Weed Technology

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Influence of weed size on herbicide interactions for Enlist™ and Roundup Ready® Xtend® technologies

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Research Article

Cite this article: Meyer CJ and Norsworthy JK (2019) Influence of weed size on herbicide interactions for Enlist™ and Roundup Ready® Xtend® technologies. Weed Technol **33**: 569–577. doi: 10.1017/wet.2019.27

Received: 3 January 2019
Revised: 25 March 2019
Accepted: 27 March 2019

Associate Editor:
Lawrence E. Steckel, University of Tennessee

Nomenclature:
2,4-D; dicamba; glufosinate; glyphosate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Watson; pitted morningglory, *Ipomoea lacunosa* L.

Keywords:
Antagonism; Colby's method; glufosinate plus glyphosate; glyphosate plus 2,4-D; glyphosate plus dicamba; herbicide interactions

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Abstract

Weed size can nfluence herbicide performance and herbicide interactions in mixtures. To control a broad range of species in soybean or cotton, POST herbicide mixtures will likely be commonplace in Roundup Ready® XtendFlex® and Enlist™ technologies. The impact of weed size on herbicide interactions that could occur in Roundup Ready XtendFlex or Enlist crops was assessed in two field experiments conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR. Combinations of glufosinate, glyphosate, dicamba, and 2,4-D were applied to either 10-cm or 30-cm weeds and evaluated for percent weed control, height reduction, and density reduction, collected 5 wk after treatment. Colby's method was used to analyze treatments for herbicide interactions for control of barnyardgrass, Palmer amaranth, and pitted morningglory. Antagonism was identified with at least one treatment on all species. Almost all treatments were antagonistic for percent weed control, height reduction, and density reduction on barnyardgrass. When glyphosate in mixture with 2,4-D or dicamba was applied to 30-cm barnyardgrass, control declined 9% for both mixtures relative to glyphosate alone. Glufosinate plus glyphosate was antagonistic when applied to both 30-cm pitted morningglory and barnyardgrass. Glufosinate plus dicamba provided less control and density reduction of Palmer amaranth than what was expected from Colby's equation. Overall, antagonism was more likely to be identified when applications were made to 30-cm weeds compared with 10-cm weeds. The utility of a given herbicide mixture will depend on the species present in the field and the size of those species at the time of application.

Introduction

Approximately 94% of soybean [*Glycine max* (L.) Merr.] and 91% of cotton (*Gossypium hirsutum* L.) hectares in the United States were planted to a variety containing an herbicide-resistance trait in 2018 (USDA-NASS 2018). The herbicide-resistance traits vary with the individual technology, but Enlist™ (Corteva Agriscience, Indianapolis, IN), LibertyLink® (BASF, Florham Park, NJ), and Bollgard II® Xtendflex® (Bayer CropScience, St. Louis, MO) technology all contain a glufosinate-resistance trait, increasing the likelihood glufosinate will be applied in mixture with synthetic auxins or glyphosate. Research has demonstrated glufosinate plus 2,4-D and glufosinate plus dicamba control of glyphosate-resistant Palmer amaranth (Merchant et al. 2013, 2014a, 2014b). However, evidence suggests glufosinate plus glyphosate and glyphosate plus a synthetic auxin are antagonistic when applied to various monocot species (Besançon et al. 2018; Flint and Barrett 1989; Meyer et al. 2017; O'Sullivan and O'Donovan 1980).

Despite the prevalence of weeds across the United States that evolved resistance to glyphosate (Heap 2018), preserving the effectiveness of glyphosate on sensitive species is still of value. For example, in multiple resistant-crop technologies (e.g., Enlist, Bollgard II XtendFlex) alternative herbicides to glyphosate, such as glufosinate, often require sequential applications or additional herbicides for effective control of grass species, including giant foxtail (*Setaria faberi* Herrm.) and johnsongrass [*Sorghum halepense* (L.) Pers.] (Meyer et al. 2015a; Wiesbrook et al. 2001). In the Midsouth, barnyardgrass is still a highly prevalent and problematic species in cotton and soybean fields (Van Wychen 2016). Although glufosinate is effective in controlling small barnyardgrass, glyphosate provides excellent control of small and large barnyardgrass (Meyer 2015a; Payne and Oliver 2000; Scott et al. 2017). Effective management of glufosinate and glyphosate is needed to mitigate the likelihood of resistance, specifically when antagonism in various mixtures may be present.

Mixtures of glyphosate plus a synthetic auxin herbicide have been reported as antagonistic when applied to monocots. Meyer et al. (2015b) observed a reduction in barnyardgrass control with glyphosate when dicamba was added to the solution. Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) also observed antagonism with mixtures of glyphosate plus dicamba and

Table 1. Herbicide information for all products used in the 2,4-D and dicamba experiments.

