Weed Technology

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Research Article

Cite this article: Randell-Singleton T, Hand LC, Vance JC, Wright-Smith HE, Culpepper AS (2024) Confirming resistance to PPO-inhibiting herbicides applied preemergence and postemergence in a Georgia Palmer amaranth population. Weed Technol. **38**(e23), 1–10. doi: 10.1017/wet.2024.12

Received: 22 June 2023
Revised: 20 October 2023
Accepted: 7 February 2024

Associate Editor:
R. Joseph Wuerffel, Syngenta

Nomenclature:
Aciflurofen; flumioxazin; fomesafen; lactofen; oxyfluorfen; trifludimoxazin; Palmer amaranth; *Amaranthus palmeri* S. Watson; cotton; *Gossypium hirsutum* L.; peanut, *Arachis hypogaea* L.; soybean, *Glycine max* (L.)

Keywords:
Herbicide resistance; residual herbicides; postemergence herbicide applications

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Confirming resistance to PPO-inhibiting herbicides applied preemergence and postemergence in a Georgia Palmer amaranth population

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Abstract

Herbicides that inhibit protoporphyrinogen oxidase (PPO) are used in more than 40 agronomic and specialty crops across Georgia to manage weeds through residual and postemergence (POST) control. In 2017, a population of Palmer amaranth exhibiting reduced sensitivity to POST applications of PPO-inhibiting herbicides was identified by the University of Georgia. Seed were collected from the site along with a known sensitive population; distance between the samples was 200 m, increasing the likelihood of similar environmental and genetic characteristics. To quantify sensitivity for both preemergence (PRE) and POST uses, 21 greenhouse dose-response assessments were conducted from 2017 to 2022. After conducting initial rate-response studies, 13 doses per herbicide were chosen for the POST experiment; field use rates of fomesafen (420 g ai ha^{-1}), lactofen (219 g ai ha^{-1}), acifluorfen (420 g ai ha^{-1}), and trifludimoxazin (25 g ai ha^{-1}) ranging from 0× to 4× the field use rate for the susceptible population, and 0× to 40× for the suspect population were applied. Herbicide treatments included adjuvants and were applied to plants 8 to 10 cm in height. Relative resistance factors (RRFs) were calculated for control ratings, mortality, and biomass, and ranged from 105 to 318, 36 to 1,477, 215 to 316, and 9 to 49 for fomesafen, lactofen, acifluorfen, and trifludimoxazin, respectively. In the PRE experiment, herbicide applications included five to nine doses of fomesafen (1× = 210 g ai ha^{-1}), flumioxazin (1× = 57 g ai ha^{-1}), oxyfluorfen (1× = 561 g ai ha^{-1}), and trifludimoxazin (1× = 38 g ai ha^{-1}); doses ranged from 0× to 6× for the suspect population and 0× to 2× for the susceptible population. Visual control, mortality, and biomass RRFs ranged from 3 to 5 for fomesafen, 21 to 31 for flumioxazin, 6 to 22 for oxyfluorfen, and 8 to 38 for trifludimoxazin. Results confirm that a Georgia Palmer amaranth population is resistant to PPO-inhibiting herbicides applied both PRE and POST.

Introduction

A familiar pest to many farmers and weed scientists, Palmer amaranth is a persistent weedy presence across U.S. agronomic and specialty crops. Native to the arid southwestern U.S. and northern Mexico, populations have spread beyond these regions through expansion of crop landscapes and movements associated with modern agriculture (Roberts and Florentine 2022; Steckel 2007; Ward et al. 2013; Webster and Nichols 2012). Palmer amaranth was first noted outside its native habitat in 1915 in Virginia, and as of 2020, its presence has been documented in at least 27 of the 48 continental U.S. states (USDA-APHIS 2020). By 2022, 28 states reported that Palmer amaranth was one of the most common and troublesome weeds in agronomic or vegetable cropping systems (Van Wychen 2022).

