Weed Technology 2016 30:366–376

Evaluation of Herbicide Programs for Use in a 2,4-D–Resistant Soybean Technology for Control of Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*)

M. Ryan Miller and Jason K. Norsworthy*

Two separate field experiments were conducted over a 2-yr period in Fayetteville, AR, during 2012 and 2013 to (1) evaluate POST herbicide programs utilizing a premixture of dimethylamine (DMA) salt of glyphosate + choline salt of 2,4-D in a soybean line resistant to 2,4-D, glyphosate, and glufosinate and (2) determine efficacy of herbicide programs that begin with PRE residual herbicides followed by POST applications of 2,4-D choline + glyphosate DMA on glyphosate-resistant Palmer amaranth. In the first experiment, POST applications alone that incorporated the use of residual herbicides with the glyphosate + 2,4-D premixture provided 93 to 99% control of Palmer amaranth at the end of the season. In the second experiment, the use of flumioxazin, flumioxazin + chlorimuron methyl, *S*-metolachlor + fomesafen, or sulfentrazone + chloransulam applied PRE provided 94 to 98% early-season Palmer amaranth control. Early-season control helped maintain a high level of Palmer amaranth control throughout the growing season, in turn resulting in fewer reproductive Palmer amaranth plants present at soybean harvest compared to most other treatments. Although no differences in soybean yield were observed among treated plots, it was evident that herbicide programs should begin with PRE residual herbicides followed by POST applications of glyphosate + 2,4-D mixed with residual herbicides to minimize late-season escapes and reduce the likelihood of contributions to the soil seedbank. Dependent upon management decisions, the best stewardship of this technology will likely rely on the use multiple effective mechanisms of action incorporated into a fully integrated weed management system.

Nomenclature: 2,4-D choline; chlorimuron methyl; chloransulam; flumioxazin; fomesafen; glyphosate; glufosinate; sulfentrazone; *S*-metolachlor; Palmer amaranth, *Amaranthus palmeri* S. Wats.; soybean, *Glycine max* (L.) Merr.

Key words: 2,4-D–resistant soybean; herbicide programs; transgenic soybean; weed control.

Dos experimentos de campo fueron realizados separados durante un período de dos años en Fayetteville, AR, durante 2012 y 2013 para (1) evaluar programas de herbicidas POST utilizando una premezcla de sal dimethylamine (DMA) de glyphosate + sal choline de 2,4-D con una línea de soja resistente a 2,4-D, glyphosate, y glufosinate y (2) determinar la eficacia de programas de herbicidas que inician con herbicidas PRE residuales seguidos por aplicaciones POST de 2,4-D choline + glyphosate DMA para el control de *Amaranthus palmeri* resistente a glyphosate. En el primer experimento, aplicaciones POST solas que incorporaron el uso de herbicidas residuales con la premezcla de glyphosate + 2,4-D brindaron 93 a 99% de control de *A. palmeri* al final de la temporada. En el segundo experimento, el uso de flumioxazin, flumioxazin + chlorimuron methyl, *S*-metolachlor + fomesafen, o sulfentrazone + chloransulam aplicados PRE brindaron 94 a 98% de control de *A. palmeri* temprano durante la temporada de crecimiento. El control temprano en la temporada ayudó a mantener un alto nivel de control de *A. palmeri* a lo largo de la temporada de crecimiento, lo que resultó un menos plantas de *A. palmeri* en estado reproductivo al momento de la cosecha de la soja, al compararse con la mayoría de los otros tratamientos. Aunque no se observaron diferencias en el rendimiento de la soja entre parcelas tratadas, fue evidente que los programas de herbicidas deberían iniciar con herbicidas residuales PRE seguidos de aplicaciones POST de mezclas de glyphosate + 2,4-D con herbicidas residuales para minimizar los escapes tarde en la temporada y así poder reducir la probabilidad de contribuciones al banco de semillas del suelo. Dependiendo de las decisiones de manejo, la mejor forma de preservar esta tecnología será probablemente el depender del uso de múltiples mecanismos de acción efectivos que deben ser incorporados en un sistema de manejo de malezas totalmente integrado.

In 1996, glyphosate-resistant soybean technology was introduced into the marketplace (Roundup Ready®, Monsanto, St. Louis, MO) and was rapidly adopted by growers in the midsouthern United States resulting in a complete transformation of weed control programs (Norsworthy et al. 2012). As

DOI: 10.1614/WT-D-15-00129.1

* Graduate Research Assistant and Professor, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701. Corresponding author's E-mail: mrm032@uark.edu

a result, sole reliance on total POST herbicide programs with glyphosate as the primary component became a common practice (Norsworthy et al. 2012; Young 2006). As glyphosate alone quickly became a standard weed control program, the use of PRE and POST residual herbicides was quickly forgotten (Culpepper and York 1998; Nuti et al. 2003; Wilcut et al. 2003). Therefore, the tremendous selection pressure on weeds treated with glyphosate has resulted in the evolution of glyphosate-resistant weed species (Culpepper et al. 2006; Norsworthy et al. 2008; Powles and Yu 2010). Currently, 32 weed species have been reported as glyphosate-resistant (Heap 2015).

