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Growth Stage Affects Dose Response of Selected Glyphosate-Resistant Weeds to Premix of 2,4-D Choline and Glyphosate (Enlist Duo™ Herbicide*)

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Abstract

With the intent to control glyphosate–resistant and hard to control weeds, a formulation of 2,4-D choline (24.4%) and glyphosate (22.1%) (Enlist Duo™ herbicide) (Note 1) has been developed recently to be used post-emergence in corn and soybean tolerant to Enlist Duo™ in the United States. Dose response studies were conducted under greenhouse conditions for the evaluation of effective rates of Enlist Duo™ to control glyphosate-resistant common waterhemp (*Amaranths rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), and kochia [*Kochia scoparia* (L.) Schrad] and to determine the effect of growth stage of these weeds on the efficacy of Enlist Duo™. Three parameter log-logistic models were used to develop dose response curves. Glyphosate-resistant giant ragweed was the most sensitive of the three weed species, followed by common waterhemp, and kochia. Based on the visual control or injury estimates, the Enlist Duo™ rates required for 90% control (ED_{90}) of common waterhemp, giant ragweed, and kochia were 1179, 825, and 4,382 g ae ha^{-1}, respectively, for 10-cm tall plants compared to 2,480, 1,101, and 5,305 g ae ha^{-1}, respectively, for 20-cm tall plants at 21 days after treatment (DAT). The ED_{90} values calculated on the basis of percent shoot biomass reduction and visual control or injury estimates were usually similar at 21 DAT. The greenhouse studies indicate that Enlist Duo™ can effectively control less than or equal to 20-cm tall glyphosate-resistant giant ragweed and less than or equal to 10-cm tall glyphosate-resistant common waterhemp at the recommended rate (1,640 g ae ha^{-1}).

Keywords: broadleaf weeds, Enlist Duo™, resistance management, weed growth stage, 2,4-D choline and glyphosate

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1. Introduction

Simplified and economical weed management programs based on continuous and sole reliance on a single herbicide or herbicide(s) with the same mode(s) of action have resulted in the evolution of herbicide-resistant weeds (Beckie, 2011; VanGessel, 2001). The widespread cultivation of glyphosate-resistant crops since their introduction and commercialization in 1990’s and an almost exclusive reliance on glyphosate for POST weed control has raised issues of glyphosate-resistant weeds (Culpepper et al., 2006; Powles et al., 1998; VanGessel, 2001). As of 2014, 34 weed species have been confirmed resistant to glyphosate worldwide (Heap, 2015). Six weed species, including 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 in Nebraska, USA (Jhala, 2015).

Management of glyphosate-resistant weeds is the most significant predicament for growers (Culpepper et al., 2008; Johnson et al., 2009; Norsworthy et al., 2008; Price et al., 2011, 2012). Several weed management strategies have been recommended for controlling glyphosate-resistant weeds, including the use of tillage, cover crops, crop rotation, residual PRE herbicides, tank-mixing glyphosate with other modes of action herbicides, herbicide rotation or use of herbicides with different modes of action, and planting cultivars or crops resistant to herbicides other than glyphosate (Aulakh et al., 2011, 2012; Beckie, 2006; Norsworthy et al., 2012; Wilson et al., 2007). In the southeastern United States, growers experiencing problems with glyphosate-resistant weeds, have adopted alternative herbicides, hand weeding, and tillage which, however lead to higher production costs, loss of valuable topsoil, and decline in the area under no-till production systems (Aulakh et al., 2012, 2013; Price et al., 2011; Sosnoskie & Culpepper, 2014). Nevertheless, there is also a pressing need for novel herbicide-resistant technologies in addition with other weed management strategies to control glyphosate-resistant weeds.

