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Glyphosate-Resistant Weed Control and Soybean Injury in Response to Different PPO-Inhibiting Herbicides

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Abstract

In Nebraska, 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) as well as acetolactate synthase (ALS)-inhibitor-resistant weeds occur in many soybean fields where herbicides from these modes-of-action have been frequently used in the past. Currently, the protoporphyrinogen oxidase (PPO)-inhibitors are the only effective herbicides for POST control of both glyphosate- and ALS-inhibitor-resistant weeds in soybean. Greenhouse experiments were conducted in 2014 to evaluate the efficacy of PPO-inhibitors applied POST for the control of three glyphosate-resistant (GR) weeds and potential for soybean injury, when applied at two growth stages. All herbicide treatments controlled 10- and 20-cm tall GR common waterhemp ≥ 95% at 21 DAT. GR giant ragweed and kochia were controlled 86 to 99% when treated at 10-cm height and 78 to 92% at 20-cm height by 21 DAT. Herbicide treatments reduced shoot biomass in the three GR weeds 88 to 100% when treated at 10-cm height and 73 to 100% when treated at 20-cm height, at 21 DAT. Soybean injury and shoot biomass data revealed that acifluorfen and lactofen were more injurious (≥ 17%), whereas fomesafen, and fomesafen plus glyphosate were relatively safer (< 10% injury). Overall, fomesafen and fomesafen plus glyphosate caused least injury to soybean and were more effective in controlling GR common waterhemp, giant ragweed, and kochia compared with acifluorfen and lactofen.

Keywords: herbicide resistance, protoporphyrinogen oxidase, resistance management, soybean injury, weed control

1. Introduction

In the United States, glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.) were introduced for commercial cultivation in 1996, 1997, and 1998, respectively. Consequently, weed management in the GR crops became entirely dependent on the POST applications of glyphosate only. The glyphosate usage in these crops increased from 4.2 million kg in 1996 to 50 million kg in 2012 (Food & Water Watch, 2013; USDA-NASS, 2014). Unfortunately, overreliance on glyphosate alone for postemergence (POST) weed control in GR crops resulted in evolution of glyphosate-resistant weeds (Culpepper et al., 2006; Powles et al., 1998; VanGessel, 2001). As of 2015, 15 weed species have been confirmed resistant to glyphosate in the United States (Heap, 2015).

In the midwestern United States, glyphosate has been intensively used on a vast acreage predominantly as a preplant and POST herbicide in GR corn and soybean (Duke, 2005; Owen & Zelaya, 2005). In Nebraska, six weed species that includes common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp (*Amaranths rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), horseweed [*Conyza canadensis* (L.) Cronq.], kochia [*Kochia scoparia* (L.) Schrad], and Palmer amaranth [*Amaranthus palmeri* S. Wats.] have been confirmed resistant to glyphosate (Chahal et al., 2015a; Jhala, 2015). The occurrence of GR weeds is a serious concern, particularly for midwestern soybean growers, because of limited effective POST herbicide options. Additionally, most of the GR weeds in the midwest are also resistant to ALS-inhibitors such as chlorimuron-ethyl, imazamox, imazaquin, imazethapyr, thifensulfuron-methyl (Legleiter & Bradley, 2008; Sarangi et al., 2015).

Management of GR weeds is a serious challenge that often results in high weed management costs (Beckie, 2011). Weed interference frequently results in significant economic losses due to reduced crop yields. For example, in Missouri and Ohio, one giant ragweed plant per m^{-2} reduced soybean yield more than 45% (Baysinger & Sims, 1991; Webster et al., 1994). Similarly, Hager et al. (2002) reported up to 43% reduction in soybean yield when common waterhemp interference was allowed to persist 4 wk or longer after soybean unifoliate leaf expansion. Previous research indicated several strategies for controlling GR weeds, including the use of tillage, cover crops, crop rotation, residual preemergence (PRE) herbicides, tank-mixing glyphosate with other mode-of-action herbicides, and planting cultivars or crops resistant to herbicides other than glyphosate (Aulakh et al., 2011, 2012, and 2013; Aulakh, 2013; Aulakh & Jhala, 2015; Beckie, 2006; Chahal et al., 2015b; Chahal & Jhala, 2015; Norsworthy et al., 2012; Wilson et al., 2007).