The physiological and biological characteristics of Palmer amaranth have contributed to its rapid spread across regions, along with its rise as an impactful weed of agriculture production. As a dioecious and highly fecund species, possessing the ability to produce 600,000 seeds per plant, Palmer amaranth is a significant contributor to the soil weed seed bank (Keeley et al. 1987; Ward et al. 2013; Webster and Grey 2015). Seedlings rapidly emerge when favorable conditions arise and quickly complete their life cycle in response to changing environmental conditions (Ehleringer 1985; Jha et al. 2010a,b; Kistner and Hatfield 2018; Steckel et al. 2004). Once established, Palmer amaranth plants are extremely competitive with crop plants for resources (sunlight, water, nutrients, space, pollinating insects) through high photosynthetic rates, fast vegetative growth, diaheliotropism, and prolific root production (Capinera 2005;

Culpepper et al. 2010; Ehleringer 1985; Ehleringer and Forseth 1980; Horak and Loughin 2000; Ward 2013). Population spread is currently limited to areas in which enough growing degree days can be accumulated to complete its life cycle, however, as climatic patterns in rainfall and temperature shift in the future, Palmer amaranth will continue to exploit these changes, expand its habitat, and lengthen its growing season (Kistner and Hatfield 2018; Runquist et al. 2019). Because research has confirmed the aggressive ability of Palmer amaranth present during the growing season to negatively impact yield, its presence in crop production fields must be avoided for a successful and economically profitable harvest (Basinger et al. 2019; Burke et al. 2007; Morgan et al. 2001; Tharp and Kells 2002).

Herbicides that inhibit protoporphyrinogen oxidase (PPO) are used for Palmer amaranth control in more than 40 Georgia cropping systems including cotton, peanuts, soybean, fruits, and vegetables (Anonymous 2013, 2015, 2019a, 2019b, 2020, 2021). Following the confirmation of glyphosate-resistant (GR) Palmer amaranth in Georgia in 2006, and subsequent identification in nearly 30 states over the next 14 yr, reliance on PPO-inhibiting herbicides has increased substantially (Culpepper et al. 2006; Heap 2023a; Salas et al. 2016; Schwartz-Lazaro et al. 2017; Sosnoskie and Culpepper 2014). PPO-inhibiting herbicides, including acifluorfen, fomesafen, flumioxazin, lactofen, and oxyfluorfen, act through the inhibition of the protoporphyrinogen oxidase enzyme, leading to a buildup of singlet oxygen that begins to break down cellular components in the presence of light (Cobb 2022; Shaner 2014). Through fast-acting contact activity when applied postemergence (POST), or residual weed control when applied preemergence (PRE), PPO-inhibiting herbicides are critical tools for Palmer amaranth management (Askew et al. 2002; Grichar 1997; Sperry et al. 2017; Sweat et al. 1998).

Like glyphosate, increased reliance on PPO-inhibiting herbicides and limited implementation of integrated control tactics led to the confirmation of PPO-resistant Palmer amaranth in Arkansas in 2014 (Salas et al. 2016). Since identifying the initial case, PPO-resistant Palmer amaranth has spread throughout the mid-South and Midwest (Heap 2023a; Montgomery et al. 2020; Oliveira et al. 2020; Wu et al. 2020). With prolific seed production, resistant Palmer amaranth can spread quickly, and by the time a farmer notices a field failure following PPO herbicide applications, the seed bank may already be filled with the resistant biotype (Salas et al. 2016; Sosnoskie et al. 2012). With each generation of Palmer amaranth subjected to PPO herbicide selection pressure, surviving plants can deposit seeds into the soil seedbank, allowing the population to become more homogenous, and increasing the potential for the spread of the resistant trait (Salas et al. 2016).