Multiple herbicide-tolerant corn and soybean cultivars have been developed using molecular techniques for addressing the growing needs to control glyphosate-resistant weeds (Green et al., 2008). It includes corn and soybean tolerant to 2,4-D choline ($C_{13}H_{19}Cl_2NO_4$) along with glyphosate ($C_8H_6Cl_2O_3$), also known as the Enlist™ weed management system (Craigmyle et al., 2013a). Newly developed 2,4-D choline is a low-volatile form of 2,4-D manufactured using Colex-D™ technology. The spectrum of weed control will be similar to glyphosate and 2,4-D, but will further provide flexibility for applying up to V8 or 76-cm tall corn and up to R2 or full flower stage of soybean. The new herbicide formulation (Enlist Duo™ herbicide) recently received an approval by the United States Environmental Protection Agency (USEPA).

Enlist Duo™ will provide an additional tool for the management of glyphosate-resistant and hard-to-control weeds in corn and soybean. Recommended application rate of Enlist Duo will be 1640 g ae/ha to 2185 g ae/ha based on the weed growth stage. Understanding of the most effective application rate with respect to weed growth stage is needed to incorporate this new herbicide formulation in POST herbicide programs for controlling glyphosate-resistant weeds in corn and soybean. Preliminary experiments conducted in Nebraska provided 99% control of glyphosate-resistant giant ragweed when 1,065 g ae/ha of 2,4-D choline was applied POST in Enlist soybean (unpublished data). The recommended rate of Enlist Duo™ herbicide applied in this experiment (1,640 g ae/ha) contains 800 and 840 g of 2,4-D choline and glyphosate, respectively. It is expected that this formulation will be equally effective for controlling other glyphosate-resistant broadleaf weeds. However, scientific literature is not yet available about the response of economically important glyphosate-resistant broadleaf weeds to this formulation. The objectives of this study were to (1) describe the dose-response relationship of Enlist Duo™ applied POST for controlling glyphosate-resistant weed species including common waterhemp, giant ragweed, and kochia, and (2) determine the effect of growth stage of weed species on dose response of Enlist Duo™. We hypothesized that Enlist Duo™ will be effective at least for controlling glyphosate-resistant weeds when applied to 10-cm tall plants at a recommended rate, but a higher rate may or may not be required to control 20-cm tall plants depending on the weed species.

2. Methods

2.1 Plant Material

Greenhouse studies were conducted at the University of Nebraska-Lincoln to determine the dose response of glyphosate-resistant weeds, including common waterhemp, giant ragweed, and kochia to Enlist Duo™ applied POST at two growth stages. The seeds of common waterhemp, giant ragweed, and kochia were collected from three different sites with confirmed glyphosate-resistance in Nebraska in last five years. When screened with glyphosate at 1,680 g ae ha^{-1} (2× rate) before conducting this study, the survival frequency of these three glyphosate-resistant weeds exceeded 90%. Weed seeds were sown in 28 × 54 × 6 cm germination trays containing potting mix (Berger BM1 potting mix, Berger Peat Moss Ltd, Quebec, Canada). Seedlings at the cotyledon to first true-leaf stage were transplanted in 10-cm-diam plastic pots containing a 3:1 mixture of potting mix and soil. Plants were supplied with adequate nutrients and water, and kept in greenhouse at 30/20 ^{o}C day/night temperature and 16-h photoperiod.

2.2 Herbicide Treatments

Herbicide treatments included 8 rates (0, 0.25×, 0.50×, 0.75×, 1.0×, 1.5×, 2.0×, and 2.5×), where, 1× = recommended field rate of the formulation of 2,4-D choline and glyphosate (1,640 g ae ha^{-1}). Selected glyphosate-resistant weeds were treated when they were 10-and 20-cm tall. The recommended adjuvant, liquid ammonium sulfate (N-PAK®AMS Liquid, Winfield Solutions, LLC, St. Paul MN 55164) was mixed with each treatment at 2.5% v/v. Herbicide treatments were prepared in distilled water and applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale MN 56045) fitted with an 8001E nozzle (Teejet, Spraying Systems Co, Wheaton IL 60187) calibrated to deliver 140 L ha^{-1} carrier volume at 207 kPa. After herbicide treatment, plants were returned to the greenhouse.