Before the commercialization of GR soybean, ALS- and PPO-inhibitors were the primary weed management tools for POST broadleaf weed control in soybean. In recent years, the use of PPO-inhibitors has increased, especially for controlling glyphosate- and ALS-resistant weeds in soybean (Legleiter et al., 2009). The PPO enzyme has a crucial role in the biosynthesis of heme and chlorophyll in the plant (Beale & Weinstein, 1990). The inhibition of PPO enzyme results in the production of proto IX which, in the presence of sunlight, causes the cell membrane disruption and ultimately leads to plant death (Jacobs et al., 1991; J. M. Jacobs & N. J. Jacobs, 1982, 1993; Lee & Duke, 1994; Lee et al., 2000; Matsunaka, 1976).

Since the weed species differ in their response to herbicides, different PPO-inhibitors may differ in their efficacy of weed control. Therefore, a knowledge of efficacy of different PPO-inhibitors in terms of degree of control of different GR weed species and potential for soybean injury is needed to help soybean growers make sound weed management decisions. The objectives of this study were to 1) evaluate the efficacy of various PPO-inhibitors applied POST in controlling 10- and 20-cm tall GR common waterhemp, giant ragweed, and kochia, and 2) to investigate the potential for GR soybean injury with PPO-inhibitors applied POST at 3- or 5-trifoliate soybean stages. We hypothesized that PPO-inhibitors will differ in their efficacy for controlling different weed species and level of soybean injury when applied at two different growth stages.

2. Materials and Methods

Greenhouse experiments were conducted in 2014 at the University of Nebraska-Lincoln, Lincoln, NE to evaluate the control of three GR weed species and potential for GR soybean injury in response to different PPO-inhibitors applied POST. The seeds of GR weed species used in this study were collected from three different locations in Nebraska (Sandell et al., 2011, 2012; Sarangi et al., 2014). Weed seeds were planted in 10 cm-diameter pots containing a 3:1 mixture of potting mix (Berger BM1 potting mix, Berger Peat Moss Ltd, Quebec, Canada) and soil collected from a field near Lincoln, NE with known history of no herbicide use for last five years. Soybean seeds were planted in 20 cm-diameter pots containing the same 3:1 mixture. Plants were supplied with adequate nutrients and water, and kept in greenhouse at 30/20 ^{o}C day/night temperature and 16-h photoperiod.

The experimental design was a factorial of six herbicide treatments, four plant species, and two growth stages. Pots were arranged in a completely randomized design with four replications and experiment was conducted twice for the consistency of results. A single plant per pot was considered as an experimental unit. Herbicide treatments included five PPO-inhibitors (Table 1) and a nontreated control. Selected GR weeds (common waterhemp, giant ragweed, and kochia) were treated with the herbicides when 10- and 20-cm tall while soybean was treated at 3-and 5-trifoliate stages. The recommended adjuvant, liquid ammonium sulphate (N-PAK®AMS Liquid, Winfield Solutions, LLC, St. Paul MN 55164) was mixed at 2.0% v/v with acifluorfen, fomesafen, and fomesafen plus glyphosate and at 4% v/v with lactofen. Crop-oil-concentrate was added to each herbicide treatment at 0.625% v/v basis. Herbicide treatments were prepared in distilled water and applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale MN 56045) fitted with an 8002E nozzle (Teejet, Spraying Systems Co, Wheaton IL 60187) calibrated to deliver 187 L ha^{-1} carrier volume at 207 kPa. After the herbicide application, plants were returned to the greenhouse.

Table 1. Herbicide treatments, products, and rates used in a greenhouse experiment conducted in Nebraska in 2014

Herbicide common name[a]	Herbicide trade name	Rate	Manufacturer
		g ai ha^{-1}	
Acifluorfen	Ultra blazer	420	United Phosphorus, King of Prussia, PA 19406
Fomesafen	Flexstar	264	Syngenta Crop Protection, Inc. Greensboro, NC 27419
Fomesafen + glyphosate	Flexstar GT	1,380	Syngenta Crop Protection, Inc.
Lactofen[b]	Cobra	220	Valent U.S.A. Corporation, Walnut Creek, CA 94596-8025
Lactofen[c]	Phoenix	220	Valent U.S.A. Corporation

Note. [a]AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) was added at 2% v/v to acifluorfen, fomesafen, and fomesafen plus glyphosate and at 4% v/v with lactofen. Crop-oil-concentrate was added to each treatment at 0.625% v/v basis.

