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Trifludimoxazin mixtures for preplant burndown weed control in soybean

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Research Article

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Nomenclature:
Glufosinate; glyphosate; paraquat; saflufenacil; trifludimoxazin; giant ragweed, *Ambrosia trifida* L.; horseweed, *Erigeron canadensis* L.; waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; soybean, *Glycine max* (L.) Merr.

Keywords:
Additivity; antagonism; synergism

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Abstract

Trifludimoxazin is a novel protoporphyrinogen oxidase (PPO)-inhibiting herbicide currently under development for foliar and residual control of several problematic weeds in preplant applications for soybean production. Field experiments were conducted in 2017 and 2018 to evaluate the foliar efficacy of trifludimoxazin applied alone and in combination with other herbicides on waterhemp, giant ragweed, and horseweed. Foliar applications of trifludimoxazin alone at 12.5 or 25.0 g ai ha^{-1} were highly efficacious on glyphosate-resistant waterhemp (94% to 99% control) and moderately effective on giant ragweed (78% to 79% control) and resulted in minor efficacy on horseweed (≤20% control). Combinations of trifludimoxazin with glufosinate, glyphosate, paraquat, or saflufenacil remained highly effective (≥91% control) on waterhemp and giant ragweed. All herbicide mixtures with trifludimoxazin applied to horseweed were classified as additive interactions. Greenhouse experiments and Isobole analysis indicated that trifludimoxazin mixtures with glyphosate and glufosinate on waterhemp and giant ragweed were additive. Mixtures of trifludimoxazin + paraquat were slightly antagonistic under greenhouse conditions when applied to either waterhemp or giant ragweed, whereas trifludimoxazin + saflufenacil was synergistic when applied to giant ragweed. Overall, trifludimoxazin applied alone at 12.5 or 25.0 g ha^{-1} is effective for managing waterhemp and, to an extent, giant ragweed, but not horseweed, in preplant burndown applications. Furthermore, the addition of glufosinate, glyphosate, paraquat, or saflufenacil to applications of trifludimoxazin does not appreciably reduce weed control for these mixtures. As such, applications of trifludimoxazin alone and in combination with these herbicides may be utilized for effective preplant management of several problematic weeds in soybean.

Introduction

In Indiana, waterhemp, giant ragweed, and horseweed are among the most problematic weeds in soybean production (Gibson et al. 2005). In the eastern Corn Belt, giant ragweed and waterhemp emergence can begin in mid-March and mid-April, respectively, and continues throughout much of the soybean growing season (Heneghan 2016; Johnson et al. 2007). Horseweed, in contrast, can grow as a winter annual or summer annual and is capable of germination and emergence almost year-round, depending on geography (Buhler and Owen 1997). Soybean yield loss resulting from weed competition varies by species, but season-long interference has been documented to reduce soybean grain yields by 56% with waterhemp, 77% with giant ragweed, and as much as 90% with horseweed (Bensch et al. 2003; Bruce and Kells 1990; Webster et al. 1994). As a result, effective management approaches are necessary to minimize crop yield loss resulting from competition from these weeds.

Effective weed management often begins with planting crops into weed-free fields. While tillage has historically been an effective means for reducing competition from winter annuals and early-germinating summer annual weeds, adoption of reduced- or no-till practices predominates, with ~70% of U.S. soybean producers implementing some manner of conservation tillage (Claassen et al. 2018). A reduction in tillage intensity can facilitate increased diversity among weeds that are present (Murphy et al. 2006), and use of nonselective herbicides for preplant weed management in soybean has become commonplace (Lanie et al. 1994). Historically, glyphosate has been the most common nonselective herbicide used for preplant vegetation management; however, glyphosate resistance has been problematic in a number of species, including glyphosate-resistant waterhemp, giant ragweed, and horseweed in Indiana (Davis et al. 2008; Givens et al. 2009; Harre et al. 2017; Heap 2024). The challenge in managing these herbicide-resistant weeds has led to the use of other nonselective herbicides, such as paraquat and glufosinate, to manage resistant weed biotypes (Eubank et al. 2008). In addition to diversification of herbicides used, mixtures of herbicides can be implemented to improve the spectrum of weeds controlled. This practice is especially useful when using selective

Table 1. Sources of herbicides used for field and greenhouse experiments.

Common name	Trade name	Manufacturer	Manufacturer website
Glufosinate	Liberty®	BASF (Research Triangle Park, NC, USA)	http://www.basf.com/
Glyphosate	Roundup PowerMAX®	Bayer Crop Science (St. Louis, MO, USA)	http://www.bayer.com/
Paraquat	Gramoxone®	Syngenta Crop Protection (Greensboro, NC, USA)	http://www.syngenta.com/
Saflufenacil	Sharpen®	BASF	http://www.basf.com/
Trifludimoxazin	Tirexor®	BASF	http://www.basf.com/

herbicides like 2,4-D, dicamba, or saflufenacil, particularly when glyphosate-resistant weeds are present (Eubank et al. 2013; Robinson et al. 2012; Spaunhorst and Bradley 2013).