The evolution of glyphosate-resistant Palmer amaranth has been particularly problematic for many crop producers in the United States. Palmer amaranth is an erect, rapid-growing plant that has been listed as the most troublesome weed in soybean and cotton (*Gossypium hirsutum* L.) in the midsouthern United States (Riar et al. 2013), ultimately forcing weed management decisions to be focused on controlling this troublesome weed. While Palmer amaranth is the most threatening weed for midsouthern U.S. soybean growers today, numerous other weed species also have been identified as problematic. In a survey of crop consultants across Arkansas, Louisiana, Mississippi, and Tennessee, species including Palmer amaranth, morningglory (*Ipomoea* spp.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], horseweed [*Conyza canadensis* (L.) Cronq.], prickly sida (*Sida spinosa* L.), broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster], hemp sesbania [*Sesbania herbacea* (P. Mill.) McVaugh], johnsongrass (*Sorghum halepense* L.), yellow nutsedge (*Cyperus esculentus* L.), and sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] were listed as the most problematic weeds in soybean (Riar et al. 2013). Among these weeds, several have evolved glyphosate resistance, such as Palmer amaranth, horseweed, and johnsongrass (Heap 2015).

In terms of production acreage, during the 2014 production season, 94% of the soybean acreage in the United States was planted with herbicide-resistant soybean cultivars (USDA NASS 2014). However, this acreage was not planted entirely to glyphosate-resistant soybean as traits that provide resistance to an over-the-top application of glufosinate (LibertyLink®, Bayer CropScience, Research Triangle Park, NC) and sulfonylurea (STS®, DuPont, Wilmington, DE) represent a portion of the acreage planted today. As a result of glyphosate-resistant weed species, researchers have promoted the use of alterative herbicide resistance traits and overlaying multiple residual herbicides for effective season-long weed control (Jha and Norsworthy 2009; Neve et al. 2011; Norsworthy et al. 2012). While several herbicide mechanisms of action are effective when applied PRE on glyphosate-resistant Palmer amaranth, very few effective POST options remain (Scott et al. 2014). Effective POST options are further limited by the increasing documentation of Palmer amaranth with multiple resistance to glyphosate and acetolactate synthase (ALS)-inhibiting herbicides (Bagavathiannan and Norsworthy 2013; Heap 2015).

In conventional and glyphosate-resistant soybean, effective POST herbicide options currently consist of several protoporphyrinogen oxidase (PPO)-inhibiting herbicides. In addition to the option of a PPO herbicide, glufosinate can be used to provide POST control of glyphosate-resistant Palmer amaranth in glufosinate-resistant soybean (Riar et al. 2013; Scott et al. 2014). However, these limited POST herbicide options must be applied to small Palmer amaranth to be effective (Tharp et al. 1999; Norsworthy et al. 2012). Therefore, it is apparent that as herbicide-resistant Palmer amaranth and other herbicide-resistant weeds continue to spread across major production areas, new technologies are required for effective control.

Currently, 2,4-D, a synthetic auxin herbicide in the phenoxy chemical family, is used for selective broadleaf weed control in grass crops and noncrop areas. Following decades of availability in the marketplace, recent attention has grown around using 2,4-D as an effective alternative mechanism of action for the control of glyphosate-resistant broadleaf weed species, primarily due to the development of 2,4-D–resistant crops (Loux 2008). Chehal and Johnson (2012) reported that the addition of 2,4-D to glyphosate provided 99% control of glyphosate-resistant horseweed compared to only 12% with glyphosate alone. In a similar study, 2,4-D added to glufosinate provided an increased level of common waterhemp (*Amaranthus rudis* Sauer) control compared to herbicide treatments consisting of glufosinate only (Craigmyle et al. 2013).

While 2,4-D is an effective broadleaf herbicide, its efficacy on grass species is generally poor. However, combining it with glyphosate or glufosinate can increase the spectrum of control. Tank-mixing herbicides, such 2,4-D, glyphosate, glufosinate, or a combination of these, allows for multiple mechanisms of action to be applied, which is a central component of resistance management (Norsworthy et al. 2012). Use of new technologies that permit these and other herbicides to be applied within crops will diversify current weed management programs. An example of this would be development of herbicide premixtures for in-crop use in new herbicide-resistance technologies. Therefore, as new herbicide-resistant weed populations evolve, an important resistance management tactic will consist of using multiple herbicide-resistant crops or traits (Green et al. 2008).

The introduction of 2,4-D choline-, glyphosate-, and glufosinate-resistant soybean (Enlist™ soybean, Dow Agrosciences, Indianapolis, IN) and 2,4-D choline + glyphosate DMA (Enlist Duo™ herbicide, Dow Agrosciences) is intended to provide growers with a novel technological tool capable of controlling difficult-to-manage, herbicide-resistant weed species, including glyphosate-resistant Palmer amaranth. This new soybean technology represents a three-gene herbicide-resistant trait package, which provides resistance to foliar applications of 2,4-D choline, glyphosate, and glufosinate. While auxin-type herbicides have historically had a high potential for drift, the 2,4-D choline + glyphosate herbicide premixture (Colex-D™, Dow AgroSciences) is designed to reduce physical and vapor drift of the herbicide premixture.

Applications of 2,4-D, glyphosate, and glufosinate alone or tank-mixed represent broad-spectrum POST herbicides that have the potential to control nine of the 10 most problematic weeds in midsouthern U.S. soybean (Riar et al. 2013; Scott et al. 2014). As observed with glyphosate, overreliance on total POST herbicide programs fails to provide consistent season-long control and places high selection pressure on weeds. As a result, new herbicide label recommendations will encourage growers to rotate mechanisms of action along with inclusion of PRE followed by POST herbicide applications, overlaying of residual herbicides, and incorporating integrated weed management strategies (Anonymous 2015).

