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Steppig et. al: Trifludimoxazin Foliar Control

Common Target Site Resistance Mutations for PPO-Inhibiting Herbicides in Waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) Do Not Confer Cross-Resistance to Trifludimoxazin

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# Abstract

Trifludimoxazin is a PPO-inhibiting herbicide currently under development for preplant burndown and soil residual weed control in soybean [Glycine max (L.) Merr.] and other crops. Greenhouse dose response experiments with foliar applications of trifludimoxazin, fomesafen, and saflufenacil were conducted on susceptible and PPO-inhibitor resistant (PPO-R) waterhemp [Amaranthus tuberculatus (Moq.) Sauer] and Palmer amaranth (Amaranthus palmeri S. Watson) biotypes. These PPO-R biotypes contained the PPO2 target site (TS) mutations  $\Delta G210$  (A. tuberculatus and A. palmeri), R128G (A. tuberculatus), and V361A (A. palmeri). The R/S ratios for fomesafen and saflufenacil ranged from 2.0 to 9.2 across all PPO-R biotypes. In contrast, the response of known PPO inhibitor-susceptible and -resistant biotypes to trifludimoxazin did not differ within each Amaranthus species. In 2017 and 2018 experiments at the Meigs and Davis Purdue Agriculture Centers were conducted in fields with native A. tuberculatus populations comprised of 3% and 30% PPO-R plants ( $\Delta$ G210 mutation), respectively. At Meigs in 2018, A. tuberculatus control following foliar applications of fomesafen, lactofen, saflufenacil, and trifludimoxazin was greater than 95%. When averaged across the other three site-years, applications of 25 g ai ha<sup>-1</sup> trifludimoxazin resulted in 95% control of A. tuberculatus 28 DAA, while applications of fomesafen (343 g ai ha<sup>-1</sup>), lactofen (219 g ai ha<sup>-1</sup>), or saflufenacil (25.0 or 50 g ai ha<sup>-1</sup>), resulted in 80 to 88% control. Thus, at these relative application rates, the foliar efficacy of trifludimoxazin was comparable or greater on A. tuberculatus, when compared to other commercial PPO inhibitors, even in populations where low frequencies of PPO inhibitorresistant plants exist. The lack of cross resistance for common PPO2 TS mutations to trifludimoxazin, and the level of foliar field efficacy observed on populations containing PPO-R individuals suggests that trifludimoxazin may be a valuable herbicide in an integrated approach for managing herbicide-resistant Amaranthus weeds.

**Keywords:**  $\Delta$ G210 mutation, herbicide resistance, novel herbicide active ingredients, protoporphyrinogen oxidase, R128G mutation, resistance management, V361A mutation

# Introduction

Weeds belonging to the *Amaranthaceae* family continue to be among the most common and problematic weeds in soybean [*Glycine max* (L.) Merr.] production systems in the United States (US) (Van Wychen 2019). Two especially pernicious examples within this family include waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] and Palmer amaranth (*Amaranthus palmeri* S. Watson). Season-long competition from *A. tuberculatus* and *A. palmeri* in soybean can cause yield losses of 56% and 79%, respectively, justifying the implementation of effective management strategies targeting these species (Bensch et al. 2003). While crop yield losses decrease as time of crop-weed interference is diminished, high fecundity in both species means that any number of plants allowed to reach reproductive maturity will replenish the soil seedbank and provide additional management challenges in subsequent years (Korres et al. 2018a; Korres et al. 2018b). Unfortunately, conditions favoring germination of both species coincide with much of the timeframe in which US soybeans are grown, necessitating season-long management strategies to be implemented (Hartzler et al. 1999; Jha and Norsworthy 2009; Steckel et al. 2001). As such, integrating several tactics which reduce weed competition and seed production is a common recommendation (Norsworthy et al. 2012).

While tillage can be an effective method for reducing densities of *Amaranthus* weeds early in the growing season, the aforementioned wide window of germination of these species necessitates additional management inputs for seedlings emerging later in the year (Oryokot et al. 1997) Additionally, reduced- and no-till practices have greatly increased since the 1990s, with approximately 70% of soybean hectares in the US subjected to some degree of conservation tillage (Claasen et al. 2018). Consequently, the reliance on chemical weed control methods remains high, and weed management programs which include both preemergence and postemergence herbicide applications have shown to be effective and economical in soybean (Farmer et al. 2017; Legleiter et al. 2009; Swinton and Van Deynze 2017). While preemergence herbicide applications in a preplant burndown or a subsequent postemergence herbicide application is often necessary to eliminate weeds that emerge during the growing season (Hager et al. 2002).