The efficacy of these herbicide mixtures is paramount, as a variety of outcomes regarding plant response are possible following their co-application. Specifically, the three most common responses are synergy, additivity, and antagonism (Colby 1967). For weed control, additivity and synergy are both desirable outcomes, as plant response following the co-application of multiple herbicides is equal to or greater than the expected response of each herbicide applied independently (Flint et al. 1988). Utilizing additive or synergistic mixtures can improve the spectrum of weeds controlled, while simultaneously reducing time and monetary inputs associated with multiple successive herbicide applications (Hatzios and Penner 1985). Moreover, synergistic combinations are particularly beneficial in providing high levels of weed control with reduced herbicide rates, as well as improved control of herbicide-resistant weed biotypes (Walsh et al. 2012). Conversely, reductions in herbicide efficacy because of antagonism between two co-applied herbicides can result in a failed herbicide application. Optimizing herbicide use patterns to control herbicide-resistant weeds has arguably never been more important, as there are more than 500 unique cases of herbicide resistance encompassing more than 270 species and 21 herbicide modes of action (MOAs) (Heap 2024).

Trifludimoxazin is a novel protoporphyrinogen oxidase (PPO)-inhibiting herbicide currently under development for preplant applications in a number of crops, including soybean, corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.) (Asher et al. 2020; Findley et al. 2020). Previous reports have indicated that trifludimoxazin may be applied either alone or in combination with other herbicides for broad-spectrum control of several problematic weed species, including those that are resistant to commercial PPO inhibitors (Findley et al. 2020). Scientific literature on the efficacy of trifludimoxazin alone or in mixture with other standard herbicides used in preplant applications is scant. Therefore our objectives were to (1) determine the efficacy of foliar applications of trifludimoxazin compared with glufosinate, glyphosate, paraquat, and saflufenacil and (2) investigate potential mixture interactions between trifludimoxazin and the other four herbicides when applied to waterhemp, giant ragweed, or horseweed.

Materials and Methods

Field Efficacy

Three field experiments were conducted in 2017 and 2018 utilizing foliar applications of trifludimoxazin alone (12.5 or 50 g ai ha^{-1}) and in combination with glyphosate (870 g ae ha^{-1}), glufosinate (590 g ai ha^{-1}), paraquat (840 g ai ha^{-1}), or saflufenacil (25 g ai ha^{-1}) on waterhemp, giant ragweed, and horseweed. Information regarding herbicide manufacturers for products used can be found in Table 1. Experiments were established in fallow field areas at locations with endemic near monocultures of each target weed species. Waterhemp and horseweed experiments were conducted near Brookston, IN (40.58°N, 86.77°W), with native populations of both species having high levels of resistance to glyphosate. Giant ragweed experiments were conducted at the Throckmorton Purdue Agriculture Center near Lafayette, IN (40.29°N, 86.90°W). Experiments implemented plots measuring 3 × 9 m, arranged in a randomized complete-block design (RCBD) with four replicates.

Herbicide treatments were applied using a CO_2-pressured backpack sprayer with a 2-m handheld spray boom equipped with four flat-fan TeeJet® XR8002 spray tips (TeeJet® Technologies, Glendale Heights, IL, USA) calibrated to deliver 140 L ha^{-1} at 276 kPa. In addition to the aforementioned herbicides, methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL, USA) and ammonium sulfate (N-Pak AMS Liquid, Winfield Solutions, St. Paul, MN, USA) were added to each treatment at 1% v/v and 1% w/w, respectively, as each is either required or permitted for the labeled use of each product. Relatively large weeds were targeted for each species in an effort to elicit sublethal response in weeds, as applications of individual herbicides resulting in approximately 50% control are most useful for analyzing herbicide interactions (Colby 1967; Meyer and Norsworthy 2019). Applications were performed when average weed height was 15 to 20 cm for waterhemp and 20 to 25 cm for giant ragweed and horseweed. Four randomly selected plants within each plot measuring 18 cm (waterhemp) or 23 cm (giant ragweed and horseweed) were marked at the time of application for further evaluation. The average density of waterhemp, giant ragweed, and horseweed was 450, 100, and 400 plants m^{-2}, respectively. Owing to the high weed density within plots, vegetation immediately surrounding the marked plants was manually removed prior to application to facilitate adequate herbicide coverage on marked plants during application and to reduce localized competition after application. Visual estimates of control for whole plots, in addition to marked plants within each plot, were assessed at 3, 7, 14, and 21 or 28 d after application (DAA) using a 0 (no control) to 100 (complete plant death) scale. Waterhemp and horseweed experiments were terminated at 28 DAA, but data collection for giant ragweed experiments was concluded at 21 DAA owing to high levels of biomass accumulation in nontreated plots at that timing. Following the final visual evaluation, plant height was recorded in the marked plants within each plot, and aboveground biomass was collected by clipping the plants at the soil surface. Plants harvested for biomass evaluation were oven-dried at 60 C for 7 d, then weighed. Both height and biomass data were converted to relative percentages of the height or weight from the nontreated plot within each replicate.