The purpose of this research was to evaluate the effectiveness of a 2,4-D choline + glyphosate DMA premixture alone and as part of a herbicide program containing residual herbicides in order to develop effective recommendations associated with this trait for soybean growers located in the midsouthern United States. It was hypothesized that the 2,4-D choline + glyphosate premixture applied in conjunction with residual herbicides will provide greater season-long weed control compared to programs that rely on POST nonresidual applications alone. The objectives of this research were to (1) determine the efficacy of POST applications of the 2,4-D choline + glyphosate premixture alone and in mixture with other herbicides registered for use in soybean and (2) evaluate the efficacy of herbicide programs that begin with PRE residual herbicides followed by POST herbicide applications utilizing the 2,4-D choline + glyphosate premixture in resistant soybean to control glyphosate-resistant Palmer amaranth and other weeds.

Materials and Methods

Two separate field experiments were conducted in 2012 and 2013 at the University of Arkansas Agricultural Research and Extension Center located in Fayetteville, AR. The soils in both experiments included a mixture of Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) and a Leaf silt loam (fine, mixtured, active, thermic Typic Albaqults). In both years, fields were prepared through disking and cultivating prior to planting. Immediately following field preparation, beds were formed on 0.92-m centers. Each experimental plot contained four rows resulting in an overall plot size of 3.7 m wide by 7.62 m long. In both years, an experimental 2,4-D, glyphosate, glufosinate–resistant soybean (Enlist™ soybean) line was planted at an approximate 2-cm depth at 120,000 seed ha^{-1} using a tractor-mounted 7200 MaxEmerge planter. Throughout each growing season, plots were irrigated four to six times using furrow irrigation and standard soybean production practices typical for the region were used. Herbicide treatments were applied with a CO_2-pressurized backpack sprayer fitted with 1100015 AIXR flat-fan nozzles (Teejet

Table 1. Herbicide information for all products used in experiments.

Herbicide trade name	Herbicide common name	Rate[a]	Manufacturer
		g ae or ai ha^{-1}	
Classic	Chlorimuron	22	DuPont Crop Protection, Wilmington, DE
Enlist Duo	2,4-D choline + glyphosate DMA	532 + 560, 800 + 840, or 1,065 + 1,120	Dow AgroSciences LLC, Indianapolis, IN
Flexstar	Fomesafen	263	Syngenta Crop Protection LLC, Greensboro, NC
Liberty	Glufosinate	594	Bayer CropScience, Research Triangle Park, NC
Prefix	Fomesafen + *S*-metolachlor	1540	Syngenta Crop Protection LLC, Greensboro, NC
Sonic	Chloransulam + sulfentrazone	147	Dow AgroSciences LLC, Indianapolis, IN
Valor	Flumioxazin	63 or 71	Valent U.S.A. Corporation, Walnut Creek, CA
Warrant	Acetochlor	1,260	Monsanto Co., St. Louis, MO

[a] All treatments contained an ammonium sulfate-containing adjuvant at 2.5% v/v (AMS-supreme™, Drexel Chemical Company, Memphis, TN).

Technologies, Springfield, IL) calibrated to deliver 140 L ha^{-1} at 4.8 km hr^{-1} at 276 kPa.

Efficacy of POST-Only Programs Utilizing a 2,4-D + Glyphosate Premixture. The experiment was conducted in a randomized complete block design with four replications. Herbicide programs evaluated included 2,4-D choline + glyphosate at 532 + 560, 800 + 840, or 1,065 + 1,120 g ae ha^{-1} early POST (EPOST); 2,4-D choline + glyphosate at 532 + 560, 800 + 840, or 1,065 + 1,120 g ae ha^{-1} EPOST followed by (fb) 2,4-D choline + glyphosate at 532 + 560, 800 + 840, or 1,065 + 1,120 g ae ha^{-1} mid-POST (MPOST). Treatments were applied alone or in combination with acetochlor, fomesafen, *S*-metolachlor + fomesafen, or glufosinate. (Refer to Table 1 for a complete list of products used and Tables 2 and 3 for a complete list of treatments.) Planting occurred on May 25, 2012, and May 20, 2013. EPOST treatments were applied on June 13, 2012, and June 20, 2013, at the V3 growth stage and MPOST treatments were applied on July 3, 2012, and July 2, 2013, at the V6 growth stage. Weeds evaluated included glyphosate-resistant Palmer amaranth, large crabgrass, and carpetweed. In both years, Palmer amaranth height ranged from 15 to 20 cm and 19 to 25 cm at a density of 51 to 59 plants m^{-2} at the EPOST and MPOST application timings, respectively. Large crabgrass height ranged from 4 to 7 cm and 10 to 11 cm at a density of 7 to 8 plants m^{-2} at the EPOST and MPOST application timings, respectively. Additionally, carpetweed height ranged from 1 to 2 cm and 1 to 3 cm at a density of 7 to 8 plants m^{-2} at the EPOST and MPOST applications timings, respectively. Visible crop injury and weed control were rated on a scale of 0 to 100%, where 0 was no control or no crop injury and 100 represented complete control or total crop death. The total density of reproductive Palmer amaranth plants per square meter, both male and female, was determined at the time of crop maturity. In the nontreated control, stand counts were recorded for 1 m of row whereas stand counts in treated plots were between the two center rows for the length of the treated plot.