[b]Lactofen: Cobra.

[c]Lactofen: Phoenix.

Weed control and soybean injury were assessed visually at 7, 14, and 21 d after treatment (DAT) using a scale ranging from 0 (no control or injury) to 100% (complete control or death). Visual control or injury estimates were based on symptoms such as chlorosis, necrosis, crinkling, and stunting of the treated plants compared with the nontreated control plants. The aboveground shoot biomass of each weed species and soybean was harvested at 21 DAT, oven-dried for 96 h at 65 C, and the dry weight was determined.

3. Statistical Analysis

The shoot biomass data of three GR weeds and GR soybean were converted into percent shoot biomass reduction compared to the nontreated control (Wortman, 2014):

$$Percent\ shoot\ biomass\ reduction = [(\bar{C} - B) / \bar{C}] \times 100 \quad (1)$$

Where, $\bar{C}$ is the mean shoot biomass of the four nontreated control replicates, and *B* is the shoot biomass of a treated individual plant.

Data were subjected to ANOVA, separately by plant species i.e. weed or soybean and growth stage, using the GLIMMIX procedure in SAS (SAS version 9.3, SAS Institute Inc, Cary, NC). Experimental run, herbicide treatment, and their interactions were considered fixed effects while the replication and its interactions with fixed effects were considered random effects. Where the ANOVA indicated significant main or interaction effects, means were separated at $P \leq 0.05$ using Fisher's protected LSD test. Multiple means comparisons were made using "adj=sim" option in lsmestimate statement in GLIMMIX procedure.

4. Results and Discussion

Experiment run and its interaction with herbicide treatment were not significant, therefore, data were combined across experiment runs.

For soybean injury, the herbicide treatment main effect was significant ($P = 0.034$). Injury ranged from 5 to 23%, depending on herbicide treatment, over the three wk period following herbicide application (Table 2). Injury symptoms were typified by foliar chlorosis, crinkling, necrosis, and plant height reduction. Two lactofen formulations produced similar levels of soybean injury, therefore, their means were averaged. Acifluorfen and lactofen injured soybean > 17%, however, fomesafen, and fomesafen plus glyphosate were relatively safer with < 10% injury. Previous researchers have reported similar levels of soybean injury by PPO-inhibitors with fomesafen causing less injury than acifluorfen or lactofen (Ellis & Griffin, 2003; Hager et al., 2003; Harris et al., 1991; Higgins et al., 1988; Johnson et al., 2002). Soybean shoot biomass reduction, averaged over two growth stages due to non-significant differences, corresponded with visual control differences among herbicide treatments at 21 DAT (Table 2).

Table 2. Herbicide treatments response on soybean injury at 7, 14, 21 d after treatment (DAT), and shoot biomass reduction at 21 DAT in a greenhouse experiment conducted in Nebraska in 2014[a].

Herbicide treatment	Soybean injury			Soybean biomass reduction[c]
	7 DAT[b]	14 DAT[b]	21 DAT[b]	21 DAT[b]
	%			%
Nontreated control[d]	-	-	-	0 b
Aciflourfen	23 a	21 a	20 a	28 a
Fomesafen	9 b	8 b	8 b	4 b
Fomesafen + glyphosate	6 b	7 b	5 b	7 b
Lactofen[e]	22 a	20 a	17 a	24 a

Note. [a]Experimental run-by-treatment and treatment-by-soybean growth stage interaction was not significant; therefore, soybean injury and biomass data were averaged over experimental runs and soybean growth stages.

[b]Abbreviations: DAT, days after treatment.

[c]Soybean shoot biomass data represent percent reduction compared with the nontreated control (6.8 g plant^{-1}, averaged over two growth stages).

[d]The percent control (0%) data of nontreated control were not included in analysis.

[e]Means averaged over two lactofen formulations.

