

Control of giant ragweed (*Ambrosia trifida*) in mesotrione-resistant soybean

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Research Article

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Abstract

Preemergence applications of mesotrione, an herbicide that inhibits 4-hydroxyphenolpyruvate dioxygenase (HPPD), have recently gained regulatory approval in soybean varieties with appropriate traits. Giant ragweed is an extremely competitive broadleaf weed, and biotypes resistant to acetolactate synthase inhibitors (ALS-R) can be particularly difficult to manage with soil-residual herbicides in soybean production. This study investigated control of giant ragweed from preemergence applications of cloransulam (32 g ai ha⁻¹), metribuzin (315 g ai ha⁻¹), and S-metolachlor (1,600 g ai ha⁻¹) in a factorial design with and without mesotrione (177 g ai ha⁻¹) at two different sites over 2 yr. Treatments with mesotrione were also compared with two commercial premix products: sulfentrazone (283 g ai ha⁻¹) and cloransulam (37 g ai ha⁻¹), and chlorimuron (19 g ai ha⁻¹), flumioxazin (69 g ai ha⁻¹), and pyroxasulfone (87 g ai ha⁻¹). At 42 d after planting, control and biomass reduction of giant ragweed were greater in treatments with mesotrione than any treatment without mesotrione. Giant ragweed biomass was reduced by 84% in treatments with mesotrione, whereas treatments without mesotrione did not reduce biomass relative to the nontreated. Following these preemergence applications, sequential herbicide treatments utilizing postemergence applications of glufosinate (655 g ai ha⁻¹) plus fomesafen (266 g ai ha⁻¹) and S-metolachlor (1,217 g ai ha⁻¹) resulted in at least 97% control of giant ragweed at 42 d after planting, which was greater than sequential applications of glufosinate alone in 3 of 4 site-years. Preemergence applications of mesotrione can be an impactful addition to soybean herbicide programs designed to manage giant ragweed, with the potential to improve weed control and delay the onset of herbicide resistance by providing an additional effective herbicide site of action.

Introduction

Giant ragweed is a dicotyledonous annual broadleaf in the *Asteraceae* family. Though giant ragweed is native to North America with a core range in central Ohio, the species has spread throughout parts of Asia and Europe (Hovick et al. 2018; Montagnani et al. 2017). Giant ragweed has an early germination period that continues throughout the growing season, high genetic diversity and germination polymorphism, and can be extremely competitive with crops like soybean (Abul-Fatih and Bazzaz 1979; Bassett and Crompton 1982; Baysinger and Sims 1991; Schutte et al. 2012). As few as one giant ragweed plant per square meter has been shown to reduce soybean yields by up to 77%, due in part to the ability of giant ragweed to rapidly accumulate biomass and reach heights of up to 6 m (Bassett and Crompton 1982; Webster et al. 1994). Considered by many growers to be among the most troublesome weeds in soybean production, giant ragweed appears to be spreading westward across the Corn Belt, and the number of infested row-crop acres has been increasing (Barnes et al. 2004; Gibson et al. 2006; Harre et al. 2017; Regnier et al. 2016). Additionally, giant ragweed is adapting to agricultural environments and targeted control practices through higher reproductive allocation, altered emergence patterns, and the evolution of herbicide-resistance mechanisms, reflecting the high phenotypic plasticity that is often associated with this species (Abul-Fatih and Bazzaz 1979; Albert et al. 2011; Hovick et al. 2018; Patzoldt and Tranel 2002; Stachler 2008).

Several families of herbicides that inhibit the acetolactate synthase (ALS) enzyme are effective for foliar and soil-residual control of giant ragweed, though multiple applications of these herbicides coupled with other herbicide modes of action and nonchemical management tactics are often necessary for season-long control of heavy infestations in soybean fields (Baysinger and Sims 1992; Franey and Hart 1999; Ganie et al. 2016; Johnson et al. 2007; Taylor et al. 2002). The substantial dependence on ALS inhibitors, such as cloransulam, for control of giant ragweed has contributed to the evolution and proliferation of biotypes resistant to ALS inhibitors (ALS-R) (Jasieniuk et al. 1996; Norsworthy et al. 2012; Tranel and Wright 2002). The presence of ALS-R giant ragweed was first documented in 1998 (Patzoldt and Tranel 2002) and has since been confirmed in seven US states and Ontario, Canada (Heap 2022). These biotypes exhibit

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high-level cross-resistance to at least three classes of ALS-inhibiting herbicides (sulfonyleureas, imidazolinones, and triazolopyrimidines) without incurring a fitness cost (Marion et al. 2017; Patzoldt and Tranel 2002).

