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Response of Soybean (*Glycine max* (L.) Merr.) and Weed Control with Postemergence Herbicides and Combinations of Cytokinin Mixtures

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Abstract: A field study was conducted in 2015 and 2016 in Stoneville, MS, to evaluate the influence of cytokinin products on soybean injury and weed control when combined with common POST soybean herbicide treatments. Cytokinin treatments included no cytokinin mixture and two formulated cytokinin mixtures (kinetin-1 and kinetin-2) applied at 0.000227 kg ai ha^{-1}. Herbicide treatments were no herbicide, glyphosate at 1.37 kg ae ha^{-1} alone and in combination with *S*-metolachlor at 1.42 kg ai ha^{-1} or fomesafen 0.395 kg ai ha^{-1}. The addition of cytokinin treatments had no impact on soybean injury, plant height, or yield. Glyphosate plus fomesafen provided the greatest level of Palmer amaranth control, between 84 and 67%., 7 days and 28 days after treatment, respectively. Barnyardgrass control with glyphosate plus fomesafen was antagonized by one of two cytokinin products. To prevent possible reductions in herbicide efficacy, tank mixtures with cytokinin products should not be applied to soybean in POST herbicide applications.

Keywords: cytokinins; kinetin mixtures; plant health management

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1. Introduction

Even though there are numerous weeds that impact soybean production in the southern U.S., Palmer amaranth [*Amaranthus palmeri* L. Wats.] has been ranked as one of the most troublesome weeds in the southern U.S. since the 1970s [1–3]. By 2013, Palmer amaranth ranked the most troublesome weed of soybean in three southern U.S. states [4]. Palmer amaranth has increased in severity, in part because of herbicide resistance. In 2004, Georgia reported the first glyphosate-resistant (GR) Palmer amaranth [5], followed by Arkansas in 2005 [6]. In Mississippi, GR Palmer amaranth was documented in 2008 [7]. In all three states, the reports of GR Palmer amaranth were the result of soybean farmers almost exclusive reliance on glyphosate to manage troublesome weeds using in season POST applications. Moreover, the increased widespread resistance within Palmer amaranth populations occurred due to pollination from male genotypes spreading the resistance mechanism [7].

Barnyardgrass is also a problematic weed of U.S. soybean [8,9]. Similar to Palmer amaranth, barnyardgrass is considered to be a more problematic weed as a result of herbicide resistance. Tennessee was the first state to confirm GR barnyardgrass in the U.S. [10]. In a model based on Arkansas' cotton (*Gossypium hirisutum* (L.))-growing region, Bagavathiannan et al. [11] predicted GR barnyardgrass will develop by 2022 following five annual glyphosate applications in continuous GR cotton [*Gossypium hirsutum* L.]. One potential cultural mechanism to reduce the likelihood of resistance development would be crop rotation. By rotating to GR corn (*Zea mays* (L.)) or glufosinate-resistant cotton, resistance could be delayed 6 y [11]. In Mississippi, barnyardgrass has a history of resistance to multiple herbicide mode of action (MOA) [7,12]. With the state's close proximity to Tennessee, researchers in Mississippi have collected and tested barnyardgrass samples for possible glyphosate resistance [13].

Various herbicides can be utilized to manage GR Palmer amaranth in soybean given the loss of glyphosate as a viable control option [14]. Protoporphyrinogen oxidase (PPO) inhibitors such as fomesafen are used for PRE and POST control of Palmer amaranth in soybean production systems [15]. Given that Palmer amaranth is an important broadleaf weed species, researchers have documented the control of additional broadleaf weeds following POST applications of fomesafen. Bond et al. [16] and Norsworthy et al. [6] reported 96 and 100% GR Palmer amaranth control, respectively, with fomesafen at 0.420 kg ai ha^{-1}. In addition, Stephenson et al. [17] documented common cocklebur (*Xanthium strumarium* L.), prickly sida (*Sida spinosa* L.), and Palmer amaranth control with fomesafen.

Cytokinins occur naturally in plants and are responsible for cell division and enlargement as well as the formation of flowers and fruit [18]. Cytokinins have been reported to increase soybean cell proliferation in tissue culture [19]. Kinetin, a specific cytokinin, has been reported to reverse the effect of NaCl on tobacco [*Nicotiana tabacum* (L.)] leaves, which mimics water stress, when applied in solution to a leaf disc tissue culture [20]. In general, cytokinin mixtures are available as plant growth regulators (PGRs) for use in multiple crops, and labeling for formulated cytokinin mixtures claims these products have the ability to improve vigor, promote root and shoot growth, reduce stress, and slow leaf aging [21,22]. However, data supporting the label claims and general benefits of applying cytokinin mixtures are limited, especially in row crop production systems. Most research detailing the effects of kinetin and additional cytokinin mixtures has been conducted in tissue culture situations and not following the application to plants in field settings.