Herbicide Programs using PRE Herbicides fb POST Applications of 2,4-D + Glyphosate. To evaluate herbicide programs utilizing a premixture of 2,4-D choline + glyphosate, field experiments were conducted to determine the efficacy of the premixture alone and as part of a complete herbicide program that utilized PRE residual herbicides on problematic weeds in soybean. The experiment was conducted during the summers of 2012 and 2013 in a randomized complete block design with four replications. Herbicide programs evaluated included PRE applications of sulfentrazone + chloransulam, flumioxazin, chlorimuron, flumioxazin + chlorimuron, or no PRE followed by POST applications of 2,4-D choline + glyphosate alone or in combination with glufosinate. (Refer to Table 1 for a complete list of products used and Tables 4 and 5 for a complete list of treatments.) Treatments contained an ammonium sulfate–containing adjuvant at 2.5%

https://doi.org/10.1614/WT-D-15-00129.1 Published online by Cambridge University Press

Table 2. Efficacy of POST herbicide programs containing 2,4-D + glyphosate on Palmer amaranth in soybean.[ab]

Treatment	Timing	Rate	Palmer amaranth control		
			14 DAEP	14 DAMP	End of season
		g ae or ai ha^{-1}	—————————%—————————		
2,4-D choline + glyphosate	EPOST	532 + 560	71 cd	62 C	65 e
2,4-D choline + glyphosate	EPOST	800 + 840	77 bcd	62 c	70 de
2,4-D choline + glyphosate	EPOST	1,065 + 1,120	89 ab	87 ab	89 abc
2,4-D choline + glyphosate fb	EPOST	532 + 560	64 d	84 b	81 cd
2,4-D choline + glyphosate	MPOST	532 + 560			
2,4-D choline + glyphosate fb	EPOST	800 + 840	82 abc	92 ab	94 ab
2,4-D choline + glyphosate	MPOST	800 + 840			
2,4-D choline + glyphosate fb	EPOST	1,065 + 1,120	88 ab	96 ab	95 a
2,4-D choline + glyphosate	MPOST	1,065 + 1,120			
2,4-D choline + glyphosate + acetochlor fb	EPOST	800 + 840 + 1,260	82 abc	94 ab	99 a
2,4-D choline + glyphosate	MPOST	800 + 840			
2,4-D choline + glyphosate + fomesafen fb	EPOST	800 + 840 + 263	93 a	92 ab	93 abc
2,4-D choline + glyphosate	MPOST	800 + 840			
2,4-D choline + glyphosate + *S*-metolachlor + fomesafen fb	EPOST	800 + 840 + 1,540	95 a	98 a	97 a
2,4-D choline + glyphosate	MPOST	800 + 840			
2,4-D choline + glyphosate + glufosinate fb	EPOST	532 + 560 + 594	76 d	87 ab	82 bcd
2,4-D choline + glyphosate + glufosinate	MPOST	532 + 560 + 594			
2,4-D choline + glyphosate + glufosinate fb	EPOST	800 + 840 + 594	75 d	86 ab	81 cd
2,4-D choline + glyphosate + glufosinate	MPOST	800 + 840 + 594			
Contrast					
EPOST alone vs. EPOST fb MPOST			—	***	***
Residual POST vs. no residual POST			—	**	**

[a] Abbreviations: DAEP, days after early-POST application; DAMP, days after mid-POST application; EPOST, early POST; fb, followed by; MPOST, mid-POST.

[b] Means within columns followed by different letters are significantly different using Fisher's protected LSD ($\alpha = 0.05$).

** $P \leq 0.01$ according to orthogonal contrasts.

*** $P \leq 0.001$ according to orthogonal contrasts.

v/v. Planting occurred on May 25, 2012, and May 20, 2013, with PRE treatments applied immediately after planting was completed and occurred on the same day as planting in each year of the experiment. EPOST treatments were applied on June 13, 2012, and June 20, 2013, at the V3 growth stage and MPOST treatments were applied on July 3, 2012, and July 2, 2013, at the V6 growth stage. Weeds evaluated included glyphosate-resistant Palmer amaranth, large crabgrass, and carpetweed. No weeds were present at the time of the PRE application due to cultivation events during field preparation and bedding. In both years, Palmer amaranth height ranged from 10 to 14 cm and 13 to 15 cm with a density of 49 to 54 plants m^{-2} at the EPOST and MPOST application timings, respectively. Large crabgrass height ranged from 4 to 5 cm and 5 to 6 cm with a density of 1 to 2 plants m^{-2} at the EPOST and MPOST application timings, respectively, and average carpetweed height ranged from 1 to 2 cm with a density of 4 to 5 plants m^{-2} at the EPOST and MPOST application timings. Data collection for visible crop injury, weed control, and reproductive Palmer amaranth densities were collected as previously described.

Statistical Analyses. In both experiments, data were subjected to ANOVA using the MIXED procedure in JMP (JMP, Version 11; SAS Institute Inc, Cary, NC 27513). Herbicide treatments were analyzed as fixed effects while replication and year were analyzed as random effects. Where the ANOVA indicated significant differences, means were separated with Fisher's protected LSD ($\alpha = 0.05$) and orthogonal contrasts were used for preplanned comparisons.