Giant ragweed resistance to ALS inhibitors severely impacts effective management with preemergence, soil-residual herbicides (Loux et al. 2022; Taylor et al. 2002). In soybean production, these ALS-R biotypes were controlled with postemergence applications of glyphosate until the evolution and subsequent spread of glyphosate-resistant biotypes in 2004, and multiple-resistant biotypes in 2006 (Gower et al. 2003; Heap 2022; Stachler 2008). In 2016, a survey reported the suspicion and/or confirmation of giant ragweed with some form of herbicide resistance in 57% of responding midwestern counties, with multiple-resistant biotypes reported in 12 states (Regnier et al. 2016). A study conducted on giant ragweed populations collected from fields throughout the State of Indiana identified glyphosate-resistant giant ragweed plants in 83% of the fields where ALS-R biotypes were found (Harre et al. 2017). Despite the widespread distribution of ALS-R biotypes, the use of ALS inhibitors for partial control of giant ragweed may still be warranted, as most populations appear to be segregating for resistance (Boe 2019; Harre et al. 2017). In fact, biotypes susceptible to ALS inhibitors outnumbered resistant biotypes in more than 70% of the fields sampled in Indiana (Harre et al. 2017).

Though several postemergence herbicides such as protoporphyrin oxidase (PPO) inhibitors, auxin mimics, and glufosinate are still effective for control of multiple-resistant giant ragweed (Barnett et al. 2013; Jhala et al. 2014; Kaur et al. 2014; Loux et al. 2022; Norsworthy et al. 2010; Vink et al. 2012), maintaining giant ragweed control through the critical weed-free period of 8 to 10 wk after soybean emergence is difficult without the use of effective soil-residual herbicides (Baysinger and Sims 1991). In corn production, 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides have been used for soil-residual control of several broadleaf weed species including giant ragweed (Givens et al. 2009; Mitchell et al. 2001; Sutton et al. 2002). In these systems, the HPPD inhibitor mesotrione is often applied preemergence in combination with atrazine, a photosystem II inhibitor. This strategy typically results in greater than 80% control of giant ragweed (Belfry and Sikkema 2015; Bollman et al. 2006; Loux et al. 2011; Soltani et al. 2011).

Preemergence applications of mesotrione have received federal approval for use in soybean varieties with genetically engineered resistance to HPPD inhibitors (Anonymous 2021; USDA-APHIS 2013a; 2013b). Resistance is conferred by the insertion of a mutant HPPD gene derived either from oat (*Avena sativa* L.) in 'SYHT0H2' cultivars (Hawkes et al. 2015; Hipskind et al. 2012; USDA-APHIS 2013b), or from *Pseudomonas fluorescens* in LibertyLink®-GT27® varieties (Boudec et al. 2001; USDA-APHIS 2013a). Expression of HPPD enzymes derived from these mutant HPPD genes have a lower binding affinity for HPPD-inhibiting herbicides (Boudec et al. 2001; Hawkes et al. 2015), endowing soybean with resistance to rates of mesotrione similar to those used in corn production. Applying multiple, effective herbicide modes of action is one of the most effective tools for slowing the onset of herbicide resistance (Jasieniuk et al. 1996; Norsworthy et al. 2012; Young 2006). As such, the joint application of HPPD inhibitors with other effective soybean preemergence herbicides has the potential to increase giant ragweed control and decrease selection for herbicide resistance in giant ragweed. However, it should likewise be noted that the use of HPPD inhibitors in soybean may

reduce the efficacy of herbicide rotation as a resistance-management strategy when these chemistries are also used in the rotational cropping systems.

No research evaluating mesotrione applied preemergence in soybean for soil-residual control of giant ragweed is present in the literature. Because mesotrione has been an effective tool for management of this weed in corn production, the co-application of this herbicide with other soybean preemergence herbicides may be a robust control tactic in future weed management programs. Therefore, the primary objectives of this study were to determine the extent of giant ragweed management and response of HPPD inhibitor-resistant soybean to (i) preemergence applications of mesotrione alone and in combination with cloransulam, metribuzin, and/or S-metolachlor for control of predominantly susceptible or ALS-R giant ragweed populations, and (ii) sequential herbicide combinations (i.e., preemergence followed by post-emergence) utilizing either glufosinate or glufosinate plus fomesafen and S-metolachlor applied postemergence.