Tank mixtures with multiple herbicide MOA offer the potential to increase weed control and reduce application costs [23]. In some specific instances, these combinations can result in synergistic, antagonistic, or additive effects [24]. Synergism occurs when the total response of the components is greater than the sum of the individuals [24]. Antagonism occurs when the sum is less than the response of the individual components [24]. The components could be herbicides, foliar fertilizers, water, or any other components [25–30].

Reports of herbicide-by-herbicide interactions are common in the literature. Minton et al. [31] reported barnyardgrass control was antagonized when quizalofop or sethoxydim were combined with lactofen. Starke and Oliver [28] documented antagonism on entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula* (Gray)) but not on pitted morningglory (*Ipomoea lacunose* (L.)) control when fomesafen and glyphosate were combined. In addition, water has been reported to antagonize herbicides because of the cations present in hard water. Stable complexes are formed when glyphosate bonds with di- and trivalent cations and have been reported to result in glyphosate antagonism [32–34].

Research detailing interactions between herbicides and cytokinin mixtures is limited. Additionally, labeling of formulated cytokinin mixtures does not mention mixtures with additional products, such as herbicides, beyond outlining the use of surfactants [21,22]. Cytokinins have previously been hypothesized as products that could reduce the injury associated with flooding in corn [35]. In addition, a patent exists for a 1:1 mixture of glyphosate and kinetin to reduce glyphosate phytotoxicity [36]. In order to reduce application costs by limiting the number of trips across the field, growers may combine POST herbicides and cytokinin mixtures. A field study was conducted to evaluate the influence on crop response and weed control of adding foliar cytokinin mixtures to POST soybean herbicide applications. This study was conducted parallel to Lawrence et al. [37], as part of a larger soybean research project [38].

2. Materials and Methods

2.1. Experimental Site Description

A field study was conducted at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, USA in 2015 and 2016 to evaluate combinations of cytokinin mixtures and POST herbicides in soybean. The study was performed at two sites in 2015 (2015-A and 2015-B) and 2016 (2016-A and 2016-B). Coordinates, soil series, description, pH, and organic matter (OM) for each siteyear are presented in (Table 1). The experimental sites were known to be heavily infested with barnyardgrass and Palmer amaranth. Each site was conventionally tilled prior to planting to stimulate weed germination and ensure uniform emergence. 'Asgrow 4632' (Monsanto Company, St. Louis, MO, USA) mid maturity group IV soybean were used in all siteyears and sowed with a John Deere small-plot air planter (John Deere 1730, Deer and Company, One John Deere Place, Moline, IL, USA) at a rate of 320,000 seed ha^{-1}. The general plot size consisted of four rows of planted soybean (4.0 m wide) by 9.1 m in length separated by a fallow alley.

Table 1. Global positioning system (GPS) coordinates, soil series, soil description, soil pH, and soil organic matter (OM) for weed control studies conducted in Stoneville, MS during 2015 and 2016 to determine the response of tank mixtures containing cytokinins and POST herbicide treatments in soybean.

Siteyear	Coordinates	Soil Series	Description	pH	OM
				1:2 (*v*:*v*)	%
2015-A	33°26′29.18″ N, 90°54′41.92″ W	Dundee very fine sandy loam	Fine-silty, mixed, active, thermic Typic Endoqualfs	6.1	1.2
2015-B	33°24′21.94″ N, 90°55′31.27″ W	Newellton silty clay	Clayey over loamy, smectitic overmixed, superactive, nonacid, thermic Fluvaquentic Epiaquepts	6.9	1.6
2016-A	33°26′28.33″ N, 90°54′23.67″ W	Commerce sandyclay loam	Fine-silty, mixed, superactive, nonacid, thermic FluvaquenticEndoaquepts	6.8	1.6
2016-B	33°24′21.94″ N, 90°55′31.27″ W	Newellton silty clay	Clayey over loamy, smectitic overmixed, superactive, nonacid, thermic Fluvaquentic Epiaquepts	6.9	1.6

