This is a “preproof” accepted article for Weed Science. This version may be subject to change in the production process, *and does not include access to supplementary material*.
DOI: 10.1017/wsc.2024.103



# 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

Nicholas R. Steppig[1], William G. Johnson[2], Bryan G. Young[2]

[1]Former Graduate Research Assistant (ORCID 0009-0005-7467-6921), Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA; [2]Professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA

**Author for correspondence:** Nicholas R. Steppig; nicksteppig17@gmail.com

**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 ΔG210 (*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 (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 three site-years, applications of 25 g ai $ha^{-1}$ trifludimoxazin resulted in 95% control of *A. tuberculatus* 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 to other commercial PPO inhibitors, even in populations where low frequencies of PPO inhibitor-resistant 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:** ΔG210 mutation, herbicide resistance, novel herbicide active ingredients, protoporphyrinogen oxidase, R128G mutation, resistance management, V361A mutation


https://doi.org/10.1017/wsc.2024.103

## 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 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).


https://doi.org/10.1017/wsc.2024.103

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 (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* (Rangani et al. 2019; Nie et al. 2023). As the


https://doi.org/10.1017/wsc.2024.103

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 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 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 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 County, Indiana, respectively (Spaunhorst et al. 2019).


https://doi.org/10.1017/wsc.2024.103

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-$cm^3$ 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 $ha^{-1}$ 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 $ha^{-1}$), saflufenacil (0 to 125 g ai $ha^{-1}$, labeled 1X use rate = 25 g ai $ha^{-1}$), or fomesafen (0 to 1320 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 $v^{-1}$. 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 1100 µmol $m^{-2}$ $s^{-1}$ 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


https://doi.org/10.1017/wsc.2024.103

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):

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

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 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 ΔG210 target-site mutation among individual plants at the Meigs and Davis populations, respectively. 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 $ha^{-1}$ 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 $ha^{-1}$ at 276 kPa. Herbicide treatments included


https://doi.org/10.1017/wsc.2024.103

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, Precision Laboratories, Waukegan, IL) was added to each treatment at 1% v $v^{-1}$ 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 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 ten-fold 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 $GR_{50}$ values indicated that *Amaranthus* biotypes containing target-site PPO resistance mutations (ΔG210 or R128G in *A. tuberculatus* and ΔG210 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


https://doi.org/10.1017/wsc.2024.103

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. 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, 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 target-site mutations which confer robust resistance to saflufenacil.

In contrast to fomesafen or 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) (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


https://doi.org/10.1017/wsc.2024.103

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 ≥ 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 $ha^{-1}$), lactofen, or fomesafen, resulted 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


https://doi.org/10.1017/wsc.2024.103

were less effective compared to 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 to 25 g $ha^{-1}$ 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 $ha^{-1}$ 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, 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 ≥ 93% *A. tuberculatus* control, and 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 three 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-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


https://doi.org/10.1017/wsc.2024.103

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.


## Funding

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.


## Competing Interests

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

## References


Asher BS, Dotray PA, Liebl RA, Kelling JW, Udeigwe TK, Reed JD, Keller KE, Bowe SJ, Aldridge RB, Simon A (2020) Vertical mobility and cotton tolerance to trifludimoxazin, a new protoporphyrinogen oxidase-inhibiting herbicide, in three West Texas soils. Weed Technol 35:144-148

Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37-43

Claassen R, Bownam M, McFadden J, Smith D, Wallander S (2018) Tillage intensity and conservation cropping in the United States. USDA: EIB-197. 27 p

Dieleman JA, Mortenson DA, Martin AR (1999) Influence of velvetleaf (*Abutilon theophrasti*) and common sunflower (*Helianthus annus*) density variation on weed management outcomes. Weed Technol 47:81-89

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen LP, Matsumoto H (1991) Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 39: 465-473




https://doi.org/10.1017/wsc.2024.103

Falk JS, Shoup DE, Al-Khatib K, Peterson DE (2006) Protox-resistant waterhemp (*Amaranthus rudis*) response to herbicides applied at different growth stages. Weed Sci 54:793-799

