19 kg ae ha ⁻¹	Dow AgroSciences, Indianapolis, IN, http://www.dowagro.com
Glufosinate	Liberty® 280 SL	0.59 kg ai ha ⁻¹	Bayer CropScience, Research Triangle Park, NC, https://www.cropscience.bayer.com
S-metolachlor	Dual MAGNUM®	1.09 kg ai ha ⁻¹	Syngenta Crop Protection, Greensboro, NC, https://www.syngenta.com
Acetochlor	Warrant®	1.26 kg ai ha ⁻¹	Monsanto Company, St. Louis, MO, http://www.monsanto.com
2,4-D choline	Enlist One™	1.07 kg ae ha ⁻¹	Dow AgroSciences
Glyphosate	Roundup PowerMAX®	1.12 kg ae ha ⁻¹	Monsanto Company

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2.3 STATISTICAL ANALYSIS. A univariate analysis was performed on all responses in order to test for stable variance. No data sets were transformed as transformation did not increase stabilization. Data sets were analyzed using PROC MIXED with pdmix 800 macro included [28] and treatments were separated by Fisher's Protected LSD at an alpha level of 0.05 using SAS 9.4 software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513).

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3. RESULTS AND DISCUSSION

For Palmer amaranth, Russian-thistle, and kochia control, trials were analyzed independently due to a significant year effect ($P < 0.05$) across all possible year combinations. Within year (2013, 2014, and 2015), 10 to 15 and 20 to 30 cm Palmer amaranth ratings were combined due to no difference in control based on weed height at application ($P > 0.05$). In 2013, 10 to 15 and 20 to 30 cm Russian-thistle and kochia ratings were combined due to no difference in control based on weed height at application ($P > 0.05$). All other control ratings were analyzed independently due to a significant weed height effect ($P < 0.05$).

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3.1 PALMER AMARANTH CONTROL. In 2013, 2,4-D choline + glyphosate at 1.64 kg ae ha⁻¹, 2,4-D choline + glyphosate at 2.19 kg ae ha⁻¹, 2,4-D choline + glyphosate + S-metolachlor, and 2,4-D choline + glyphosate + acetochlor controlled 3 to 5 cm Palmer amaranth 95 to 98% 21 DAT while glufosinate alone controlled Palmer amaranth 58% (Table 3). For 10 to 30 cm Palmer amaranth, 2,4-D choline + glyphosate at 2.19 kg ha⁻¹, 2,4-D choline + glyphosate + S-metolachlor, and 2,4-D choline + glyphosate + acetochlor controlled Palmer amaranth the greatest (71 to 77%) while glufosinate alone provided the least control (5%).

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In 2014, 2,4-D choline + glyphosate at 1.64 kg ha⁻¹ + glufosinate, 2,4-D choline + glyphosate at 2.19 kg ha⁻¹ + glufosinate, and 2,4-D choline + glyphosate + acetochlor controlled 10 to 30 cm Palmer amaranth 88 to 90% 21 DAT while glufosinate alone achieved the least control (54%) (Table 3). In 2015, 2,4-D choline + glyphosate at 2.19 kg ha⁻¹, 2,4-D choline + glyphosate + S-metolachlor, and 2,4-D choline + glyphosate + acetochlor achieved the greatest Palmer amaranth control (97 to 98%) while glyphosate alone achieved the least control (82%) (Table 3). 2,4-D choline + glyphosate at 2.19 kg ha⁻¹ and 2,4-D choline + glyphosate + S-metolachlor achieved the greatest control (86 to 87%) of 10 to 30 cm Palmer amaranth while glufosinate alone achieved the least control (44%).

Table 3. Influence of weed height and herbicide treatment on Palmer amaranth control 21 days after treatment in 2013, 2014, and 2015 near Lubbock, TX^a.