Materials and Methods

Field Experiments

Two field experiments were conducted in 2018 and 2019 at two sites near West Lafayette, Indiana: the Throckmorton Purdue Agriculture Center (TPAC; 40.17° N, 86.54° W), and a commercial field, TIP-1 (40.26° N, 87.04° W). The field at TPAC (pH 6.6, 2.9% organic matter) consisted primarily of a Toronto-Millbrook silt loam complex, whereas the TIP-1 field (pH 5.7, 1.8% organic matter) was a combination of Mahalasville, Treaty and Rainsville silt loams. The frequencies of ALS-R giant ragweed to susceptible biotypes at TPAC and TIP-1 prior to initiating this research were 10% and 70%, respectively (N.T. Harre, unpublished data).

Each experimental area was sprayed with paraquat (Gramoxone 2.0 SL®; Syngenta Crop Protection, Greensboro, NC) followed by a combination of disking and field cultivating prior to planting. Soybean was planted at 346,000 seeds ha⁻¹ in 76-cm rows at a depth of 2.5 to 5 cm. A 'SYHT0H2' soybean cultivar (maturity group 3; Syngenta) (Hipskind et al. 2012) was planted in May of 2018, and due to the deregulation of LibertyLink®-GT27® varieties, 'Stine 33GA13' soybeans (USDA-APHIS 2013a) were planted in June of 2019 after a wet spring that delayed field operations. Plots were 3m by 9m with herbicides applied to the center two rows of each four-row plot using a CO₂-pressurized backpack sprayer and a 2-m handheld spray boom equipped with extended-range, flat-fan nozzles (XR 8002VS; TeeJet® Spraying Systems, Wheaton, IL) delivering 140 L ha⁻¹ at 207 kPa. All preemergence herbicides were applied immediately following planting.

Two experiments were initiated at each site, with either preemergence-only or sequential (preemergence/postemergence) applications. The preemergence-only experiment used a factorial design to evaluate the addition of mesotrione (177 g ai ha⁻¹) to other soybean preemergence herbicide programs that included cloransulam (32 g ai ha⁻¹), metribuzin (315 g ai ha⁻¹), and/or S-metolachlor (1,600 g ai ha⁻¹) for soil-residual control of giant ragweed and soybean response (Table 1). The second experiment was a factorial of preemergence and postemergence herbicide treatments applied sequentially. Both experiments included two commercial herbicide premixes commonly recommended for preemergence control of giant ragweed in soybean: sulfentrazone (283 g ai ha⁻¹) and cloransulam (37 g ai ha⁻¹), and chlorimuron

Table 1. Sources of herbicides used in field experiments.

Common name	Trade name	Manufacturer	Manufacturer location	Manufacturer website
Mesotrione	Callisto®	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com
Cloransulam	Firstate®	Corteva Agriscience	Indianapolis, IN	www.corteva.com
Metribuzin	Tricor® DF	UPL NA Inc.	King of Prussia, PA	www.upl-ltd.com
S-metolachlor	Dual Magnum®	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com
Sulfentrazone + cloransulam	Authority® First DF	FMC Corp.	Philadelphia, PA	www.fmc.com
Chlorimuron + flumioxazin + pyroxasulfone	Fierce® XLT	Valent USA Corp.	Walnut Creek, CA	www.valent.com
Glufosinate	Liberty® 280 SL	BASF Corp.	Research Triangle Park, NC	www.basf.com
S-metolachlor + fomesafen	Prefix®	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com

(19 g ai ha⁻¹), flumioxazin (69 g ai ha⁻¹), and pyroxasulfone (87 g ai ha⁻¹). The postemergence application included either glufosinate (655 g ai ha⁻¹) or glufosinate plus fomesafen (266 g ai ha⁻¹) and S-metolachlor (1,217 g ai ha⁻¹). The postemergence applications were made at 21 d after planting (DAP) when soybeans were at the V2 growth stage and included ammonium sulfate (N-PAK® AMS Liquid; Winfield Solutions, LLC, St. Paul, MN) at 10 g L⁻¹.

