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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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Research Article

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Abstract

Trifludimoxazin is a protoporphyrinogen oxidase (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 ΔG210 (*A. tuberculatus* and *A. palmeri*), R128G (*A. tuberculatus*), and V361A (*A. palmeri*). The resistant/susceptible (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 PPO-R 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 composed of 3% and 30% PPO-R plants (ΔG210 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 3 site-years, applications of 25 g ai ha^{-1} trifludimoxazin resulted in 95% control of *A. tuberculatus* at 28 DAA, while applications of fomesafen (343 g ai ha^{-1}), lactofen (219 g ai ha^{-1}), or saflufenacil (25.0 or 50 g ai ha^{-1}), 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 with other commercial PPO inhibitors, even in populations where low frequencies of PPO-R 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 suggest that trifludimoxazin may be a valuable herbicide in an integrated approach for managing herbicide-resistant *Amaranthus* weeds.

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 (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. 2019, 2018). Unfortunately, conditions favoring germination of both species coincide with much of the time frame in which U.S. soybeans are grown, necessitating season-long management strategies to be implemented (Hartzler et al. 1999; Jha and Norsworthy 2009; Steckel et al. 2002). As such, integrating several tactics that 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 United States subjected to some degree of conservation tillage (Claassen et al. 2018). Consequently, the reliance on chemical weed control methods remains high, and weed management programs that include both preemergence and postemergence herbicide applications have been 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 can provide control of *Amaranthus* weeds for several weeks following crop emergence, a foliar application 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 diphenyl ethers, *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 acetolactate synthase (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 (ΔG210) 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* (Nie et al. 2023; Rangani et al. 2019). 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 (TS) mutations that confer resistance to commercial PPO-inhibiting herbicides (Armel et al. 2017). At present, no research has been published that examines the relative activity of trifludimoxazin on *Amaranthus* weeds compared with other commercial PPO inhibitors. Therefore, experiments were conducted to address two research objectives: evaluate the effect of select PPO TS 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 TS mutations to *PPX2* conferring resistance to commercial PPO inhibitors (PPO-R). *Amaranthus tuberculatus* biotypes were generated from a field population collected in 2016 from Gibson County, Indiana, where the population was segregating for ΔG210 and R128G resistance mutations. Parent plants from homozygous individuals within this population were crossed to produce homozygous lines for wild-type, ΔG210, and R128G biotypes (Steppig et al. 2017). *Amaranthus palmeri* biotypes included PPO-sensitive and PPO-R (ΔG210) populations collected in 2013 from Washington and Daviess counties, 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 by herbicide by herbicide rate) factorial in a randomized complete block design (RCBD), with eight 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, Agawam, MA), and transplanted into 164-cm^3 Cone-tainers (Ray Leach SC-10 Super Cell Cone-tainer™, Stuewe & Sons, Tangent, OR) filled with a 2:1 mixture of potting soil and sand once seedlings reached the 1-leaf stage. Plants were watered daily and fertilized with a micro- and macronutrient fertilizer (Jack's Classic Professional 20-20-20, JR Peters, 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, Hollandale MN) calibrated to deliver 140 L ha^{-1} at 207 kPa via an even-fan

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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^{-1}), saflufenacil (0 to 125 g ai ha^{-1}, labeled 1X use rate = 25 g ai ha^{-1}), or fomesafen (0 to 1,320 g ai ha^{-1}, labeled 1X use rate = 343 g ai ha^{-1}) with methylated seed oil (MSO Ultra™, Precision Laboratories, Waukegan, IL) added to each herbicide treatment at 1% v/v. Rates were evenly spaced along a $\log_{3.5}$ or $\log_5$ 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 1,100 µmol m^{-2} s^{-1} photon flux during daylight hours, and day/night temperatures of 30 and 25 C, respectively. Following herbicide application, plants were rearranged spatially every 3 d 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 d 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 d at 60C, and data were normalized according to the nontreated check within each biotype/herbicide combination. Data were analyzed using a four-parameter log-logistic model (Equation 1):