With GR Palmer amaranth being Georgia's most troublesome pest in all of agriculture, coupled with its ubiquitous presence throughout the state, its management must be at the forefront of a farmer's weed management plan (Culpepper et al. 2020). Therefore, because of their effectiveness and use flexibility, PPO-inhibiting herbicides have become critical components of weed management programs across Georgia cropping systems (Bryant and Ethredge 2022a,b; Culpepper and Singleton 2023; Hand 2022). Additionally, when markets are profitable, crop rotations may be replaced with successive plantings of the same crop (Livingston et al. 2015), further subjecting Palmer amaranth populations to multiple years of selection pressure from the most effective herbicide chemistries; often these are the PPO-inhibiting herbicides. Once resistance does occur in Georgia, its ability to spread could be rapid due to intense use of the chemistry across the agricultural landscape coupled with small average field sizes, which facilitates continuous movement between fields (Norsworthy et al. 2012; Thill and Mallory-Smith 1997). To preserve the utility of these herbicides, efforts must be made to quickly identify and manage Palmer amaranth populations not controlled by PPO-inhibiting herbicides across the state while continuing to implement sound, diversified weed management programs that reduce selection pressure within and across cropping systems.

In 2017, a Georgia soybean farmer reported a failure in Palmer amaranth control to the University of Georgia Extension service following applications of two PPO-inhibiting herbicides for 3 yr in a row. During each growing season, flumioxazin was applied PRE, and fomesafen was applied POST, placing immense selection pressure on Palmer amaranth that was present in the field. This site was unique, in that the production field was split into two different management areas, both under the same center-pivot irrigation system; one used for soybean and the other for cotton (Figure 1). After conducting a field bioassay, Extension service personnel determined that Palmer amaranth was not effectively controlled in the soybean field with fomesafen, whereas 200 m away plants in the cotton field were completely controlled, indicating the presence of two different biotypes within the same field. Therefore, the objectives of this research were to 1) quantify the sensitivity of this Georgia Palmer amaranth population to multiple PPO-inhibiting herbicides through dose-response assessments applied PRE or POST, and 2) calculate levels of resistance if applicable.

Materials and Methods

Seed Collection, Verification of Sensitive Population, and Heritability of Suspect Resistant Population

In fall 2017, inflorescences were collected from an estimated 30 mature female Palmer amaranth plants that had escaped the initial field screening, where fomesafen was applied POST within the soybean management area. To understand the ability of the Palmer amaranth to tolerate the herbicide in the field, fomesafen was applied at 280 (1× field rate), 560, 1,120, 2,240, and 2,800 g ai ha^{-1} in mixture with a nonionic surfactant at 0.25% v/v, with treatments arranged in a randomized complete block design and replicated four times. Samples from surviving plants were placed in the University of Georgia Weed Science Greenhouse in Tifton, GA, where they were dried down under greenhouse lights, threshed, and cleaned by hand to separate seeds, following methods outlined by Sosnoskie et al. (2012) and Wise et al. (2009). Collected seeds were combined into a composite sample, labeled as F_1, and placed into cool storage (1 C) for at least 6 wk to break dormancy (Buhler and Hoffman 1999; Culpepper et al. 2006). At the same time inflorescences were collected from herbicide survivors in the soybean field, an estimated 50 mature seed heads were also collected from female Palmer amaranth plants located in the cotton weed management area, known to be susceptible to PPO-inhibiting herbicides. After processing the seeds following the same method stated above, they were labeled as "susceptible" and placed in the cooler under the same conditions as previously described.