Frequency of Resistance to ALS Inhibitors

Both experiments were conducted at two different field sites based on the expected prevalence of giant ragweed biotypes resistant to ALS inhibitors. The only mechanism known to confer ALS-R in giant ragweed is a single-nucleotide polymorphism in the *ALS* gene (Trp754Leu) (Marion et al. 2017; Patzoldt and Tranel 2002). Thus, a high-throughput molecular assay designed by Harre et al. (2017) was appropriate for resistance screening (Délye et al. 2015; Yu and Powles 2014). In 2018, 16% and 71% of the plants sampled in the nontreated plots of each experiment were resistant to ALS inhibitors at TPAC and TIP-1, respectively. In 2019, the experiments were conducted in a different field at TPAC where the frequency of ALS-R was considerably greater (57%) than the year prior, though it remained similar at TIP-1 (73%).

Data Collection

The area within the center two soybean rows was evaluated. Soybean injury and giant ragweed control were visually rated at 14, 21, and 42 DAP on a scale of 0 to 100%, with 0 being no injury and 100 being plant death. Ratings were also taken at 28 and 35 DAP in the sequential experiment. Soybean stand counts were taken at 21 DAP by counting the number of plants in 1 m of each of the center two rows in each plot, and the average count per meter of row was analyzed. Giant ragweed density counts were taken at 21 and 42 DAP, and biomass was collected at 42 DAP and oven-dried at 50 C until the weight was constant. Density counts were taken by randomly placing a 0.5-m² quadrat at two different locations between the center two soybean rows in each plot. These locations were marked with wire flags, and the quadrats were placed in the same locations for the 21 and 42 DAP counts. Biomass was harvested from the same quadrat area used for density counts. Density and biomass data were combined over locations within each plot, yielding a total measured area of 1 m² per plot for analysis. Both biomass and density measurements for each treated plot were analyzed as a percent reduction compared with the nontreated. Other weed species such as fall panicum (*Panicum dichotomiflorum* Michx.), ivyleaf morningglory (*Ipomoea hederacea* Jacq.), and velvetleaf (*Abutilon theophrasti* Medik.) were

present in the experiments, but these infestations were minor relative to giant ragweed (data not presented).

Experimental Design and Analysis

Treatments were arranged in a randomized complete block design with four replications. Each experiment was conducted twice at both sites over the 2018 and 2019 growing seasons. Data were tested for normality and constancy of variance using PROC UNIVARIATE in SAS® 9.4 (SAS Institute Inc., Cary, NC), or through visual inspection of histograms and quantile-quantile (Q-Q) plots of the residuals, and plots of residuals vs. fitted values. All data were subjected to ANOVA using PROC GLIMMIX in SAS®. Treatment means were separated using Tukey-Kramer's HSD at an alpha level of 0.05.

In the preemergence-only experiment, fixed effects included a factorial of residual herbicide treatments, the addition of mesotrione, site, and year. Data were combined over sites, years, and residual herbicides other than mesotrione when these effects and their associated interactions were not significant ($P > 0.05$). Orthogonal contrasts were used to compare the combined efficacy of all treatments that included mesotrione with the commercial standards. In the sequential experiment, fixed effects included a factorial of preemergence and postemergence herbicide treatment, year, and site. Similarly, data were combined over years, sites, and treatments where appropriate.

Results and Discussion

Soybean Injury

Preemergence herbicide treatments did not cause soybean stand loss in either experiment (data not presented). In prior research evaluating soybean varieties that expressed the mutant *HPPD* gene from *Pseudomonas fluorescens* (*hppdPfw336*), soybean biomass was reduced by up to 25% after a preemergence application of 210 g ai ha⁻¹ of mesotrione (Schultz et al. 2015). In the present study, both soybean varieties showed robust resistance to preemergence applications of mesotrione at the rate used in these experiments (177 g ai ha⁻¹). Though soybean biomass and yield were not evaluated, no stunting or bleaching symptomology consistent with injury from an HPPD inhibitor was observed in either experiment in treatments with mesotrione. Data for general soybean injury (stunting, chlorosis, leaf malformation) were combined over sites and separated by year based on ANOVA. In 2018, an average of 11% soybean injury was observed from applications of the premix of chlorimuron, flumioxazin, and pyroxasulfone at 14 DAP, though injury from this treatment was less than 6% at subsequent rating timings. This level of injury at 14 DAP was greater than all other treatments, where less than 5%