Table 3. Influence of POST herbicide programs containing 2,4-D + glyphosate on reproductive Palmer amaranth density at soybean maturity and soybean yield.[ab]

Treatment	Timing	Rate	Palmer amaranth	Yield
		g ae or ai ha^{-1}	plants m^{-2}	kg ha^{-1}
Nontreated	—	—	—	1,980 b
2,4-D choline + glyphosate	EPOST	532 + 560	8.0 a	4,090 a
2,4-D choline + glyphosate	EPOST	800 + 840	6.5 a	3,860 a
2,4-D choline + glyphosate	EPOST	1,065 + 1,120	1.5 b	3,620 a
2,4-D choline + glyphosate fb	EPOST	532 + 560	2.1 b	3,400 a
2,4-D choline + glyphosate	MPOST	532 + 560		
2,4-D choline + glyphosate fb	EPOST	800 + 840	0.5 b	3,490 a
2,4-D choline + glyphosate	MPOST	800 + 840		
2,4-D choline + glyphosate fb	EPOST	1,065 + 1,120	0.6 b	3,820 a
2,4-D choline + glyphosate	MPOST	1,065 + 1,120		
2,4-D choline + glyphosate + acetochlor fb	EPOST	800 + 840 + 1,260	0.1 b	4,050 a
2,4-D choline + glyphosate	MPOST	800 + 840		
2,4-D choline + glyphosate + fomesafen fb	EPOST	800 + 840 + 263	0.6 b	4,240 a
2,4-D choline + glyphosate	MPOST	800 + 840		
2,4-D choline + glyphosate + *S*-metolachlor + fomesafen fb	EPOST	800 + 840 + 1,540	0.3 b	4,030 a
2,4-D choline + glyphosate	MPOST	800 + 840		
2,4-D choline + glyphosate + glufosinate fb	EPOST	532 + 560 + 594	2.8 b	3,930 a
2,4-D choline + glyphosate + glufosinate	MPOST	532 + 560 + 594		
2,4-D choline + glyphosate + glufosinate fb	EPOST	800 + 840 + 594	2.8 b	3,770 a
2,4-D choline + glyphosate + glufosinate	MPOST	800 + 840 + 594		
Contrast				
PRE fb MPOST vs. EPOST fb MPOST			—	NS

[a] Abbreviations: EPOST, early POST; MPOST, mid-POST; fb, followed by; NS, nonsignificant according to orthogonal contrasts.

[b] Means within columns followed by different letters are significantly different using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Efficacy of POST-Only Programs Utilizing a 2,4-D + Glyphosate Premixture. Across all ratings, herbicide programs provided $\geq$ 95% control of large crabgrass and carpetweed and no program exhibited > 5% soybean injury (data not shown). POST herbicide programs, 14 d after the EPOST application (DAEP), provided 64 to 95% control of Palmer amaranth (Table 2). The lowest level of Palmer amaranth control 14 DAEP was observed when the premixture of 2,4-D choline + glyphosate at 532 + 560 g ae ha^{-1} was applied alone, whereas the greatest level of control was observed when the premixture was applied at a rate of 2,4-D choline + glyphosate at 800 + 840 g ae ha^{-1} alone or tank-mixed with residual herbicides. Adequate moisture required for residual herbicide activation was received in each year. Consequently, 14 d after the MPOST (DAMP) applications, herbicide programs that utilized a residual herbicide in the EPOST application provided greater control ($P \leq 0.01$) compared to programs that did not include a residual herbicide in a POST applied mixture (Table 2). Additionally, herbicide programs that utilized two POST applications (EPOST fb MPOST) provided greater ($P \leq 0.0001$) control of Palmer amaranth compared to programs that relied on a single EPOST application 14 DAMP. These findings are likely attributed to the wide emergence period of Palmer amaranth (Jha and Norsworthy 2009). Thus, it is recommended that multiple effective herbicide applications be used to control weeds that emerge over an extended period (Reddy and Norsworthy 2010).

The greatest level of Palmer amaranth control 14 DAMP was observed with the highest rate of 2,4-D + glyphosate premixture applied alone or in conjunction with residual herbicides. However, contrast analysis of Palmer amaranth at the end of season revealed superior control when multiple effective POST herbicides were applied rather than a single POST program (Table 2). Additionally, POST herbicide programs that utilized residual herbicides provided greater Palmer amaranth control compared to programs that did not, which was consistent with previous findings (Neve et al. 2011).

https://doi.org/10.1614/WT-D-15-00129.1 Published online by Cambridge University Press

Table 4. Effect of 2,4-D + glyphosate-containing herbicide programs on Palmer amaranth in soybean.[ab]

Treatment	Timing	Rate	Palmer amaranth control		
			14 DAEP	14 DAMP	End of season
		g ae or ai ha^{-1}	%		
Flumioxazin fb	PRE	71	94 ab	90 abc	87 ab
2,4-D choline + glyphosate	MPOST	800 + 840			
Flumioxazin + chlorimuron fb	PRE	63 + 22	95 ab	90 ab	90 a
2,4-D choline + glyphosate	MPOST	800 + 840			
Fomesafen + *S*-metolachlor fb	PRE	1,540	98 a	95 a	87 ab
2,4-D choline + glyphosate	MPOST	800 + 840			
Sulfentrazone + chloransulam fb	PRE	147	95 ab	88 abc	86 ab
2,4-D choline + glyphosate	MPOST	532 + 560			
Sulfentrazone + chloransulam fb	PRE	147	94 ab	85 bc	85 ab
2,4-D choline + glyphosate	MPOST	800 + 840			
Sulfentrazone + chloransulam fb	PRE	147	95 ab	91 ab	87 ab
2,4-D choline + glyphosate	MPOST	1,065 + 1,120			
Sulfentrazone + chloransulam fb	PRE	147	96 ab	87 bc	86 ab
2,4-D choline + glyphosate + glufosinate	MPOST	800 + 840 + 594			
2,4-D choline + glyphosate fb	EPOST	532 + 560	69 c	66 d	68 c
2,4-D choline + glyphosate	MPOST	532 + 560			
2,4-D choline + glyphosate fb	EPOST	800 + 840	86 b	82 c	80 b
2,4-D choline + glyphosate	MPOST	800 + 840			
Contrasts					
PRE alone vs. EPOST alone			***	—	—
PRE fb MPOST vs. EPOST fb MPOST			—	—	***