Before beginning dose-response studies in the greenhouse, the sensitivity of the susceptible Palmer amaranth population to PPO-inhibiting herbicides used in the studies was confirmed in both the field and greenhouse (data not reported). The resistance trait must be heritable based on the criteria for confirmation of herbicide-resistant weeds (Heap 2023b). Therefore, seeds collected from survivors in the field and the initial bioassay greenhouse

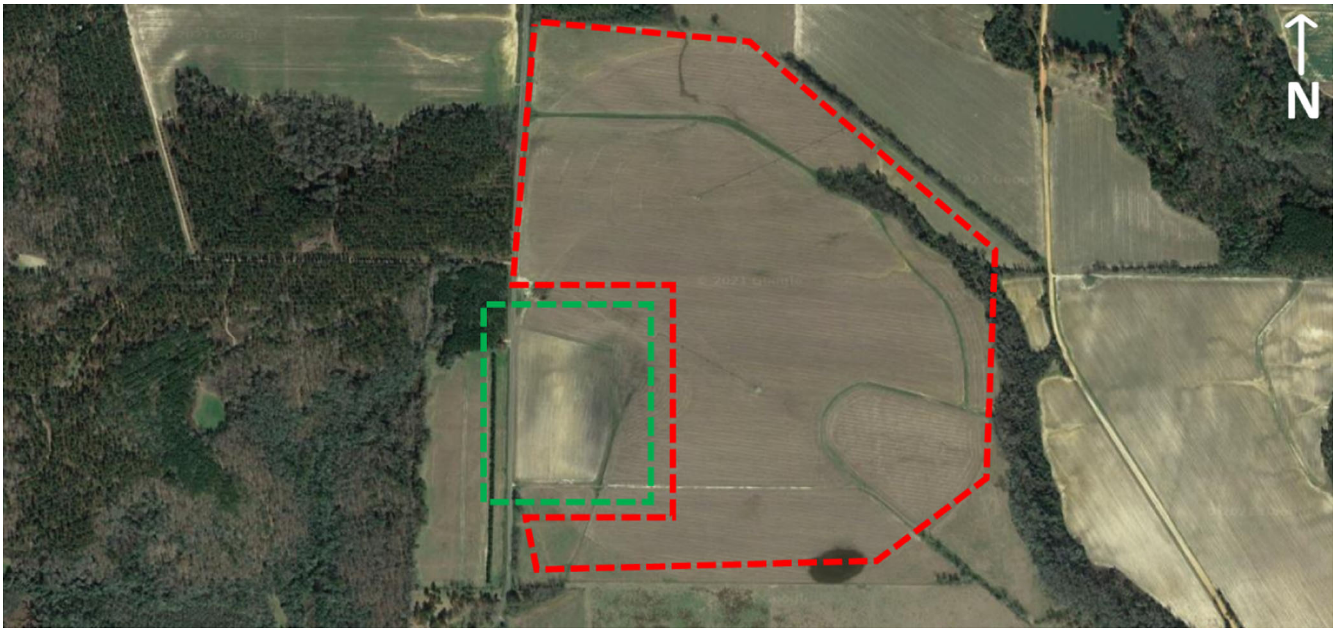

Figure 1. Collection site of Palmer amaranth populations for assessment. The suspected resistant population was collected from the area highlighted in red, and the known susceptible population was collected from the area highlighted in green.

experiment were used for dose-response assessments to ensure this assumption was met.

Postemergence Studies

A greenhouse bioassay was initiated during spring 2018. Palmer amaranth seeds from both sites were planted in round pots (10 cm diameter, 10 cm deep) filled with premoistened Miracle-Gro Moisture Control potting mix (Scotts Miracle-Gro, Marysville, OH). At planting, seeds were scattered over the surface of the soil and incorporated to a 0.6-cm depth by hand, to ensure proper seed-to-soil contact. Pots were then placed under overhead lights (Philips 1000w Agrolite XT; Atlanta, GA; 1,621 µmol/s, 130,000 lumens) set to a 12-h photoperiod. Prior to emergence, all pots were lightly overhead irrigated by hand to ensure consistent moisture, without pushing seed deep into the potting media; all pots were subirrigated once emergence began to ensure plants were not broken or injured. Based on local methodology developed for previous research, greenhouse temperatures were maintained at 35 C until Palmer amaranth emergence, then temperatures were reduced to 30 C for the remainder of the study. Because a known seed amount was not used in this bioassay, emerged plants were thinned daily to ensure four evenly spaced healthy plants that were consistent in height, remained in each pot by the time emergence ceased. Prior to herbicide application, 30 g of Super Rainbow Plant Food 10-10-10 (Nutrien, Loveland, CO) was sprinkled around the base of the plants and covered with 5 cm of soil, which also provided stabilization for the seedlings to move through the spray chamber.