Visual estimates of control and height/biomass reduction data were subjected to ANOVA using the PROC GLIMMIX procedure

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in SAS (version 9.4; SAS Institute, Cary, NC, USA), and significant means were separated using Tukey's HSD ($\alpha = 0.05$). Herbicide treatment was considered a fixed effect, whereas year and replication were treated as random effects. Data were analyzed separately by species and combined over years as a result of nonsignificant Treatment × Year interaction within species. Colby's method was used to evaluate interactions between trifludimoxazin and the other four herbicides for the data collected at the final evaluation timing. Assessment via Colby's method requires the calculation of expected control values for combinations of herbicides using the following equation:

$$E = (X + Y) - \left[\frac{(XY)}{100}\right] \quad [1]$$

where E is the expected level of control when two herbicides are applied in mixture and X and Y represent the control observed from each herbicide applied individually. Control values observed for mixtures in the field were compared with the calculated expected values via a two-sided t-test ($\alpha = 0.05$), where a significant deviation of the observed value from the expected value indicated either synergism or antagonism (Lancaster et al. 2019; Walsh et al. 2012).

Greenhouse Isobole Analysis

Greenhouse experiments were conducted to further characterize the interaction of trifludimoxazin and glufosinate, glyphosate, paraquat, or saflufenacil on waterhemp and giant ragweed using the Isobole method (Akobundu et al. 1975; Berenbaum 1989; Tammes 1964). In general, Colby's method for analysis of herbicide interactions is appropriate for field research where the number of treatments can be limited, whereas the Isobole method provides a more complete analysis of the herbicide interaction across a more robust response range. However, the Isobole method requires preliminary herbicide dose–response experiments and large sets of herbicide dose interactions, which may be reasonable only with the smaller experimental units found in controlled environment experiments.

Isobole methodology was adapted from Armel et al. (2007), who utilized a concentration addition (CA) joint action reference model (Abendroth et al. 2011; Cedergreen 2014; Cedergreen et al. 2008) to create isobolograms predicting the efficacy of herbicide combinations based on the relative potencies of their component parts. This iteration of the Isobole method assumes that the efficacy of a mixture of two herbicides, at a fixed ratio (based on relative potency), is equal to the efficacy of the individual components, unless the herbicides are acting antagonistically or synergistically. To assess potential antagonistic or synergistic interactions with this method, several doses of each herbicide were applied alone, and the rate required for each herbicide to elicit a 50% response level (GR_{50} value) was calculated. The GR_{50} values were plotted on an x-y coordinate graph, and an "independent action line" was created by connecting the values for each herbicide. The independent action line indicated the infinite combination of doses of each of the herbicides that should provide a 50% response for additive interactions. Additionally, herbicide combinations were applied at fixed ratios based on the relative potencies of the individual components of the mixture, as determined by preliminary experiments (Armel et al. 2007).

Preliminary dose–response assays were conducted to determine the relative potency of each herbicide evaluated compared with trifludimoxazin using five rates of each herbicide. Data were subjected to nonlinear regression using a four-parameter log-logistic model,

Table 2. Relative potency, compared to trifludimoxazin, of herbicides applied to waterhemp and giant ragweed in greenhouse experiments, based on calculated GR_{50} values from preliminary dose–response assays and analysis via four-parameter log-logistic regression.

	Herbicide			
Weed species	Glufosinate	Glyphosate	Paraquat	Saflufenacil
Waterhemp	20:1	300:1	30:1	0.75:1
Giant ragweed	35:1	65:1	30:1	0.3:1

$$f(x) = c + \frac{d - c}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [2]$$

where b is the slope of the curve, c is the lower asymptote, d is the upper asymptote, and e is the GR_{50} value, via the DRC package in R (version 3.6.2; Knezevic et al. 2007). GR_{50} values from glufosinate, glyphosate, paraquat, and saflufenacil were compared with trifludimoxazin to elucidate the relative potency of each herbicide (Table 2) and rate structures for subsequent interaction experiments were based on the calculated potencies.

Seeds from a waterhemp population susceptible to both glyphosate and PPO inhibitors were sown in 25 × 50-cm greenhouse flats containing commercial potting mix (Fafard Germinating Mix, Sun Gro Horticulture, Agawa, MA, USA). Seedlings were transplanted to 164-cm^3 cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers, Stuewe and Sons, Tangent, OR, USA) filled with a 2:1 mixture of potting soil and sand when seedlings reached the 1-leaf stage and were allowed to grow until the 4- to 6-leaf stage (6 cm average height). Giant ragweed seeds were stratified in a 3:1 mixture of sand to soil for 4 wk following the methodology described by Westhoven et al. (2008) to alleviate dormancy. After a 4-wk stratification, seeds were sown in greenhouse flats containing commercial potting mix, similar to waterhemp. Following germination and expansion of cotyledons, seedlings were transplanted to square, 10 × 10-cm pots filled with a 2:1 mixture of potting soil and sand. Seedlings were allowed to grow until 4 true leaves were fully expanded (6 cm average height), at which point, herbicide applications were made. Both waterhemp and giant ragweed were watered daily and fertilized weekly using a micro- and macronutrient fertilizer (Jack's Classic Professional 20-20-20, JR Peters, Allentown, PA, USA) throughout the course of the experiments.