Weed control ratings 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 plant death). Visual weed control estimates were based on symptoms such as chlorosis, necrosis, stunting, and death of the treated plants or weeds compared with nontreated control plants (Ganie et al., 2015; Sarangi et al., 2014). Aboveground shoot biomass of each weed species was harvested at 21 DAT, oven-dried for 96 h at 65 °C, and the weight was determined.

2.3 Experimental Design and Statistical Analysis

Experimental design was a factorial of eight rates of Enlist Duo™ and two weed growth stages (10- and 20-cm tall). Pots were arranged in a completely randomized design with four replications and the experiment was repeated twice for the consistency of results. A single plant per pot was considered as an experimental unit.

The shoot biomass data were converted into percent shoot biomass reduction compared with the nontreated control (Wortman, 2014):

$$\textit{Percent shoot biomass reduction} = \left[\left(\overline{C} - B\right) / \overline{C}\right] \times 100 \tag{1}$$

Where, $\overline{C}$ is the mean shoot biomass of the four nontreated control replicates, and B is the shoot biomass of a treated individual experimental unit.

Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS (SAS version 9.3, SAS Institute Inc, Cary, NC) to test for treatment-by-experiment interaction. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Fisher's protected LSD test. Visual weed control or injury estimate and shoot biomass reduction (as a percentage compared to the nontreated control) data were regressed over herbicide treatments using the three-parameter log-logistic model (Seefeldt et al., 1995).

$$Y = D/1 + \exp\left[B - (\log(X) - \log(E))\right] \tag{2}$$

Where, Y is the response variables (percent weed control estimates or percent reduction in shoot biomass), D is the upper limit, B is the slope of the line, E is the dose resulting in a 50% or 90% control (known as ED_{50} or ED_{90}), and X is the herbicide rate. Analyses of dose–response curves were performed separately for each weed species and ED_{50} and ED_{90} values were determined using the *drc* package (drc 1.2, Christian Ritz and Jens Strebig, R2.5, Kurt Hornik, online) in software R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (Ritz & Streibig, 2005). The regression parameters were obtained using the nonlinear least-squares function of the statistical software R and graphical presentation was generated using the same software. The *anova* function was used to perform the lack of fit test and the p-value of ≥ 0.05 indicates the acceptable description of the data by the fitted non-linear model.

3. Results and Discussion

Treatment-by-experiment interaction was not significant; therefore, data were pooled over two experiments and combined data are presented. A test of lack of fit at the 95% level was non-significant for any of the curves tested, indicating fitted models were correct and regression parameters along with ED_{50} and ED_{90} values were determined (Tables 1 and 2).

3.1 Common Waterhemp

Growth stage of glyphosate-resistant common waterhemp at the time of herbicide application affected control and shoot biomass reduction ($P < 0.0001$). At the recommended rate (1,640 g ae ha^{-1}) of Enlist Duo™ herbicide, common waterhemp was controlled 90 and 62% for 10- and 20-cm tall plants, respectively, at 14 DAT (data not shown). At 21 DAT, common waterhemp control increased to 95% for 10-cm tall plants compared to 80% for 20-cm tall plants (Figure 1A, Table 1). Higher rates (≥ 2,480 g ae ha^{-1}) were required to achieve 90% control of 20-cm tall common waterhemp plants. Several studies have reported that the height of weed species at the time of POST herbicide application is an important factor determining the level of control achieved (Chahal et al., 2014; Cordes et al., 2004; Craigmyle et al., 2013b).