2.2. Experimental Treatments and Design

The study was designed as a two-factor factorial within a randomized complete block with four replications. Factor A was herbicide treatment (n = 4) and consisted of no herbicide, glyphosate (N-(phosphonomethyl)glycine) at 1.36 kg ha-1 alone and in combination with *S*-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1S)-2-methoxy-1-methyethyl acetamide) at 1.42 kg ha-1, and fomesafen (5-2-chloro-4-(trifluoromethyl)phenoxy-N-(methylsulfonyl)-2-nitrobenzamide) at 0.375 kg ha-1. Factor B was cytokinin mixture (n = 3) included as a tank mix component with each of the herbicide treatment applications listed above and consisted of no cytokinin mixture, kinetin-1 (as 0.000227 kg ha-1 of Ascend, WinField Solutions, LLC, St. Paul, MN, USA), and kinetin-2 (as 0.000227 kg ha-1 of Radiate, Loveland Products, Inc., Greely, CO, USA). All treatments were applied with a tractor-mounted sprayer calibrated to deliver 140 L ha-1 at 248 kPa fitted with extended range flat-fan (XR10002 TeeJet, IL, USA) nozzles at the V3 soybean growth stage, when unrolled leaflets were present on the first through the fourth node.

2.3. Experimental Data Collection

Visible estimates of soybean injury and weed control were recorded on a scale from 0 to 100% with 0 representing no injury or control and 100 representing soybean death or complete weed control from within each plot area [39]. Soybean injury was evaluated 3, 7, 14, 21, and 28 d after treatment (DAT) and control of Palmer amaranth and barnyardgrass was evaluated 7, 14, 21, and 28 DAT. Heights of five soybean plants in each plot were measured from the ground to the uppermost node 14 DAT and at maturity. Soybean plots were harvested using a small-plot combine (Kincaid Equipment, Haven, KS, USA) on September 25 and October 5 in 2015, and September 16 and October 12 in 2016. Yield was adjusted to 13% moisture content.

2.4. Experimental Data Analysis

Square roots of visible injury and control estimates were arcsine transformed prior to data analyses. The transformation did not improve the homogeneity of the variance based on visual inspection of plotted residuals; therefore, nontransformed data were used for analyses. Soybean injury and weed control data were analyzed utilizing the augmented mixed-model methodology previously detailed by [40]. Data for soybean height and yield were subjected to ANOVA using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with siteyear, replication (nested within siteyear), and treatment-by-rep interactions listed as the random effect parameters [41]. Least square means were calculated and mean separation ($\alpha \leq 0.05$) was produced using PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings [42]. When injury and weed control data did not return a significant synergistic or antagonistic effect [40], the data were analyzed as previously described for soybean height and yield.

3. Results

No synergistic or antagonistic effects were detected for soybean injury regardless of evaluation interval. The main effect of cytokinin product did not influence soybean injury; however, a main effect of herbicide treatment was detected 3, 7, and 14 DAT (Table 2). Injury was at least 5% greater with glyphosate plus fomesafen compared with other treatments 3, 7 and 14 DAT (Table 2). By 21 and 28 DAT, soybean injury was $\leq$ 1% across all herbicide treatments (data not presented).

Table 2. Soybean injury 3, 7, and 14 d after treatment (DAT) and Palmer amaranth control 7, 14, 21, and 28 DAT with tank mixtures of POST herbicide treatments and cytokinin products applied at the V3 growth stage in Stoneville, MS, in 2015 and 2016 †.

Herbicide Treatment	Rate	Injury			Palmer Amaranth Control			
		3 DAT	7 DAT	14 DAT	7 DAT	14 DAT	21 DAT	28 DAT
	kg ae or ai ha^{-1}				%			
None	-	0 c	0 c	0 b	0 c	0 c	0 c	0 c
Glyphosate	1.37	1 c	0 c	1 b	65 b	63 b	62 b	58 b
Glyphosate plus fomesafen	1.37 + 0.395	15 a	12 a	6 a	84 a	82 a	78 a	67 a
Glyphosate plus *S*-metolachlor	1.37 + 1.42	6 b	6 b	1 b	64 b	68 b	63 b	61 b
p-value	-	0.0039	0.0343	0.0001	0.0021	0.0001	0.0001	0.0001

† Data were pooled over four siteyears and three cytokinin products (none, kinetin-1, kinetin-2). Cytokinins were as follows: as 0.000227 kg ai ha^{-1} of each of kinetin-1 (as Ascend) and kinetin-2 (as Radiate). Means followed by the same letter for each parameter and/or evaluation are not different at $\alpha \leq 0.05$.