[a] Abbreviations: DAEP, days after early-POST application; DAMP, days after mid-POST application; EPOST, early POST; MPOST, mid-POST; fb, followed by.

[b] Means within columns followed by different letters are significantly different using Fisher's protected LSD ($\alpha = 0.05$).

*** $P \leq 0.001$ according to orthogonal contrasts.

Palmer amaranth control at the end of season for the sequential applications of 2,4-D choline + glyphosate was comparable to the same sequential applications that included a residual herbicide in one of the applications. A residual herbicide in one or both of the 2,4-D choline + glyphosate applications will likely provide an additional effective herbicide mode of action for managing glyphosate-resistant Palmer amaranth, which is the current recommendation for this technology (Anonymous 2015).

The most aggressive herbicide programs, containing multiple effective modes of actions in conjunction with residual herbicides, also resulted in having the lowest density of reproductive Palmer amaranth plants at the end of the season, with densities ranging from 0.1 to 2.8 plants m^{-2} (Table 3). Although seed production was not evaluated, it is noteworthy that one or more reproductive plants were found in one or more plots of all experimental treatments, meaning that the risk for seed production by these escapes existed (data not shown). Many of the plants present at maturity were small in size and likely emerged following the final herbicide application. Less aggressive, single EPOST applications of 2,4-D choline + glyphosate at 532 + 560 or 800 + 840 g ha^{-1}, had the highest density of reproductive Palmer amaranth plants at the end of the season measuring 8.0 and 6.5 plants m^{-2}, respectively. These less aggressive herbicide programs will not be recommended (Anonymous 2015).

Soybean in herbicide-treated plots averaged grain yields $\geq$ 3,490 kg ha^{-1}, which were significantly higher than the nontreated control, which yielded 1,980 kg ha^{-1} (Table 3). Furthermore, there were no differences in yields observed among any of the herbicide treatments.

Results from this study indicate that the higher rates of the 2,4-D + glyphosate premixture alone will provide effective weed control, even when Palmer amaranth size was at the maximum

https://doi.org/10.1614/WT-D-15-00129.1 Published online by Cambridge University Press

Table 5. Influence of 2,4-D + glyphosate-containing herbicide programs on reproductive Palmer amaranth density at soybean maturity and soybean yield.[ab]

Treatment	Timing	Rate	Palmer amaranth	Yield
		g ae or ai ha^{-1}	plants m^{-2}	kg ha^{-1}
Nontreated		—	—	2,210 b
Flumioxazin fb	PRE	71	2.9 b	2,970 ab
2,4-D choline + glyphosate	MPOST	800 + 840		
Flumioxazin + chlorimuron fb	PRE	63 + 22	1.9 b	2,900 ab
2,4-D choline + glyphosate	MPOST	800 + 840		
Fomesafen + *S*-metolachlor fb	PRE	1,540	1.3 b	3,190 a
2,4-D choline + glyphosate	MPOST	800 + 840		
Sulfentrazone + chloransulam fb	PRE	147	1.9 b	3,210 a
2,4-D choline + glyphosate	MPOST	532 + 560		
Sulfentrazone + chloransulam fb	PRE	147	2.1 b	3,300 a
2,4-D choline + glyphosate	MPOST	800 + 840		
Sulfentrazone + chloransulam fb	PRE	147	2.6 b	3,150 a
2,4-D choline + glyphosate	MPOST	1,065 + 1,120		
Sulfentrazone + chloransulam fb	PRE	147	1.3 b	3,020 a
2,4-D choline + glyphosate + glufosinate	MPOST	800 + 840 + 594		
2,4-D choline + glyphosate fb	EPOST	532 + 560	6.9 a	3,060 a
2,4-D choline + glyphosate	MPOST	532 + 560		
2,4-D choline + glyphosate fb	EPOST	800 + 840	4.4 ab	2,860 ab
2,4-D choline + glyphosate	MPOST	800 + 840		
Contrast				
PRE fb MPOST vs. EPOST fb MPOST			—	NS

[a] Abbreviations: EPOST, early POST; MPOST, mid-POST; fb, followed by; NS, nonsignificant according to orthogonal contrasts.
[b] Means within columns followed by different letters are significantly different using Fisher's protected LSD ($\alpha = 0.05$).

recommended height (15 cm) for this technology, and adding a residual herbicide did not further improve control. However, diverse herbicide programs for controlling glyphosate-resistant Palmer amaranth is an important herbicide-resistance management strategy (Norsworthy et al. 2012). Additionally, full labeled-use rates should always be used to achieve the greatest level of possible control and reduce the likelihood for the evolution of resistance. These results further displayed the high level of weed control this new technology is capable of providing despite the larger than recommended weed size at the time of application. Nevertheless, emphasis should be placed on a zero-tolerance weed threshold (Norsworthy et al. 2014a), herbicides should be applied at or less than the recommended weed height, and programs should not begin with an EPOST or MPOST application but rather start prior to planting with the application of residual herbicides in conjunction with nonchemical weed management tactics.