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have been used for several decades, and include multiple chemical families such as diphenylethers, N-phenyl-imides, N-phenyl-oxadiazolones, and N-phenyl-triazinones (HRAC 2020; Salas et al. 2016). The PPO enzyme catalyzes the last common step in the tetrapyrrole synthesis pathway, whereby protoporphyrinogen IX is oxidized to form protoporphyrin IX. When PPO is inhibited, protoporphyrinogen IX accumulates in the chloroplast and is transported to the cytoplasm, where the molecule oxidizes spontaneously to form protoporphyrin IX (Lee and Duke 1994). The subsequent accumulation of protoporphyrin IX in the cytoplasm generates reactive oxygen species in the presence of light, resulting in lipid peroxidation and rapid cell death in susceptible species (Duke et al. 1991). Herbicides that inhibit PPO can exhibit both preemergence and postemergence activity on weeds, present low risk of toxicity to humans, and are capable of being used at lower rates relative to many other herbicides (Hao et al. 2011). These favorable properties, in addition to high levels of activity on glyphosate- and ALS-resistant biotypes of *Amaranthus* weed species, have contributed to more frequent applications of PPO inhibitors in recent years (Salas et al. 2016; USDA-NASS 2020).

Interestingly, while PPO-inhibiting herbicides have been used since the 1960s, evolution of resistance to this chemical family has been slow to evolve. The first instance of resistance to PPO inhibitors was documented in an *A. tuberculatus* population from Kansas in 2001 (Shoup et al. 2003). Resistance in *A. tuberculatus* and *A. palmeri* populations was determined to be the result of an insensitive target site caused by the loss of a glycine residue at the amino acid position 210 ( $\Delta$ G210) of the *PPX2* gene, which codes for production of PPO (Patzoldt et al. 2006; Salas et al. 2016). Subsequently, additional mutations to *PPX2* have been shown to confer resistance to PPO inhibitors in both species. One such mutation includes substitutions to the amino acid position 128 of the wild-type gene, where the native arginine residue is altered. Several different iterations of this mutation have been documented to be possible in *Amaranthus* species, but only arginine-to-glycine (R128G), arginine-to-methionine (R128M) and arginine-to-isoleucine (R128I) isoforms have been confirmed to confer resistance to PPO-inhibiting herbicides (Giacomini et al. 2017; Nie et al. 2019). More recently, additional amino acid substitutions to *PPX2* (G399A and V361A) have been shown to endow resistance to several foliar-applied PPO inhibitors in *A. palmeri* (Rangani et al. 2019; Nie et al. 2023). As the

distribution and frequency of these mutations continue to increase, new management strategies will need to be adopted in order to effectively manage *Amaranthus* populations.

Trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione] is a novel PPO-inhibiting herbicide belonging to the N-phenyl-imide family. Trifludimoxazin is currently being developed with projected use as a preplant burndown herbicide in conventional soybean, corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.), and for vegetation management in chemical fallow areas (Asher et al. 2020; PMRA 2020). Foliar application of trifludimoxazin has been reported to control *Amaranthus* biotypes with target site mutations that confer resistance to commercial PPOinhibiting herbicides (Armel et al. 2017). At present, no research has been published that examines the relative activity of trifludimoxazin on *Amaranthus* weeds compared to other commercial PPO-inhibitors. Therefore, experiments were conducted to address two research objectives: evaluate the effect of select PPO target-site mutations in *A. tuberculatus* and *A. palmeri* on foliar efficacy of trifludimoxazin, fomesafen, and saflufenacil; and investigate the foliar efficacy of trifludimoxazin relative to other PPO-inhibiting herbicides under field conditions, when applied to *A. tuberculatus*.