The application rates of Enlist Duo™ required for 50 and 90% control of common waterhemp at 21 DAT were 339 and 1,179 g ae ha^{-1}, respectively, for 10-cm tall plants compared to 484 and 2,480 g ae ha^{-1}, respectively, for 20-cm tall plants (Table 1). Similarly, Robinson et al. (2012) reported poor control (< 50%) of 20- to 30-cm tall common waterhemp with 2,4-D (280 g ae ha^{-1}) and higher rates (1,120 g ae ha^{-1}) were required to achieve > 90% control. Spaunhorst and Bradley (2013) reported 30 to 40% control of 15- to 30-cm tall glyphosate-resistant common waterhemp compared to 62% control of 7.5-cm tall plants with a tank-mixture of dicamba and glyphosate. The rates required for 50 and 90% shoot biomass reduction were 303 and 883 g ae ha^{-1}, respectively, for 10-cm tall plants compared to 388 and 2,668 g ae ha^{-1}, respectively, for 20-cm tall plants (Figure 1B, Table 2). Thus, effective rates determined on the basis of shoot biomass reduction were usually similar to those determined on the basis of visual control estimates.

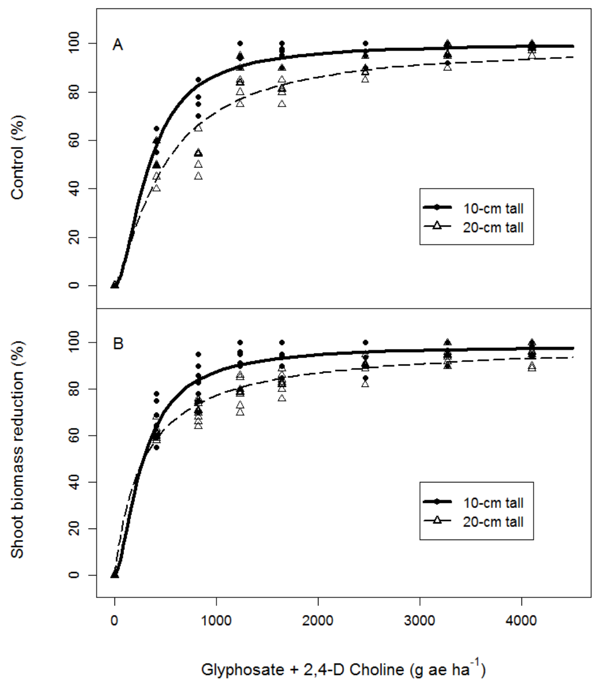

Figure 1. Glyphosate-resistant common waterhemp (A) control of 10- and 20-cm tall plants on the basis of visual injury ratings at 21 d after treatment (DAT), and (B) percent shoot biomass reduction of 10- and 20-cm tall plants at 21 DAT in a greenhouse dose response study with a formulation of 2,4-D choline and glyphosate at University of Nebraska-Lincoln

3.2 Giant Ragweed

Growth stage of giant ragweed significantly affected herbicide efficacy ($P < 0.0001$). Dose response curve indicated ≥ 90% control of 10-cm tall giant ragweed even with a lower than the recommended rate (1,640 g ae ha^{-1}) at 21DAT (Figure 2A), while the 20-cm tall giant ragweed was controlled 87 to 93% at the recommended rate at 21 DAT. Similarly, Vink et al. (2012) reported > 90% control of 2- to 17-cm tall giant ragweed with 2,4-D ester (500 g ae ha^{-1}) applied alone, indicating the sensitivity of giant ragweed to 2,4-D. Higher level of giant ragweed control has been reported in a previous study despite the plant height being higher than recommended. For example, Robinson et al. (2012) reported ≥ 99% control of 26- to 46-cm tall giant ragweed with 2,4-D (280 g ae ha^{-1}) applied alone or tank-mixed with glyphosate (1,120 g ae ha^{-1}).