Data for Palmer amaranth control indicated no synergistic or antagonistic effects. Additionally, the main effect of cytokinin product was not significant for Palmer amaranth control. A main effect of herbicide treatment was detected for Palmer amaranth control at all evaluations (Table 1). Glyphosate plus fomesafen provided 84 and 67% control of Palmer amaranth 7 and 28 DAT, respectively (Table 2). Glyphosate alone or in combination with *S*-metolachlor did not control Palmer amaranth > 68% at any evaluation interval (Table 2). Across all evaluations, Palmer amaranth control was at least 6% greater with glyphosate plus fomesafen compared with other herbicide treatments (Table 2). Glyphosate alone controlled Palmer amaranth 58 to 65% across all evaluation intervals (Table 2), confirming the populations of Palmer amaranth contained GR individuals.

An antagonistic effect was detected on barnyardgrass control 14 DAT when kinetin-1 was combined with glyphosate plus fomesafen (Table 3). The addition of kinetin-1 to glyphosate plus fomesafen caused a 9% reduction in barnyardgrass control compared with glyphosate plus fomesafen or with no cytokinin in the mixture (Table 3). Across all other evaluation intervals, a main effect of herbicide treatment was detected for barnyardgrass control (Table 4). Glyphosate alone controlled more barnyardgrass than other herbicide treatments 7 DAT (Table 4). By 21 and 28 DAT, glyphosate plus *S*-metolachlor controlled barnyardgrass greatest (Table 4). Glyphosate plus fomesafen provided 9 and 6% less barnyardgrass control 7 and 21 DAT, respectively, compared with glyphosate alone (Table 4). Barnyardgrass control 28 DAT with glyphosate plus fomesafen was comparable with glyphosate alone (Table 4).

Table 3. Antagonistic responses for barnyardgrass control 14 d after treatment (DAT) with tank mixtures of POST herbicide treatments and cytokinin products applied at the V3 growth stage to soybean in Stoneville, MS, during 2015 and 2016 †,‡.

Herbicide Treatment	Rate	Cytokinin Tank Mix Component ††					
		Kinetin-1			Kinetin-2		
		Expected	Observed	*p*-value	Expected	Observed	*p*-Value
	kg ae or ai ha^{-1}	%			%		
Glyphosate	1.37	89	87	0.5290	89	88	0.7514
Glyphosate plus fomesafen	1.37 + 0.395	82	73 *	0.0047	82	81	0.8016
Glyphosate plus *S*-metolachlor	1.37 + 1.42	91	87	0.1781	91	91	0.9686

† Expected values for each cytokinin product are the same due to a lack of herbicidal activity from the cytokinin tank mixtures; therefore, values are the percent weed control without a cytokinin product. ‡ Asterisks denote antagonistic responses between herbicide treatment and cytokinin product when $\alpha \leq 0.05$. †† Applications were made with kinetin-1 (as 0.000227 kg ai ha^{-1} of Ascend) and kinetin-2 (as 0.000227 kg ai ha^{-1} of Radiate) as tank mixtures with each of the herbicide treatments. The *p*-value nested within each cytokinin product denotes significant differences between observed and expected values within each corresponding cytokinin product.

Table 4. Barnyardgrass control 7, 21 and 28 d after treatment (DAT), soybean plant height 14 DAT, mature plant height, and yield that resulted from soybean receiving tank mixtures of POST herbicide treatments and cytokinins applied at the V3 growth stage in Stoneville, MS, during 2015 and 2016 †.

Herbicide Treatment	Rate	Barnyardgrass Control			Soybean Plant Height		
		7 DAT	21 DAT	28 DAT	14 DAT	Maturity	Yield
	kg ae or ai ha^{-1}		%			cm	kg ha^{-1}
None	-	0 d	0 d	0 c	40 a	100	2674 b
Glyphosate	1.37	91 a	86 b	83 b	37 b	96	3499 a
Glyphosate plus fomesafen	1.37 + 0.395	82 c	80 c	79 b	36 b	97	3640 a
Glyphosate plus *S*-metolachlor	1.37 + 1.42	86 b	92 a	89 a	36 b	97	3525 a
p-value	-	0.0078	0.0035	0.0001	0.0001	0.1293	0.0478

† Data were pooled over four siteyears and three cytokinin products (none, kinetin-1, kinetin-2). Means followed by the same letter for each parameter and/or evaluation are not different at $\alpha \leq 0.05$. Applications were made with kinetin-1 (as 0.000227 kg ai ha^{-1} of Ascend) and kinetin-2 (as 0.000227 kg ai ha^{-1} of Radiate) as tank mixtures with each of the herbicide treatments.