#### **Materials and Methods**

### Cross-Resistance to Trifludimoxazin

A greenhouse experiment was conducted to evaluate whole plant response to foliar applications of trifludimoxazin, saflufenacil, and fomesafen, on three *A. tuberculatus* and three *A. palmeri* biotypes. Each species included a known PPO-susceptible biotype in addition to two biotypes with target-site mutations to *PPX2* conferring resistance to commercial PPO inhibitors (PPO-R). *Amaranthus tuberculatus* biotypes were generated from a field population collected from Gibson County, Indiana in 2016, where the population was segregating for  $\Delta$ G210 and R128G resistance mutations. Parent plants from homozygous individuals within this population were crossed to produce homozygous lines for wild-type,  $\Delta$ G210, and R128G biotypes (Steppig et al. 2017). *Amaranthus palmeri* biotypes included PPO-sensitive and PPO-R ( $\Delta$ G210) populations collected in 2013 from Washington and Daviess County, Indiana, respectively (Spaunhorst et al. 2019).

Additionally, an *A. palmeri* biotype from Alabama with the V361A PPO-R mutation was included (Nie et al. 2023). In contrast to *A. tuberculatus* biotypes used for evaluation, *A. palmeri* biotypes were all from field populations with unknown segregation for respective resistance mutations.

For each weed species the experiment was designed as a three-factor (biotype x herbicide x herbicide rate) factorial on a randomized complete block design (RCBD), with 8 replications, and repeated once. Seeds from each biotype were sown in greenhouse flats measuring 25 by 50 cm, containing commercial potting mix (Fafard Germinating Mix; Sun Gro Horticulture, Agawa, MA), and transplanted into 164-cm<sup>3</sup> cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers; Stuewe & Sons, Tangent, OR) filled with a 2:1 mixture of potting soil and sand once seedlings reached the one-leaf stage. Plants were watered daily and fertilized with a micro- and macronutrient fertilizer (Jack's Classic Professional 20-20-20, JR Peters Inc., Allentown PA) weekly until they reached the 4- to 6-leaf stage (5 to 7.5 cm in height), at which time herbicide applications were made using a track-mounted research sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale MN) calibrated to deliver 140 L ha<sup>-1</sup> at 207 kPa via an even-fan XR8002E nozzle (TeeJet Technologies, Glendale Heights, IL). Herbicide treatments included eight rates of trifludimoxazin (0 to 62.5 g ai  $ha^{-1}$ , projected labeled 1X use rate = 12.5 to 25 g ai ha<sup>-1</sup>), saflufenacil (0 to 125 g ai ha<sup>-1</sup>, labeled 1X use rate = 25 g ai ha<sup>-1</sup>), or fomesafen (0 to 1320 g ai ha<sup>-1</sup>, labeled 1X use rate = 343 g ai ha<sup>-1</sup>) with methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) added to each herbicide treatment at 1% v v<sup>-1</sup>. Rates were evenly spaced along a log<sub>35</sub> or log<sub>5</sub> scale and selected based on results from preliminary studies (data not shown).

Greenhouse environmental conditions included a 16:8 h light:dark photoperiod, where natural light was supplemented with high-pressure sodium bulbs delivering 1100  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> photon flux during daylight hours, and day/night temperatures of 30 and 25C, respectively. Following herbicide application, plants were rearranged spatially every three days to reduce environmental effects resulting from spatial variation within the greenhouse (Wallihan and Garber 1971). Visual estimates of *A. tuberculatus* and *A. palmeri* control were recorded at 3, 7, and 14 days after application (DAA) utilizing a 0 to 100 scale, where 0 = no control and 100 = complete plant death. At 14 DAA, shoot biomass was harvested by clipping plants at the soil

surface. Collected plant tissue was oven-dried for 3 days at 60C, and data were normalized according to the non-treated check within each biotype/herbicide combination. Data were analyzed using a four-parameter log-logistic model (Equation 1):

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

where *b* is the slope of the curve, *c* is the lower asymptote, *d* is the upper asymptote, and *e* is the herbicide rate required to produce 50% control or biomass reduction (i.e.  $GR_{50}$  value), via the *drc* package in R software v. 3.6.2 (Knezevic et. al 2007). Data were pooled over runs due to a lack of treatment by run interaction, as determined by analysis of variance (ANOVA,  $\alpha = 0.05$ ). Calculated  $GR_{50}$  values were used to quantify resistance indices for resistant biotypes within each herbicide and *Amaranthus* species. Additionally,  $GR_{50}$  values within species and herbicides were compared using the *compParm* function, which runs pairwise t-tests to determine whether model parameters (in this case, *e*) differ (Ritz et. al 2015).