Herbicide applications were made using a track-mounted research sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN, USA) calibrated to deliver 140 L ha^{-1} at 207 kPa with an even flat-fan TeeJet® XR8002E (TeeJet® Technologies) spray tip. For waterhemp experiments, six rates of trifludimoxazin (0 to 1.6 g), glufosinate (0 to 32 g), glyphosate (0 to 480 g), paraquat (0 to 48 g), and saflufenacil (0 to 1.2 g) were applied alone and in combinations of each herbicide based on the relative potency of each herbicide (Table 2). In giant ragweed experiments, trifludimoxazin (0 to 13.5 g), glufosinate (0 to 473 g), glyphosate (0 to 878 g), paraquat (0 to 405 g), and saflufenacil (0 to 4.05 g) plus combinations were performed. All herbicide treatments included methylated seed oil (MSO Ultra) and ammonium sulfate (N-Pak AMS Liquid) 1% v/v and 1% w/w, respectively.

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Table 3. Calculated GR_{50} values from greenhouse experiments as determined by nonlinear regression using a log-logistic four-parameter model.

Herbicide	GR_{50} value (±95% CI)	
	Waterhemp	Giant ragweed
	g ai/ae ha^{-1}	
Trifludimoxazin	0.17 (0.12–0.21)	0.92 (0.63–1.21)
Glufosinate	43.6 (11.3–75.9)	49.2 (38.9–59.7)
Glyphosate	66.8 (41.4–92.2)	45.5 (33.4–57.6)
Paraquat	9.91 (8.49–11.3)	23.6 (16.8–30.4)
Saflufenacil	0.15 (0.13–0.17)	0.38 (0.21–0.44)
Trifludimoxazin + glufosinate	7.60 (6.20–9.00)	21.2 (9.30–33.2)
Trifludimoxazin + glyphosate	37.0 (27.8–46.2)	37.5 (18.9–56.2)
Trifludimoxazin + paraquat	4.27 (3.70–4.85)	17.9 (13.7–22.2)
Trifludimoxazin + saflufenacil	0.17 (0.15–0.18)	0.38 (0.27–0.48)

Experiments were conducted utilizing a two-factor (Herbicide × Rate) factorial, RCBD, with 10 replicates, and repeated once for each species. Visual estimates of control were made at 3, 7, and 14 DAA utilizing a 0 to 100 scale, as described previously. At 14 DAA, aboveground biomass was collected by clipping plants at the soil surface. Collected plant tissue was oven-dried for 7 d at 60 C, and data were normalized according to the nontreated check within each species–herbicide combination. Biomass data were analyzed via four-parameter log-logistic regression using Equation 2 to calculate GR_{50} values for each herbicide or herbicide combination (Table 3), with data pooled over runs due to a lack of Treatment × Run interaction, as determined by ANOVA ($\alpha = 0.05$). Isobolograms were created, as previously described, using the GR_{50} values for individual herbicides to create a line of independent action for each herbicide combination. Calculated GR_{50} values, along with 95% confidence intervals, for herbicide combinations were partitioned proportionally into each component part according to the relative rates of each herbicide used within a mixture. These values were then plotted on the same graph as the independent action line for each herbicide combination within species. Interactions were classified based on the relative position of the GR_{50} values for herbicide combinations in comparison with the independent action line, where antagonism was indicated by a value above the line, synergy below the line, and additivity when the value did not deviate from the line.

Results and Discussion

Waterhemp

Trends in control of marked plants reflected observations on the whole-plot level, with, generally speaking, higher control in marked plants relative to the whole plot. Lower control on the whole-plot level can likely be attributed to reduced herbicide coverage as a result of the high weed density and plant height at application. Marked plants were more uniform in height at herbicide application relative to plants across the entire plot and were used to determine biomass and height reductions compared with nontreated checks. Although both whole-plot data and marked plant data are presented, discussion herein pertains only to marked plant data.

Foliar applications of trifludimoxazin alone in the field translated to rapid and near-complete control of waterhemp with a high frequency of glyphosate-resistant individuals within the population. By 3 DAA, control of marked waterhemp plants was 95% and 96% for trifludimoxazin applied at 12.5 and 25.0 g ha^{-1}, respectively (Table 4). The rapid onset of observed symptomology was similar to the quick-acting contact activity displayed in treatments containing saflufenacil or paraquat, where control on marked plants was 89% and 97%, respectively, at 3 DAA (Table 4). In contrast, applications of glufosinate (32%) and glyphosate (5%) were in the early stages of symptom development at 3 DAA. At later evaluation timings, similar trends were observed, with applications of trifludimoxazin and paraquat providing 94% to 100% control of marked plants 28 DAA (Table 4). Waterhemp regrowth following saflufenacil treatment was observed over the course of the experiment, ultimately resulting in less control (81%) at 28 DAA than the peak activity at 3 DAA (Table 4). Applications of glufosinate resulted in low levels (36%) of waterhemp control at 28 DAA, consistent with previous research that has demonstrated reduced glufosinate efficacy in relatively taller weeds, such as those targeted in the present study (Barnett et al. 2013; Steckel et al. 1997). As anticipated, applications of glyphosate alone remained the least effective herbicide treatment for the glyphosate-resistant population evaluated in this experiment, providing 12% control of marked waterhemp plants at 28 DAA.