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

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 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 3% and 30% frequency of the ΔG210 TS mutation among individual plants in the Meigs and Davis populations, respectively. Experimental units were 3 by 9 m plots arranged in an RCBD with four replications. Experiments were established in fallow field areas under continuous no-till management, with existing vegetation controlled before trial initiation via application of 840 g ai ha^{-1} paraquat (Gramoxone® SL 2.0, Syngenta Crop Protection, 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) calibrated to deliver 140 L ha^{-1} at 276 kPa. Herbicide treatments included trifludimoxazin (6.25, 12.5, and 25.0 g ha^{-1}), saflufenacil (25 and 50 g ha^{-1}), 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^{-1}). Additionally, fomesafen (343 g ha^{-1}), lactofen (219 g ha^{-1}), and flumioxazin (71.5 g ha^{-1}), were included as commercial standards at labeled 1X field use rates. Methylated seed oil (MSO Ultra™) was added to each treatment at 1% v/v 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^2, and proportions of live versus dead plants were 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 v. 9.4 (SAS Institute, Cary, NC), and significant means were separated using Tukey's HSD ($\alpha = 0.05$). At Meigs in 2018, different trends were observed in control data compared with 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 nonsignificant treatment by site-year interaction.

Results and Discussion

Cross-Resistance to Trifludimoxazin

The relative efficacy of applications of trifludimoxazin was approximately 2- or 10-fold higher compared with applications of saflufenacil or fomesafen, respectively, in the susceptible biotype of *A. tuberculatus* (Table 1; Figure 1). In susceptible *A. palmeri*, applications of both trifludimoxazin (approximately 7-fold) and saflufenacil (approximately 12-fold) resulted in higher efficacy relative to fomesafen applications (Table 1). Comparisons of calculated GR_{50} values indicated that *Amaranthus* biotypes containing TS PPO-resistance mutations (ΔG210 or R128G in *A. tuberculatus* and ΔG210 or V361A in *A. palmeri*) were less sensitive to both saflufenacil (resistant/susceptible [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 demonstrating 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 TS resistance mutations, when compared with fomesafen (Wu et al. 2020). Thus, evidence of cross-resistance to PPO herbicides resulting from TS mutations in *Amaranthus* weeds is more complex than previously thought, and response is dependent on the specific PPO-inhibiting herbicide

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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[a].

Species	Biotype[b]	GR_{50} (±SE) for shoot biomass		
		Fomesafen	Saflufenacil	Trifludimoxazin
		g ai ha^{-1}		
A. 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 (± 0.08)
	R128G	19.3 (± 3.60)	0.79 (± 0.13)	0.27 (± 0.04)
A. 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 (± 0.08)	0.31 (± 0.09)

[a]Herbicides were applied to plants 5 to 7.5 cm in height and biomass was collected 14 d after treatment. GR_{50}, the herbicide rate required to reduce shoot biomass by 50%.
[b]ΔG210, *A. tuberculatus or A. palmeri* resistant via ΔG210 mutation; R128G, *A. tuberculatus* resistant via R128G mutation; V361A, *A. palmeri* resistant via V361A mutation.