Once Palmer amaranth plants averaged 8 to 10 cm in height, treatments were applied. Each pot represented one experimental unit and all pots were arranged in a randomized complete block design; four replications were included. Treatments consisted of a POST application of either fomesafen (Reflex®; Syngenta Crop Protection, Greensboro, NC) at 210, 420 (1×), 840, or 1,682 g ai ha^{-1}; lactofen (Cobra®; Valent USA, Walnut Creek, CA) at 106, 219 (1×), 438, or 876 g ai ha^{-1}; or acifluorfen (Ultra Blazer®; UPL Inc., King of Prussia, PA) at 210, 420 (1×), 840, or 1,682 g ai ha^{-1}. All herbicide applications included adjuvants (crop oil concentrate at 1% v/v for lactofen; nonionic surfactant at 0.25% v/v for fomesafen and acifluorfen). Treatments were applied using a spray chamber set to deliver 140 L ha^{-1} at 165 kPa, using a single 8002 Teejet Even flat spray nozzle (Spraying Systems Co., Glendale Heights, IL). At the conclusion of data collection, two plants surviving the highest application rate for each herbicide within each replication were replanted into large pots (25 cm diameter, 25 cm deep) using the same potting media as previously described. These plants were fertilized, irrigated as needed, and allowed to interbreed during pollination. At plant maturity, female inflorescences were harvested, processed, and labeled as F_2.

Dose-response assessments were initiated on the F_2 and susceptible Palmer amaranth populations during spring 2020 and were repeated in fall 2020. Supplemental treatments were added to the range of application rates through two additional experimental runs, both conducted during spring 2022, for a total of four experimental runs (Ritz et al. 2015). Plants from both populations were established in pots using the materials and methods as previously described and were arranged in a randomized complete block design; five to eight replications were included in each experimental run. Evaluated herbicides included fomesafen, lactofen, acifluorfen, and trifludimoxazin (BASF Corporation, Research Triangle Park, NC), each with a recommended field use rate (×) of 420, 219, 420, and 25 g ai ha^{-1}, respectively. For the F_2 plants, 13 treatments were included that represented doses from 0× to 40× the field use rate for each herbicide, while the susceptible plants were treated with 13 doses ranging from 0× to 4× the recommended field use rate. All POST herbicide applications included the use of adjuvants as recommended by the label, with nonionic surfactant (0.25% v/v) included for fomesafen and acifluorfen applications, crop oil concentrate (1% v/v) included for lactofen applications, and methylated seed oil (1% v/v) included for trifludimoxazin applications. All herbicide

https://doi.org/10.1017/wet.2024.12 Published online by Cambridge University Press

treatments were implemented using application methods as previously described. Care was taken to ensure that all spray equipment was cleaned using a triple rinse procedure with ammonia between herbicides, and that there was no risk of contamination via particle drift to plants between herbicide applications. Once herbicide treatments were applied, plants were returned to the greenhouse, where they were placed back under greenhouse lights and subirrigated for the remainder of the study. Following the completion of data collection, several Palmer amaranth plants from the F_2 population surviving the two highest application rates from each herbicide were repotted, placed back into the greenhouse, and allowed to grow and interbreed until maturity. Female inflorescences were processed and stored as previously described; this seed collection was labeled as F_3.

