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## Enlist Weed Control Systems for Controlling Horseweed (Conyza canadensis) in Enlist Soybean

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#### Authors' contributions

This work was carried out in collaboration between all authors. Authors DMS and KKR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KKR, LAC, JME, LLG, RAH and LCW coordinated trial implementation and data submission. Author DMS managed the literature searches. All authors read and approved the final manuscript.

#### Article Information

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**Original Research Article** 

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### ABSTRACT

**Aims:** Evaluate glyphosate-resistant *Conyza canadensis* control with Enlist weed control systems that include sequential applications of burndown application prior to Enlist soybean (*Glycine max* (L.) Merr.) planting followed by an early postemergence application to Enlist soybean.

**Study Design:** Studies conducted as randomized complete block with 4 replications.

**Place and Duration of Study:** Twenty-one field studies were conducted between 2014 and 2015 growing seasons across soybean production areas in the United States.

**Methodology:** Prior to planting, burndown applications of glyphosate, glyphosate + 2,4-D choline, glufosinate, or glufosinate + 2,4-D choline were applied with and without sulfentrazone + cloransulam. At the V3 growth stage of Enlist soybean, postemergence applications of glyphosate, 2,4-D choline + glyphosate, glufosinate, 2,4-D choline + glufosinate or glyphosate + dicamba were applied according to the defined sequential program. Visual control ratings of weed control were taken at 4 weeks after each application.

**Results:** *Conyza canadensis* control at 4 weeks after the burndown application (28 DABA) was 54% for glyphosate, 97% for glyphosate + dicamba, 93% for 2,4-D choline + glyphosate, 85% for

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glufosinate, 92% for 2,4-D choline + glufosinate. Applying a second application of 2,4-D choline + glyphosate, 2,4-D choline + glufosinate or glyphosate + dicamba resulted in  $\geq$ 95% control of *Conyza canadensis* 28 days after sequential application. The addition of sulfentrazone + cloransulam to the first application provided more consistent control at both observation dates. **Conclusion:** Enlist E3 soybean enabled burndown applications or postemergence applications of 2,4-D choline Colex-D + glufosinate or glyphosate that provided >95% *Conyza canadensis* control. Residual herbicides sulfentrazone + cloransulam can be included in the burndown application to provide residual control. Early post-emergence applications of 2,4-D choline + glyphosate or glufosinate can be utilized to provide control of any surviving or newly emerged *Conyza canadensis*.

Keywords: Enlist E3; 2,4-D choline; Conyza canadensis; horseweed.

#### 1. INTRODUCTION

Conyza canadensis L., known as horseweed or marestail, is a member of the Asteraceae family that is native to North America. It can follow either a winter or summer annual life cycle. In northern regions, horseweed most typically emerges in the fall, overwinters as a basal rosette and bolts in the spring [1]. In southern regions, horseweed emerges predominately in the spring where it generally remains as a rosette briefly before bolting [2,3]. Mature plants can reach heights of 1.8 m. In the Midwest United States, horseweed will typically begin to flower at the end of July and disperse seed from August to October. A single horseweed plant can produce over 200,000 seeds with seed production being proportional to plant height [4.5.6]. Horseweed seeds are dispersed primarily by the wind but dispersal via water can also occur. Regehr and Bazzaz [4] reported seed deposition up to 122 m into a corn field downwind from the edge of a field occupied by horseweed plants. Dauer et al. [6] reported 99% of horseweed seed landed within 100 m of the source while 1% of horseweed seed dispersed at least 500 m from source populations.

Weed species composition and density change in response to crop management practices. Adoption of no-till or reduced tillage systems for soybean (*Glycine max* (L.) Merr.) production is commonly associated with a change in predominate weed species found in the soybean fields. Winter annuals previously controlled with tillage must be controlled by other methods such as herbicides in no-till systems [7]. No-till systems favor small seeded broadleaf and grass weeds which can germinate on the soil surface under crop residue. Populations of large seeded weeds that require deeper soil placement decline over time in no-till fields [8,9]. Regeher and Bazzaz [4] considered horseweed a successional winter annual that rapidly infests undisturbed sites such as no-till or abandoned fields. Horseweed is well adapted to no-till soybean fields as there is no seed dormancy, 95% of seeds germinate within 0 to 0.5 cm of the soil surface, seed production is prolific, and seeds are wind-dispersed across fields [4,10,11].