# Field Efficacy

Field experiments were conducted at the Meigs Horticulture Research Farm (Meigs), near Lafayette, IN (40.28°N, 86.88°W) and at the Davis Purdue Agriculture Center (Davis), near Farmland, IN (40.25°N, 85.15°W) in 2017 and 2018 to evaluate the efficacy of foliar applications of trifludimoxazin and other PPO-inhibiting herbicides on *A. tuberculatus*. Field sites were selected based on the presence of endemic populations of *A. tuberculatus*. Previous statewide herbicide resistance screens utilizing a TaqMan qPCR assay, as described by Wuerffel et. al (2015), determined a 3 and 30% frequency of the  $\Delta$ G210 target-site mutation among individual plants at the Meigs and Davis populations. Experimental units were 3 by 9 m plots arranged in a RCBD with four replications. Experiments were established in fallow field areas under continuous no-till management, with existing vegetation controlled prior to trial initiation via application of 840 g ai ha<sup>-1</sup> paraquat (Gramoxone SL 2.0, Syngenta Crop Protection LLC, Greensboro, NC).

Herbicides were applied when average *A. tuberculatus* plants measured between 5 and 10 cm in height. Applications were performed utilizing a  $CO_2$ -pressured backpack sprayer with a 2-m handheld spray boom equipped with four flat-fan XR8002 nozzles (TeeJet Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha<sup>-1</sup> at 276 kPa. Herbicide treatments included

trifludimoxazin (6.25, 12.5, and 25.0 g ha<sup>-1</sup>), saflufenacil (25 and 50 g ha<sup>-1</sup>), and trifludimoxazin plus saflufenacil (6.25 + 25.0, 6.25 + 50.0, 12.5 + 25.0, 12.5 + 50.0, 25.0 + 25.0, and 25.0 + 50.0 g ha<sup>-1</sup>). Additionally, fomesafen (343 g ha<sup>-1</sup>), lactofen (219 g ha<sup>-1</sup>), and flumioxazin (71.5 g ha<sup>-1</sup>), were included as commercial standards at labeled 1X field-use rates. Methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) was added to each treatment at 1% v v<sup>-1</sup> as either required or permitted for the labeled use of each product. Visual estimates of *A. tuberculatus* control were collected at 3, 7, 14, 21 and 28 DAA. At 28 DAA, weed densities were assessed from two random quadrats within each plot measuring 0.5 m<sup>2</sup>, and proportions of live versus dead plants recorded to calculate the percentage of surviving plants in each plot. Plants were considered alive if green tissue was present from apical or axillary shoot meristems, whereas dead plants had no regrowth. Plants emerging after herbicide applications were not considered for the evaluation.

Data were subjected to ANOVA via PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC), and significant means separated using Tukey's HSD ( $\alpha = 0.05$ ). At Meigs in 2018, different trends were observed in control data compared to the other site-years. As a result, this site-year was analyzed separately, with herbicide treatment considered a fixed effect, and replication a random effect. Data from Davis (2017 and 2018) and Meigs (2017) were analyzed similarly, with site-year included as a random effect in the model and replication nested within site-year. Data for Davis (both years) and Meigs in 2017 were combined due to a non-significant treatment by site-year interaction.