Herbicide common name	Herbicide trade name	Rate	Manufacturer	Address	Website	Adjuvant[ab]
		g ai or g ae ha^{-1}				
Glyphosate	Durango	840	Corteva Agriscience	Indianapolis, IN	www.corteva.us	
Glufosinate	Liberty	595	Bayer CropScience LP	Research Triangle Park, NC	www.bayercropscienceus.com	
2,4-D	Weedar	1,065	Nufarm Inc.	Burr Ridge, IL	www.nufarm.com/US/Home	
Glyphosate + 2,4-D	Enlist Duo	840 + 785	Corteva Agriscience	Indianapolis, IN	www.corteva.us	
S-metolachlor	Dual Magnum	1,390	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	
Glyphosate	Roundup PowerMax	865	Monsanto Co.	St. Louis, MO	www.monsanto.com	
Dicamba	Clarity	560	BASF Corp.	Research Triangle Park, NC	www.basf.com	NIS

[a]Abbreviation: NIS, nonionic surfactant.
[b]Adjuvant rates: NIS, 0.25% v/v.

Table 2. Weed sizes and densities of barnyardgrass, Palmer amaranth, and pitted morningglory at two herbicide application timings in the 2,4-D experiment evaluated in 2015 and 2016.

	2015			2016		
	Weed height			Weed height		
Species	~10	~30	Density	~10	~30	Density
	—cm—		plants m^{-2}	—cm—		plants m^{-2}
Barnyardgrass	11	29	5	8	25	24
Palmer amaranth	12	21	4	5	22	7
Pitted morningglory	15	25	4	6	19	1

Table 3. Weed sizes and densities of barnyardgrass, Palmer amaranth, and pitted morningglory at both herbicide application timings[a] in the dicamba experiment evaluated in 2015 and 2016.

	2015			2016		
	Weed height			Weed height		
Species	~10	~30	Density	~10	~30	Density
	—cm—		plants m^{-2}	—cm—		plants m^{-2}
Barnyardgrass	11	32	8	8	25	22
Palmer amaranth	13	31	2	5	22	5
Pitted morningglory	13	33	2	6	19	2

[a]First and second application timing to approximately 10- and 30-cm weeds, respectively.

glyphosate plus 2,4-D on species including johnsongrass and wild oat (*Avena fatua* L.). However, the identification of antagonism depended on the specific rates and species in question.

A common and relatively straightforward technique to evaluate herbicide interactions, particularly when evaluating many different herbicides in a field setting, is Colby's method (Colby 1967). Colby's method calculates an expected value for a mixture on the basis of the performance of the individual herbicides alone. However, when one herbicide has no POST activity on a given species, (e.g., 2,4-D on barnyardgrass), Colby's method is not suitable for making comparisons between the observed and expected values. When Colby's method is not applicable, typically a significant reduction in herbicidal activity of the mixture (e.g., glyphosate plus 2,4-D), compared with the herbicide with activity alone (e.g., glyphosate) is considered antagonism. Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) considered significant deviations of the mixtures from the products alone as an antagonistic interaction.

A wide range of variables can influence interactions that occur between two herbicides, and the size of the weed can also play a role. Antagonism between glufosinate and glyphosate was more likely to be identified when applied to large weeds compared with small weeds (Miller et al. 2015). Similarly, the antagonistic interaction between clethodim and an acetolactate synthase inhibitor was more severe when applied to six- to eight-leaf goosegrass [*Eleusine indica* (L.) Gaertn.] compared with three- to four-leaf goosegrass (Burke et al. 2002). Identifying herbicide interactions may be more likely on weed sizes beyond the range listed on herbicide labels, but understanding if antagonism is present and how mixtures perform on various weed sizes is important for selecting optimum herbicide mixtures.

Effective POST mixtures are needed in Enlist and Roundup Ready® Xtend crops to control a broad weed spectrum and minimize evolution of resistance, especially when controlling herbicide-resistant populations of weeds such as Palmer amaranth and barnyardgrass. Therefore, our objective was to identify the impact of weed size on the potential for antagonism in 2,4-D- and dicamba-resistant cropping systems using products labeled or recommended in those systems.