In both the bioassay and dose-response studies, Palmer amaranth control was assessed using a 0% (no plant injury) to 100% (complete plant death) visual scale to determine the impact that each herbicide and application rate had on plant injury and subsequent control, beginning 2 d after treatment (DAT) and continuing daily until the study was completed at 8 DAT when plant mortality plateaued in the sensitive population. To further quantify treatment differences, mortality (the number of alive or dead plants) was assessed in each plot when visual control plateaued (8 DAT). At this time, each plant was then removed at the soil level, and a plot weight of all combined plants was recorded for biomass assessments. All mortality and biomass assessments were converted to a reduction relative to the nontreated control for analysis.

Preemergence Studies

To develop procedures and better understand initial Palmer amaranth population responses to PPO-inhibiting herbicides applied PRE, a greenhouse bioassay was initiated during spring 2021. Plastic greenhouse flats (46 cm long by 25 cm wide by 7 cm deep) were filled with soil collected from a field at the University of Georgia known to be absent of Palmer amaranth. Soil was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudult) consisting of 85.80% sand, 0.80% silt, and 13.40% clay. Before it was placed into the flat, all soil was sieved to remove large rocks, plant debris, and weedy tubers. Once filled, flats were placed in the greenhouse under lights set to a 24-h photoperiod for at least 48 h before study initiation allowing soil in each flat to dry. During this time, greenhouse temperature was maintained at 35 C to facilitate soil drying, to later ensure uniform soil moisture across flats. At 24 h prior to initiating the study, each soil flat received 500 to 750 mL of subirrigation to restore soil moisture content and ensure consistency across flats. To prevent possible contamination between individual flats, greenhouse tables were covered with a grid structure constructed out of polyvinyl chloride pipe and covered in plastic, which allowed each flat to be placed in an individual cell for the entirety of the study (Figure 2). On the day of study initiation, 0.19 g of Palmer amaranth seeds (approximately 250 seeds) from the F_2 or the susceptible population were spread over each respective flat. Seeds were secured in place by spreading a thin layer (0.3 cm) of soil over the seed, which minimized disturbance during treatment application and ensured good seed-to-soil contact.

Flats were arranged in a randomized complete block design, and included four replications (one replication per greenhouse table). Herbicide treatments in the PRE bioassay included five doses of fomesafen ranging from 26 to 420 g ha^{-1} and five doses of flumioxazin (Valor® EZ; Valent USA) ranging from 5 to 86 g ha^{-1} applied to the F_2 and susceptible populations; a nontreated control was also included for each population. Herbicide treatments were applied in the spray chamber following methods previously described, and placed back into the greenhouse, where each flat immediately received 0.75 cm of overhead irrigation for herbicide activation. This activation event was applied using a watering can to ensure all irrigation was accurately applied to the individual flat. Following overhead activation, all irrigation needs were met through subirrigation for the remainder of the study. Subirrigation was implemented (250 to 750 mL per flat) as needed to ensure that soil did not dry out, and preventing formation of a soil crust and herbicide movement up and down in the soil. At study completion, surviving Palmer amaranth plants from the two highest application rates from each herbicide were replanted and allowed to grow until maturity, when the seeds were collected, processed, and labeled as F_3.

Figure 2. Greenhouse design for preemergence dose-response screening studies. Each flat filled with soil was placed in an individual cell for subirrigation to prevent herbicide contamination between flats from water movement.

Dose-response assessments were conducted from 2021 to 2022 and included six experimental runs. Study initiation, including soil preparation and seed planting, occurred as previously described, with Palmer amaranth seeds from the F_2 and susceptible population included to quantify plant response. Seeds from the F_3 population were also used in the final two experimental runs to supplement a low F_2 seed supply. The experimental design was a randomized complete block design with four replications per study. Treatments included five to nine doses each of fomesafen (2.59 to 630 g ha^{-1}), flumioxazin (0.078 to 171 g ha^{-1}), oxyfluorfen (0.769 to 3,366 g ha^{-1}), and trifludimoxazin (0.052 to 228 g ha^{-1}) for the F_2/F_3 and susceptible population. The 1× field use rate was 210, 57, 561, and 38 g ha^{-1} for each herbicide, respectively.