Table 2. Efficacy of preemergence herbicides with or without mesotrione on giant ragweed 42 d after planting at two sites in Indiana.^a

Herbicide	Control ^b		Density reduction 2018 ^c					
	Without mesotrione		With mesotrione		Without mesotrione		With mesotrione	
None	–		82	a	–		86	a
Cloransulam	20	bc	86	a	52	ab	77	a
Metribuzin	4	d	82	a	9	bc	91	a
S-metolachlor	3	d	83	a	7	c	76	a
Cloransulam + metribuzin	29	b	87	a	69	a	76	a
Cloransulam + S-metolachlor	23	bc	88	a	59	a	82	a
Metribuzin + S-metolachlor	12	d	82	a	13	bc	77	a
Cloransulam + metribuzin + S-metolachlor	30	b	90	a	53	ab	76	a

^aMeans within a column under the same heading that are followed by the same letter are not different according to Tukey-Kramer's HSD ($\alpha = 0.05$).

^bControl ratings were combined over data collected in 2018 and 2019.

^cDensity reduction in each treatment was determined relative to the nontreated.

injury was observed at each evaluation timing (data not presented). In 2019, soybean injury was less than 6% in all treatments and at all rating timings (data not presented). The difference in injury between years may be attributed to variable environmental conditions or the differential response to PPO-inhibiting herbicides inherent to some soybean varieties (Dayan et al. 1997). Soybean genetics related to mesotrione resistance did not lead to the difference in injury between years.

Similar to the preemergence-only experiment, 11% injury was observed in 2018 from preemergence applications of the premix of chlorimuron, flumioxazin, and pyroxasulfone at 14 DAP in the sequential experiment, and less than 6% injury was observed in all treatments in 2019 (data not presented). For evaluations in the sequential experiment between 28 and 42 DAP, soybean injury data were combined over years, sites, and preemergence treatments. Injury at 28 DAP (1 wk after the postemergence application) was greater in treatments that included glufosinate plus fomesafen and S-metolachlor (17%) than treatments that only included glufosinate (6%) ($t_1 = 16.82$, $P < 0.0001$). Soybean injury remained evident in treatments with glufosinate plus fomesafen and S-metolachlor at the 42 DAP rating (5%), which was greater than in treatments where only glufosinate was applied post-emergence (1%) ($t_1 = 11.88$, $P < 0.0001$). Injury in the former treatment was consistent with applications of fomesafen (bronzing and spray droplet-sized necrotic lesions) (Hager et al. 2003; Legleiter and Bradley 2008).

Giant Ragweed Efficacy: Preemergence-Only Experiment

In the preemergence-only experiment at 21 DAP, control of giant ragweed was 80% or greater across years and sites in treatments with mesotrione (data not presented). Efficacy in many treatments had declined by 42 DAP, resulting in the greatest differences between treatments at the 42 DAP timing. Thus, further discussion of the efficacy of these treatments on giant ragweed will consist of data collected at 42 DAP. The interaction between site and treatment was not significant for any response variable, so all data were combined over sites. Control data were also combined over years. Among the factorial herbicide treatments, giant ragweed control was greater in treatments that included cloransulam (20% to 30%) than treatments that only included metribuzin and/or S-metolachlor (3% to 12%) (Table 2). Giant ragweed control was improved with the addition of mesotrione, including mesotrione alone, ranging from 82% to 90% (Table 2). Despite the factorial interaction of mesotrione with the other residual herbicides, the

effect of mesotrione ($F_{1,206} = 2,078$) was greater than the effect of the other herbicides ($F_{7,206} = 11$), resulting in greater control in treatments with mesotrione (85%) compared with those same treatments without mesotrione (17%) ($F_{1,107} = 1,621$, $P < 0.0001$). Including additional preemergence herbicides with alternative modes of action did not increase giant ragweed control beyond mesotrione alone (Table 2). However, the addition of these herbicide mode-of-action groups should at least partially reduce the risk of giant ragweed evolving resistance to mesotrione.