Materials and Methods

Two field experiments were conducted at Northeast Research and Extension Center in Keiser, AR (35.66927°N, 90.08105°W), in 2015, and both experiments were repeated in 2016. The two experiments (hereafter referred to as the 2,4-D and dicamba experiments) had randomized, complete block designs with two factors: herbicide treatment and weed size. Each experiment included four replications and each experiment was repeated twice. All herbicide products used in the experiments are listed in Table 1. In the 2,4-D experiment, herbicide products and combinations that could occur in the Enlist™ crop systems (i.e., Liberty® [BASF], Durango® [Dow Agrosciences], and Enlist Duo® [Dow Agrosciences] herbicides) were evaluated on weed populations at two application timings. The first herbicide application occurred when weeds were approximately 10-cm tall and the second application was made when they were 30-cm tall (Table 2). Weed sizes were determined by measuring three plants for each species in the nontreated check plots. When the tallest species was approximately 10- and 30-cm tall, applications were made.

For the dicamba experiment, products associated with the Roundup Xtend® cropping system (e.g., Liberty, Roundup PowerMax® II, and Clarity® [BASF] herbicides) were also

https://doi.org/10.1017/wet.2019.27 Published online by Cambridge University Press

Table 4. Application dates, times, and weather conditions at the time of application for the 2,4-D and dicamba experiments.

Year	Timing[a]	2,4-D experiment[b]				Dicamba experiment			
		Application date	Time	Temp	RH	Application date	Time	Temp	RH
	cm			C	%			C	%
2015	10	July 16	8:30 AM	30	79	July 16	8:00 AM	36	77
	30	July 28	10:00 AM	32	75	July 28	3:00 PM	35	76
2016	10	June 29	8:45 AM	26	75	June 29	9:30 AM	26	75
	30	July 18	1:30 PM	33	52	July 18	2:15 PM	33	52

[a]Timing of herbicide application based on plant height.
[b]Abbreviations: Temp, temperature; RH, relative humidity.

applied to two weed sizes (Table 3). Some herbicide treatments (e.g., glufosinate plus glyphosate) were used in both experiments; however, different herbicide products for the same active ingredient were used (e.g., Roundup PowerMax and Durango herbicides). Changes in formulation and adjuvant load may affect mixture efficacy and herbicide interactions (Kudsk and Mathiassen 2004; Nalewaja and Matysiak 1992).

When trials were initiated in 2015, Xtendimax® and Engenia® herbicides (i.e., dicamba), now registered in Roundup Ready Xtend crops, were not commercially available. Thus, a commercially available diglycoamine formulation of dicamba was used (Table 1). For the 2,4-D experiment, a premix of glyphosate dimethylamine (DMA) plus 2,4-D choline was used (Enlist Duo herbicide). However, no stand-alone product of 2,4-D choline (e.g., Enlist One herbicide) was available and a 2,4-D amine formulation was used when needed.

Experiments were established on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) with a pH of 6.7 and 1.7% organic matter. Plot size was 3.9 × 9.1 m in both years. In 2015, a DeKalb® (Monsanto, St. Louis, MO) DD1246 SmartStax® corn (*Zea mays* L.) hybrid was planted at 86,500 seeds ha^{-1} in rows 97-cm wide in the trial area to mimic a crop canopy. In 2016, a DeKalb DKC46-36RIB SmartStax hybrid was planted at 101,000 seeds ha^{-1}. Fertilizer and lime were applied on the basis of soil test results and according to University of Arkansas recommendations; however, no nitrogen was applied to the corn in an effort to keep the crop from outcompeting the weeds. A SmartStax hybrid was selected for these experiments because it was commercially available and able to tolerate POST applications of 2,4-D, dicamba, glufosinate, glyphosate, and *S*-metolachlor. Plantings occurred on June 16, 2015, and June 10, 2016. The first herbicide application occurred approximately 4 wk after trial establishment, when weeds were approximately 10-cm tall (Table 4). Corn was at or near V5 at the time of the first application and V8 at the time of the second application, in both years. Furrow irrigation to soil saturation was used as needed throughout the growing season.

When an herbicide was applied in mixture, it was applied at the same rate when applied alone. If the treatment contained dicamba, a nonionic surfactant (NIS) at 0.25% (v/v) (Induce, Helena Chemical, Collierville, TN) was added to the solution. Any reference to dicamba alone refers to a solution of dicamba plus NIS. If dicamba was mixed with a glyphosate product, no NIS was added, because of adjuvants present in the glyphosate product. A CO_2-pressurized backpack sprayer was used to spray all herbicide treatments. At the time of application, the sprayer was calibrated to deliver 141 L ha^{-1} spray volume at 276 kPa with application made 4.8 km h^{-1}. Nozzles were spaced 51 cm apart and the boom was equipped with TeeJet 110015 Air Induction Extended Range nozzle tips (TeeJet Technologies, Springfield, IL). One day after experimental treatments were applied, a blanket application of *S*-metolachlor was made to all plots unless a plot had already received an application of *S*-metolachlor as part of the experimental treatment. *S*-metolachlor was applied across the whole trial to suppress new weed emergence.