Weeds response to herbicide treatments was highly variable. At 7 DAT, lactofen, and aciflourfen controlled 10-cm tall GR common waterhemp ≥ 91% (Table 3). With fomesafen and fomesafen plus glyphosate, control was 86 and 84%, respectively. The 20-cm tall common waterhemp was controlled 82 to 89% without differences among herbicide treatments (Table 3). Similar treatment differences were observed at 14 DAT with control ranging from 88 to 98% for both common waterhemp growth stages. By 21 DAT, common waterhemp was controlled 95 to 100% depending on herbicide treatment (Table 3). The shoot biomass data at 21 DAT also corresponded with the common waterhemp control achieved at that time (Table 3). Hager et al. (2003) observed highly variable common waterhemp control (57 to 95%) with early-POST and POST applications of acifluorfen, fomesafen, and lactofen at different rates and found lactofen more effective than aciflourfen and fomesafen.

Table 3. Glyphosate-resistant common waterhemp control at 7, 14, and 21 d after treatment (DAT) and shoot biomass reduction at 21 DAT in a greenhouse experiment conducted in Nebraska in 2014[a].

Herbicide treatment	Common waterhemp control						Shoot biomass reduction[c]	
	7 DAT[b]		14 DAT[b]		21 DAT[b]		21 DAT[b]	
	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm
	%						%	
Nontreated control[d]	-	-	-	-	-	-	0 b	0 c
Acifluorfen	91 ab	82 a	98 a	92 a	99 ab	99 a	98 a	90 b
Fomesafen	86 ab	86 a	93 ab	96 a	99 ab	97 a	98 a	98 a
Fomesafen + glyphosate	84 b	83 a	89 b	88 a	95 b	96 a	94 a	97 a
Lactofen (cobra)	91 ab	89 a	98 a	96 a	100 a	100 a	100 a	100 a
Lactofen (phoenix)	94 a	85 a	96 ab	95 a	98 ab	98 a	97 a	97 a

Note. [a]Experimental run-by-treatment interaction was not significant; therefore, percent visual control and shoot biomass data were averaged over experimental runs.

[b]Abbreviations: DAT, days after treatment.

[c]Shoot biomass data represent percent reduction compared with the nontreated control (3.2 g plant^{-1} for 10 cm, and 5.1 g plant^{-1} for 20 cm tall common waterhemp).

[d]The percent control (0%) data of nontreated control were not included in analysis.

At 7 DAT, GR giant ragweed control varied from 62 to 69% for 10-cm and 44 to 61% for 20-cm tall plants

(Table 4). Application of fomesafen plus glyphosate controlled 20-cm giant ragweed greater (61%) than fomesafen alone (44%). By 14 DAT, 10-cm giant ragweed was controlled 88 to 92% without differences among herbicide treatments. However, 20-cm giant ragweed control with lactofen (86%) was greater than with acifluorfen (76%). By 21 DAT, giant ragweed control ranged from 96 to 99% for 10-cm and 82 to 92% for 20-cm tall plants (Table 4). At 21 DAT, the shoot biomass reduction (> 98%) by different herbicide treatments corresponded with visual control for 10-cm tall giant ragweed. However, for 20-cm giant ragweed, lower shoot biomass reduction (< 85%) was observed compared to the visual control at 21 DAT (Table 4). Previous researchers also reported variation in the control of different giant ragweed accessions with aciflourfen, fomesafen, and lactofen (Norsworthy et al., 2011; Tylor et al., 2002).

Table 4. Glyphosate-resistant giant ragweed control at 7, 14, and 21 d after treatment (DAT) and shoot biomass reduction at 21 DAT in a greenhouse experiment conducted in Nebraska in 2014[a].

Herbicide treatment	Giant ragweed control						Shoot biomass reduction[c]	
	7 DAT[b]		14 DAT[b]		21 DAT[b]		21 DAT[b]	
	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm
	%						%	
Nontreated control[d]	-	-	-	-	-	-	0 b	0 c
Acifluorfen	68 a	51 ab	88 a	76 b	97 a	82 b	98 a	73 b
Fomesafen	66 a	44 b	91 a	83 ab	99 a	87 ab	99 a	78 b
Fomesafen + glyphosate	66 a	61 a	88 a	80 ab	96 a	88 ab	99 a	77 b
Lactofen (cobra)	62 a	55 ab	92 a	87 a	97 a	91 a	99 a	84 a
Lactofen (phoenix)	69 a	53 ab	92 a	86 a	98 a	92 a	98 a	78 b

Note. [a]Experimental run-by-treatment interaction was not significant; therefore, percent visual control and shoot biomass data were averaged over experimental runs.