# **Results and Discussion**

### Cross-Resistance to Trifludimoxazin

The relative efficacy of applications of trifludimoxazin was approximately two- or tenfold higher compared to applications of saflufenacil or fomesafen, respectively, in the susceptible biotype of *A. tuberculatus* (Table 1 and Figure 1). In susceptible *A. palmeri*, applications of both trifludimoxazin (approximately seven-fold) and saflufenacil (approximately twelve-fold) resulted in higher efficacy relative to fomesafen applications (Table 1). Comparisons of calculated GR<sub>50</sub> values indicated that *Amaranthus* biotypes containing target-site PPO resistance mutations ( $\Delta$ G210 or R128G in *A. tuberculatus* and  $\Delta$ G210 or V361A in *A. palmeri*) were less sensitive to both saflufenacil (R/S ratios of 2.0 to 2.8) and fomesafen (R/S ratios of 3.0 to 9.2), relative to the susceptible biotypes within each species (Tables 2 and 3). The greater activity of saflufenacil, relative to fomesafen, on *A. tuberculatus* and *A. palmeri* observed here is consistent with previous research which demonstrated that saflufenacil was the most efficacious of eight commercial PPO-inhibiting herbicides applied to a fomesafen-resistant population of *A. palmeri* (Salas-Perez et al. 2017).

A comparison of herbicide binding at the protein level demonstrated that saflufenacil has a higher relative affinity for the PPO enzyme, even those containing target-site resistance mutations, when compared to fomesafen (Wu et al. 2020). Thus, evidence of cross-resistance to PPO herbicides resulting from target-site mutations in *Amaranthus* weeds is more complex than previously thought, and response is dependent on the specific PPO-inhibiting herbicide applied. A partial explanation may be the extensive use of the diphenylether herbicide chemical family, such as acifluorfen, fomesafen, and lactofen, largely contributed to the evolution of mutations (e.g.  $\Delta$ G210) to PPO2 which are particularly robust against these herbicides (Rangani et al. 2019). In contrast, saflufenacil, a member of the N-phenyl-imide (formerly pyrimidinedione) family, was commercialized in 2010, is only labeled for preplant or preemergence applications, and has relatively rapid dissipation in the soil, lending to minimal selection pressure for resistance specific to saflufenacil (Grossman et al. 2012; Mueller et al. 2014). The lack of consistent selection pressure from saflufenacil may at least partially explain the dearth of targetsite mutations which confer robust resistance to saflufenacil.

In contrast to fomesafen or saflufenacil, resistant biotypes of *A. tuberculatus* ( $\Delta$ G210 or R128G) or *A. palmeri* ( $\Delta$ G210 or V361A) did not display reduced sensitivity to applications of trifludimoxazin (R/S ratios of 0.8 to 1.2) (Tables 1-3). This supports the notion that trifludimoxazin has efficacy on *Amaranthus* biotypes that are resistant to current commercial standards for PPO-inhibiting herbicides (Findley et al. 2020; Wang et al. 2019). While the activity of fomesafen and saflufenacil can be compromised by the conformational changes to the PPO enzyme resulting from target-site resistance mutations, it appears that the activity of trifludimoxazin is impacted to a lesser extent. (Wu et al. 2020). Previous research has demonstrated high levels of *in vivo* trifludimoxazin activity on bacterial PPO enzymes with several of the R128 mutant variations, including the R128I and R128M variants, which have been detected in field populations of *Amaranthus* weeds (Giacomini et al. 2017; Lillie 2019; Nie

et al. 2019). The current study demonstrates similar *in planta* efficacy of trifludimoxazin on *A*. *tuberculatus* and *A. palmeri* biotypes containing various *PPX2* target-site mutations.

While these results are promising in terms of implications for managing *A. tuberculatus* and *A. palmeri* populations that are resistant to other PPO-inhibiting herbicides via the current documented target-site mutations, there is no information on whether trifludimoxazin provides comparable activity on *Amaranthus* biotypes that possess non-target-site (NTS) mechanisms of resistance to PPO inhibitors (Tranel 2020). Recently, *A. tuberculatus* and *A. palmeri* populations with NTS-based resistance to PPO-inhibiting herbicides have been confirmed, but at present are not nearly as prevalent as those resistant via target-site mechanisms (Obenland et al. 2019; Varanasi et al. 2018). Non-target site resistance in these species is mediated by increased herbicide metabolism via cytochrome P450s and/or glutathione *S*-transferases and confers cross-resistance among PPO-inhibiting herbicides (Jugulam and Shyam 2019; Varanasi et al. 2019). As target-site mutations are currently the most prevalent mechanism of resistance to PPO inhibitors, however, trifludimoxazin may be a valuable herbicide for managing biotypes of *Amaranthus* weeds with target-site PPO mutations, in addition to biotypes that are resistant to glyphosate, ALS inhibitors, and other herbicides.