Weed control ratings and data on weed heights and densities were collected 5 wk after treatment (WAT) for barnyardgrass, Palmer amaranth, and pitted morningglory. Weed control was visually evaluated by comparing treated plots with nontreated check plots. Weeds were rated by species on a scale of 0 (no control) to 100% (complete death of all plants). Weed densities for each species were determined by counting individuals in two 1-m^2 quadrats. If one or fewer individuals were counted in at least one of the quadrats, all the individuals in the plot were counted. Height measurements of three individuals of each species were collected in each plot.

To use Colby's method on height and density assessments, data for each plot were converted to a percentage of the nontreated check. For ease of discussion, heights and densities are presented as height and density reductions, so a 100% reduction (0 plants m^{-2} or 0 cm) correlates with 100% visual control (complete death of all plants). Colby's method (Colby 1967) was used to assess herbicide interactions and requires the calculation of an expected value, as calculated by Equation 1:

$$E = (X + Y) - (XY)/100 \quad [1]$$

where E is the expected level of control of a given species when two herbicides are applied in a mixture, and variables X and Y represent the level of control of a given weed species provided by each herbicide applied individually. The expected value for a mixture was compared with the observed value from the field, using a two-sided *t*-test ($\alpha = 0.05$). When the expected value was significantly greater than the observed value, the mixture was considered antagonistic. If a treatment contained more than two herbicides (e.g., glufosinate plus glyphosate plus dicamba), an expected value was not calculated for the mixture. However, if one component of a three-herbicide mixture had no POST activity on a given species, an expected value was calculated from the two herbicides that had control. Thus, the expected value for barnyardgrass control for glufosinate plus glyphosate plus dicamba was equal to glufosinate plus glyphosate, because dicamba has no POST activity on barnyardgrass.

In addition to the analysis used for herbicide interactions, the data were also subjected to ANOVA using JMP Pro 13 (SAS Institute, Cary, NC). Data from both years were combined, and replication and year were included in the analysis as random effects. The results from the ANOVA were used to compare mixtures with their individual components in addition to the

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Table 5. Palmer amaranth control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D experiment.

			Control				Height reduction[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[e]	10	31				13				27			
		30	20				10				6			
2,4-D	785[e]	10	89				72				85			
		30	83				77				85			
Glufosinate	595	10	96				79				95			
		30	87				72				88			
Glyphosate + 2,4-D	840[e] + 785[e]	10	94	NS	93	NS	81	NS	74	NS	94	NS	90	NS
		30	92	^	87	NS	81	NS	78	NS	89	NS	86	NS
Glufosinate + glyphosate	595 + 840[e]	10	97	NS	97	NS	66	NS	80	NS	93	NS	96	NS
		30	89	NS	89	NS	84	NS	76	NS	86	NS	89	NS
Glufosinate + 2,4-D	595 + 785[e]	10	97	NS	99	NS	70	NS	93	NS	96	NS	99	NS
		30	99	^	98	NS	89	NS	92	NS	99	^	98	NS
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	98	NS			89	NS			98	NS		
		30	95	^			94	NS			96	NS		
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[e] + 1,390	10	98	NS			85	NS			99	NS		
		30	99	^			98	^			99	^		
LSD			6				18				10			

[a]Height and density reduction are expressed as a percentage of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]The "^" symbol indicates a mixture that provided significantly greater control than any individual component alone based on the LSD. NS indicates the mixture was similar to both herbicides alone.
[d]NS indicates the expected value was not different than the observed value. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

comparisons made using Colby's method. Comparisons from the ANOVA provided additional information to the herbicide interaction analysis by affirming that an antagonistic herbicide mixture may provide greater control than either component alone (e.g., see Table 5). Treatment means were separated using the Fisher protected LSD test ($\alpha = 0.05$).

Results and Discussion

Palmer Amaranth

2,4-D Experiment

All herbicide mixtures were considered additive for control, height reduction, and density reduction 5 WAT (Table 5). Control with glyphosate DMA alone was not greater than 31% (Table 5). Control with glyphosate DMA) alone may be of some value for control of glyphosate-resistant populations. For example, control of 30-cm Palmer amaranth with a premix of 2,4-D plus glyphosate DMA was significantly greater (92%) than control with 2,4-D alone (83%) ($p<0.05$). When 2,4-D was applied with glufosinate, control of 30-cm Palmer amaranth was also greater than control provided by either 2,4-D or glufosinate alone, indicating the mixture may provide some benefit toward delaying the onset of resistance.