Although waterhemp control under field conditions exceeded 91% for all combinations of trifludimoxazin + glufosinate, glyphosate, paraquat, or saflufenacil, several instances of antagonism occurred according to Colby's analysis (Table 5). Specifically, trifludimoxazin + glyphosate mixtures exhibited only an additive response, whereas all other combinations produced at least one instance of antagonism. These observations may practically be classified as "false antagonism" as described by Hugie et al. (2008), who note that high levels of control imparted by applications of one or both components of a mixture arithmetically limit the utility of Colby's method such that a "less than additive" (i.e., antagonistic) response is the only possibility.

Greenhouse experiments utilizing the Isobole analysis method demonstrated an additive effect for the trifludimoxazin combinations on waterhemp (Figure 1). The only exception was the combination of trifludimoxazin + paraquat, which was slightly antagonistic. The contrast between mixture interactions observed in several combinations from field and greenhouse experiments highlights the impact of herbicide rate selection and weed size at application, among other factors, which can influence the characterization of these interactions (Green 1989; Riley and Shaw 1988; Scott et al. 1998).

When considering results from both field and greenhouse experiments, trifludimoxazin applied at 12.5 or 25.0 g ha^{-1} appears to be an effective option for management of waterhemp, even when applied to plants as large as 15 to 20 cm. Additionally, although some combinations of trifludimoxazin plus field-use rates of glufosinate, paraquat, or saflufenacil were deemed antagonistic under field and greenhouse conditions, high levels of control were still attained in the field. Thus the trifludimoxazin combinations evaluated may still provide substantial utility for managing waterhemp, especially where glyphosate-resistant populations are present. Combinations of other PPO inhibitors with systemic herbicides like glyphosate can be either synergistic or antagonistic, depending on the weed species and biotype, herbicide, or rates applied (Ashigh and Hall 2010; Norris et al. 2001). One example, presented by Mellendorf et al. (2013), showed that the addition of glyphosate to saflufenacil increased control of a glyphosate-resistant population of horseweed when lower rates of saflufenacil were applied. While the same did not hold true following applications of higher rates of saflufenacil with glyphosate, the efficacy of saflufenacil was not reduced as a result of adding

Table 4. Average waterhemp control from field experiments conducted near Brookston, IN, in 2017 and 2018.[a,b]

		Visual control estimate				
		3 DAA		28 DAA		
Trifludimoxazin	Tank-mix herbicide[c]	Marked plants	Whole plot	Marked plants	Whole plot	Biomass reduction
g ai ha^{-1}		—————————%—————————				% of NTC
12.5	—	95 a	83 a	94 a	87 a	95 a
25.0	—	96 a	85 a	99 a	89 a	95 a
—	Glufosinate	32 b	28 b	36 b	41 b	58 b
—	Glyphosate	5 c	7 b	12 b	22 b	23 c
—	Paraquat	97 a	95 a	100 a	93 a	97 a
—	Saflufenacil	89 a	73 a	81 a	76 a	89 a
12.5	Glufosinate	90 a	85 a	91 a	73 a	92 a
25.0	Glufosinate	94 a	91 a	99 a	81 a	94 a
12.5	Glyphosate	91 a	85 a	92 a	82 a	89 a
25.0	Glyphosate	96 a	83 a	97 a	91 a	96 a
12.5	Paraquat	98 a	95 a	100 a	86 a	97 a
25.0	Paraquat	97 a	97 a	100 a	94 a	97 a
12.5	Saflufenacil	96 a	85 a	95 a	76 a	94 a
25.0	Saflufenacil	97 a	87 a	98 a	83 a	97 a
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: DAA, days after application; NTC, nontreated check.
[b]Means within a column followed by the same letter do not differ according to Tukey's HSD ($\alpha = 0.05$).
[c]Rates for tank-mix herbicides: glufosinate, 590 g ai ha^{-1}; glyphosate, 870 g ae ha^{-1}; saflufenacil, 25 g ai ha^{-1}; paraquat, 840 g ai ha^{-1}.

Table 5. Tank-mix interactions as determined by analysis via Colby's method for marked waterhemp plants in field experiments conducted near Brookston, IN, in 2017 and 2018.[a,b]

		Control 28 DAA				Biomass reduction			
Trifludimoxazin rate	Tank-mix herbicide	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.
——g ai/ae ha^{-1}——		—— % ——				—— % ——			
12.5	12.5	94				95			
25.0	25.0	99				95			
—	—	36				58			
—	—	12				23			
—	Glufosinate	100				97			
—	Glyphosate	81				89			
12.5	Paraquat	91	95	0.5187	Add.	**92**	**98**	**0.0194**	**Ant.**
25.0	Saflufenacil	95	99	0.4780	Add.	94	98	0.7039	Add.
12.5	Glufosinate	92	95	0.5778	Add.	89	92	0.6027	Add.
25.0	Glufosinate	97	99	0.4177	Add.	96	92	0.3476	Add.
12.5	Glyphosate	100	100	0.9876	Add.	97	100	**<0.0001**	**Ant.**
25.0	Glyphosate	100	100	0.9264	Add.	**97**	**100**	**0.0010**	**Ant.**
12.5	Paraquat	95	99	0.1707	Add.	94	99	0.0554	Add.
25.0	Paraquat	98	99	0.2602	Add.	**97**	**99**	**0.0043**	**Ant.**