The application rates of Enlist Duo™ required for 50 and 90% control were 350 and 825 g ae ha^{-1}, respectively, for 10-cm tall plants compared to 324 and 1,101 g ae ha^{-1}, respectively, for 20-cm tall plants (Figure 2A, Table 1). Similarly, the rates required for 50 and 90% shoot biomass reduction in 10-cm tall plants were 201 and 805 g ae ha^{-1}, respectively, compared to 281 and 1142 g ae ha^{-1}, respectively, for 20-cm tall plants (Figure 2B, Table 2). Results of this study suggested that Enlist Duo™ herbicide is very effective for controlling glyphosate-resistant giant ragweed. This might be due to giant ragweed's sensitivity to phenoxy herbicides (Robinson et al., 2012; Vink et al., 2012). Recently, Kaur et al. (2014) and Jhala et al. (2014a) reported 99% control of glyphosate-resistant giant ragweed in Nebraska with herbicide programs that included preplant application of 2,4-D in soybean.

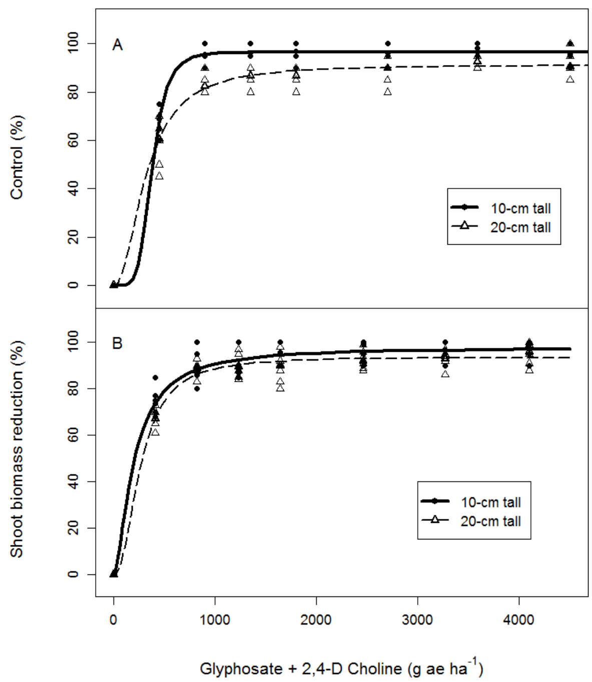

Figure 2. Glyphosate-resistant giant ragweed (A) control of 10- and 20-cm tall plants on the basis of visual injury ratings at 21 d after treatment (DAT), and (B) percent shoot biomass reduction of 10- and 20-cm tall plants at 21 DAT in a greenhouse dose response study with a formulation of 2,4-D choline and glyphosate at University of Nebraska-Lincoln

3.3 Kochia

Similar to common waterhemp and giant ragweed, visual control and percent shoot biomass reduction of glyphosate-resistant kochia were significantly affected by the growth stage ($P < 0.0001$). At 21 DAT, 10- and 20-cm tall kochia were controlled 59 to 87% and 43 to 66%, respectively, at the recommended rate (Figure 3A). Regardless of growth stage and application rate, ≥ 90% control was not achieved.

Although the shoot biomass curve indicated 90% reduction at 3,704 g ae ha^{-1} for 10-cm tall kochia (Figure 3C), the ED_{90} value for visual control estimates has a limited biological meaning because 90% control was never achieved regardless of growth stage (Figure 3A, Table 2). Results indicate that the recommended rate of Enlist Duo™ can provide up to 87% control of 10-cm tall kochia, but is less effective for controlling 20-cm tall plants. Wicks et al. (1994) also reported 75% control of 2- to 10-cm tall glyphosate susceptible kochia and 29% control of 10- to 20-cm tall plants with 2,4-D ester applied alone. Kochia leaves are pubescent and also have crystalline epicuticular wax which may play an important role in reducing retention and absorption of 2,4-D (Harbour et al. 2003).