Overall, control of giant ragweed with mesotrione in this experiment was similar to what other researchers have found in corn production. In a study summarizing data over 2 yr across four midwestern states, control from the highest rate of mesotrione (210 g ai ha⁻¹) ranged from approximately 75% to 90% (Bollman et al. 2006), similar to what has been reported by other research groups (Belfry and Sikkema 2015; Loux et al. 2011). In agreement with these prior studies, control of giant ragweed in treatments with mesotrione was 82% to 90% in this study (Table 2).

In 2018, the density of giant ragweed was reduced more with cloransulam than with S-metolachlor (Table 2). Giant ragweed density was similar between treatments with cloransulam and treatments with mesotrione, despite mesotrione resulting in greater control (Table 2). Whereas the number of giant ragweed plants may have been similar between these treatments in 2018, many plants in treatments where mesotrione was applied had emerged later in the season than plants in treatments without mesotrione, which is demonstrated in the subsequent comparison of biomass data. In 2019, density data were combined over herbicide treatments, as mesotrione was the only significant main effect and there was no interaction between the main effects. Giant ragweed density was reduced by 86% in treatments with mesotrione relative to the nontreated, whereas treatments without mesotrione reduced density by 32% (Table 3). Biomass data were combined over years, sites, and herbicides other than mesotrione. Giant ragweed biomass was reduced by 84% in treatments with mesotrione, whereas treatments without mesotrione increased biomass by 34% relative to the nontreated (Table 3). Although an increase in biomass may seem counterintuitive, these other herbicides reduced the competition from other weed species present in the trial (data not presented), allowing the surviving giant ragweed plants to grow more vigorously in these plots compared with the nontreated plots.

The efficacy of mesotrione for control of giant ragweed was also compared with two soybean herbicide premixes that are

Table 3. Combined efficacy of preemergence herbicides for control of giant ragweed 42 d after planting at two sites in Indiana.^a

Herbicide treatments ^b	Density reduction 2019 ^c		Biomass reduction ^d	
	%			
Without mesotrione	32	b	-34	b
With mesotrione	86	a	84	a

^aMeans within a column are not different according to Tukey-Kramer's HSD ($\alpha = 0.05$).

^bData from preemergence herbicide treatments were combined into two groups based on the inclusion of mesotrione.

^cDensity and biomass reduction were determined relative to the nontreated.

^dBiomass reduction was calculated from data collected in 2018 and 2019. Negative values indicate that giant ragweed biomass was greater in treated plots compared with the nontreated.

Table 4. Efficacy of mesotrione combinations on giant ragweed 42 d after planting compared with two commercial standard herbicide premixes at two sites in Indiana in 2018 and 2019.

Herbicide	Control	Density reduction ^a	Biomass reduction
		%	
Mesotrione mixtures ^b	85	83	84
Sulfentrazone + cloransulam	22	45	-38
Chlorimuron + flumioxazin + pyroxasulfone	43	53	-10
Contrast 1 ^c	***	***	***
Contrast 2	***	***	***

^aDensity and biomass reduction were determined relative to the nontreated. Negative values indicate that giant ragweed density and/or biomass were greater in treated plots compared with the nontreated.

^bMeans pooled over all treatments that contained mesotrione.

^cOrthogonal contrast 1: mesotrione mixtures vs. sulfentrazone + cloransulam; orthogonal contrast 2: mesotrione mixtures vs. chlorimuron + flumioxazin + pyroxasulfone. Significance designated as *** = $P < 0.001$.

considered to be commercial standards for residual control of giant ragweed: sulfentrazone and cloransulam, and chlorimuron, flumioxazin, and pyroxasulfone. Orthogonal contrasts were used to determine that giant ragweed control, density reduction, and biomass reduction were greater for the pooled mesotrione treatments compared with each of the commercial premixes at 42 DAP (Table 4). Across all of the herbicide treatments in the preemergence-only experiment, only those treatments that included mesotrione reduced giant ragweed biomass relative to the nontreated plots (Tables 3 and 4).