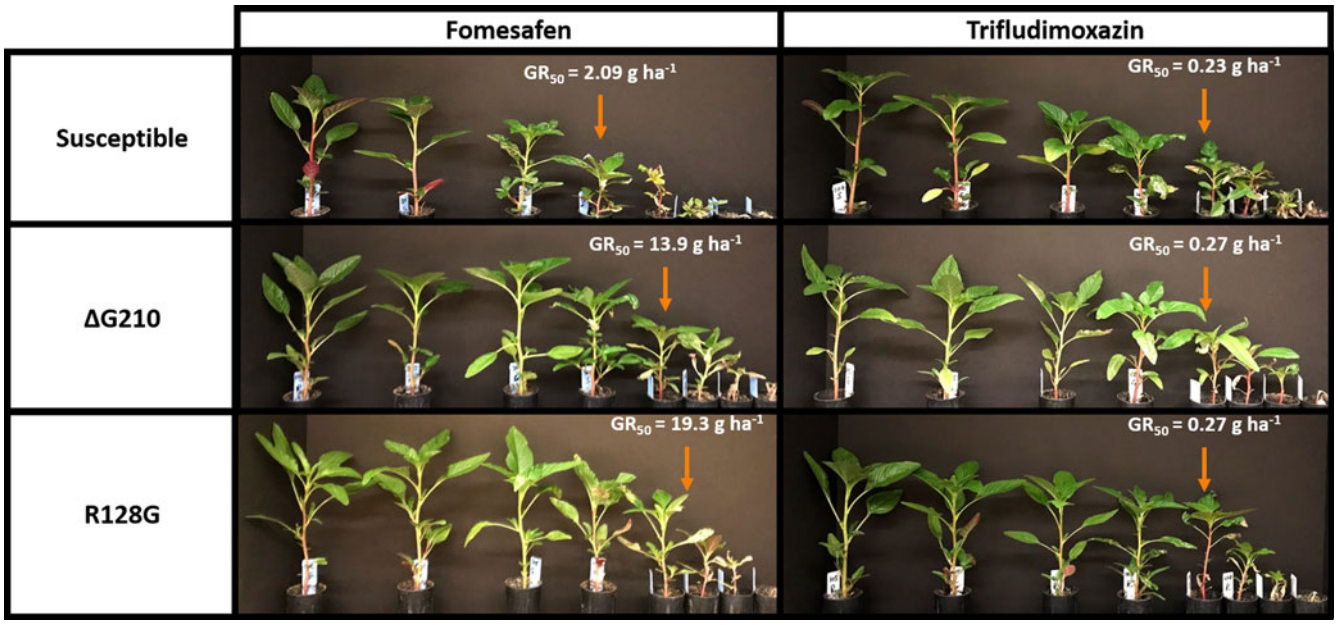

Figure 1. Comparison of dose response following applications of fomesafen and trifludimoxazin, applied to protoporphyrinogen oxidase–susceptible (PPO-S) and the protoporphyrinogen oxidase–resistant (PPO-R) (ΔG210 and R128G) biotypes of *Amaranthus tuberculatus*.

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. ΔG210) 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, leading to minimal selection pressure for resistance specific to saflufenacil (Grossman et al. 2010; Mueller et al. 2014). The lack of consistent selection pressure from saflufenacil may at least partially explain the dearth of TS mutations that confer robust resistance to saflufenacil.

Resistant biotypes of *A. tuberculatus* (ΔG210 or R128G) or *A. palmeri* (ΔG210 or V361A) did not display reduced sensitivity to applications of trifludimoxazin (R/S ratios of 0.8 to 1.2), in contrast to fomesafen or saflufenacil (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 activities of fomesafen and saflufenacil can be compromised by the conformational changes to the PPO enzyme resulting from TS 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* TS 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 TS 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 TS mechanisms (Obenland et al. 2019; Varanasi et al. 2018). NTS resistance in these species is mediated by increased herbicide metabolism via cytochrome P450s and/or glutathione *S*-transferases and confers

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Table 2. Results from pairwise *t*-tests comparing model parameter *e* (GR_{50} value), within *Amaranthus* species and herbicide, via the *compParm* function in the DRC package in R[a].

Species	Herbicide	Biotype[b]	Comparison results		
			Estimate	SE	P-value
Amaranthus tuberculatus	Fomesafen	Susceptible vs. ΔG210	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. ΔG210	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. ΔG210	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. ΔG210	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. ΔG210	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. ΔG210	1.006	0.473	0.990
		Susceptible vs. V361A	1.326	0.442	0.462
		ΔG210 vs. V361A	1.318	0.682	0.641

[a]GR_{50}, the herbicide rate required to reduce shoot biomass by 50%.
[b]ΔG210, *A. tuberculatus* or *A. palmeri* resistant via ΔG210 mutation; R128G, *A. tuberculatus* resistant via R128G mutation; V361A, *A. palmeri* resistant via V361A mutation.