Farmer JA, Bradley KW, Young BG, Steckel LE, Johnson WG, Norsworthy JK, Davis VM, Loux MM (2017) Influence of tillage method on management of Amaranthus species in soybean. Weed Technol 31:10-20

Findley D, Youmans CC, Bowe S, (2020) Tirexor (trifludimoxazin): next generation burndown update – US. Proceedings of the 2020 Weed Science Society of America Annual Meeting. Maui, HI: Weed Science Society of America

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BT, Schott RC, Tranel PJ (2017) Two new PPX2 mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559-1563

Grossman K, Niggeweg R, Christiansen N, Looser R, Ehrhardt T (2010) The herbicide saflufenacil (Kixor™) is a new inhibitor of protoporphyrinogen ix oxidase activity. Weed Sci 58:1-9

Hager AG, Wax LM, Bollero GM, Stoller EW (2002) Influence of diphenylether herbicide application rate and timing on common waterhemp (*Amaranthus rudis*) control in soybean (*Glycine max*) Weed Technol 17:14-20

Hager AG, Wax LM, Bollero GA, Simmons FW (2002) Common waterhemp (*Amaranthus rudis* Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.). Crop Prot 21:277-283

Hager AG, Wax LM, Bollero GA, Stoller EW (2003) Influence of diphenylether herbicide application rate and timing on common waterhemp (*Amaranthus rudis*) control in soybean (*Glycine max*). Weed Technol 17:14

Hao GF, Zuo Y, Yang SH, Yang GF (2011) Protoporphyrinogen oxidase inhibitor: an ideal target for herbicide discovery. Chimia 65:961-969

Hartzler RG, Buhler DD, Stoltenberg DE (1999) Emergence characteristics of four annual weed species. Weed Sci 47:578-584

Hausman NE, Tranel PJ, Riechers DE, Hager AG (2016) Responses of waterhemp (*Amaranthus tuberculatus*) population resistant to HPPD-inhibiting herbicides in foliar-applied herbicides. Weed Technol 30:106-115


https://doi.org/10.1017/wsc.2024.103

Heap I (2020) International survey of herbicide resistant weeds. http://www.weedscience.org. Accessed December 14, 2020

[HRAC] Herbicide Resistance Action Committee (2020) HRAC mode of action classification 2020. https://hracglobal.com/files/HRAC_Revised_MOA_Classification_Herbicides_Poster.pdf Accessed December 15, 2020

Jha P, Norsworthy JK (2009) Soybean canopy and tillage effects on emergence of Palmer amaranth (*Amaranthus palmeri*) from a natural seed bank. Weed Sci 57:644-651

Jugulam M, Shyam C (2019) Non-target-site resistance to herbicides: recent developments. Plants 8:1-16

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840-848

Korres NE, Norsworthy JK, Youmg BG, Reynolds DB, Johnson WG, Conley SP, Smeda RJ, Mueller TC, Spaunhorst DJ, Gage KL, Loux M, Kruger GR, Bagavathiannan MV (2018a) Effects of Palmer Amaranth (*Amaranthus palmeri*) establishment time and distance from the crop row on biological and phenological characteristics of the weed: implications on soybean yield. Weed Sci 67:126-136

Korres NE, Norsworthy JK, Mauromoustakos A, Williams MM (2018b) Soybean density and Palmer amaranth (*Amaranthus palmeri*) establishment time: effects on weed biology, crop yield, and economic returns. Weed Sci 68:468-475

Lee HJ, Duke SO (1994) Protoporphyrinogen IX-oxidizing activities involved in the mod of action of peroxidizing herbicides. J Agric Food Chem 42:2610-2618

Lillie KJ (2019) Characterization of PPO-inhibitor resistance in waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*). M.S. thesis. Urbana, IL: University of Illinois at Urbana-Champaign. 93 p

Legleiter TR, Bradley KW, Massey RE (2009) Glyphosate-resistant waterhemp (*Amaranthus rudis*) control and economic returns with herbicide programs in soybean. Weed Technol 23:54-61

Morichetti S Ferrell J, MacDonald G, Sellers B, Rowland D (2012) Weed management and peanut response from applications of saflufenacil. Weed Technol 26:261-266