Dicamba Experiment

Two mixtures were identified as antagonistic for Palmer amaranth control 5 WAT in the dicamba experiment when applied to 30-cm weeds: glufosinate plus glyphosate potassium (K) and glufosinate plus dicamba (Table 6). Antagonism was identified on Palmer amaranth for glufosinate plus glyphosate K, whereas no antagonism was reported for glufosinate plus glyphosate DMA in the 2,4-D experiment (Table 5). It should be reiterated that this is a population apparently segregating for glyphosate resistance, and control with glyphosate alone was low (approximately 30%), which affects the calculated expected values for the mixture. Another explanation for the discrepancy between trials may be attributed to the formulations of glyphosate used in each experiment. There are subtle differences in the performance of glyphosate salts alone (Kudsk and Mathiassen 2002, 2004) and differences in herbicide interactions for different glyphosate formulations applied in mixtures with other herbicides have also been identified (Kudsk and Mathiassen 2004; Nalewaja and Matysiak 1992).

Percent control and density reduction were antagonistic for glufosinate plus dicamba, with observed values being 9% and 10% less than expected values, respectively. No herbicide mixtures were antagonistic when applied to 10-cm Palmer amaranth. One of the treatments identified as antagonistic applied to 30-cm weeds (glufosinate plus dicamba) was additive and resulted in greater control than either glufosinate or dicamba alone when applied to 10-cm weeds. Thus, identification of a specific herbicide interaction appears to depend on weed size. Dicamba plus glufosinate plus glyphosate K plus *S*-metolachlor resulted in 99% control, height reduction, and density reduction. The four-way mixture resulted in the greatest control among all treatments, including dicamba plus glufosinate plus glyphosate K. *S*-metolachlor is not known to have POST activity, and all plots received an application of *S*-metolachlor 24 h after experimental treatments were applied, except for the treatment that already contained *S*-metolachlor. Thus, the improvement in control is likely due to the adjuvants in the *S*-metolachlor product (Dual Magnum, Syngenta, Basel Switzerland).

Pitted Morningglory

2,4-D Experiment

The only mixture considered antagonistic for control of pitted morningglory in the 2,4-D experiment was glufosinate plus glyphosate DMA applied to 30-cm weeds, where 91% control

https://doi.org/10.1017/wet.2019.27 Published online by Cambridge University Press

Table 6. Palmer amaranth control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the dicamba experiment.

			Control				Height reduction[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	32				13				17			
		30	24				11				6			
Dicamba	560[e]	10	93				89				84			
		30	85				53				74			
Glufosinate	595	10	93				75				90			
		30	84				71				85			
Glyphosate + dicamba	865[e] + 560[e]	10	95	NS	97	NS	80	NS	90	NS	90	NS	84	NS
		30	87	NS	91	NS	71	NS	57	NS	83	NS	78	NS
Glufosinate + glyphosate	595 + 865[e]	10	94	NS	95	NS	73	NS	77	NS	84	NS	81	NS
		30	81	NS	89	*	74	NS	75	NS	79	NS	89	NS
Glufosinate + dicamba	595 + 560[e]	10	99	^	99	NS	99	NS	98	NS	98	NS	93	NS
		30	89	NS	98	*	80	NS	93	NS	88	NS	98	*
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560	10	99	^			99	NS			99	NS		
		30	92	^			86	^			90	NS		
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1,390	10	100	^			98	NS			99	NS		
		30	99	^			99	^			99	^		
LSD			6				15				11			

[a]Height and density reduction are expressed as a percentage of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]The "^" symbol indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.
[d]The "*" symbol denotes significant antagonism based on a two-sided *t*-test between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) − (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

was 8% lower than the expected value (Table 7). The same treatment was antagonistic for density reduction, where the observed value was 7% lower than the expected value. Glufosinate and 2,4-D alone provided 91% or greater control of pitted morningglory, depending on weed size, and control was not improved when another herbicide was added.

Pitted morningglory density was lower in 2016 (1 plant m^{-2}) compared with 2015 (4 plants m^{-2}), which could have affected results, because there were fewer individuals exposed to the herbicide treatments. However, trends were similar in both years. Higher weed densities can increase the risk of herbicide failure and increase the likelihood of resistance. Although a mixture may not improve control of one species, herbicides are commonly mixed to broaden spectrum of activity or improve control of other species. In addition, the use of two herbicides with different sites of action in mixture is an effective tactic to delay the onset of resistance (Bagavathiannan et al. 2014; Norsworthy et al. 2012).