Herbicide Programs using PRE Herbicides fb POST Applications of 2,4-D + Glyphosate. Throughout the experiment and across rating times, herbicide programs provided $\geq$ 95% control of large crabgrass and carpetweed and no program exhibited > 5% soybean injury (data not shown). Orthogonal contrast analysis, 14 DAEP application, revealed a difference ($P \leq 0.0001$) between programs that utilized a PRE herbicide compared to programs that relied solely on POST-applied herbicides for early-season Palmer amaranth control. Herbicide programs that utilized flumioxazin, flumioxazin + chlorimuron, *S*-metolachlor + fomesafen, or sulfentrazone + chloransulam PRE provided 94 to 98% control of Palmer amaranth at the 14 DAEP timing (Table 4). Alternatively, total POST programs comprised of 532 + 560 or 800 + 840 g ha^{-1} of the 2,4-D choline + glyphosate premixture provided only 69 and 86% control of Palmer amaranth, respectively. The importance of weed management programs beginning with PRE residual herbicides was evident based on herbicide program performance for the 14 DAMP treatments.

https://doi.org/10.1614/WT-D-15-00129.1 Published online by Cambridge University Press

In programs where a PRE herbicide was applied followed by a MPOST application of 2,4-D choline + glyphosate, Palmer amaranth control ranged from 85 to 95% (Table 4). Sequential applications of 2,4-D choline + glyphosate provided only 66 to 82% control at 14 DAMP. While the highest rate of 2,4-D choline + glyphosate used in this study provided a similar level of Palmer amaranth control to the PRE treatments 14 DAEP and 14 DAMP, complete reliance on POST herbicides is a poor herbicide-resistance management strategy (Norsworthy et al. 2012), and efforts must be made to discourage total-POST adoption by growers.

At the end of the season and prior to harvest, Palmer amaranth control was the greatest with herbicide programs that contained PRE fb MPOST applications compared to programs that excluded the use of PRE herbicides at the beginning of the season ($P \leq 0.0001$) (Table 4). These results are consistent with previous findings, which reported that season-long residual control is needed as a result of the rapid growth and ability of Palmer amaranth to emerge throughout the growing season (DeVore et al. 2013). In herbicide programs where PRE fb MPOST herbicide applications were deployed, Palmer amaranth control ranged from 85 to 90% at the end of season (Table 4). In contrast, sequential POST applications of 2,4-D choline + glyphosate at either 532 + 560 or 800 + 840 g ha^{-1} resulted in no better than 80% Palmer amaranth control late in the year.

No differences in soybean yield occurred among the programs evaluated, and all programs outyielded the nontreated control (Table 5). Differences in density of reproductive Palmer amaranth present in plots at soybean maturity were detected among the herbicide programs evaluated. Sequential POST-only applications had the highest density of Palmer amaranth present at soybean maturity (Table 5). In contrast, programs that utilized flumioxazin, flumioxazin + chlorimuron, *S*-metolachlor + fomesafen, or sulfentrazone + chloransulam PRE fb 2,4-D choline + glyphosate, with or without the addition of glufosinate, had 1.3 to 2.9 reproductive Palmer amaranth m^{-2}. Whereas in POST-only programs, Palmer amaranth density at soybean maturity ranged from 4.4 to 6.9 Palmer amaranth plants m^{-2}.

While few late-emerging Palmer amaranth plants may be perceived as being harmless, previous research has reported that late-season Palmer amaranth seedlings are capable of seed production within 30 d after emergence (Jha and Norsworthy 2009). Previous research has also reported that weeds left in the field at the time of harvest have the potential to enter harvesting machinery and be distributed across the field (Walsh and Powles 2014). Thus, leaving weeds in the field prior to harvest can result in spreading viable weed seeds across the field. This practice will not only lead to increasing weed populations in that field, but will also negatively impact sustainable weed management. In a study evaluating harvest weed seed control, $\geq$ 99% of Palmer amaranth seeds were retained on the plant at the time of soybean maturity (Norsworthy et al. 2014b), indicating a large amount of seed could potentially enter the harvesting equipment and distribute these seed across the field during harvest. Additionally, previous research found that the escape of a single female Palmer amaranth plant in cotton resulted in a 95 to 100% field infestation after a 3-yr period (Norsworthy et al. 2014a).

Conclusions and Practical Implications. In summary, POST applications of the 2,4-D choline + glyphosate premixture displayed a high level of weed control including control of glyphosate-resistant Palmer amaranth. Additionally, growers should make timely applications prior to weeds approaching the maximum recommended height. Crop injury was not observed to be problematic, indicating a high level of soybean tolerance to POST applications of 2,4-D, glyphosate, and glufosinate. Herbicide programs evaluated that began with herbicides, or contained residual herbicides, provided the most consistent season-long weed control and resulted in the lowest density of reproductive Palmer amaranth plants prior to harvest. Results further indicated that diverse weed control programs in herbicide-resistant soybean need to begin with the use of residual herbicides applied PRE. Therefore, we fail to reject our hypothesis that the 2,4-D choline + glyphosate premixture applied in conjunction with residual herbicides will provide greater season-long weed control than programs that rely on POST non-residual applications alone. Significant emphasis should also be placed on the use of multiple effective mechanisms of action in order to reduce the risk of herbicide resistance evolution and practice the best stewardship of this new technology. For the best protection of any new herbicide-resistant technology it is vital that growers practice integrated weed management strategies and best management practices such as proper application timing and utilizing PRE fb POST residual herbicides in combination with deployment of nonchemical weed control tactics.