Beginning at 7 DAT and continuing weekly through 28 DAT, the residual activity and effectiveness of the herbicides were visually assessed through control evaluations using the same scale as previously described. Furthermore, to further quantify differences in residual activity, emergence was assessed weekly by counting the number of emerged living plants in each plot. Biomass was collected on each plot 21 to 28 DAT by counting and

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removing the aboveground plant material and recording a collective weight. Data assessments on emergence and biomass were converted to a reduction relative to the nontreated control for analysis.

Statistical Analysis

Interactions between treatments, experiment runs, and generations of Palmer amaranth populations (PRE studies only) were evaluated to determine whether combining data across studies was appropriate, and due to no significance, all data for analysis were combined within each herbicide and application timing. To determine the response of Palmer amaranth to PRE and POST-applied PPO-inhibiting herbicides, regression analysis was performed on visual control, emergence (PRE only), mortality (POST only), and biomass reductions. As suggested by Thornley and Johnson (1990), each response variable was fit to a three-parameter sigmoidal curve using SigmaPlot software (version 15.0; Systat Software Inc., San Jose, CA), where y is the response from the population, a is the upper limit, b is the slope, and c represents the effective herbicide concentration for 50% inhibition (EC_{50} rate) (Equation 1):

$$y = a/\{1 + \exp[-(\text{rate} - c)/b]\} \quad (1)$$

Furthermore, the same equation was used on the response of each population by replication, with the resulting parameter estimates subjected to an ANOVA using the GLIMMIX procedure with SAS Enterprise Guide 8.3 software (SAS Institute, Cary, NC). Relative resistance factor (RRF) was calculated by dividing the EC_{50} for the F_2/F_3 populations by the EC_{50} for the susceptible population. To determine whether emergence between populations differed in the PRE study, emergence count data were subjected to an ANOVA using the GLIMMIX procedure with SAS Enterprise Guide software.

Results and Discussion

Postemergence Dose-Response Assessment Results

At the 1× field application rate, visual control of the suspect population was 46%, 34%, 46%, and 91% with fomesafen, lactofen, acifluorfen, and trifludimoxazin, respectively, compared with 95%, 85%, 96%, and 99% in the sensitive population, at 8 DAT when control was at its maximum (Figure 3). A significantly higher dose of fomesafen ($P = 0.0027$), lactofen ($P = 0.0353$), acifluorfen ($P < 0.0001$), and trifludimoxazin ($P = 0.0007$) was necessary to reach 50% visual control of the suspect population compared with the susceptible population; RRF values indicate a level of resistance of 318×, 1,248×, 316×, and 10×, for those herbicides, respectively (Table 1). Considering the criteria for confirming herbicide resistance, the suspect population exhibited a high level of resistance (RRF ≥10) when assessing visual control, for all herbicides evaluated (Heap 2023b). Results indicate that this population can no longer be effectively controlled with practical, field-recommended rates of fomesafen, lactofen, acifluorfen, or trifludimoxazin and is resistant to PPO-inhibiting herbicides. These results are consistent with published studies confirming PPO-resistance in Palmer amaranth from POST applications, although this study did not focus on isolating the mechanism of resistance (Heap 2023b; Montgomery et al. 2020; Oliveira et al. 2020; Wu et al. 2020).