Dicamba Experiment

Similar to the 2,4-D experiment, the mixture of glufosinate plus glyphosate (in this experiment, glyphosate K) was considered antagonistic for pitted morningglory control when applied to 30-cm weeds (Table 8). Glufosinate plus dicamba was antagonistic for percent control and density reduction when applied to 30-cm weeds, but not 10-cm weeds. Glufosinate plus dicamba was also antagonistic for Palmer amaranth control and density reduction, indicating this mixture may have reduced performance on dicot weeds relative to what would be expected on the basis of the performance of the herbicides alone. It is not clear why dicamba may antagonize glufosinate, or vice versa, and more research is needed to identify a mechanism for such antagonism.

Barnyardgrass

2,4-D Experiment

All herbicide mixtures evaluated with Colby's method were antagonistic for barnyardgrass control and density reduction at both weed sizes (Table 9). All treatments were also antagonistic for height reduction, except for glufosinate plus glyphosate and 2,4-D plus glufosinate plus glyphosate K plus *S*-metolachlor applied to 10-cm weeds, which was additive. As explained previously, expected values for the treatments containing three and four herbicides were calculated for barnyardgrass from the two herbicides that have POST activity (glufosinate and glyphosate). When glyphosate DMA was applied as a premix with 2,4-D, a reduction in barnyardgrass control was observed relative to glyphosate DMA alone for both application timings (i.e., 10- and 30-cm weeds). Flint and Barrett (1989) reported greater shoot and root fresh weights for johnsongrass treated with glyphosate plus 2,4-D, compared with glyphosate alone. In addition, 2,4-D increased the rate of glyphosate required to kill three monocot species when applied as a mixture (O'Sullivan and O'Donovan 1980). The premix of glyphosate DMA plus 2,4-D was also antagonistic for height and density reduction when applied to 30-cm barnyardgrass.

Dicamba Experiment

All herbicide mixtures evaluated with Colby's method were antagonistic for barnyardgrass control and height reduction (Table 10). All mixtures were also antagonistic for density reduction, except dicamba plus glufosinate plus glyphosate K plus *S*-metolachlor, which was additive. Much like the antagonism observed with glyphosate DMA plus 2,4-D in the 2,4-D experiment, a reduction in barnyardgrass control was observed when glyphosate K plus dicamba was applied to 30-cm weeds, compared with glyphosate K alone. Meyer et al. (2015b) observed a reduction

https://doi.org/10.1017/wet.2019.27 Published online by Cambridge University Press

Table 7. Pitted morningglory control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D experiment.

			Control				Height reduction[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[e]	10	84				68				81			
		30	65				30				47			
2,4-D	785[e]	10	94				98				98			
		30	91				97				98			
Glufosinate	595	10	97				97				94			
		30	95				96				94			
Glyphosate + 2,4-D	840[e] + 785[e]	10	98	NS	99	NS	98	NS	99	NS	97	NS	99	NS
		30	96	NS	96	NS	86	NS	96	NS	95	NS	98	NS
Glufosinate + glyphosate	595 + 840[e]	10	97	NS	99	NS	88	NS	97	NS	97	NS	98	NS
		30	91	NS	98	*	86	NS	90	NS	89	NS	96	*
Glufosinate + 2,4-D	595 + 785[e]	10	95	NS	99	NS	94	NS	99	NS	93	NS	99	NS
		30	95	NS	99	NS	94	NS	99	NS	96	NS	99	NS
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	97	NS			94	NS			94	NS		
		30	93	NS			92	NS			93	NS		
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[e] + 1,390	10	95	NS			90	NS			94	NS		
		30	96	NS			89	NS			98	NS		
LSD			6				12				8			

[a]Height and density reduction are expressed as a percentage of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]NS indicates the mixture was similar to both of the herbicides alone based on the LSD.
[d]The "*" symbol denotes significant antagonism based on a two-sided *t*-test between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) − (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

Table 8. Pitted morningglory control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the dicamba experiment.

			Control				Height reduction[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	87				66				85			
		30	72				54				57			
Dicamba	560[e]	10	92				91				91			
		30	90				88				89			
Glufosinate	595	10	95				99				99			
		30	89				97				99			
Glyphosate + dicamba	865[e] + 560[e]	10	96	NS	98	NS	90	NS	96	NS	91	NS	98	NS
		30	90	NS	92	NS	93	NS	95	NS	97	^	95	NS
Glufosinate + glyphosate	595 + 865[e]	10	96	NS	99	NS	95	NS	99	NS	96	NS	99	NS
		30	87	NS	96	*	87	NS	98	NS	96	NS	99	NS
Glufosinate + dicamba	595 + 560[e]	10	98	NS	99	NS	97	NS	99	NS	99	NS	99	NS
		30	89	NS	98	*	94	NS	99	NS	94	NS	99	*
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560[e]	10	98	NS			97	NS			97	NS		
		30	94	NS			84	∨			94	NS		
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1,390	10	97	NS			95	NS			97	NS		
		30	95	NS			96	NS			97	NS		
LSD			8				11				7			