Glyphosate-resistant (GR) soybean varieties were introduced in 1996 and by 2006 were planted on >90% of soybean acres in the United States [12]. GR soybean enabled growers to simplify weed management, reduce time and labor inputs, reduce tillage, and reduce herbicide costs compared to weed control practices used before the introduction of glyphosate-resistant crops [12,13]. Young [13] concluded that growers abandoned the principles of sound weed and herbicide-resistance management by relying exclusively on glyphosate for weed control. In notill or reduced tillage soybean production, alvphosate was utilized to control winter annual weeds including horseweed prior to planting crops followed by a one or two postemergence applications of glyphosate during the cropping season to control summer annual broadleaf and grass weeds. After just 3 years of continuous use of glyphosate for weed control in continuous GR soybean, the first instance of glyphosate resistance in horseweed was found in Delaware 2000 [14]. Between 2001 and 2016 in glyphosate-resistant populations of horseweed were reported in 23 states across most crop production areas of the US [15]. GR horseweed can be highly competitive particularly in high densities and impact soybean yield. Eubank et al. [16] reported that soybean yields horseweed plots not treated with herbicide were reduced by 73 to 90% compared to plots treated with 2,4-D + glyphosate 4 weeks before planting.

Herbicides for foliar control of glyphosateresistant horseweed in burndown applications prior to soybean planting include 2,4-D, dicamba, cloransulam, chlorimuron, and saflufenacil [17,18]. Effective herbicide control options for controlling horseweed after glyphosate-resistant soybean emergence are currently limited to cloransulam and chlorimuron. Glufosinateresistant soybean allows glufosinate to be used for control horseweed after soybean emergence.

Horseweed control programs have focused on pre-plant control with herbicides. Horseweed with resistance to both ALS and glyphosate was reported as early as 2003 [15,19]. Loux [17] has suggested that horseweed weed control programs should assume multiple resistance and recommended a three step management program be implemented that includes: 1) fall application of 2,4-D; 2) effective burndown application in spring with 2,4-D ester or dicamba tank mixed with glyphosate or saflufenacil; and 3) include a herbicide with residual activity on horseweed in the burndown application. Addition of 2,4-D to glyphosate in the burndown application is effective for control of GR horseweed. Due to potential injury to soybean a period 7 to 30 days between 2,4-D application and planting is required depending on 2,4-D rate and formulation [18]. Dicamba can be used in burndown applications but requires a plant- back interval of 14 days for 280 g ae ha-1 and 28 days for 560 g ae ha-1 formulation [18]. Furthermore, Loux [17] suggests the use of glufosinate resistant soybean to allow application of glufosinate over the top of the crop for control of horseweed plants less than15 cm in height.

Enlist E3 soybean is a new herbicide-resistant trait jointly developed by Dow AgroSciences and MS Technologies that provides tolerance to 2,4-D, glyphosate and glufosinate [20]. Enlist E3 soybean express the aryloxyalkanoate dioxygenase-12 (AAD-12), double mutant 5-enolpyruvylshikimate-3-phosate synthase (2mEPSPS), and phosphinothricin acetyltransferase (PAT) proteins. AAD-12 protein provides tolerance to 2,4-D by rapid metabolic inactivation [21]. The 2mEPSPS protein has a decreased sensitivity to the herbicide glyphosate making the plant resistant to glyphosate [22]. The PAT enzyme acetylates the primary amino group of glufosinate rendering it inactive [20]. Enlist E3 soybean has tolerance to pre-emergence and postemergence applications of 2,4-D.

Enlist Duo® herbicide with Colex-D® Technology is a proprietary blend of 2,4-D choline and

glyphosate dimethylamine registered for postemergence use on Enlist E3<sup>™</sup> sovbean [23]. The registered use rates of Enlist Duo in the United States are 1640 or 2185 g ae ha<sup>-1</sup>, which contain 800 + 840 or 1065 + 1120 g ae ha<sup>-1</sup>, respectively, of 2,4-D choline and glyphosate dimethylamine. When GR weeds are present, the recommended rate is 2185 g ae ha<sup>-1</sup> rate. Enlist Duo may be applied as a burndown application to control weeds prior to planting with no plant-back restrictions. A maximum of two postemergence applications may be applied from soybean emergence to R2 growth stage.

Glufosinate products that allow applications over the top of glufosinate resistant soybean can be sprayed over the top of Enlist E3 soybean up to the R1 growth stage. Loux [17] recommended glufosinate in the burndown application and postemergence in glufosinate resistant soybean. Glufosinate at 0.47 or 0.59 kg ai ha-1 average control was 92% at 28 d after treatment [16]. However, Eubank et al. [16] suggested that lower control occurred under high density due to poor spray coverage. Steckel et al. [24] found glufosinate control of GR horseweed was temperature dependent as control provided by 0.47 kg ai ha<sup>-1</sup> applied at 30, 21 and 14 d before planting was 55, 53 and 88% when the 5-d average daily temperature prior to application was 8, 15 and 18 C, respectively.