Mueller TC, Boswell BW, Mueller SS, Steckel LE (2014) Dissipation of fomesafen, saflufenacil, sulfentrazone, and flumioxazin from a Tennessee soil under field conditions. Weed Sci 62:664-671

Nie H, Mansfield BC, Harre NT, Young JM, Steppig NR, Young BG (2019) Investigating target-site resistance mechanism to the PPO-inhibiting herbicide fomesafen in waterhemp and interspecific hybridization of *Amaranthus* species using next generation sequencing. Pest Manag Sci 75:3235-3244

Nie H, Harre NT, Young BG (2023) A new V361A mutation in *Amaranthus palmeri* PPX2 associated with PPO-inhibiting herbicide resistance. Plants 12:1886

Norsworthy JK, Ward SM, Shaw DR, Llwewllyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Obenland OA, Ma R, O'Brien SR, Lygin AV, Riechers DE (2019) Carfentrazone-ethyl resistance in an *Amaranthus tuberculatus* population is not mediated by amino acid alterations in the PPO2 protein. PLoS ONE 14:1-15

Oryokot JOE, Murphy SD, Swanton CJ (1997) Effect of tillage and corn on pigweed (*Amaranthus spp.*) seedling emergence and density. Weed Sci 45:120-126

Patzoldt WL, Hager AG, McCormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. Proc Natl Acad Sci USA 103:12329-12334

[PMRA] Pest Management Regulatory Agency (2020) Proposed registration decision PRD2020-15, trifludimoxazin, Vulcaris and Voraxor. https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/consultations/proposed-registration-decisions/2020/trifludimoxazin-vulcarus-voraxor/document.html. Accessed December 15, 2020

Rangani G, Salas-Perez RA, Aponte RA, Knapp M, Craig IR, Mietzner T, Langaro AC, Noguera MM, Porri A, Burgos NR (2019) A novel single-site mutation in the catalytic domain of protoporphyrinogen oxidase ix (PPO) confers resistance to PPO-inhibiting herbicides. Front Plant Sci 10:1-12


https://doi.org/10.1017/wsc.2024.103

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS ONE 10(12):e0146021

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72:864-869

Salas-Perez RA, Burgos NR, Rangani G, Singh S, Refatti JP, Piveta L, Tranel PJ, Mauromoustakos A, Scott RC (2017) Frequency of Gly-210 deletion mutation among protoporphyrinogen oxidase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) populations. Weed Sci 65:718-731

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145-150

Spaunhorst DJ, Nie H, Todd JR, Young JM, Young BG, Johnson WG (2019) Confirmation of herbicide resistance mutations Trp574Leu, ΔG210, and EPSPS gene amplification and control of multiple herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) with chlorimuron-ethyl, fomesafen, and glyphosate. PLoS ONE 14:1-12

Steckel LE, Sprague CL, Hager AG (2002) Common waterhemp (*Amaranthus rudis*) control in corn (*Zea mays*) with single preemergence and sequential applications of residual herbicides. Weed Technol 16:755-761

Steppig NR, Willingham SD, Young BG (2018) Evaluation of trifludimoxazin alone and in combination with saflufenacil for soil-residual control of PPO inhibitor-resistant tall waterhemp. Proceedings of the 2018 North Central Weed Science Society Annual Meeting. Milwaukee, WI: North Central Weed Science Society

Steppig NR, Mansfield BC, Nie H, Young JM, Young BG (2017) Presence of an alternative mechanism of resistance to PPO-inhibiting herbicides in tall waterhemp populations from Indiana, Illinois, Iowa, Missouri, and Minnesota. Proceedings of the 2017 North Central Weed Science Society Annual Meeting. St. Louis, MO: North Central Weed Science Society

Swinton SM, Van Deynze B (2017) Hoes to herbicides: economics of evolving weed management in the United States. Eur J Dev Res 29: 560-574

Tranel PJ (2020) Herbicide resistance in *Amaranthus tuberculatus*. Pest Manag Sci 77:43-54


https://doi.org/10.1017/wsc.2024.103

[USDA-NASS] US Department of Agriculture, National Agriculture Statistics Survey (2020) Agricultural Chemical Use Program. Soybeans. https://quickstats.nass.usda.gov/. Accessed 4/25/2021