[a]Height and density reduction are expressed as a percentage of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]The "^" symbol indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.
[d]The "*" symbol denotes significant antagonism based on a two-sided *t*-test between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) − (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

in barnyardgrass control from mixtures of glyphosate plus dicamba. Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) identified antagonism of glyphosate plus dicamba when applied to monocot species. Glyphosate K had similar control and density reduction as all other treatments when applied to 10-cm weeds, except for dicamba alone. However, when applied to 30-cm weeds, glyphosate K alone provided greater control (91%) and density reduction (86%) than all other treatments except for 2,4-D plus glufosinate plus glyphosate K plus *S*-metolachlor.

https://doi.org/10.1017/wet.2019.27 Published online by Cambridge University Press

Table 9. Barnyardgrass control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D experiment.

			Control				Height reductionb[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[e]	10	97				74				97			
		30	93				72				95			
2,4-D	785[e]	10	0				0				9			
		30	0				3				5			
Glufosinate	595	10	96				66				92			
		30	84				57				82			
Glyphosate + 2,4-D	840[e] + 785[e]	10	91	∨			74	NS			94	NS		
		30	84	∨			53	∨			85	∨		
Glufosinate + glyphosate	595 + 840[e]	10	98	NS	99	*	74	NS	86	NS	91	NS	99	*
		30	87	∨	99	*	69	NS	88	*	95	NS	99	*
Glufosinate + 2,4-D	595 + 785[e]	10	94	NS			65	NS			92	NS		
		30	82	NS			57	NS			96	NS		
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	94	NS	99	*	72	NS	86	*	88	∨	99	*
		30	84	∨	99	*	57	NS	88	*	88	∨	99	*
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[e] + 1,390	10	93	NS	99	*	78	NS	86	NS	95	NS	99	*
		30	91	NS	99	*	65	NS	88	*	86	∨	99	*
LSD			5				16				7			

[a]Height and density reduction are expressed as a percentage of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]The "^" symbol indicates a mixture that provided significantly less control compared to at least one of the herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.
[d]The "*" symbol denotes significant antagonism based on a two-sided *t*-test between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) − (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

Table 10. Barnyardgrass control 5 wk after treatment, height reduction, and density reduction as affected by herbicide treatment and weed size for the dicamba experiment

			Control				Height reduction[a]				Density reduction[a]			
Common name	Rate	Size	Obs[b]	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]	Obs	P value[c]	Exp	P value[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	96				74				92			
		30	91				71				86			
Dicamba	560[e]	10	0				2				4			
		30	0				4				8			
Glufosinate	595	10	95				66				84			
		30	78				57				63			
Glyphosate + dicamba	865[e] + 560[e]	10	93	NS			52	∨			86	NS		
		30	82	∨			64	NS			68	∨		
Glufosinate + glyphosate	595 + 865[e]	10	95	NS	99	*	60	∨	92	*	86	NS	98	*
		30	85	∨	98	*	69	NS	87	*	66	∨	92	*
Glufosinate + dicamba	595 + 560[e]	10	95	NS			74	NS			93	NS		
		30	82	NS			67	NS			60	NS		
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560	10	95	NS	99	*	64	NS	92	*	89	NS	98	*
		30	85	∨	98	*	61	NS	87	*	63	∨	92	*
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1,390	10	95	NS	99	*	63	NS	92	*	86	NS	98	*
		30	89	NS	97	*	72	NS	87	*	80	NS	92	NS
LSD			5				11				14			

[a]Height and density reduction are expressed as a percent of the nontreated control.
[b]Abbreviations: Exp, expected value; NS, not significant; Obs, observed value.
[c]The "^" symbol indicates a mixture that provided significantly less control compared to at least one of the herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.
[d]The "*" symbol denotes significant antagonism based on a two-sided *t*-test between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) − (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.
[e]Rate is in g ae ha^{-1}.

Practical Implications

Antagonism was identified on all three species investigated in this experiment (i.e., barnyardgrass, Palmer amaranth, and pitted morningglory) but was dependent on the herbicide mixture, weed size, and parameter evaluated (e.g., weed density). Antagonism is more likely to be identified when herbicide mixtures are applied to larger weeds (Burke et al. 2002; Miller et al. 2015). Antagonism was more common when the herbicide mixtures were evaluated on barnyardgrass, compared with the dicot species. Some mixtures had a significant reduction in barnyardgrass control relative to

https://doi.org/10.1017/wet.2019.27 Published online by Cambridge University Press

one of its components (e.g., glyphosate plus 2,4-D, glufosinate plus glyphosate, and glufosinate plus glyphosate plus dicamba applied to 30-cm weeds).