# Field Efficacy

As PPO-inhibiting herbicides induce symptomology rapidly following foliar application (Hager et al. 2003; Hausman et al. 2016), the highest level of *A. tuberculatus* control was observed 7 DAA, followed by slight, and gradual decreases in control for some treatments at later evaluations (Table 4 and Supplementary Table 1) Combined across all site-years, *A. tuberculatus* control was  $\geq$  93% at 7 DAA, regardless of herbicide treatment applied (Supplementary Table 1). At 14 DAA, differences between herbicide treatments were more defined, where applications of saflufenacil (25 or 50 g ha<sup>-1</sup>), lactofen, or fomesafen, resulted in 88% to 90% control (Supplementary Table 1). *Amaranthus tuberculatus* control following applications of all other herbicide treatments was  $\geq$  94% at the same evaluation timing (Supplementary Table 1).

Herbicide treatment differences at 28 DAA for the combined sites were more pronounced as *A. tuberculatus* regrowth progressed for treatments exhibiting lower levels of efficacy at 14 DAA. Applications of 6.25, 12.5 or 25 ga ha<sup>-1</sup> trifludimoxazin resulted in 88%, 91%, and 95% *A. tuberculatus* control at 28 DAA, respectively (Table 4). Applications of both rates of saflufenacil

were less effective compared to applications of 12.5 or 25 g ha<sup>-1</sup> trifludimoxazin, resulting in 80% and 83% control when applied at 25 and 50 g ha<sup>-1</sup>, respectively (Table 4), similar to previous results with *A. palmeri* (Morichetti et al. 2012). Applications of lactofen (87% control) were less efficacious compared to 25 g ha<sup>-1</sup> trifludimoxazin, but control of *A. tuberculatus* with fomesafen (88%) and flumioxazin (94%) were similar compared to trifludimoxazin at any rate (Table 4). The levels of control achieved with lactofen and fomesafen were similar to those obtained in previous research on *A. tuberculatus* when applied at similar rates and growth stages (Hager et al. 2003).

Reduced control of *A. tuberculatus* following applications of 6.25 g ha<sup>-1</sup> trifludimoxazin, saflufenacil (both rates), or lactofen was primarily a result of regrowth following herbicide treatment. These results were reflected in plant mortality data collected at 28 DAA, where dead plants accounted for 85%, 78%, 84%, and 87% of *A. tuberculatus*, following applications of trifludimoxazin, saflufenacil, or lactofen, respectively (Table 4). Applications of trifludimoxazin plus saflufenacil were highly efficacious regardless of rate, resulting in  $\geq$  93% *A. tuberculatus* control, and mortality  $\geq$  96% (Table 4). Control of *A. tuberculatus*, including plant mortality, was  $\geq$  95% at 28 DAA at Meigs (2018), with no differences attributable to herbicide treatment (Table 4). High levels of weed control observed at this site-year are likely attributable to the lower density (14 plants m<sup>-2</sup>) of *A. tuberculatus* relative to the other three site years (62 plants m<sup>-2</sup>, averaged across site-years) (data not shown) (Dieleman et al. 1999). Overall, the foliar efficacy of trifludimoxazin at 6.25, 12.5, and 25 g ai ha<sup>-1</sup> on *A. tuberculatus* in these field experiments was high, even when applied to populations where TS mutations conferring resistance to PPO-inhibiting herbicides were present.

Herbicide applications in the present study were performed on small weeds, consistent with recommendations for field use of many herbicides. This may have contributed to the relatively high levels of *A. tuberculatus* control observed even at Davis, where approximately 30% of the plants were PPO-resistant (Falk et al. 2006). The predicted use pattern for trifludimoxazin targets preplant applications prior to soybean, corn, cotton, and other crops, alone, and in combination with saflufenacil (Asher et al. 2020; Findley et al. 2020). As such, the utility of trifludimoxazin for managing *Amaranthus* populations may have the most benefit in double-crop soybean in the southern United States Corn Belt where *A. tuberculatus* is a common preplant weed challenge, or in the southern United States, where emerged *Amaranthus* weeds are

more likely to be present prior to planting full-season crops. Other research has demonstrated that trifludimoxazin has soil-residual activity on *Amaranthus* weeds, and that activity is increased when trifludimoxazin and saflufenacil are combined (Steppig et al. 2018). Based on these results, trifludimoxazin and combinations of trifludimoxazin plus saflufenacil, may be a highly effective option for early-season weed management, particularly where weeds resistant to glyphosate and other herbicide sites of action are prevalent.