Table 3. Resistant/susceptible (R/S) ratios calculated using GR_{50} values for each species and biotype, within herbicide[a].

Species	Biotype[b]	R/S ratio		
		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

[a]GR_{50}, the herbicide rate required to reduce shoot biomass by 50%.
[b]ΔG210, *A. tuberculatus* or *A. palmeri* resistant via ΔG210 mutation; R128G, *A. tuberculatus* resistant via R128G mutation; V361A, *A. palmeri* resistant via V361A mutation.

cross-resistance among PPO-inhibiting herbicides (Jugulam and Shyam 2019; Varanasi et al. 2019). As TS 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 TS 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 at 7 DAA, followed by slight, and gradual decreases in control for some treatments at later evaluations (Table 4; Supplementary Table 1) Combined across all site-years, *A. tuberculatus* control was ≥93% at 7 DAA, regardless of herbicide treatment applied (Supplementary Table 1). At 14 DAA, differences between herbicide treatments were more defined, with applications of saflufenacil (25 or 50 g ha^{-1}), lactofen, or fomesafen resulting in 88% to 90% control (Supplementary Table 1). *Amaranthus tuberculatus* control following applications of all other herbicide treatments was ≥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^{-1} 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 with applications of 12.5 or 25 g ha^{-1} trifludimoxazin, resulting in 80% and 83% control when applied at 25 and 50 g ha^{-1}, respectively (Table 4), similar to previous results with *A. palmeri* (Morichetti et al. 2012). Applications of lactofen (87% control) were less efficacious compared with 25 g ha^{-1} trifludimoxazin, but control of *A. tuberculatus* with fomesafen (88%) and flumioxazin (94%) were similar compared with 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^{-1} 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, when 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 ≥93% *A. tuberculatus* control and mortality

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Table 4. Efficacy of foliar application of protoporphyrinogen oxidase (PPO)-inhibiting herbicides on *Amaranthus tuberculatus* at 28 d after application (DAA) for field experiments conducted at the Davis Purdue Agriculture Center and Meigs Horticulture Research Farm (Meigs) in 2017 and 2018[a].

		A. tuberculatus control at 28 DAA[c]			
		Combined field sites	Meigs 2018	Combined field sites	Meigs 2018
Herbicide[b]	Rate	Visual control estimate		Plant mortality[d]	
	g ai ha^{-1}	%			
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 a	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 a	99
Trifludimoxazin + saflufenacil	6.25 + 50.0	97 a	99	97 a	97
Trifludimoxazin + saflufenacil	12.5 + 25.0	93 ab	99	95 ab	99
Trifludimoxazin + saflufenacil	12.5 + 50.0	95 ab	99	99 a	98
Trifludimoxazin + saflufenacil	25.0 + 25.0	96 a	99	96 a	99
Trifludimoxazin + saflufenacil	25.0 + 50.0	96 a	99	99 a	99
Lactofen	219	87 cd	99	87 c	98
Fomesafen	343	88 bcd	95	90 bc	92
Flumioxazin	71.5	94 abc	99	97 a	98

[a]Frequency of *A. tuberculatus* individuals with resistance to PPO-inhibiting herbicides via the ΔG210 mutation was approximately 30% and 3% at Davis Purdue Agricultural Center and Meigs, respectively.
[b]Herbicide treatments included methylated seed oil, applied at 1% v/v.
[c]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 ($\alpha = 0.05$). No significant differences in visual estimates of control or plant mortality at 28 DAA were observed between herbicide treatments at Meigs in 2018 ($\alpha = 0.05$).
[d]*Amaranthus tuberculatus* plants from two 0.5-m^2 quadrats in each plot were counted at 28 DAA, 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.

≥96% (Table 4). Control of *A. tuberculatus*, including plant mortality, was ≥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^{-2}) of *A. tuberculatus* relative to the other 3 site-years (62 plants m^{-2}, 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^{-1} 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-R (Falk et al. 2006). The predicted use pattern for trifludimoxazin targets preplant applications before 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 U.S. 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 before 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.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2024.103

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