Van Wychen L (2019) 2019 Survey of the most common and troublesome weeds in broadleaf crops, fruits and vegetables in the United States and Canada. Weed Science Society of America Survey Dataset. Available: https://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx

Varanasi VK, Brabham C, Norsworthy JK (2018) Confirmation and characterization of non-target site resistance to fomesafen in Palmer amaranth (*Amaranthus palmeri*) Weed Sci 66:702-709

Varanasi V, Brabham C, Korres N, Norsworthy J (2019) Nontarget site resistance in Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] confers cross-resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Technol 33:349-354

Wallihan EF, Garber MJ (1971) Efficiency of glasshouse pot experiments rotating versus stationary benches. Plant Physiol 48:789-791

Wang D, Zhang R, Ismail I, Xue Z, Liang L, Yu S, Wen X, Xi Z (2019) Design, herbicidal activity, and QSAR analysis of clycloalka[d]quinazoline-2,4-dione-benzoxazinones as protoporphyrinogen ix oxidase inhibitors. J Agric Food Chem 67:9254-9364

Wu C, Goldsmith MR, Pawlak J, Feng P, Smith S, Navarro S, Perez-Jones A (2020) Differences in efficacy, resistance mechanism and target protein interaction between two PPO inhibitors in Palmer amaranth (*Amaranthus palmeri*) Weed Sci 68:105-115

Wuerffel RJ, Young JM, Lee RM, Tranel PJ, Lightfoot DA, Young BG (2015) Distribution of the ΔG210 protoporphyrinogen oxidase mutation in Illinois waterhemp (*Amaranthus tuberculatus*) and an improved molecular method for detection. Weed Sci 63:839-845

Wuerffel RJ, Young JM, Matthews JL, Young BG (2015) Characterization of PPO-inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting herbicides. Weed Sci 63:511-521


https://doi.org/10.1017/wsc.2024.103

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 | $GR_{50}$ (±SE) for shoot biomass | | |
|---|---|---|---|---|
| | | Fomesafen | Saflufenacil | Trifludimoxazin |
| | | g ai $ha^{-1}$ | | |
| *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 (± 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 (± 0.08) | 0.31 (± 0.09) |

[a] Herbicides were applied to plants 5 to 7.5 cm in height and biomass was collected 14 days after treatment.

[b] Abbreviations: $GR_{50}$: the herbicide rate required to reduce shoot biomass by 50%; ΔG210, *Amaranthus tuberculatus or Amaranthus palmeri* resistant via ΔG210 mutation; R128G, *Amaranthus tuberculatus* resistant via R128G mutation; SE, standard error; V361A, *Amaranthus palmeri* resistant via V361A mutation.

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].

| | | | Comparison results | | |
|---|---|---|---|---|---|
| Species | Herbicide | Biotype | Estimate | Standard error | 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] Abbreviation: $GR_{50}$: the herbicide rate required to reduce shoot biomass by 50%; ΔG210, *Amaranthus tuberculatus* or *Amaranthus palmeri* resistant via ΔG210 mutation; R128G, *Amaranthus tuberculatus* resistant via R128G mutation; V361A, *Amaranthus palmeri* resistant via V361A mutation.

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

| Species | Biotype | 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] Abbreviation: $GR_{50}$: the herbicide rate required to reduce shoot biomass by 50%; ΔG210, *Amaranthus tuberculatus* or *Amaranthus palmeri* resistant via ΔG210 mutation; R128G, *Amaranthus tuberculatus* resistant via R128G mutation; V361A, *Amaranthus palmeri* resistant via V361A mutation.

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[a].

| | | *Amaranthus tuberculatus* control 28 DAA | | | | | |
|---|---|---|---|---|---|---|---|
| | | Combined field sites[c] | | Meigs 2018[d] | Combined field sites | | Meigs 2018[e] |
| Herbicide[b] | Rate | Visual control estimate | | | Plant mortality[f] | | |
| | 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$^{-1}$.