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# **Competing Interests**

Author N.R. Steppig is currently employed by BASF Corporation. No competing interests have been declared.

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Table 1. Calculated  $GR_{50}$  values for foliar applications of fomesafen, saflufenacil, and trifludimoxazin applied to three biotypes of *Amaranthus tuberculatus* and *Amaranthus palmeri* under greenhouse conditions<sup>a</sup>.

		$GR_{50}$ (±SE) for shoot biomass			
Species	Biotype	Fomesafen	Saflufenacil	Trifludimoxazin	
			g ai ha <sup>-1</sup> $$		
Amaranthus tuberculatus	Susceptible	2.09 (± 0.59)	0.40 (± 0.15)	0.23 (± 0.07)	
	ΔG210	13.9 (± 2.67)	0.96 (± 0.21)	$0.27~(\pm 0.08)$	
	R128G	19.3 (± 3.60)	0.79 (± 0.13)	0.27 (± 0.04)	
Amaranthus palmeri	Susceptible	2.72 (± 0.60)	0.22 (± 0.03)	0.41 (± 0.08)	
	ΔG210	12.3 (± 3.50)	0.62 (± 0.13)	0.41 (± 0.18)	
	V361A	8.15 (± 2.46)	$0.44 \ (\pm 0.08)$	0.31 (± 0.09)	

<sup>a</sup> Herbicides were applied to plants 5 to 7.5 cm in height and biomass was collected 14 days after treatment.

<sup>b</sup> Abbreviations: GR<sub>50</sub>: the herbicide rate required to reduce shoot biomass by 50%;  $\Delta$ G210, *Amaranthus tuberculatus or Amaranthus palmeri* resistant via  $\Delta$ G210 mutation; R128G, *Amaranthus tuberculatus* resistant via R128G mutation; SE, standard error; V361A, *Amaranthus palmeri* resistant via V361A mutation.

			Comparison results		
Species	Herbicide	Biotype	Estimate	Standard error	P-value
Amaranthus tuberculatus	Fomesafen	Susceptible vs. $\Delta$ G210	0.151	0.052	< 0.001
		Susceptible vs. R128G	0.109	0.037	< 0.001
		ΔG210 vs. R128G	0.718	0.193	0.145
	Saflufenacil	Susceptible vs. $\Delta$ G210	0.419	0.182	0.002
		Susceptible vs. R128G	0.510	0.209	0.019
		ΔG210 vs. R128G	1.217	0.340	0.525
	Trifludimoxazin	Susceptible vs. $\Delta$ G210	0.875	0.284	0.685
		Susceptible vs. R128G	0.869	0.322	0.674
		ΔG210 vs. R128G	1.512	0.630	0.417
Amaranthus palmeri	Fomesafen	Susceptible vs. $\Delta$ G210	0.321	0.108	< 0.001
		Susceptible vs. V361A	0.522	0.170	0.005
		ΔG210 vs. V361A	1.627	0.604	0.300
	Saflufenacil	Susceptible vs. $\Delta$ G210	0.292	0.105	< 0.001
		Susceptible vs. V361A	0.515	0.118	< 0.001
		ΔG210 vs. V361A	1.764	0.703	0.279
	Trifludimoxazin	Susceptible vs. $\Delta$ G210	1.006	0.473	0.990
		Susceptible vs. V361A	1.326	0.442	0.462
		ΔG210 vs. V361A	1.318	0.682	0.641

Table 2. Results from pairwise t-tests comparing model parameter e (GR<sub>50</sub> value), within *Amaranthus* species and herbicide, via the *compParm* function in the *drc* package in R<sup>a</sup>.

<sup>a</sup> Abbreviation:  $GR_{50}$ : the herbicide rate required to reduce shoot biomass by 50%;  $\Delta G210$ , *Amaranthus tuberculatus* or *Amaranthus palmeri* resistant via  $\Delta G210$  mutation; R128G, *Amaranthus tuberculatus* resistant via R128G mutation; V361A, *Amaranthus palmeri* resistant via V361A mutation.