Herbicide main effects were detected for soybean height 14 DAT, mature soybean height, and soybean yield (Table 4). Pooled across cytokinin mixtures, soybean heights 14 DAT and at maturity were greater for the no herbicide treatment compared with treatments that received a herbicide (Table 4). Height differences were attributed to a severe infestation of Palmer amaranth and barnyardgrass, increasing competition for sunlight necessary for photosynthesis during vegetative growth [43]. Pooled across cytokinin mixtures, treatments containing a herbicide produced greater soybean yields than the no herbicide treatment (Table 4).

4. Discussion

Crop injury that results from POST applications of agricultural pesticides is a common occurrence. Reducing the crop injury with the addition of products in tank mix combinations could be a valuable strategy for farmers, and reduce the need for additional trips across a field. However, the addition of some products in tank mix combinations should be researched to verify that weed control is not reduced by the addition of products to already effective herbicide treatments. Bronzing and necrosis of soybean plant tissues following POST fomesafen applications has been well-documented [44,45]. However, even though some crop injury to soybean can be expected as a result of POST fomesafen applications as either a stand-alone treatment or in combination with additional herbicides, weed control of troublesome, GR weeds is still effective. Everman et al. [46], Whitaker et al. [14], Barkley et al. [47], and Miller and Norsworthy [48] all observed Palmer amaranth control after PRE or POST applications of fomesafen. Fomesafen does not have residual grass activity and as a result, to effectively manage potentially GR barnyardgrass *S*-metolachlor was included in the current research studies. Moreover, since glyphosate is a POST herbicide lacking residual control, it should be expected that the residual control from *S*-metolachlor would control barnyardgrass better than glyphosate alone 28 DAT [15,49].

Testing for herbicide interactions as well as determining a level of antagonism associated with specific herbicide products can be difficult. Various statistical techniques to test for herbicide interactions in mixtures with additional compounds have previously been outlined in the literature. Colby's method has been one of the more popular tests and was most recently used to detail antagonism of volunteer GR corn control in dicamba-resistant soybean [50]. Blouin et al. [40] developed the nonlinear model to test for interactions used by Webster et al. [45] in evaluating a safening interaction on rice [*Oryza sativa* (L.)] treated with clomazone plus bensulfuron or halosulfuron. After expanding on the nonlinear model, Blouin et al. [40] created the augmented mixed-model methodology utilized by Fish et al. [51] to determine synergism and antagonism between propanil and imazamox on red rice (*Oryza sativa* (L.)) and barnyardgrass control. Reports of herbicide-by-herbicide or -water interactions are abundant in the literature. Minton et al. [31] reported barnyardgrass control was antagonized when quizalofop or sethoxydim were combined with lactofen. Starke and Oliver [28] documented antagonism on entireleaf morningglory [*Ipomoea hederacea* var. *integriuscula* (Gray)] but not on pitted morningglory [*Ipomoea lacunose* (L.)] control when fomesafen and glyphosate were combined. Water may antagonize herbicides because of the cations present in hard water. Stable complexes are formed when glyphosate bonds with di- and trivalent cations, leading to glyphosate antagonism [33–35]. In the current research project, we relied on a statistical method previously developed by Blouin et al. [41]. Since the tank mixtures that contained cytokinins did not reduce soybean injury, improve soybean plant height, or increase soybean yield there was no synergism to report as a result of the tank mix components.

Barnyardgrass control with glyphosate plus fomesafen was antagonized by the addition of kinetin-1. Similar results have previously been reported, stating that blended fertilizers do not decrease soybean injury from POST herbicides [37]. However, contrary to Lawrence's [37] research these cytokinin mixtures did not influence weed control when combined with glyphosate alone or in combination with *S*-metolachlor. Future research should evaluate the possible agronomic benefit of using cytokinins as PGRs in soybean to justify the application costs. Cytokinins should not be mixed with POST soybean herbicide applications included in this research, because this research demonstrated cytokinin mixtures did not reduce soybean injury and could negatively influence control of certain weed species with these specific herbicide treatments.

5. Conclusions

The practice of mixing multiple products has become common to reduce applications on a single field. Results of this study show that cytokinins have the potential to reduce barnyardgrass control when applied with glyphosate and fomesafen. Split application should be considered for applying cytokinins in combination with glyphosate and fomesafen. When the decision to mix multiple products in a single application is made, the applicator should make sure research has been conducted on the efficacy of the mixture.

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