[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 ($\alpha = 0.05$).

[d,e] 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 ($\alpha = 0.05$).

[f] *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.

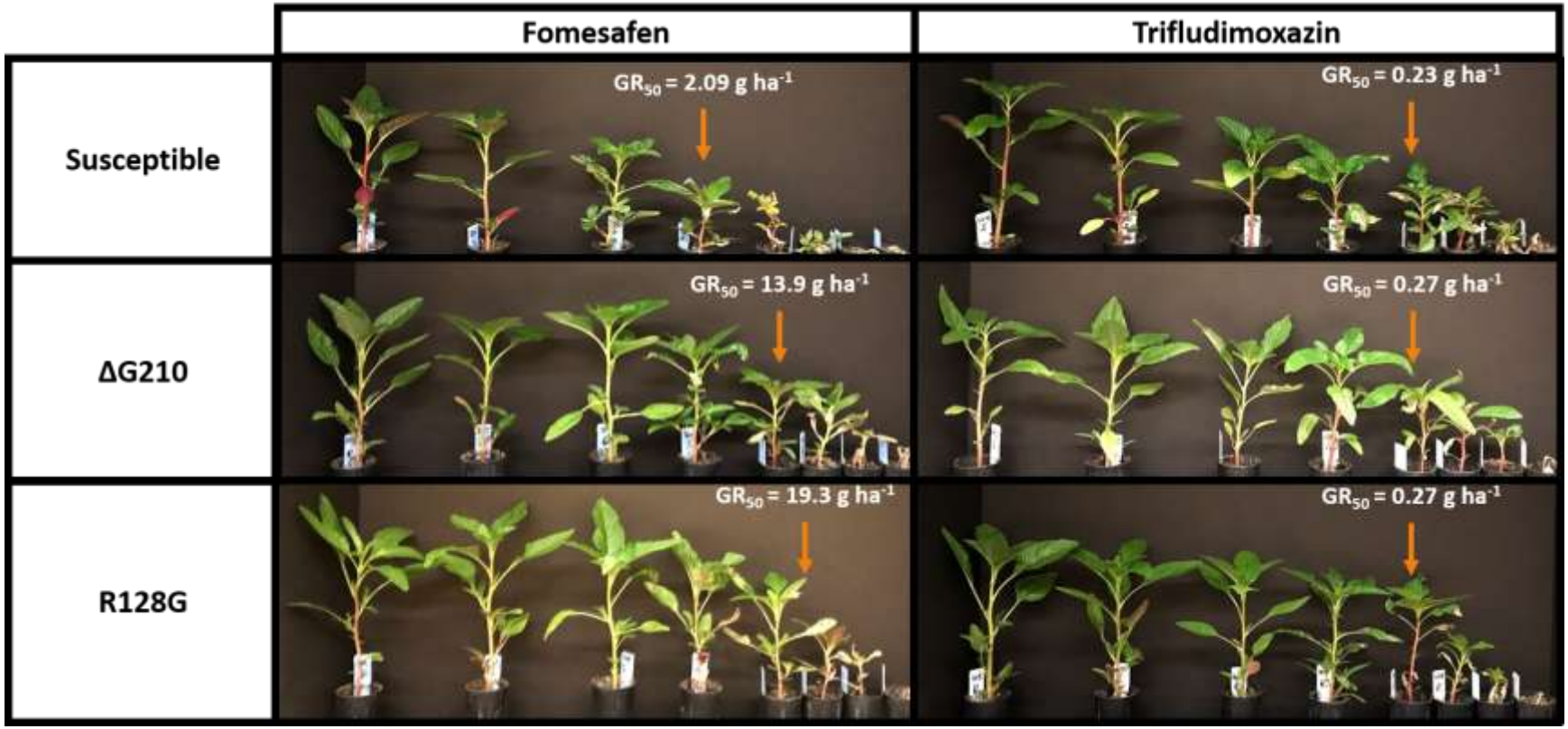


**Figure 1.** Comparison of dose response following applications of fomesafen and trifludimoxazin, applied to PPO-S and the PPO-R (ΔG210 and R128G) biotypes of *Amaranthus tuberculatus*.