[a]Abbreviations: Add., additive; Ant., antagonism; DAA, days after application; Exp., expected value; Int., interaction; Obs., observed value.
[b]Boldface indicates interactions that are not additive (i.e., antagonistic or synergistic).

glyphosate. In our results, the addition of glyphosate to trifludimoxazin similarly did not compromise the high efficacy of applications of trifludimoxazin alone. Although little information exists regarding interactions between PPO inhibitors and other contact herbicides, a recent study found that applications of reduced rates of glufosinate and lactofen or saflufenacil were synergistic when applied to waterhemp (Takano et al. 2020). Although synergy was not observed between trifludimoxazin and glufosinate using full use rates of either herbicide under field conditions, or with constant rates consistent with the relative potency of each herbicide in the greenhouse, altering the ratios of each herbicide applied in mixture may possibly result in synergism.

Giant Ragweed

Similar to results from waterhemp field experiments, the onset of trifludimoxazin activity was rapid in giant ragweed, with applications of 12.5 and 25.0 g ha^{-1} resulting in 83% and 85% control 3 DAA on marked plants, respectively (Table 6). Necrotic symptomology following trifludimoxazin applications peaked at the 7 DAA evaluation timing, with a decline in control observed at the later evaluation timings as a result of regrowth from apical and axillary meristems (Table 6). By 21 DAA, all herbicide treatments, with the exception of trifludimoxazin or glyphosate alone, resulted in near-complete control (≥99%) of marked plants (Table 6). Although analysis of height reduction via Colby's method indicated all but one herbicide combination to be antagonistic

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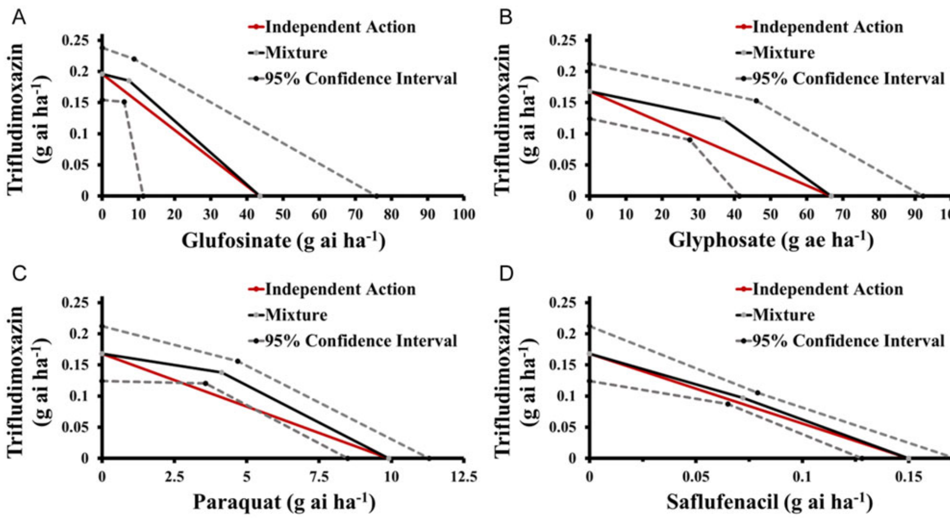

Figure 1. Isobole analysis for GR_{50} values utilizing combinations of trifludimoxazin and glufosinate (A), glyphosate (B), paraquat (C), or saflufenacil (D) applied to waterhemp. The independent action line, denoted in red, indicates combinations of each herbicide expected to elicit 50% control. Deviation of the GR_{50} value and corresponding 95% confidence interval from the independent action line indicate an antagonistic interaction for trifludimoxazin + saflufenacil, whereas all other combinations are additive.

Table 6. Giant ragweed control from field experiments conducted at Lafayette, IN, in 2017 and 2018.[a,b]

Trifludimoxazin	Tank-mix herbicide[c]	Visual control estimate 3 DAA Marked plants	Visual control estimate 3 DAA Whole plot	Visual control estimate 28 DAA Marked plants	Visual control estimate 28 DAA Whole plot	Biomass reduction
g ai ha^{-1}				%		% of NTC
12.5	—	83 a	80 ab	78 b	73 b	68 d
25.0	—	85 a	83 ab	79 b	74 b	74 cd
—	Glufosinate	53 b	54 bc	100 a	96 a	85 a–c
—	Glyphosate	25 c	25 c	79 b	67 b	76 b–d
—	Paraquat	96 a	93 a	100 a	95 a	94 a
—	Saflufenacil	92 a	87 a	100 a	98 a	89 ab
12.5	Glufosinate	78 a	73 ab	100 a	95 a	85 a–c
25.0	Glufosinate	80 a	75 ab	100 a	95 a	87 a–c
12.5	Glyphosate	82 a	79 ab	99 a	91 a	88 ab
25.0	Glyphosate	88 a	82 ab	99 a	95 a	92 a
12.5	Paraquat	96 a	96 a	100 a	99 a	93 a
25.0	Paraquat	97 a	95 a	100 a	98 a	90 ab
12.5	Saflufenacil	91 a	87 a	100 a	98 a	90 ab
25.0	Saflufenacil	93 a	87 a	100 a	98 a	92 a
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: DAA, days after application; NTC, nontreated check.
[b]Means within a column followed by the same letter do not differ according to Tukey's HSD (α = 0.05).
[c]Rates for tank-mix herbicides: glufosinate, 590 g ai ha^{-1}; glyphosate, 870 g ae ha^{-1}; saflufenacil, 25 g ai ha^{-1}; paraquat, 840 g ai ha^{-1}.