Control of giant ragweed with cloransulam was similar at both sites, despite a lower frequency of ALS-R at TPAC. Even at TPAC in 2018, when the frequency of ALS-R was 16% in the nontreated plots, control in treatments with cloransulam (but without mesotrione) was less than 40% at 42 DAP (data not presented). This was similar to what has been observed with preemergence-applied ALS inhibitors in previous research on giant ragweed populations that included ALS-R biotypes (Taylor et al. 2002). In 2018, the average density of giant ragweed in the nontreated plots at TPAC was greater than 70 plants m^2 (data not shown). Even with an assumption of 100% control of susceptible plants with cloransulam, more than 10 resistant plants per m^2 would still remain, which could result in near-complete yield loss if left unmanaged (Baysinger and Sims 1991; Webster et al. 1994). Other researchers have suggested that use of ALS inhibitors for control of ALS-R giant ragweed should be "de-emphasized" (Taylor et al. 2002). However, because this herbicide can be highly effective on

susceptible biotypes (Franeý and Hart 1999; Loux et al. 2022), cloransulam still has value for partial control of segregating populations. When supplemented with other effective preemergence herbicides like mesotrione, in conjunction with postemergence herbicides and nonchemical control tactics, the utility of ALS inhibitors like cloransulam may be sustainable (Boe 2019).

Giant Ragweed Efficacy: Sequential Experiment

Similar to the preemergence-only experiment, treatments with mesotrione in the sequential experiment generally resulted in greater control of giant ragweed at 21 DAP than treatments without mesotrione, including the commercial standard premixes (data not presented). Glufosinate is often highly effective for foliar control of giant ragweed (Kaur et al. 2014; Wiesbrook et al. 2001). In this experiment, all giant ragweed plants that had emerged by 21 DAP (10 to 15 cm) were controlled by the postemergence application of either glufosinate or glufosinate plus fomesafen and S-metolachlor. At 42 DAP, the main effects of preemergence herbicide treatment and the interaction of the preemergence treatment with the postemergence application were not significant. Therefore, data for each postemergence treatment were combined over respective preemergence treatments.

Similar trends were observed across response variables at 42 DAP. Across all site-years, control of giant ragweed at 42 DAP and biomass reduction were greater than 90%, regardless of which postemergence treatment was applied (Table 5). At both sites in 2018, giant ragweed control, density reduction, and biomass reduction were greater in treatments where glufosinate plus fomesafen and S-metolachlor were applied, compared with treatments that only included glufosinate (Table 5). The largest difference between treatments was observed in 2018, where giant ragweed density was reduced an additional 27% with the inclusion of fomesafen and S-metolachlor. In 2019, efficacy was similar between postemergence treatments at TPAC, while the inclusion of fomesafen and S-metolachlor increased the efficacy of the postemergence application at TIP-1 (Table 5). No significant precipitation was recorded at TPAC after the postemergence herbicides were applied in 2019, whereas TIP-1 received 3.7 cm of rainfall between 21 and 42 DAP (Table 6). The lack of activating rainfall at TPAC likely reduced the soil-residual activity of fomesafen at that site. Regardless, efficacy of both postemergence applications was 93% or greater across all response variables in 2019 (Table 5).

Glufosinate is rapidly degraded by soil microbes, and applications do not result in soil-residual weed control (Aulakh and Jhala 2015; Bartsch and Tebbe 1989; Takano and Dayan 2020). In the preemergence-only experiment, applications of S-metolachlor alone did not control giant ragweed (Table 2). Several studies have shown that fomesafen can be highly effective for foliar control of giant ragweed (Barnett et al. 2013; Baysinger and Sims 1992; Norsworthy et al. 2011; Taylor et al. 2002). This experiment demonstrates that soil-residual control of giant ragweed with fomesafen is also possible, given sufficient activating rainfall.

High-level resistance to foliar applications of fomesafen has been recently confirmed in a giant ragweed population from Wisconsin (Faleco et al. 2021), though the characterization of the resistance mechanism is limited thus far. Fomesafen is a diphenylether herbicide that inhibits the PPO enzyme. In common ragweed (*Ambrosia artemisiifolia* L.), an Arg98Leu mutation in the *PPX2* target-site gene has been shown to confer an 80-fold level of resistance to postemergence applications of fomesafen, and a

Table 5. Efficacy of postemergence herbicides 42 d after planting on giant ragweed at two sites near West Lafayette, IN.^{a-c}

Herbicide ^e	Control			Density reduction ^d			Biomass reduction											
	Combined 2018	TPAC 2019	TIP-1 2019	Combined 2018	TPAC 2019	TIP-1 2019	Combined 2018	TPAC 2019	TIP-1 2019									
Glufosinate	90	b	97	a	95	B	66	b	93	a	93	b	95	b	100	a	99	b
Glufosinate + fomesafen + S-metolachlor	98	a	97	a	97	A	93	a	95	a	97	a	100	a	100	a	100	a

^aAbbreviations: Tip-1, a commercial field; TPAC, Throckmorton Purdue Agriculture Center.