Upon the commercial launch of Enlist E3 soybean, 2,4-D choline, glyphosate and glufosinate can be integrated into weed control programs to provide 3 effective modes of action for glyphosate susceptible weeds and 2 effective modes of action for glyphosate-resistant weeds. The objective of this research was to determine performance of sequential weed control programs containing 2,4-D, glyphosate, and/or glufosinate in the burndown and postemergence applications for the control of GR horseweed.

#### 2. MATERIALS AND METHODS

# 2.1 Research Sites, Crop, and Weed Information

Experiments were conducted during the 2014 and 2015 growing seasons across the Midwest and mid-south regions of the United States (Table 1). Sites were selected based on previous reports of GR horseweed. Enlist E3 soybean were planted at a population of 296,400 to 447,000 seeds ha<sup>-1</sup> at 19 locations and 2 locations were left fallow for the duration of the trial. Soybeans were planted within 4 weeks of the burndown herbicide application. At the time of the burndown application, the average horseweed plant height was determined by measuring from the soil surface to the top of the plant in the untreated control plots. Horseweed plants were between 3 and 18 cm at time of the burndown application. The density of horseweed in the untreated plots were determined by counting the number of plants within a 0.09 or 1 square meter area randomly chosen in each untreated plot. Soil texture, organic matter and pH are representative of cultivated fields in each area.

#### 2.2 Herbicides

Herbicides were used within their registered or anticipated registered rates for control of horseweed in Enlist E3 soybean (Table 2). Weed control programs consisted of a burndown application applied prior to planting followed by postemergence application at the V3 growth stage. In 2014, the burndown applications were made either with or without the inclusion of cloransulam + sulfentrazone as the residual herbicide (Table 3). Herbicides applied at V3 growth stage included: 2,4-D choline + glyphosate, glufosinate, 2,4-D choline + glufosinate, glyphosate or glyphosate + dicamba depending on the burndown application (Table 3). 2,4-D choline + glyphosate was applied at 1640 g ae/ha and the maximum use rate of 2185 g ae/ha. In 2015, herbicide treatments were refined to focus on burndown applications that contained a residual herbicide followed by a postemergence application at the V3 stage of soybean (Table 4). In both years, ammonium sulfate (N-PAK, Winfield Solutions, P.O. Box 64589, St. Paul, MN 55164-0589) was added at 2.5% v/v to the treatments containing 2,4-D choline + glyphosate, glyphosate and glufosinate treatments. Ammonium sulfate was added to treatments containing glyphosate + dicamba in 2014 but not in 2015 to align with label restrictions which prohibit use of ammonium sulfate. Herbicide applications were made with compressed CO<sub>2</sub> or air pressurized backpack sprayers calibrated to deliver 140 L ha<sup>-1</sup> at 19 locations, 168 L ha<sup>-1</sup> at 2 locations and 187 L ha<sup>-1</sup> at 3 locations.

# 2.3 Plot Size, Experiment Design and Weed Control Ratings

Experiments were designed as randomized complete blocks with three or four replications.

Each plot was 4 soybean rows wide and plot length varied from 6.1 to 12.2 m depending on location. Assessment of horseweed control from the burndown herbicide treatment were made approximately 28 days after application. Following the V3 application, assessment of horseweed control were made approximately at 28 days after application. Weed control was reported based on a scale of 0 (no control in the untreated check) to 100% (complete control) as compared to the untreated plots in the replicate [25].

#### 2.4 Statistical Analysis

Data were analyzed within year as treatments in 2015 focused on burndown treatments that contained residual herbicide. A linear mixed model (ANOVA) was fit using the Ime4 package in R version 3.2.1 (The R Foundation for Statistical Computing, c/o Institute for Statistics and Mathematics, Wirtschaftsuniversität Wie, Welthandelsplatz, 1020 Vienna, Austria). Herbicide treatment was the fixed effect and trial location and reps were random effects in the model. To satisfy ANOVA assumptions, weed control ratings were subjected to a Box-Cox transformation [26] using the car package in R that determined the power transformation which maximized the log likelihood function for each response variable. However, back-transformed data are presented with mean separations based on transformed data and means rounded to whole numbers. Where the ANOVA test indicated treatment effects were significant, means were separated at P <.05 using Tukey's HSD test.