(data not shown), these observations may again be best classified as false antagonism owing to the high levels of height reduction imparted by applications of the individual herbicides. When considering visual estimates of control and biomass reduction data, additive interactions predominated for herbicide combinations with trifludimoxazin on giant ragweed. Indeed, the only interaction that was not additive was the synergistic combination of trifludimoxazin at 25.0 g ha^{-1} applied with glyphosate (Table 7).

Combinations of trifludimoxazin and glufosinate or glyphosate in the greenhouse were additive on giant ragweed, whereas mixtures with paraquat or saflufenacil were antagonistic and synergistic, respectively (Figure 2). An interesting contrast exists between field and greenhouse results, with trifludimoxazin + paraquat proving to be antagonistic when applied at sublethal rates to both smaller giant ragweed and waterhemp plants, yet high levels of efficacy were still observed when applied to large plants at field-use rates. Green (1989) stated that "antagonism defines a type of herbicide interaction, not whether a mixture is agronomically useful." This highlights the importance of considering the practical implications of calculated antagonism in the context of how herbicide mixtures will be applied under field conditions. In our research, even though antagonistic relationships have been observed, the combination of trifludimoxazin with the four herbicides on giant ragweed appears still to result in successful weed control when applied at field-use rates. Conversely, the synergy observed between trifludimoxazin and saflufenacil under greenhouse conditions implies that varying the rates of each herbicide in combination may have practical relevance in terms of

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Table 7. Mixture interactions as determined by analysis via Colby's method for marked giant ragweed plants in field experiments conducted at Lafayette, IN, in 2017 and 2018.[a,b]

		Control 21 DAA				Biomass reduction			
Trifludimoxazin rate	Tank-mix herbicide	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.
g ai/ae ha^{-1}		%				%			
12.5	12.5	78				68			
25.0	25.0	79				74			
—	—	100				85			
—	—	79				76			
—	Glufosinate	100				94			
—	Glyphosate	100				89			
12.5	Paraquat	100	100	0.9798	Add.	85	93	0.1746	Add.
25.0	Saflufenacil	100	100	0.9913	Add.	87	95	0.1613	Add.
12.5	Glufosinate	99	97	0.1028	Add.	88	91	0.5658	Add.
25.0	Glufosinate	**99**	**96**	**0.0237**	**Syn.**	92	93	0.6819	Add.
12.5	Glyphosate	100	100	0.9955	Add.	93	97	0.0798	Add.
25.0	Glyphosate	100	100	0.9801	Add.	90	98	0.0923	Add.
12.5	Paraquat	100	100	0.3506	Add.	90	94	0.4206	Add.
25.0	Paraquat	100	100	0.8516	Add.	92	96	0.2024	Add.

[a]Abbreviations: Add., additive; Ant., antagonism; DAA, days after application; Exp., expected value; Int., interaction; Obs., observed value.
[b]Boldface indicates interactions that are not additive (i.e., antagonistic or synergistic).

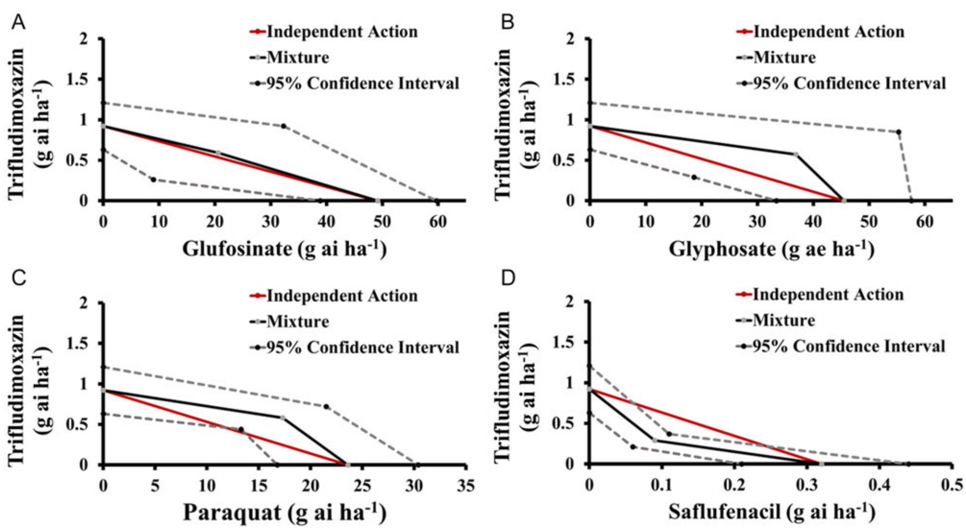

Figure 2. Isobole analysis for GR_{50} values utilizing combinations of trifludimoxazin and glufosinate (A), glyphosate (B), paraquat (C), or saflufenacil (D) applied to giant ragweed. Deviation of the GR_{50} value and corresponding 95% confidence interval from the independent action line indicate antagonism and synergism for combinations of trifludimoxazin + paraquat and trifludimoxazin + saflufenacil. Combinations of trifludimoxazin + glufosinate or glyphosate are additive.

giant ragweed control. Future research investigating different ratios of trifludimoxazin + saflufenacil may help elucidate the synergistic interaction between these two herbicides.