^bPostemergence herbicides were applied at 21 d after planting, and means were calculated from data combined over preemergence herbicide treatments.

^cMeans within a column with the same letter are not different according to Tukey-Kramer's HSD ($\alpha = 0.05$).

^dDensity and biomass reduction were determined relative to the nontreated.

^eAll postemergence applications included ammonium sulfate at 9.53 kg ha⁻¹.

Table 6. Weekly rainfall accumulation and average temperature at two Indiana field sites.^{a,b}

WAP	2018				2019			
	TPAC	TIP-1	TPAC	TIP-1	TPAC	TIP-1	TPAC	TIP-1
	cm		C		cm		C	
1	4.6	4.8	26	24	2.1	3.3	21	21
2	1.3	2	21	21	0.8	1.7	19	18
3	6.4	5.2	23	22	6.9	7.6	21	20
4	1.5	3.1	26	25	0	0	25	25
5	7.5	3	22	21	0	3.3	26	25
6	0.1	2.6	26	26	0	0.4	25	25

^aData for TPAC and TIP-1 were generated by weather stations at the Throckmorton Purdue Agricultural Center and the Purdue University Airport (KLAFA), respectively.

^bAbbreviations: Tip-1, a commercial field; TPAC, Throckmorton Purdue Agriculture Center; WAP, wk after planting.

10-fold level of resistance to the PPO inhibitor flumioxazin applied preemergence (Rousonelos et al. 2012). The efficacy of fomesafen for soil-residual control of PPO inhibitor-resistant giant ragweed has not yet been evaluated, though resistance to both preemergence and postemergence applications of fomesafen has been documented in several weed species (Heap 2022; Lillie et al. 2020). Whether preemergence-applied PPO inhibitors are still effective for control of PPO inhibitor-resistant weeds is highly dependent on species and resistance mechanism, in addition to the specific rate and type of PPO inhibitor applied (Copeland et al. 2018; Lillie et al. 2020; Wuerffel et al. 2015).

Overall, these results demonstrate that mesotrione can be more effective than many existing soybean herbicides for preemergence control of multiple-resistant giant ragweed. A survey of giant ragweed infestations in Indiana indicated that biotypes susceptible to ALS inhibitors outnumber resistant biotypes in nearly half of ALS-R populations (Harre et al. 2017), and only one instance of resistance to PPO inhibitors has been reported globally (Faleco et al. 2021), though not yet confirmed in a published research article. Mesotrione, cloransulam, and fomesafen can all contribute soil-residual control of giant ragweed populations that are segregating for resistance, which is important for managing weeds throughout the critical weed-free period of soybean. Sequential herbicide applications can be utilized in addition to nonchemical weed management tactics such as reduced tillage (Harrison et al. 2003), crop rotation (Goplen et al. 2017; Regnier et al. 2016), and cover crops (Regnier et al. 2016) to control giant ragweed throughout the soybean growing season and reduce selection for herbicide resistance. Integration of these management strategies is essential for sustainable management of this extremely competitive species with a propensity to develop resistance to herbicides.

Practical Implications

Soybean growers have few herbicide options for soil-residual control of giant ragweed. This research demonstrates that mesotrione applied preemergence in soybean varieties having appropriate traits can result in more effective residual control of giant ragweed than many herbicides currently available for use in soybean. Furthermore, sequential applications of foliar herbicides that target smaller weeds typically result in greater control and a lower propensity for resistance development. Greater soil-residual control allows more time for growers to make sequential herbicide applications to appropriately sized weeds, partially mitigating the challenges associated with managing a large number of hectares. Ultimately, when applied in combination with other soil-residual herbicides such as cloransulam and as a part of sequential application programs with foliar herbicides such as glufosinate and fomesafen, mesotrione can be used to control giant ragweed throughout the soybean growing season and reduce the selection for herbicide-resistant biotypes through the integration of multiple, effective modes of action. Conversely, excessive reliance on HPPD inhibitors to control weeds in additional rotational crops could serve to accelerate the development of resistant weed species if this technology is not stewarded appropriately.

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