#### 3. RESULTS AND DISCUSSION

Current glyphosate rate recommendations are 840 g ae/ha for control of horseweed less than 30 cm in height [27]. In 2014 trials, glyphosate at 1680 g ae ha<sup>-1</sup> provided between 25 and 58% control 28 d after burndown application (DABA) (Table 5). Based on level of glyphosate control 28 DABA, four sites were categorized as having high resistance (<40% control), five as moderate resistance (50-75% control), and one as low resistance (75 to 85% control). A range in level of resistance is consistent with previous research where GR horseweed treated with 1,100 g ae ha<sup>-1</sup> had 45% control in one trials and 80% control in second trial [14]. Control of a glyphosate-resistant population in Mississippi averaged 74% at 4 weeks after treatment with 1250 g ae ha<sup>-1</sup> [16]. Level of resistance increased with growth stage with 1,680 g ae ha<sup>-1</sup> rate providing 80-90% control of 5 leaf plants, 45-50% control of 13-15 leaf plants, and 40 to 50% control of 25 to 30 leaf plants [28]. Field populations of GR horseweed will vary in response to glyphosate depending on the progression of resistance in the field. Plant response can range from individuals being completely controlled by glyphosate to severe stunting with multiple stalks forming from the base of plant, to stunting of plant height for a period of time after application, to no visual injury from glyphosate application.

Presence of horseweed biotypes with multiple resistance to glyphosate and cloransulam at trial locations influence the interpretation of the data from the 2014 experiments. Comparison of horseweed control between glyphosate and glyphosate + cloransulam + sulfentrazone at each site was made to determine potential presence of multiple resistance (Table 5). Presence of multiple populations of glyphosateand cloransulam-resistant horseweed in Ontario, Canada, was based on survival following applications of glyphosate 900 g at ha<sup>-1</sup> + cloransulam 17.5 g ai ha<sup>-1</sup> [29]. Trainer et al. [19] reported 90% reduction of biomass in susceptible populations with cloransulam at 2 g ae ha<sup>-1</sup>. Since cloransulam + sulfentrazone treatments in this research contained 25 g ai ha-1 of cloransulam, greater than 85% control would be expected in the absence of ALS resistance. The combination of glyphosate + cloransulam + sulfentrazone provided >90% control of horseweed at only one of the eight locations (Table 5). At the other 7 locations, glyphosate + cloransulam + sulfentrazone provided <76% 28 DABA. These data support Loux's [17] position that multiple resistance should be assumed unless the population is known to be ALS susceptible.

In 2014, burndown application of glyphosate provided 45% control 28 DABA across eight locations (Table 6). The addition of cloransulam + sulfentrazone to glyphosate increased average control of horseweed populations with resistance to ALS and glyphosate to 73%. 2,4-D choline + glyphosate at low and high rates provided greater than 90% control and addition of cloransulam + sulfentrazone did not significantly affect control, regardless of resistance biotypes. This is in agreement with the research of Eubank et al. [16] and Kruger et al. [30] who found 2,4-D Simpson et al.; JEAI, 15(6): 1-13, 2017; Article no.JEAI.31813

alone or with glyphosate provide >90% control of GR horseweed.

Glufosinate treatments applied at burndown provided 89% control 28 DABA at 8 locations (Table 6). Previous research indicates that temperature and time of day may affect control with glufosinate treatments [31]. Analysis of control data by replication and trial revealed no trend for time of day or temperature to affect level of control reported (data not shown). Addition of cloransulam + sulfentrazone to treatments containing glufosinate improved the consistency in control and increased control to greater than 95%. Addition of 2,4-D choline to glufosinate in burndown slightly improved overall control (94-95%) at 28 DABA. Eubank et al. [16] and Steckel et al. [24] also showed the addition of 2,4-D to glufosinate improved control of GR horseweed.

Following the V3 applications, all treatments except the glyphosate treatments provided >92% control of horseweed at 28 days after postemergence application (DAPA). Horseweed control was not impacted by the addition of cloransulam + sulfentrazone in the burndown application compared to herbicide treatments without residual herbicide. Addition of 2,4-D choline with glufosinate in either the first or second application timing did tend to numerically increase the level of weed control.