The addition of the 2,4-D–resistant soybean technology will increase the choice of herbicide traits and herbicide mechanisms of action available for soybean producers. Although not every potential herbicide program was evaluated in these studies, the results not only indicated the strength of this new technology but also displayed the importance of aggressive herbicide programs that provide consistent season-long weed control. Future studies are needed to fully evaluate the most effective herbicide programs and application timings for the control of Palmer amaranth and other problematic weeds typically found in soybean. These studies aim to serve as a starting point in the development of effective weed management programs for this herbicide-resistant soybean technology and further research should be performed to combine non-chemical strategies in conjunction with harvest-weed seed methods.

Acknowledgments

The support for this research provided by Dow AgroSciences LLC is appreciated.

Literature Cited

Anonymous (2015) Enlist Duo™ product label. 62719-649 Dow AgroSciences. Indianapolis, IN: Dow AgroSciences LLC. 10 p

Bagavathiannan MV, Norsworthy JK (2013) Occurrence of arable weeds in roadside habitats: implications for herbicide resistance management. Weed Sci 53:163 [Abstract]

Chahal GS, Johnson WG (2012) Influence of glyphosate or glufosinate combinations with growth regulator herbicides and other agrochemicals in controlling glyphosate-resistant weeds. Weed Technol 26:638–643

Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of weed height and glufosinate plus 2,4-D combinations on weed control in soybean with resistance to 2,4-D. Weed Technol 27:271–280

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620–626

Culpepper AS, York AC (1998) Weed management in glyphosate-tolerant cotton. J Cotton Sci 2:174–185

DeVore JD, Norsworthy JK, Brye KR (2013) Influence of deep tillage, a rye cover crop, and various soybean production systems on Palmer amaranth emergence in soybean. Weed Technol 27:263–270

Green JM, Hazel CB, Forney DR, Pugh LM (2008) New multiple herbicide crop resistance and formulation technology to augment the utility of glyphosate. Pest Manag Sci 64:332–339

Heap I (2015) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.com. Accessed April 1, 2015

Jha P, Norsworthy JK (2009) Soybean canopy and tillage effects on emergence of Palmer amaranth (*Amaranthus palmeri*) from a natural seed bank. Weed Sci. 57:644–651

Loux M (2008) Can the DHT trait solve all of our glyphosate resistance problems? Proc North Cent Weed Sci Soc 62:12

Neve P, Norsworthy JK, Smith KL, Zelaya IA (2011) Modelling evolution and management of glyphosate resistance in *Amaranthus palmeri*. Weed Res 51:99–112

Norsworthy JK, Griffith G, Griffin T, Bagavathiannan M, Gbur EE (2014a) In-field movement of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and its impact on cotton lint yield: evidence supporting a zero-threshold strategy. Weed Technol 62:237–249

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

Norsworthy JK, Walsh MJ, Bagavathiannan MV, Bradley KW, Steckel L, Kruger G, Loux MM, Eubank T, Davis V, Johnson W, Young B, Powles S (2014b) Harvest weed seed control: testing Australian seedbank management tactics in USA soybean. Presentation 250 *in* Proceedings of the Weed Science Society of America. Vancouver, BC, Canada: Weed Science Society of America

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60(Special Issue):31–62

Nuti R, York A, Bacheler J, Edmisten K (2003) Pest control costs and returns in conventional and transgenic cotton management systems. Page 2262 *in* Proceedings of the Beltwide Cotton Conference. Memphis, TN: National Cotton Council of America

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347

Reddy KN, Norsworthy JK (2010) Glyphosate-resistant crop production systems: impact on weed species shifts. Pages 174–177 *in* Nandula VK, ed. Glyphosate Resistance in Crops and Weeds: History, Development, and Management. Hoboken, NJ: J. Wiley

Riar DS, Norsworthy JK, Steckel LE, Stephenson DO, Eubank TW, Scott RC (2013) Assessment of weed management practices and problematic weeds in the Midsouth United States—soybean: a consultant's perspective. Weed Technol 27:612–622

Scott RC, Barber LT, Boyd JW, Norsworthy JK, Burgos N (2014) Recommended Chemicals for Weed and Brush Control. Little Rock, AR: The University of Arkansas Division of Agriculture Cooperative Extension Service, Miscellaneous Publication 44. Pp 38–57
Tharp BE, Shabenberger O, Kells JJ (1999) Response of annual weed species to glufosinate and glyphosate. Weed Technol 13:541–545
[USDA, NASS] United States Department of Agriculture, National Agricultural Statistics Service (2014) http://www.nass.usda.gov. Accessed April 3, 2015
Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301–307
Wilcut JW, Hayes RL, Nichols RL (2003) A Beltwide regional economic assessment of weed management systems in non-transgenic and transgenic cotton. Page 2260 *in* Proceedings of the Beltwide Cotton Conference. Memphis, TN: National Cotton Council of America

Received August 18, 2015, and approved October 24, 2015.

Associate Editor for this paper: Jason Bond, Mississippi State University.