Treatments	Rate	2013		2014	2015	
		3 to 5 cm	10 to 30 cm	10 to 30 cm	3 to 5 cm	10 to 30 cm
	kg ae or ai ha ⁻¹	----- % -----				
2,4-D choline + glyphosate	1.64	95 ab	66 b	80 bc	94 c	79 b
2,4-D choline + glyphosate	2.19	96 ab	77 a	82 b	97 ab	87 a
2,4-D choline + glyphosate + glufosinate	1.64 + 0.59	80 cd	48 cd	90 a	95 bc	66 c
2,4-D choline + glyphosate + glufosinate	2.19 + 0.59	78 d	54 c	90 a	95 bc	69 c
2,4-D choline + glyphosate + S-metolachlor	2.19 + 1.09	98 a	71 ab	79 bc	98 a	86 a
2,4-D choline + glyphosate + acetochlor	2.19 + 1.26	95 ab	72 ab	75 c	98 a	81 b
2,4-D choline + glufosinate	1.07 + 0.59	86 cd	44 d	88 a	94 c	64 c
Glyphosate	1.12	88 bc	63 b	74 c	82 d	59 d
Glufosinate	0.59	58 e	5 e	54 d	93 c	44 e

^aMeans within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at P < 0.05. Data pooled for 10 to 15 cm and 20 to 30 cm Palmer amaranth control ratings within each year.

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119 **3.2 RUSSIAN-THISTLE CONTROL.** In 2013 at 21 DAT, all treatments controlled 3 to 5 cm Russian-thistle 96 to 99% with
 120 the exception of glufosinate alone, which controlled this weed 75% (Table 4). All treatments achieved similar control (81 to
 121 85%) of 10 to 30 cm Russian-thistle with the exception of 2,4-D choline + glyphosate at 1.64 kg ha⁻¹ alone (70%),
 122 glyphosate alone (34%), and glufosinate alone (28%).

123 In 2014 at 21 DAT, all treatments controlled 3 to 5 cm Russian-thistle 95 to 100% and 20 to 30 cm Russian-thistle 71 to
 124 76% with the exception of glyphosate alone (61%) and glufosinate alone (23%) (Table 4). Glyphosate alone controlled 3
 125 to 5 and 20 to 30 cm Russian-thistle 69 and 61%, respectively, while glufosinate alone controlled 3 to 5 and 20 to 30 cm
 126 Russian-thistle 0 and 23%, respectively. In 2015, 2,4-D choline + glyphosate at 2.19 kg ha⁻¹ and 2,4-D choline +
 127 glyphosate + S-metolachlor achieved the greatest 10 to 15 cm Russian-thistle control (81 to 88%) 21 DAT while
 128 glufosinate alone achieved the least control (16%) (Table 4).

Table 4. Influence of weed height at application and herbicide treatment on Russian-thistle control 21 days after treatment in 2013, 2014, and 2015 near Lubbock, TX.^a

Russian-thistle control

Treatments	Rate	2013		2014		2015	
		3 to 5 cm	10 to 30 cm	3 to 5 cm	20 to 30 cm	10 to 15 cm	20 to 30 cm
	kg ae or ai ha ⁻¹	----- % -----					
2,4-D choline + glyphosate	1.64	96 ab	70 b	97 ab	71 a	100 a	70 cd
2,4-D choline + glyphosate	2.19	99 ab	85 a	100 a	75 a	100 a	88 a
2,4-D choline + glyphosate + glufosinate	1.64 + 0.59	96 b	84 a	95 b	73 a	99 ab	68 d
2,4-D choline + glyphosate + glufosinate	2.19 + 0.59	97 ab	85 a	99 a	73 a	100 a	74 cd
2,4-D choline + glyphosate + S-metolachlor	2.19 + 1.09	99 ab	83 a	100 a	75 a	100 a	81 ab
2,4-D choline + glyphosate + acetochlor	2.19 + 1.26	98 ab	81 a	98 a	76 a	100 a	70 cd
2,4-D choline + glufosinate	1.07 + 0.59	99 a	81 a	100 a	73 a	100 a	70 cd
Glyphosate	1.12	98 ab	34 c	69 c	61 b	99 a	75 bc
Glufosinate	0.59	75 c	28 c	0 d	23 c	98 b	16 e

^aMeans within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at P < 0.05. In 2013, 10 to 15 and 20 to 30 cm Russian-thistle control ratings were combined due to no weed height effect (P > 0.05).

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130 **3.3 KOCHIA CONTROL.** In 2013 at 21 DAT, all treatments controlled 3 to 5 cm kochia 95 to 100% with the exception of
131 glufosinate alone, which controlled this weed 79% (Table 5). All treatments achieved 76 to 90% control of 10 to 30 cm
132 kochia with the exception of glufosinate alone (49%). In 2014, all treatments controlled 3 to 5 cm kochia 97 to 99% with
133 the exception of glufosinate alone, which only controlled this weed 3% (Table 5). 2,4-D choline + glyphosate at 2.19 kg ha⁻¹,
134 2,4-D choline + glyphosate at 2.19 kg ha⁻¹ + glufosinate, 2,4-D choline + glyphosate + S-metolachlor, and 2,4-D choline
135 + glyphosate + acetochlor achieved the greatest 20 to 30 cm kochia control (84 to 90%) while glufosinate alone controlled
136 this weed the least (53%).