In 2015, only herbicide treatments containing a residual herbicide were included, except glyphosate alone which was used to establish the level of glyphosate resistance present. Similar to 2014, control provided by glyphosate at 1680 g ae ha<sup>-1</sup> in 2015 varied by location with five sites averaging less than 55% control and seven sites averaging between 63 and 74% control at 28 DABA (Table 7). Horseweed control was >90% control at 4 locations when cloransulam + sulfentrazone was added, which may be the result of glyphosate resistance but no ALS resistance at those locations. Horseweed control with glyphosate + cloransulam + sulfentrazone was < 85% perhaps the result of resistance to glyphosate and cloransulam at the other eight locations. Since the presence or absence of ALS resistance could significantly influence the level of horseweed control in weed control programs that include cloransulam, the 2015 data were split into two subsets and presented as glyphosate-resistant and glyphosate- + ALS-resistant.

Year	Closest city	Soil chara	cteristics		Ар	plication & planti	ing dates Horseweed <sup>a</sup>		
		Texture	ОΜ <sup>ь</sup>	рΗ	Burndown date	Planting date	Postemergence date	Average height	Average density
			%	_				cm	No. m <sup>-1</sup>
2014	Queenstown, MD	Silt loam	2	5.8	June 4	June 17	July 17	15	4
2014	Whitestown, IN	Silt loam	3.4	6.5	May 2	May 27	June 20	10	33
2014	Novelty, MO	Silt loam	3.4	6.8	May 21	May 21	June 25	10	9
2014	Lincoln, NE	Silt loam	3.2	5.6	May 16	May 29	June 23	9	20
2014	Princeton, KY	Silt loam	2.6	6.7	May 8	fallow	June 21	4	NR
2014	Greenville, MS	Silt loam	1.4	6.3	April 9	May 1	May 26	18	11
2014	Portageville, MO	Sandy loam	1.4	5	April 25	May 9	May 28	15	22
2014	Knoxville, TN	Loam	1.6	6	May 3	fallow	May 28	10	10
2015	Georgetown, DE	Sandy loam	2.1	6.2	May 22	NR	June 19	NR	4
2015	Veedersburg, IN	Loam	2.9	7	May 13	May 21	June 20	5	120
2015	Veedersburg, IN	Sandy loam	1.7	5.5	May 13	May 21	June 20	13	10
2015	Pendleton, IN	Clay loam	2.8	7	May 12	May 20	June 20	NR	60
2015	Lebanon, IN	Loam	2.4	6.8	May 13	May 26	June 24	13	10
2015	Novelty, MO	Silt loam	3.5	6.4	May 1	May 13	June 17	5	11
2015	Lincoln, NE	Silt loam	3.6	5.4	April 29	May 22	June 20	5	20
2015	Princeton, KY	Silt loam	2.6	6.7	May 1	May 14	June 14	18	NR
2015	Murphysboro, IL	loam	1.7	5.7	May 1	May 13	June 24	15	16
2015	Greenville, MS	Silt loam	1.4	6.3	April 8		May 28	3	65
2015	Columbus Grove, OH	Clay loam	1.9	6.4	April 29	May 12	June 19	5	1
2015	South Charleston, OH	Silty clay loam	3	6	April 14	May 7	June 3	NR	NR
2015	Portageville, MO	Sandy loam	1.4	5	April 22	May 5	May 28	15	12

Table 1. Location, soil information, application dates, planting date, and weed height and density of GR horseweed in 2014 and 2015 experiments

<sup>a</sup>Average horseweed size and density at time of burndown herbicide application. <sup>b</sup>Abbreviations: OM, organic matter; NR, not reported

Trade name	Common name	Rate <sup>ab</sup>	Manufacturer	Manufacturer location
Enlist Duo®	2,4-D choline + glyphosate DMA	800 + 840 or 1065 + 1120	Dow AgroSciences	Indianapolis, IN
Sonic®	sulfentrazone + cloransulam	25 + 195	Dow AgroSciences	Indianapolis, IN
Durango® DMA	glyphosate DMA	1120	Dow AgroSciences	Indianapolis, IN
Roundup PowerMax	glyphosate DMA	1680	Monsanto	St. Louis, MO
Liberty 280SL	glufosinate	542	Bayer Crop Science	Research Triangle Park, NC
Clarity	dicamba	560	BASF Corp.	Research Triangle Park, NC
Valor XLT	chlorimuron + flumioxazin	21.6 + 62.7	Valent USA Corp.	Walnut Creek, CA 94596
	2,4-D choline	1065	Dow AgroSciences	Indianapolis, IN 46268

#### Table 2. Herbicides, rates and manufacturers

<sup>a</sup>Rates for 2,4-D choline, glyphosate DMA, glufosinate and dicamba are given in g ae ha<sup>1</sup>. All other rates are in g ai ha<sup>1</sup> <sup>b</sup>For premix herbicides the rate of individual active is in parenthesis