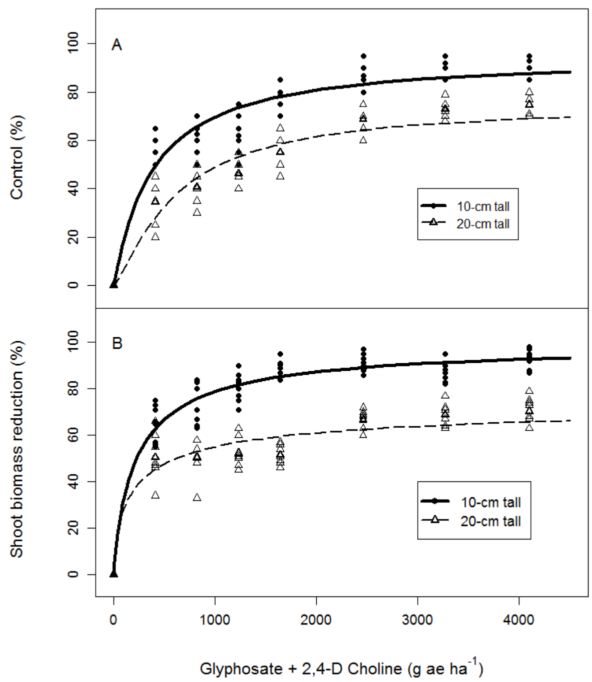

Figure 3. Glyphosate-resistant kochia (A) control of 10- and 20-cm tall plants on the basis of visual injury ratings at 21 d after treatment (DAT), and (B) percent shoot biomass reduction of 10- and 20-cm tall plants at 21 DAT in a greenhouse dose response study with a formulation of 2,4-D choline and glyphosate at University of Nebraska-Lincoln

4. Conclusions

This greenhouse study showed that Enlist Duo™ is effective for controlling glyphosate-resistant common waterhemp and giant ragweed; however, the effective rates (ED_{90}) varied with the weed species and growth stage. Glyphosate-resistant giant ragweed was the most sensitive, with ≥ 90% control achieved regardless of growth stage, while 10-cm tall glyphosate-resistant common waterhemp was controlled by the recommended rate, though higher rates (≥ 2,480 g ae ha^{-1}) were required to control 20-cm tall plants. Of the three glyphosate-resistant broadleaf weeds studied, kochia was the least sensitive as 90% control was not achieved regardless of growth stage. Herbicide labels report optimal efficacy at a specific growth stage for different weed species, and several studies have reported reduced efficacy of POST herbicides as weed size increases (Everitt & Keeling, 2007; Robinson et al., 2012).

Managing glyphosate-resistant weeds is a serious concern for profitable crop production in few countries, including Canada and United States. Additionally, with the evolution of multiple-herbicide-resistant weeds, such as common waterhemp (Bell et al., 2013; Sarangi et al., 2014), kochia (Beckie et al., 2013), and Palmer amaranth (Jhala et al., 2014b), growers' options for effective POST herbicides have dwindled (Tranel et al., 2011). New multiple herbicide-resistant crop technologies can diversify existing herbicide programs by bringing together conventional herbicide chemistries, some of which, such as 2,4-D, are not labeled for POST application in soybean (Craigmyle et al., 2013a, 2013b). Enlist Duo™ has the potential to control selected glyphosate-resistant weeds, including common waterhemp and giant ragweed. However, over reliance on any herbicide may result in the evolution of resistant weeds. In fact, common waterhemp resistant to 2,4-D has been confirmed in Nebraska (Bernards et al., 2012). Therefore, to avoid selection pressure of herbicide(s) with the same mode(s) of action, growers should adopt integrated weed management approach that include the use of residual herbicides, tank mixing herbicides with different modes of action, and rotation of herbicide-resistant crop technologies in conjunction with cultural and mechanical weed control methods (Aulakh & Jhala, 2015; Beckie, 2011; Chahal & Jhala, 2015; Ganie et al., 2015; Norsworthy et al., 2012). The response of tested weed species in terms of visual control estimates and shoot biomass reduction to different rates of formulation of 2,4-D choline and glyphosate observed in this greenhouse study might be different under field conditions. Therefore, field efficacy trials are required to determine the effect of spray parameters and environmental conditions on efficacy of this herbicide for control of glyphosate-resistant weeds.