Table 5. Influence of weed height at time of application and herbicide treatment on kochia control 21 days after treatment in 2013, 2014, and 2015 near Lubbock, TX^a.

Treatment	Rate	Kochia control			
		2013		2014	
		3 to 5 cm	10 to 30 cm	3 to 5 cm	20 to 30 cm
	kg ae or ai ha ⁻¹	----- % -----			

2,4-D choline + glyphosate	1.64	98 a	76 b	98 a	76 d
2,4-D choline + glyphosate	2.19	98 a	90 a	98 a	90 abc
2,4-D choline + glyphosate + glufosinate	1.64 + 0.59	95 a	84 ab	97 a	81 d
2,4-D choline + glyphosate + glufosinate	2.19 + 0.59	95 a	88 ab	99 a	84 a-d
2,4-D choline + glyphosate + S-metolachlor	2.19 + 1.09	100 a	85 ab	98 a	90 ab
2,4-D choline + glyphosate + acetochlor	2.19 + 1.26	98 a	86 ab	98 a	91 a
2,4-D choline + glufosinate	1.07 + 0.59	98 a	77 ab	98 a	83 bcd
Glyphosate	1.12	100 a	79 ab	88 b	82 cd
Glufosinate	0.59	79 b	49 c	3 c	53 e

^a Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD at $P < 0.05$. In 2013, 10 to 15 and 20 to 30 cm kochia control ratings were combined due to no weed height effect ($P > 0.05$). Kochia was not evaluated in 2015.

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138 Similarly, Everitt and Keeling [29] found that 2,4-D at 0.56 and 1.12 kg ha⁻¹ controlled 3 to 8 cm horseweed at least 92%
 139 28 DAT; however, reduced horseweed control was observed with these same rates of 2,4-D when applied to 10 to 15 cm
 140 and 25 to 46 cm-tall horseweed. A comparable response to 2,4-D also has been reported with other weed species such
 141 as red morningglory (*Ipomoea coccinea* L.) and dogfennel [*Eupatorium capillifolium* (Lam.) Small] [30]. Siebert et al.
 142 observed 100% control of 30 cm red morningglory; however, a 6 to 19% reduction in control was observed when 2,4-D
 143 was applied to 60 cm plants. Dogfennel control was reduced from 85 to 70 to 6% when applications of 2,4-D and dicamba
 144 were applied to plants 36, 72, and 154 cm in height, respectively [31].

145 Regardless of weed size, treatments that included 2,4-D choline were the most successful. Among these treatments, 2,4-
 146 D choline + glufosinate and 2,4-D choline + glyphosate + glufosinate achieved the greatest levels of weed control.
 147 Glyphosate alone applications were inconsistent, especially for larger weeds and glufosinate alone performed poorly
 148 across weed species with the exception of 3 to 5 cm Palmer amaranth in 2015 and 10 to 15 cm Russian-thistle in 2015.

149 4. CONCLUSION

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151 The greatest level of weed control for all three weed species was achieved at the 3 to 5 cm timing; however, weed size
 152 was most critical for Palmer amaranth and Russian-thistle compared to kochia. Averaged over all three years, Palmer
 153 amaranth control decreased from 93 to 74% following treatments that included 2,4-D choline when applied to plants 3 to 5
 154 and 10 to 30 cm, respectively. For Russian-thistle, control decreased from 98 to 78% following treatments that included
 155 2,4-D choline when applied to plants 3 to 5 and 10 to 30 cm, respectively. For kochia, control decreased from 98 to 84%
 156 following treatments that included 2,4-D choline when applied to plant 3 to 5 and 10 to 30 cm, respectively.

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COMPETING INTERESTS DISCLAIMER:

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162 Authors have declared that no competing interests exist. The products used for this research are commonly
163 and predominantly use products in our area of research and country. There is absolutely no conflict of interest
164 between the authors and producers of the products because we do not intend to use these products as an
165 avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the
166 producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW