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Efficacy of Selected Herbicide Programs in 2,4-D Tolerant Cotton (*Gossypium hirsutum* L.)

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Abstract

The use of transgenic crops has grown significantly over the past couple of decades. Many agronomic crops produced today are tolerant to glyphosate. Glyphosate-tolerant crops were commercially introduced in 1996, and, about nine years later, glyphosate-resistant Palmer amaranth was confirmed in Georgia. Glyphosate-resistant weeds arose from reliance on postemergence only glyphosate programs to control weeds in crops. New transgenic traits for glufosinate and 2,4-D choline have been developed, and evaluations of stacked traits and concurrent use of multiple herbicides have provided additional tools in the management of glyphosate-resistant weeds. Field experiments were conducted in 2012 and 2013 at the Edisto Research and Education Center near Blackville, SC, USA to determine the efficacy of 2,4-D-based herbicide programs in transgenic cotton tolerant to 2,4-D choline, glyphosate, and glufosinate. The treatments provided good to excellent Palmer amaranth and pitted morningglory control in 2012 and 2013. Seed cotton yields across treatments ranged from 0 to 2057 kg ha^{-1}. This new trait technology package in cotton permits in-season postemergence use of 2,4-D choline, a herbicide mode of action not previously used postemergence in cotton, which can control resistant weeds, including Palmer amaranth if applied at the proper growth stage.

Keywords

Glyphosate, 2,4-D Tolerant Cotton, Resistant Weeds, Glufosinate, 2,4-D Choline, Weed Control

1. Introduction

In 1996, cotton and other crops tolerant to the herbicide glyphosate were commercialized and released to the market [1]. The adoption and use of genetically modified crops have increased dramatically over the past 20 years in corn (*Zea mays* L.), soybean (*Glycine max* L.), and cotton. For example, in 2016, 93% of the USA cotton hectares were planted with seed containing tolerance to the herbicide glyphosate [2] [3]. Initially, glyphosate was very effective in managing a broad spectrum of weeds; however, reliance on a single site of action over a broad geographic area dramatically increased the selection pressure which led to the selection and spread of several glyphosate-resistant weed biotypes in cotton [4] [5].

Palmer amaranth (*Amaranthus palmeri* S. Wats) is a warm season annual dioecious broadleaf plant [6]. Currently, the most common and troublesome weed in cotton in the Southern US is Palmer amaranth due to its rapid growth rate, prolific seed production, and high competitiveness [5] [6] [7]. Cotton lint yield can be drastically reduced by the presence of Palmer amaranth [8] [9] [10]. Previous research has shown that lint yields can be reduced from as few as 1 to 2 Palmer amaranth plants per row meter [8]. Palmer amaranth competition not only causes yield losses, but it can also increase the cost of production and its large stature can impede the harvesting efficiency of cotton [9].

Palmer amaranth has previously developed resistance to several herbicide groups, including to the dinitroanilines, ALS-inhibitors, and glyphosate groups [5]. Dinitroaniline resistant Palmer amaranth biotypes were confirmed in 1992 in South Carolina [11]. In a peanut field in Georgia, Palmer amaranth biotypes were confirmed to be resistant to ALS-inhibitor herbicides in 2000 [12]. The over reliance on glyphosate postemergence only programs has led to rapid selection and spread of resistant Palmer amaranth biotypes throughout the Southern US and is causing huge economic losses in these crops. Therefore, glyphosate-resistant biotypes of Palmer amaranth have spread rapidly since their confirmation in 2005 [4] [13].

Synthetic auxin herbicides, such as 2,4-D, selectively control broadleaf weeds, including Palmer amaranth [14]. Auxinic herbicides cause uncontrolled increases in cell wall growth and cell division which leads to abnormal development in the meristematic tissue in susceptible plants [15]. Dow AgroSciences has developed a new salt formulation of 2,4-D called 2,4-D choline that is significantly less volatile than previous salt formulations. Enlist Duo™ is a prepackaged mixture of 0.19 kg ae L^{-1} of 2,4-D choline and 0.2 kg ae L^{-1} of glyphosate [16].

Dow AgroSciences has recently developed and released transgenic cotton varieties with genetic tolerance to the postemergence applications of 2,4-D, glyphosate, and glufosinate. The availability of 2,4-D choline and glufosinate for in-crop postemergence applications will aid in effective management of glyphosate-resistant Palmer amaranth and other broadleaf weeds in these transgenic varieties. Previous research has shown that timely applications of 2,4-D can effectively control a range of broadleaf weed species including common lambsquarters (*Chenopodium album* L.), common waterhemp (*Amaranthus rudis* Sauer.), giant ragweed (*Ambrosia trifida* L.), Palmer amaranth, annual morningglory (*Ipomoea* spp.) and velvetleaf (*Abutilon theophrasti* Medik) [17] [18] [19]. This will provide cotton growers with additional modes of action for troublesome broadleaf weeds.

The Enlist Duo herbicide, in combination with at-plant and postemergence soil residual herbicides, will be beneficial in managing existing populations of herbicide-resistant broadleaf weeds in cotton. Previously, herbicide programs evaluated that began with an at-plant residual herbicide provided the most consistent season-long weed control and resulted in the lowest density of reproductive Palmer amaranth plants prior to harvest [19]. The 2,4-D choline herbicide will aid control of Palmer amaranth biotypes resistant to multiple modes-of-action and help delay or prevent the development of additional resistant biotypes. Because this technology is new, very little research has been conducted on the efficacy of in-season postemergence 2,4-D choline based herbicide programs on economically important weeds in 2,4-D tolerant cotton. Therefore, research was initiated to evaluate selected 2,4-D choline based herbicide combinations on weed control and their effects on 2,4-D tolerant cotton growth and yield.

2. Materials and Methods

Field experiments were conducted on a Varina sandy loam (pH of 6.1 and organic matter of 1.3%), (fine, kaolinitic, thermic Plinthic Paleudults) at the Clemson University Edisto Research and Education Center (33.36˚N, −81.32˚W) located near Blackville, SC, USA in 2012 and 2013 to determine the efficacy of 2,4-D based herbicide programs for weed control in 2,4-D tolerant cotton. The cotton variety "pDAB4468" (Dow AgroSciences; Indianapolis, IN, USA) was seeded using a four-row Almaco cone plot planter (Almaco, Nevada, IA, USA) 1.25 cm deep in rows spaced 96 cm apart on 15 June 2012 and 21 June 2013 in a conventionally-tilled soil at 10 seed m^{-1}. Treated plot dimensions were two rows wide and 9.4 m long. In both study years, soybean was the previous year's crop grown at each location.

The experimental design was a randomized complete block of four row plots with four replications. All field maintenance processes, such as fertilizing, defoliation, and insect control, were followed according to recommended production practices for cotton in South Carolina [20]. The middle two rows represented the treated area and outside rows were used as an untreated control. In the two studies conducted in 2012 and 2013, the herbicide treatments are presented in **Table 1**. The application rates selected for the 2,4-D choline and glyphosate (Enlist Duo) treatments were based on the use rates recommended on the herbicide label [16]. The remaining treatments in the studies were selected based on the standard Extension herbicide programs used in cotton production in South Carolina. Herbicides were applied in water using a CO_2 pressurized backpack sprayer which delivered 140 L ha^{-1} at 235 kPa via a four-nozzle boom fitted with Turbo TeeJet® 11002 Induction Flat Spray nozzles (Teejet, Spraying Systems Co.,

Table 1. Herbicide treatments, application timing, and rates for 2,4-D based herbicide program evaluations.

No.	Treatment[a]	Timing[b]	Rate[c]	Product Name
			kg ai ha^{-1} or kg ae ha^{-1}	
1	Untreated Check	---	---	---
	fomesafen	PRE	0.28	Reflex
2	2,4-choline + glyphosate	POST1	0.55 + 0.55	Enlist Duo
	2,4-choline + glyphosate	POST2	0.55 + 0.55	Enlist Duo
	fomesafen	PRE	0.28	Reflex
3	2,4 choline + glyphosate	POST1	0.82 + 0.82	Enlist Duo
	2,4 choline + glyphosate	POST2	0.82 + 0.82	Enlist Duo
	fomesafen	PRE	0.28	Reflex
4	2,4 choline + glyphosate+ glufosinate	POST1	0.82 + 0.82 + 0.59	Enlist Duo + Liberty
	2,4 choline + glyphosate	POST2	0.82 + 0.82	Enlist Duo
	fomesafen	PRE	0.28	Reflex
5	2,4 choline + glyphosate+ glufosinate	POST1	0.82 + 0.82 + 0.59	Enlist Duo + Liberty
	glufosinate	POST2	0.59	Liberty
	fomesafen	PRE	0.28	Reflex
6	2,4 choline + glyphosate + acetochlor	POST1	0.82 + 0.82 + 1.26	Enlist Duo + Warrant
	2,4 choline + glyphosate	POST2	0.82 + 0.82	Enlist Duo
	fomesafen	PRE	0.28	Reflex
7	glyphosate	POST1	0.84	Roundup PowerMAX
	glyphosate	POST2	0.84	Roundup PowerMAX
	fomesafen	PRE	0.28	Reflex
8	glufosinate	POST1	0.59	Liberty
	glufosinate	POST2	0.59	Liberty
	fomesafen	PRE	0.28	Reflex
9	glufosinate + acetochlor	POST1	0.59 + 1.26	Liberty + Warrant
	glyphosate	POST2	0.84	Roundup PowerMAX

[a]All POST treatments included ammonium sulfate at 2.5% v/v. [b]Treatment timing: PRE, at planting; POST1, 5 - 10 cm weeds; POST2, 21 days after POST1. [c]Active ingredients (ai) rate used for fomesafen, glufosinate, and acetochlor. Acid equivalent (ae) rate used for 2,4-D choline and glyphosate.

Wheaton, IL, USA) at a ground speed of 5 km h^{-1}.

All plots, except the untreated control, was treated with a preemergence (PRE) application of fomesafen at 0.28 kg ai ha^{-1} at planting and followed up with two postemergence herbicide combinations (POST1 and POST2). The POST1 treatments were applied 26 days after planting (DAP) when Palmer amaranth and pitted morningglory (*Ipomoea lacunosa* L.) sizes ranged from 5 to 10 cm in height. The POST2 combinations were sprayed 47 DAP when Palmer amaranth and pitted morningglory sizes ranged from 10 to 15 cm in height. Data collected included visual ratings of percent control of weeds, estimates of weed populations in plots, crop response or injury, and seed cotton yield. Visual ratings for crop injury and percent weed control were taken on a scale of 0% - 100%, with 0% indicating no effect on cotton or weed populations, respectively, and 100% indicating crop death or complete weed control, respectively. Visual ratings of control were collected 3 weeks after POST 1 (3 WAP1) and 2 weeks after POST 2 (2 WAP2). Weed species population densities were estimated at the 2 WAP2 application timing by randomly tossing a 0.5 m^2 quadrat down the middle of the 2 treated rows and each weed species present was identified and counted. The middle two treated rows were harvested using a two-row spindle type picker and weighed in kg per plot on 11 November 2012 and 11 December 2013. Harvest weights for each plot were then converted into kg per hectare.

Ratings of weed control, estimates of weed density, and cotton yield data were subjected to ANOVA using the PROC GLM procedure in SAS (SAS 9.4, SAS® Institute Inc. Cary, NC, USA), with herbicide treatment and years as the main effect and replication as the random effect. Percent weed control and population densities were combined over trial years if no significant treatment by year was observed. Means of significant main effects were separated using Fisher's Protected LSD at $P \leq 0.05$.

3. Results and Discussion

This research showed differences among treatments and treatment by year across all rating periods. Weed control and population density data were presented separately if there was a treatment by year interaction, and averaged over both years when there was no significant interaction.

The monthly precipitation accumulation and average temperature data at the experimental sites during 2012 and 2013 are presented in **Table 2**. In 2012, a total of 740 mm rainfall was received during the growing season and 693 mm rainfall was received in 2013 at the study sites.

Soil active herbicides, such as fomesafen, require a minimum amount of precipitation after application for proper activation in the soil. In June 2012, rainfall received when the studies were initiated in the field was 81 mm. However, rainfall accumulation in June 2013 was much higher (178 mm). Despite, the lower amount of precipitation received during planting in 2012, fomesafen was properly activated based on the level of weed control observed after planting in both years. During July, August, and September, precipitation accumulation was 427 and 403 mm in 2012 and 2013, respectively (**Table 2**). The highest average air temperature was observed during the month of July (29˚C in 2012 and 27˚C in 2013). However, in 2013, May and August were also comparable to July average air temperatures indicating that effects of heat stress on the plants were experienced over a much longer period than in 2012. Average air temperatures decreased rapidly after September in 2012 and 2013.

Table 2. Monthly rainfall totals and average temperatures observed at the study sites during the growing season at Edisto Research and Education Center located near Blackville, SC, USA, for the months of May through November in 2012 and 2013.

Month	Monthly Precipitation		Monthly Average Air Temperature	
	2012	2013	2012	2013
	mm		˚C	
May	173	64	24	23
June	81	178	26	27
July	97	230	29	27
August	310	134	26	27
September	20	39	24	25
October	14	5	19	20
November	45	43	12	13
Total	740	693	-	-

3.1. Palmer Amaranth Control

Estimates for control and density of Palmer amaranth resulted in some differences between treatments (**Table 3**). Palmer amaranth visual control and population density did not have a significant treatment by year interaction; as a result, data was combined for 2012 and 2013. The PRE application of fomesafen provided early season control of Palmer amaranth across all treatments ranging 91% - 100% at 3 weeks after planting, excluding the untreated check.

At 3 WAP1, the glyphosate POST1 followed by glyphosate POST2 (TRT 7) only provided 95% and 94% control at the 3 WAP1 and 2 WAP2 evaluations periods, respectively, indicating that the natural Palmer amaranth populations at the study sites were predominately sensitive to glyphosate. The addition of 2,4-D choline improved Palmer amaranth control in all treatments (TRT 2 - 4 and 5 - 6) (100% at 2 WAP2). Similarly, Joseph [21] and Miller [19] observed 93% - 100% control of Palmer amaranth with 2,4-D choline plus glyphosate in 2,4-D tolerant soybean. However, Merchant [18] observed lower values of Palmer amaranth control with 2,4-D alone. Glufosinate POST1 followed by glufosinate POST2 (TRT 8) and glufosinate plus acetochlor POST1 followed by glyphosate POST2 (TRT 9) provided excellent control of Palmer amaranth at 2 WAP2. Estimates of the Palmer amaranth population trended with the visual control ratings in all treatments with the untreated control significantly higher at 5 plants m^{-2} than the herbicide treatments (TRT 2 - 9) (**Table 3**).

3.2. Pitted Morningglory Control

There was greater variability in the pitted morningglory population counts and visual percent control between treatments compared to Palmer amaranth (**Table 4**). A treatment by year interaction was observed with the 3 WAP1 and 2 WAP2

Table 3. Palmer amaranth (AMAPA) percent visual control and population density counts as affected by herbicide treatments in 2012 & 2013.

TRT No.[a]	AMAPA control[b]		AMAPA density
	3 WAP1[c]	2 WAP2[c]	2 WAP2[c]
	%		plants m^{-2}
1	-	-	5.0 a
2	100 a	100 a	0 c
3	100 a	100 a	0 c
4	98 ab	100 a	0 c
5	99 ab	100 a	0 c
6	100 a	100 a	0 c
7	95 b	94 b	1 b
8	96 ab	100 a	0 c
9	100 a	100 a	0 c

[a]Refer to Table 1 for treatment (TRT) names and rates; [b]Means followed by the same letter do not differ significantly according to Fishers Protected LSD at 5%; [c]Palmer amaranth percent control and population density rating periods: 3 weeks after POST1 (3 WAP1) and 2 weeks after POST2 application (2 WAP2).

Table 4. Pitted morningglory (IPOLA) percent visual control and population density counts as affected by herbicide treatments for pitted morningglory in 2012 & 2013.

TRT No.[a]	IPOLA control[b]				IPOLA density[b]	
	3 WAP1[c]		2 WAP2[c]		2 WAP2[c]	
	%				plants m^{-2}	
	2012	2013	2012	2013	2012	2013
1	-	-	-	-	11.0 a	11.0 a
2	95 abc	88 abc	96 a	90 ab	1 c	0 b
3	94 a-d	83 c	98 a	80 c	1 c	0 b
4	96 ab	85 bc	99 a	87 bc	1 c	0 b
5	97 a	83 c	96 a	80 c	0 d	0 b
6	94 a-d	90 a	95 ab	88 ab	7 ab	0 b
7	89 bcd	90 a	89 c	90 ab	6 ab	0 b
8	85 d	88 abc	96 a	88 ab	2 bc	0 b
9	87 cd	88 abc	95 ab	92 a	3 bc	1 b

[a]Refer to Table 1 for treatment (TRT) names and rates; [b]Means followed by the same letter do not differ significantly according to Fishers Protected LSD at 5%; [c]Pitted morningglory percent control and population density rating periods: 3 weeks after POST1 (3 WAP1) and 2 weeks after POST2 application (2 WAP2).

pitted morningglory rating dates; therefore, data are presented by year. Treatments were sprayed with fomesafen PRE, and significant differences in control of pitted morningglory (83% to 100%) were observed between treatments at 3 weeks after planting, excluding the untreated check. At 3 WAP1, treatments

(TRT 2 - 6) that included 2,4-D choline at POST1 provided 94% to 99% control of pitted morningglory; however, in treatments without 2,4-D choline (TRT 7 - 9), control declined to 85% to 89% for 2012 (**Table 4**). In a similar study, Merchant [21] found that the control of pitted morningglory increased when an auxinic herbicide was mixed with glufosinate rather than sprayed alone. At 2 WAP2, control of pitted morningglory was greater than 90% in 2012 with all treatments (TRT 2 - 6) containing 2,4-D choline and glyphosate combinations (**Table 4**). Estimates of pitted morningglory density trended with visual ratings of percent control for 2012; however, no differences were observed between TRT 6 and 7 and the untreated control (TRT 1). In 2013, pitted morningglory densities in the untreated check (TRT 1) were significantly higher at 11 plants m^{-2} compared to the remaining herbicide treatments (TRT 2 - 9).

Significant differences among the treatments were observed with the pitted morningglory percent control ratings and population counts. Pitted morningglory control in 2013 was less than in 2012 with ranges being from 83% to 90% at 3 WAP1 (TRT 2 - 6). The 2,4-D choline + glyphosate + glufosinate and 2,4-D choline + glyphosate treatments (TRT 3 and 5) resulted in the lowest control of 83% at 3 WAP1 in 2013 (**Table 4**). Other studies have reported that auxin herbicides mixed with glufosinate provided excellent control of morningglory species [8] [22]. Culpepper [23] also observed that 2,4-DB plus glyphosate provided control of annual morningglory. Estimates of pitted morningglory density did not correspond to the visual control ratings in 2013 because control ratings took into account the climbing nature of the plant. Overall, pitted morningglory population densities across the treatments were significantly less than the untreated control (11 plants m^{-2} at 2 WAP2).

3.3. Cotton Yield

In 2012, seed cotton yield ranged from 534 to 2057 kg ha^{-1} in the untreated check (TRT 1) and 2,4-D choline + glyphosate at POST 1 and 2 (TRT 3), respectively (**Table 5**). Among the treatments, glufosinate followed by glufosinate was numerically the lowest yield at 1358 kg ha^{-1}. The seed cotton yield harvested from the plots in 2013 ranged from 0 to 647 kg ha^{-1} and was far lower overall than in 2012. Rainfall and temperature were adequate through the growing season in 2013; however, the plots were collected later than normal due to plot equipment malfunctions and conflicting scheduling resulting in boll degradation and yield loss before harvest. Although yields were much lower in 2013, the 2,4-D choline + glyphosate at POST 1 and 2 treatment (TRT 3) also had the highest yield which was similar to 2012.

4. Summary

Overall, this research showed that herbicide programs including 2,4-D choline + glyphosate and glufosinate were more effective on Palmer amaranth and pitted morningglory, than the treatments without 2,4-D choline. However, weed size

Table 5. Mean seed cotton yield as affected by selected herbicide programs in 2012 & 2013.

TRT No.[a]	Seed Cotton Yield[b] 2012	Seed Cotton Yield[b] 2013
	kg ha^{-1}	
1	534 d	0 d
2	1679 ab	7 bcd
3	2057 a	647 a
4	1660 ab	7 bcd
5	1983 ab	49 abcd
6	1541 ab	0 d
7	1554 ab	4 cd
8	1358 abc	300 ab
9	1507 ab	79 abc

[a]Refer to Table 1 for treatment (TRT) names and rates; [b]Means followed by the same letter do not differ significantly according to Fishers Protected LSD at 5%.

at the time of POST application will be a critical consideration for economical control. The application of fomesafen PRE reduced the early season emergence of Palmer amaranth and pitted morningglory. The overall success of 2,4-D choline containing POST herbicide programs is reliant on the use of a soil residual herbicide at planting plus the use of residual herbicides at each application timing (overlapping residuals). Without an at-plant PRE herbicide, weed size and density will quickly reach beyond the recommendations by the time that the over-the-top application of 2,4-D choline is completed reducing the efficacy of the herbicide application.

In 2012, the seed cotton yield was consistent across all treatments, especially in the treatments containing 2,4-D choline which indicates that cotton is very tolerant to the over-the-top herbicides in this study. The use of 2,4-D choline in the 2,4-D tolerant cotton will be very effective tool in managing broadleaf weeds and, most importantly, reduce the possibility of selecting new herbicide resistant biotype weeds. However, growers must utilize a comprehensive management program that includes a PRE herbicide, such as fomesafen, at planting followed by tank mixing residual herbicides, such as acetochlor and s-metolachlor, with each 2,4-D choline POST application (*i.e.*, overlapping residuals).

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