Horseweed

Field applications of trifludimoxazin alone were ineffective on horseweed, providing ≤20% control regardless of herbicide rate or evaluation timing (Table 8). At 28 DAA, applications of trifludimoxazin resulted in ≤10% control of marked horseweed plants, which was similar to efficacy applications of glyphosate alone (17%) or mixtures of trifludimoxazin + glyphosate (17% to 29%) (Table 8). Conversely, treatments containing glufosinate, paraquat, saflufenacil, or combinations of trifludimoxazin plus any of these herbicides were highly efficacious, providing ≥91% control of marked horseweed plants 28 DAA (Table 8). Owing to negligible activity of trifludimoxazin and an absence of interactions, save for additivity, between the other herbicides investigated, subsequent greenhouse experiments were not conducted for horseweed.

These results indicate that the foliar activity of applications of trifludimoxazin alone on horseweed is much lower when compared with saflufenacil, which is an effective herbicide for horseweed management (Mellendorf et al. 2013). Rather, the efficacy of trifludimoxazin more closely resembles that of other PPO-inhibiting herbicides, such as carfentrazone or flumioxazin, which are efficacious when applied to *Amaranthus* weeds but have low activity when foliar applications are made to horseweed (Davis et al. 2010; Shreshtha et al. 2008; Tahmasebi et al. 2018). Thus applications of trifludimoxazin alone will not be a viable option for controlling horseweed. Alternatively, because the addition of trifludimoxazin did not reduce the high levels of efficacy observed following applications of glufosinate, paraquat, or saflufenacil,

Table 8. Horseweed control from field experiments conducted near Brookston, IN, in 2017 and 2018.[a,b]

Trifludimoxazin	Tank-mix herbicide[c]	Visual control estimate 3 DAA Marked plants	3 DAA Whole plot	28 DAA Marked plants	28 DAA Whole plot	Biomass reduction
g ai ha^{-1}		%				% of NTC
12.5	—	12 cd	13 cd	9 b	10 b	15 b
25.0	—	18 cd	19 cd	10 b	13 b	17 b
—	Glufosinate	84 ab	76 b	100 a	92 a	90 a
—	Glyphosate	7 d	8 d	17 b	18 b	25 b
—	Paraquat	94 a	91 ab	94 a	78 a	87 a
—	Saflufenacil	83 ab	81 ab	98 a	92 a	88 a
12.5	Glufosinate	89 ab	89 ab	99 a	91 a	87 a
25.0	Glufosinate	90 ab	90 ab	100 a	91 a	85 a
12.5	Glyphosate	25 c	26 c	29 b	23 b	22 b
25.0	Glyphosate	22 cd	25 c	17 b	21 b	33 b
12.5	Paraquat	92 ab	90 ab	93 a	81 a	88 a
25.0	Paraquat	95 a	92 a	91 a	81 a	88 a
12.5	Saflufenacil	85 ab	87 ab	99 a	83 a	86 a
25.0	Saflufenacil	77 b	78 ab	93 a	93 a	85 a
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: DAA, days after application; NTC, nontreated check.
[b]Means within a column followed by the same letter do not differ according to Tukey's HSD (α = 0.05).
[c]Rates for tank-mix herbicides: glufosinate, 590 g ai ha^{-1}; glyphosate, 870 g ae ha^{-1}; saflufenacil, 25 g ai ha^{-1}; paraquat, 840 g ai ha^{-1}.

mixtures of trifludimoxazin with these herbicides may be utilized for effective management of horseweed, including glyphosate-resistant biotypes like those evaluated in the field studies herein.

Practical Implications

This study concludes that foliar applications of trifludimoxazin are effective for managing waterhemp (including glyphosate-resistant populations) and, to some extent, giant ragweed, but not horseweed. Mixtures of trifludimoxazin with any of the herbicides evaluated resulted in high levels of weed control for all three species under field conditions, except for trifludimoxazin + glyphosate applied to glyphosate-resistant horseweed. Where glyphosate-resistant horseweed is present, effective control can still be achieved with combinations of trifludimoxazin + glufosinate, paraquat, or saflufenacil. As such, preplant burndown applications of trifludimoxazin alone and in combination with these herbicides will be an effective management tool for several problematic weeds in soybean, and the utility of these herbicides will be especially relevant where emerged weeds exist prior to soybean planting (e.g., double-crop soybeans, delayed planting situations, and southern latitudes, where weed germination begins earlier in the season).

Competing interests. NRS is currently employed by BASF Corporation. The authors declare no conflicts of interest.

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