#### Table 3. Herbicide treatments in 2014 trials

Treatment	Burndown application		V3 application		
number	Herbicide <sup>a</sup>	Rate <sup>b</sup>	Herbicide	Rate <sup>b</sup>	
1	Glyphosate	1680	Glyphosate	1680	
2	2,4-D choline + glyphosate	800 + 840	2,4-D choline + glyphosate	800 + 840	
3	2,4-D choline + glyphosate	2185	2,4-D choline + glyphosate	2185	
4	Glufosinate	542	Glufosinate	542	
5	Glufosinate	542	2,4-D choline + glufosinate	1065 +542	
6	2,4-D choline + glufosinate	1065 + 542	Glufosinate	542	
7	2,4-D choline + glufosinate	1065 + 542	2,4-D choline + glufosinate	1065 + 542	
8	Glyphosate + dicamba	1120 + 560	Glyphosate + dicamba	1120 + 560	
9	Glyphosate + cloransulam + sulfentrazone	1680 + 25 + 195	Glyphosate	1680	
10	2,4-D choline + glyphosate + cloransulam + sulfentrazone	800 + 840 + 25 + 195	2,4-D choline + glyphosate	1650	
11	2,4-D choline + glyphosate + cloransulam + sulfentrazone	2185 + 25 + 195	2,4-D choline + glyphosate	2185	

Treatment number	Burndown applic	ation	V3 application		
	Herbicide <sup>a</sup>	Rate <sup>b</sup>	Herbicide	Rate <sup>b</sup>	
12	Glufosinate + cloransulam + sulfentrazone	542 + 25 + 195	Glufosinate	542	
13	Glufosinate + cloransulam + sulfentrazone	542 + 25 + 195	2,4-D choline + glyphosate	800 + 840	
14	Glufosinate + cloransulam + sulfentrazone	542 + 25 + 195	2,4-D choline + glyphosate	2185	
15	Untreated		untreated		

<sup>a</sup>All herbicide treatments herbicide treatments contained 2.5% ammonium sulfate except glyphosate + dicamba treatments. <sup>b</sup>Rates for 2,4-D choline, dicamba, glufosinate, and glyphosate are expressed as g ae ha<sup>-1</sup> and cloransulam and sulfentrazone rates is expressed as g ai ha<sup>-1</sup>

### Table 4. Herbicide treatments in 2015 trials

Treatment	Burndown application	V3 application		
number	Herbicide <sup>a</sup>	Rate <sup>b</sup>	Herbicide	Rate <sup>⊳</sup>
1	Glyphosate	1680	Glyphosate	1680
2	Glyphosate + cloransulam + sulfentrazone	1680 + 95 + 195	Glyphosate	1680
3	2,4-D choline + glyphosate + cloransulam + sulfentrazone	800 + 840 + 95 + 195	2,4-D choline + glyphosate	800 + 840
4	2,4-D choline + glyphosate + clorimuron + flumioxazin	800 + 840 + 21.5 + 62.8	2,4-D choline + glyphosate	800 + 840
5	2,4-D choline + glyphosate + cloransulam + sulfentrazone	1065 + 1120 + 95 + 195	2,4-D choline + glyphosate	1065 + 1120
6	Glufosinate + cloransulam + sulfentrazone	542 + 95 + 195	Glufosinate	542
7	Glufosinate + cloransulam + sulfentrazone	542 + 95 + 195	2,4-D choline + glyphosate	800 + 840
8	Glufosinate + cloransulam + sulfentrazone	542 + 95 + 195	2,4-D choline + glyphosate	1065 + 1120
9	Glyphosate + dicamba	1120 + 560	Glyphosate + dicamba	1120 + 560
10	Glyphosate + dicamba + chlorimuron + flumioxazin	1120 + 560 + 21.5 + 62.8	Glyphosate + dicamba	1120 + 560
11	Untreated		untreated	

<sup>a</sup>All herbicide treatments herbicide treatments contained 2.5% ammonium sulfate except glyphosate + dicamba treatments. <sup>b</sup>Rates for 2,4-D choline, dicamba glyphosate, and glufosinate are expressed as g ae ha<sup>-1</sup> and cloransulam, flumioxazin, and chlorimuron rates are expressed as g ai ha<sup>-1</sup>

Nearest city	Horseweed control 28 DABA			
	Glyphosate	Glyphosate + Cloransulam + Sulfentrazone		
	%	%		
Queenstown, MD	57	57 <sup>a</sup>		
Whitestown, IN	58	74 <sup>a</sup>		
Novelty, MO	35	98		
Lincoln, NE	55	76 <sup>a</sup>		
Princeton, KY	50	73 <sup>a</sup>		
Greenville, MS	35	69 <sup>a</sup>		
Portageville, MO	50	76 <sup>a</sup>		
Knoxville, TN	25	55 <sup>°</sup>		

Table 5. Level of glyphosate resistance and potential for ALS resistance based on control 28
days after burndown application (DABA) with applications of glyphosate with and without
cloransulam + sulfentrazone for 2014 locations

<sup>a</sup>Indicates population with moderate to high resistance to cloransulam

In 2015, control of GR horseweed was greater than 94% at 28 DABA when cloransulam + sulfentrazone was applied at burndown with glyphosate, 2,4-D choline + glyphosate or glufosinate (Table 8). In contrast, control of glyphosate + ALS resistant horseweed was not significantly (P<0.05) increased when cloransulam + sulfentrazone was applied with glyphosate. For control of glyphosate + ALS resistant horseweed, 2,4-D choline + glyphosate + cloransulam + sulfentrazone or glyphosate + dicamba provided greater than 94% control. Glufosinate + cloransulam + sulfentrazone averaged 89% control at 28 DABA but a sequential application of glufosinate or 2,4-D choline + glyphosate was needed to achieve >96% control at 28 DAPA of glyphosate + ALS resistant horseweed.

In response to a rapid increase of herbicide resistance in the United States, Norsworthy et al. [32] published an extensive review on reducing the risks of herbicide resistance in which twelve best practices critical for herbicide management resistance were defined. United States EPA has recently begun to mandate herbicide resistance management plans be included in re-registrations and registrations herbicide, including 2,4-D choline of glyphosate. The 2,4-D choline + glyphosate product label specifically recommends scouting fields before and after applications, applying full rates of 2,4-D choline + glyphosate, using a broad spectrum soil applied herbicide with other modes of action, use of sequential applications of herbicides with alternative modes of action and incorporating

non-chemical weed control practices as recommended [32].

Developing horseweed weed control programs requires understanding the herbicide-resistant biotypes present in the field. Control strategy for a dominantly fall germinating biotype will need to consider for weed management options in the fall and early spring. In contrast, a control strategy of spring emerging biotypes will need to consider options prior to planting in spring and throughout the sovbean growing season. A horseweed population that has both fall and spring emerging biotype will require control options in fall, early spring and during the crop life cycle. Thus, the nature of horseweed biotypes that comprise any given population may vary field by field. As an example, in 2015, two trial locations near Veedersburg, Indiana, USA were 8 km apart with one location having only fall germinating horseweed and the other location having only spring germinating horseweed. Presence of ALS resistance may impact the herbicide selection for horseweed control. Weed control programs must also consider other weeds in the fields and the need to prevent potential development of resistance in other species.

The Enlist E3 soybean enables growers to integrate up to five modes of action for a highly effective horseweed management program. The program concepts presented here align with the recommendations of Loux et al. [17] for spring burndown, use of residual herbicide and postemergence applied herbicides during the soybean life cycle to control glyphosate-resistant horseweed.

Burndown herbicide treatment <sup>ab</sup>	POST herbicide treatment	Horsewe	ed control
		28 DABA <sup>c</sup>	28 DAPA <sup>c</sup>
			%
Glyphosate	Glyphosate	45 A <sup>d</sup>	53 A
2,4-D choline + glyphosate $(L)^{c}$	2,4-D choline + glyphosate (L)	92 CD	97 C
2,4-D choline + glyphosate $(H)^{c}$	2,4-D choline + glyphosate (H)	95 CD	99 C
Glufosinate	Glufosinate	89 C	92 C
Glufosinate	2,4-D choline + glufosinate	91 CD	97 C
2,4-D choline + glufosinate	Glufosinate	94 CD	97 C
2,4-D choline + glufosinate	2,4-D choline + glufosinate	95 CD	98 C
Glyphosate + dicamba	Glyphosate + dicamba	97 CD	99 C
Glyphosate + cloransulam + sulfentrazone	Glyphosate	73 B	73 B
2,4-D choline + glyphosate + cloransulam + sulfentrazone	2,4-D choline + glyphosate (L)	97 CD	99 C
2,4-D choline + glyphosate + cloransulam + sulfentrazone	2,4-D choline + glyphosate (H)	98 D	99 C
Glufosinate + cloransulam + sulfentrazone	Glufosinate	95 CD	96 C
Glufosinate + cloransulam + sulfentrazone	2,4-D choline + glyphosate (L)	96 CD	97 C
Glufosinate + cloransulam + sulfentrazone	2,4-D choline + glyphosate (H)	95 CD	99 C

#### Table 6. Horseweed control in 2014 following burndown and V3 applications

<sup>a</sup>Herbicide programs consisted of a burndown application applied approximately 14 days before planting followed by a V3 application. <sup>b</sup>All herbicide treatments herbicide treatments contained 2.5% ammonium sulfate except glyphosate + dicamba treatments.