The current experiments evaluated herbicides at field-use rates and obtained high levels of control for certain herbicides on a given species (e.g., glyphosate K 865 g ae ha^{-1} provided 91% to 96% barnyardgrass control). Colby (1967) explained that analyzing for herbicide interactions is better when the herbicides are applied alone at a dose that provides approximately 50% control. Riley and Shaw (1988) and Scott et al. (1998) showed that synergy is more likely when applied at reduced rates and herbicide interactions can vary for two herbicides when mixed at low rates, compared with high rates. Other methods such as that proposed by Streibig and Jensen (2000) and used by Wehtje and Gilliam (2015) likely provide a more robust analysis of how two herbicides behave in a plant when mixed. However, the purpose of the current experiments was to determine if mixtures at field-use rates have reduced performance (i.e., antagonism), and additional research may be needed at reduced rates and various mixture ratios to fully understand how the herbicides evaluated behave in mixture.

Mixtures that compromise control of one species (e.g., barnyardgrass) in favor of improving control of another (e.g., Palmer amaranth) should be avoided to mitigate the likelihood of evolving resistance. Unfortunately, mixtures may often be needed to control both glyphosate-resistant Palmer amaranth and monocot species in Enlist or Bollgard II XtendFlex to avoid complete reliance on glufosinate POST. For many mixtures, control was not greater than control with one of the component herbicides alone (e.g., glufosinate plus 2,4-D vs. glufosinate alone on all species at the 10-cm height). However, it should be noted that when large weeds were present, glufosinate plus 2,4-D provided better control of Palmer amaranth (99%) than either 2,4-D or glufosinate alone. Thus, to maximize the utility and efficiency of herbicide applications in both Enlist and Roundup Ready Xtend technologies, herbicide treatments should be selected on the basis of the weed spectrum and size of those weeds.

The optimum herbicide treatment for a given scenario will depend on the crop trait technology, weeds present, and weed size. If Palmer amaranth is the dominant weed and barnyardgrass is also present but small, the preferred treatment is glufosinate (595 g ai ha^{-1}) plus 2,4-D (1,065 g ae ha^{-1}). Glufosinate plus 2,4-D performed better than glufosinate alone on large Palmer amaranth, provided two sites of action POST, and provided good control of small barnyardgrass. Weed management decisions are often driven by Palmer amaranth because it is the most troublesome weed across the Midsouth (Van Wychen 2016) and has a rapid growth rate that can quickly overcome recommended weed sizes on herbicide labels (Horak and Loughin 2000; Sellers et al. 2003). Applying glufosinate plus 2,4-D with a residual herbicide POST would further reduce the likelihood of resistance (Norsworthy et al. 2012) and is recommended for controlling many challenging weed species, such as Palmer amaranth.

If barnyardgrass, or another monocot species, is the dominant weed in soybean or cotton field, the herbicide recommendation becomes more challenging. Glufosinate alone did not provide adequate control of large barnyardgrass, and 2,4-D has no POST activity on monocot species. If no glyphosate-resistant weeds are present, an unlikely situation in the Midsouth, the recommended POST herbicide treatment would be glyphosate plus a residual herbicide, because it would provide excellent control of large and small barnyardgrass. With the prevalence of glyphosate resistance, an herbicide mixture, or sequential applications, will likely be needed to control a broad spectrum of weeds in the field. Although not evaluated in this experiment, sequential applications are a known strategy to overcome antagonism when two herbicides are mixed (Burke et al. 2002; Green 1989). Thus, if large monocots are present in the field and glyphosate-resistant weeds have not yet emerged, glyphosate plus a residual herbicide followed by glufosinate plus 2,4-D 7 to 14 days later would likely provide excellent control of all species.

Glufosinate is an invaluable weed management tool in many current herbicide-resistant crop technologies for control of monocot and dicot weeds. In these experiments, glufosinate provided comparable barnyardgrass control to glyphosate K when applied to the recommended weed size (10 cm), although glyphosate alone was the preferred treatment when large (30 cm) barnyardgrass was present in the field. Because of the widespread occurrence of glyphosate-resistant weeds, the utility of glufosinate needs to be protected by using best management practices, as outlined by Norsworthy et al. (2012). However, the effectiveness of glyphosate on many monocot species should not be ignored. In light of the antagonism identified in these experiments, both glufosinate and glyphosate need to be properly managed in the Enlist and RoundupReady Xtend technologies to enable effective weed control programs.

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Acknowledgments. Funding for this research was provided by the Arkansas Soybean Promotion Board and Bayer CropScience. No conflicts of interest have been declared.

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