Table 1. Regression parameters (Equation 2) and formulation of 2,4-D choline and glyphosate (Enlist Duo™ herbicide) doses (g ae/ha) that provided 50 and 90% weed control [ED_{50} (± SE), ED_{90} (± SE)] on the basis of visual injury ratings at 21 days after treatment (DAT) in a greenhouse dose response study at University of Nebraska-Lincoln

Glyphosate-resistant weed species	**Regression parameters (± SE)[a]**		**ED_{50} (± SE)[a]**	**ED_{90} (± SE)[a]**
			--------------g ae ha^{-1}	-----------------
Common waterhemp	B	D		
10-cm tall	1.76 (0.10)	99 (0.88)	339 (14)	1179 (59)
20-cm tall	1.34 (0.10)	99 (1.12)	484 (32)	2480 (230)
Giant ragweed				
10-cm tall	5.14 (1.65)	97 (0.51)	350 (18)	825 (186)
20-cm tall	2.08 (0.36)	91 (1.34)	324 (19)	1101 (197)
Kochia				
10-cm tall	1.04 (0.10)	100 (1.44)	378 (41)	4382 (380)[b]
20-cm tall	1.28 (0.11)	75 (1.18)	1122 (64)	5305 (531)[b]

Note. ED_{50}, effective dose required for 50% control of glyphosate-resistant weeds; ED_{90}, effective dose required for 90% control of glyphosate-resistant weeds; SE, standard error. The values in parenthesis are standard errors.

[a] Regression parameters B and D for 3-parameter log-logistic model were obtained using the nonlinear least-squares function of the statistical software R.

[b] These values have limited biological meaning because 90% control of kochia, regardless of growth stage, was not achieved even with the highest rate of Enlist Duo™ used in this study.

Table 2. Regression parameters (Equation 2) and formulation of 2,4-D choline and glyphosate (Enlist Duo™ herbicide) doses (g ae/ha) that provided 50 and 90% weed control [ED_{50} (± SE), ED_{90} (± SE)] on the basis of shoot biomass reduction at 21 days after treatment (DAT) in a greenhouse dose response study at University of Nebraska-Lincoln

Glyphosate-resistant weed species	**Regression parameters (± SE)[a]**		**ED_{50} (± SE)[a]**	**ED_{90} (± SE)[a]**
			--------------g ae ha^{-1}	-----------------
Common waterhemp	B	D		
10-cm tall	1.60 (0.3)	99 (2.17)	303 (24)	883(330)
20-cm tall	0.98(0.05)	100 (1.13)	388 (88)	2668 (205)
Giant ragweed				
10-cm tall	1.58 (0.36)	98 (1.67)	201 (32)	805 (144)
20-cm tall	2.18 (0.42)	94 (1.12)	281 (22)	1142(231)
Kochia				
10-cm tall	0.88 (0.08)	100 (1.24)	227 (32)	3704 (336)[b]
20-cm tall	0.66 (0.10)	76 (1.32)	612 (87)	5885 (1,275)[b]

Note. ED_{50}, effective dose required for 50% shoot biomass reduction of glyphosate-resistant weeds; ED_{90}, effective dose required for 90% shoot biomass reduction of glyphosate-resistant weeds; SE, standard error. The values present in parenthesis are standard errors.

[a] Regression parameters B and D for 3-parameter log-logistic model were obtained using the nonlinear least-squares function of the statistical software R.

[b] This value has limited biological meaning because 90% shoot biomass reduction of 20-cm tall kochia was not achieved even with the highest rate of 2,4-D choline plus glyphosate used in this study.

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Notes

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