[b]Abbreviations: DAT, days after treatment.

[c]Shoot biomass data represent percent reduction compared with the nontreated control (4.5 g plant^{-1} for 10 cm, and 5.7 g plant^{-1} for 20 cm tall giant ragweed).

[d]The percent control (0%) data of nontreated control were not included in analysis.

At 7 DAT, GR kochia control varied from 69 to 74% for 10-cm and 43 to 57% for 20-cm tall plants (Table 5). Herbicide treatments did not differ in control of 10-cm kochia, however, acifluorfen and lactofen controlled 20-cm kochia 8 to 14% greater than fomesafen and fomesafen plus glyphosate. At 14 DAT, fomesafen and fomesafen plus glyphosate controlled 10-cm kochia > 90%. All other herbicide treatments were similar with 83 to 86% control regardless of the growth stage of kochia. At 21 DAT, both fomesafen and fomesafen plus glyphosate controlled 10-and 20-cm kochia 99 and 80%, respectively. Lactofen and aciflourfen were similar with ≤ 90% control of 10-cm and ≤ 80% control of 20-cm tall kochia plants. In contrast, Kumar and Jha (2015) in a field study, reported 72 and 80% control of 8 to 10 cm tall kochia three wk after treatment with fomesafen and lactofen, respectively. The shoot biomass reduction of kochia at both heights was similar to their visual control estimates at 21 DAT (Table 5).

Table 5. Glyphosate-resistant kochia control at 7, 14, and 21 d after treatment (DAT) and shoot biomass reduction at 21 DAT in a greenhouse experiment conducted in Nebraska in 2014[a].

Herbicide treatment	Kochia control						Shoot biomass reduction[c]	
	7 DAT[b]		14 DAT[b]		21 DAT[b]		21 DAT[b]	
	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm	10-cm	20-cm
	%						%	
Nontreated control[d]	-	-	-	-	-	-	0 b	0 c
Acifluorfen	69 a	52 bc	84 b	83 a	86 b	78 a	88 a	78 a
Fomesafen	70 a	43 c	92 a	84 a	99 a	80 a	99 a	78 a
Fomesafen + glyphosate	71 a	44 c	94 a	86 a	99 a	80 a	99 a	77 ab
Lactofen (cobra)	73 a	57 b	85 b	86 a	91 b	80 a	98 a	73 b
Lactofen (phoenix)	74 a	57 b	84 b	84 a	90 b	80 a	96 a	73 b

Note. [a]Experimental run-by-treatment interaction was not significant; therefore, percent visual control and shoot biomass data were averaged over experimental runs.

[b]Abbreviations: DAT, days after treatment.

[c]Shoot biomass data represent percent reduction compared with the nontreated control (4.1 g plant^{-1} for 10 cm, and 6.0 g g plant^{-1} for 20 cm tall kochia).

[d]The percent control (0%) data of nontreated control were not included in analysis.

Results from this study indicate higher potential for soybean injury with acifluorfen and lactofen compared with fomesafen and fomesafen plus glyphosate. At 21 DAT, GR common waterhemp control (≥ 95%) was similar regardless of herbicide treatment and growth stage. All herbicides controlled 10-cm tall GR gaint ragweed ≥ 97%, however, for 20-cm tall GR giant ragweed control, lactofen (91%) was more effective than acifluorfen (82%). Fomesafen and fomesafen plus glyphosate were more effective on 10-cm tall kochia with 99% control compared to 86 and 90% with aciflourfen and lactofen, respectively. The 20-cm tall GR kochia was controlled ≤ 80% regardless of herbicide treatment. Overall, fomesafen and fomesafen plus glyphosate caused least injury to soybean and were highly effective in controlling GR common waterhemp, giant ragweed, and kochia compared with acifluorfen and lactofen.

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