<sup>c</sup>Abbreviations: DABA, days after burndown application; DAPA, days after postemergence application; L, 1640 g ae ha<sup>-1</sup> rate; H, 2185 g ae ha<sup>-1</sup>

<sup>d</sup>Means followed by the same letter within column are not significantly different based on Tukey SHD ( $\alpha = 0.05$ )

#### Table 7. Level of glyphosate resistance and potential for ALS resistance based on control provided by burndown application of glyphosate with and without cloransulam + sulfentrazone for 2015 locations

Nearest city	Horseweed control 28 DABA				
	Glyphosate	Glyphosate + Cloransulam + Sulfentrazone			
		%			
Veedersburg, IN	48	75 <sup>a</sup>			
Veedersburg, IN	74	72 <sup>a</sup>			
Pendleton, IN	51	56 <sup>a</sup>			
Lebanon, IN	73	75 <sup>a</sup>			
Novelty, MO	33	98			
Lincoln, NE	54	83 <sup>a</sup>			

Nearest city	Horseweed control 28 DABA			
-	Glyphosate	Glyphosate + Cloransulam + Sulfentrazone		
		%		
Princeton, KY	63	70 <sup>a</sup>		
Murphysboro, IL	41	98		
Greenville, MS	75	96		
Columbus Grove, OH	63	83 <sup>a</sup>		
South Charleston, OH	68	85 <sup>°</sup>		
Portageville, MO	68	96		

<sup>a</sup>Indicates population with moderate to high resistance to cloransulam

#### Table 8. Control of glyphosate or glyphosate + ALS resistant horseweed at 28 days after burndown application (DABA) or days after postemergence application (DAPA) in 2015

Burndown herbicide treatment <sup>ab</sup>	Post herbicide treatment		Horsewe	ed control	
		28 DABA		28 DAPA	
		Glyphosate + ALS- resistant	Glyphosate- resistant	Glyphosate + ALS- resistant	Glyphosate- resistant
			c	%	
Glyphosate	Glyphosate	61 A <sup>C</sup>	54 A	67 A	52 A
Glyphosate + cloransulam + sulfentrazone	Glyphosate	76 A	94 B	83 B	100 B
2,4-D choline + glyphosate (L) + cloransulam + sulfentrazone	2,4-D choline + glyphosate (L)	94 BC	100 B	99 C	100 B
2,4-D choline + glyphosate (L) + chlorimuron + flumioxazin	2,4-D choline + glyphosate (L)	89 B	99 B	99 C	100 B
2,4-D choline + glyphosate (H) + cloransulam + sulfentrazone	2,4-D choline + glyphosate (H)	96 BC	100 B	99 C	100 B
Glufosinate + cloransulam + sulfentrazone	Glufosinate	88 B	93 B	98 C	100 B
Glufosinate + cloransulam + sulfentrazone	2,4-D choline + glyphosate (L)	90 B	97 B	98 C	100 B
Glufosinate + cloransulam + sulfentrazone	2,4-D choline + glyphosate $(H)$	90 B	98 B	96 C	99 B
Glyphosate + dicamba	Glyphosate + dicamba	97 C	97 B	99 C	100 B
Glyphosate + dicamba + chlorimuron + flumioxazin	Glyphosate + dicamba	99 C	99 B	99C	100 B

<sup>a</sup>Herbicide programs consisted of a burndown application applied approximately 14 days before planting followed by a V3 application. <sup>b</sup>All herbicide treatments contained 2.5% ammonium sulfate except glyphosate + dicamba treatments.

<sup>c</sup>Means followed by the same letter within column are not significantly different based on Tukey SHD ( $\alpha = 0.05$ )

#### 4. CONCLUSIONS

Enlist E3 soybean enabled burndown applications or postemergence applications of 2,4-D choline + glyphosate or glufosinate that provided >95% control of glyphosate resistant horseweed. Residual herbicides sulfentrazone + cloransulam can be included in the burndown application to provide residual control. Early postemergence applications of 2,4-D choline + glyphosate or glufosinate can be utilized to provide control of any surviving or newly emerged horseweed.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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