		R/S Ratio		
Species	Biotype	Fomesafen	Saflufenacil	Trifludimoxazin
Amaranthus tuberculatus	Susceptible	-	-	-
	ΔG210	6.6	2.4	1.2
	R128G	9.2	2.0	1.2
Amaranthus palmeri	Susceptible	-	-	-
	ΔG210	4.5	2.8	1.0
	V361A	3.0	2.0	0.8

Table 3. R/S ratios calculated using GR<sub>50</sub> values for each species and biotype, within herbicide<sup>a</sup>.

<sup>a</sup> Abbreviation: GR<sub>50</sub>: the herbicide rate required to reduce shoot biomass by 50%;  $\Delta$ G210, Amaranthus tuberculatus or Amaranthus palmeri resistant via  $\Delta$ G210 mutation; R128G, Amaranthus tuberculatus resistant via R128G mutation; V361A, Amaranthus palmeri resistant via V361A mutation.

		Amaranthus tuberculatus control 28 DAA						
Herbicide <sup>b</sup>	Rate	Coml sites <sup>c</sup>	pined fi	eld Meigs 2018 <sup>d</sup>	Com	bined fie	eld Meigs 2018 <sup>e</sup>	
		Visual control estimate			Plant mortality <sup>f</sup>			
	g ai ha <sup>-1</sup>	% _			%			
Trifludimoxazin	6.25	88	bcd	96	85	cd	93	
Trifludimoxazin	12.5	91	abc	99	93	ab	99	
Trifludimoxazin	25.0	95	ab	99	96	а	99	
Saflufenacil	25.0	80	e	95	78	d	93	
Saflufenacil	50.0	83	de	99	84	cd	99	
Trifludimoxazin + saflufenacil	6.25 + 25.0	95	ab	99	96	а	99	
Trifludimoxazin + saflufenacil	6.25 + 50.0	97	а	99	97	а	97	
Trifludimoxazin + saflufenacil	12.5 + 25.0	93	ab	99	95	ab	99	
Trifludimoxazin + saflufenacil	12.5 + 50.0	95	ab	99	99	а	98	
Trifludimoxazin + saflufenacil	25.0 + 25.0	96	а	99	96	а	99	
Trifludimoxazin + saflufenacil	25.0 + 50.0	96	а	99	99	а	99	
Lactofen	219	87	cd	99	87	с	98	
Fomesafen	343	88	bcd	95	90	bc	92	
Flumioxazin	71.5	94	abc	99	97	а	98	

Table 4. Efficacy of foliar application of PPO-inhibiting herbicides on *Amaranthus tuberculatus* at 28 days after application (DAA) for field experiments conducted at the Davis Purdue Agriculture Center and Meigs Horticulture Research Farm (Meigs) in 2017 and 2018<sup>a</sup>.

<sup>a</sup> Frequency of *A. tuberculatus* individuals with resistance to PPO-inhibiting herbicides via the  $\Delta$ G210 mutation was approximately 30% and 3% at Davis Purdue Agricultural Center, and Meigs, respectively.

<sup>b</sup> Herbicide treatments included methylated seed oil, applied at 1% v v<sup>-1</sup>.

<sup>c</sup> Combined field sites include Davis (2017 and 2018) and Meigs 2017. These data exclude Meigs 2018, as dictated by a significant treatment interaction in ANOVA. Means within a column followed by the same letter are not significantly different according to Tukey's HSD ( $\alpha = 0.05$ ).

<sup>d,e</sup> No significant differences in visual estimates of control or plant mortality at 28 days after application were observed between herbicide treatments at Meigs in 2018 ( $\alpha = 0.05$ ).

<sup>f</sup> Amaranthus tuberculatus plants from two  $0.5m^2$  quadrats in each plot were counted at 28 days after application, and a proportion of plants with no green tissue at apical or axillary meristems versus total number of plants converted to a percentage of mortality.



**Figure 1.** Comparison of dose response following applications of fomesafen and trifludimoxazin, applied to PPO-S and the PPO-R ( $\Delta$ G210 and R128G) biotypes of *Amaranthus tuberculatus*.