Assessments of mortality and fresh-weight biomass were collected 8 DAT to numerically quantify differences in survival and plant growth between the two populations following herbicide applications. As expected, Palmer amaranth in both populations exhibited differential mortality based on the herbicide applied. In the susceptible population, 90% mortality or greater was achieved with an application rate of 1×, 4×, 0.5×, and 0.0625× of fomesafen, lactofen, acifluorfen, and trifludimoxazin, respectively (Supplementary Table S1). In the suspected resistant population, a similar mortality rate was achieved with a 2× rate of trifludimoxazin (91%); however, even at a 40× rate of fomesafen, lactofen, and acifluorfen, Palmer amaranth mortality was only 63%, 15%, and 91%, respectively. When plant mortality was further described with the three-parameter sigmoidal curve to determine EC_{50} values, calculated RRF values of fomesafen (105×), lactofen (36×), acifluorfen (215×), and trifludimoxazin (49×) applied POST provided further evidence for high-level resistance in the population (Table 1). Greenhouse experiments offer the ability to provide optimum growing conditions for plants while minimizing confounding effects from uncontrollable factors (rainfall, animal predation, etc.) that may influence results (Mortensen 1982; Perkins et al. 2021). Although herbicide activity is increased in this environment with ideal growing conditions, injured plants have a better opportunity to outgrow adverse impacts from herbicide applications, which may offer an explanation to the increased variability and lower calculated RRF for mortality compared to visual control.

The aboveground biomass of surviving plants was recorded collectively for each plot, and expressed as a percent reduction from the nontreated control. Results from both populations followed a similar trend to visual control and plant mortality. At a 1× field use rate following applications of all herbicides, biomass of susceptible plants was reduced by greater than 91% compared with the nontreated control (Supplementary Table S2). A similar level of control was achieved in the suspect population following a 20× rate of acifluorfen and a 2× rate of trifludimoxazin; a reduction in biomass never exceeded 85% for fomesafen and 64% for lactofen following applications up to 40× the recommended rate for both herbicides. Calculated RRF biomass reduction values indicate a level of resistance of 238×, 1,477×, 273×, and 9× for fomesafen, lactofen, acifluorfen, and trifludimoxazin, respectively, which was similar to the values obtained via visual observation (Table 1).

Preemergence Dose-Assessment Studies

Initial greenhouse bioassays provided an opportunity to test implementation procedures and understand the sensitivity of the two populations in order to set the full range of doses for evaluation with each PRE herbicide. For dose-response studies, estimates of visual Palmer amaranth control were collected 21 to 28 DAT when plant injury was at a maximum. At a 1× field use rate, all herbicides controlled (100%) susceptible Palmer amaranth, while at the same rate, control of plants from the suspect population was 79%, 76%, 95%, and 99% with fomesafen, flumioxazin, oxyfluorfen, and trifludimoxazin, respectively (Figure 4). For fomesafen and flumioxazin, control of the suspect population exceeded 90% only once a 3× rate was applied. Comparisons between the dose (EC_{50}) required to inhibit 50% of each population indicate RRF levels of 3×, 31×, 6×, and 8× for fomesafen, flumioxazin, oxyfluorfen, and trifludimoxazin, respectively (Table 2).

To further quantify control, emergence was assessed weekly. Previously published research suggests that although Palmer

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Table 1. Herbicide dose required for 50% Palmer amaranth control, mortality, and biomass reduction in the susceptible and suspected resistant populations.[a,b]

	Control			Mortality			Biomass		
	Susceptible	Suspect		Susceptible	Suspect		Susceptible	Suspect	
	EC_{50}	EC_{50}	RRF	EC_{50}	EC_{50}	RRF	EC_{50}	EC_{50}	RRF
Fomesafen	7.64	2,428.34	318	61.11	6,421.73	105	4.8	1,360.01	238
Lactofen	0.7	874.46	1,248	107.27	3,811.15	36	0.7	1,037.9	1,477
Acifluorfen	5.02	1,585.02	316	27.89	5,993.13	215	2.56	698.92	273
Trifludimoxazin	0.08	0.75	10	0.09	4.14	49	0.08	0.69	9

[a]Abbreviations: EC_{50}, effective concentration for 50% inhibition; POST, postemergence; PPO, protoporphyrinogen oxidase; RRF, relative resistance factor.
[b]The RRF for each assessment was calculated based on the response of the respective population following POST applications of PPO-inhibiting herbicides.

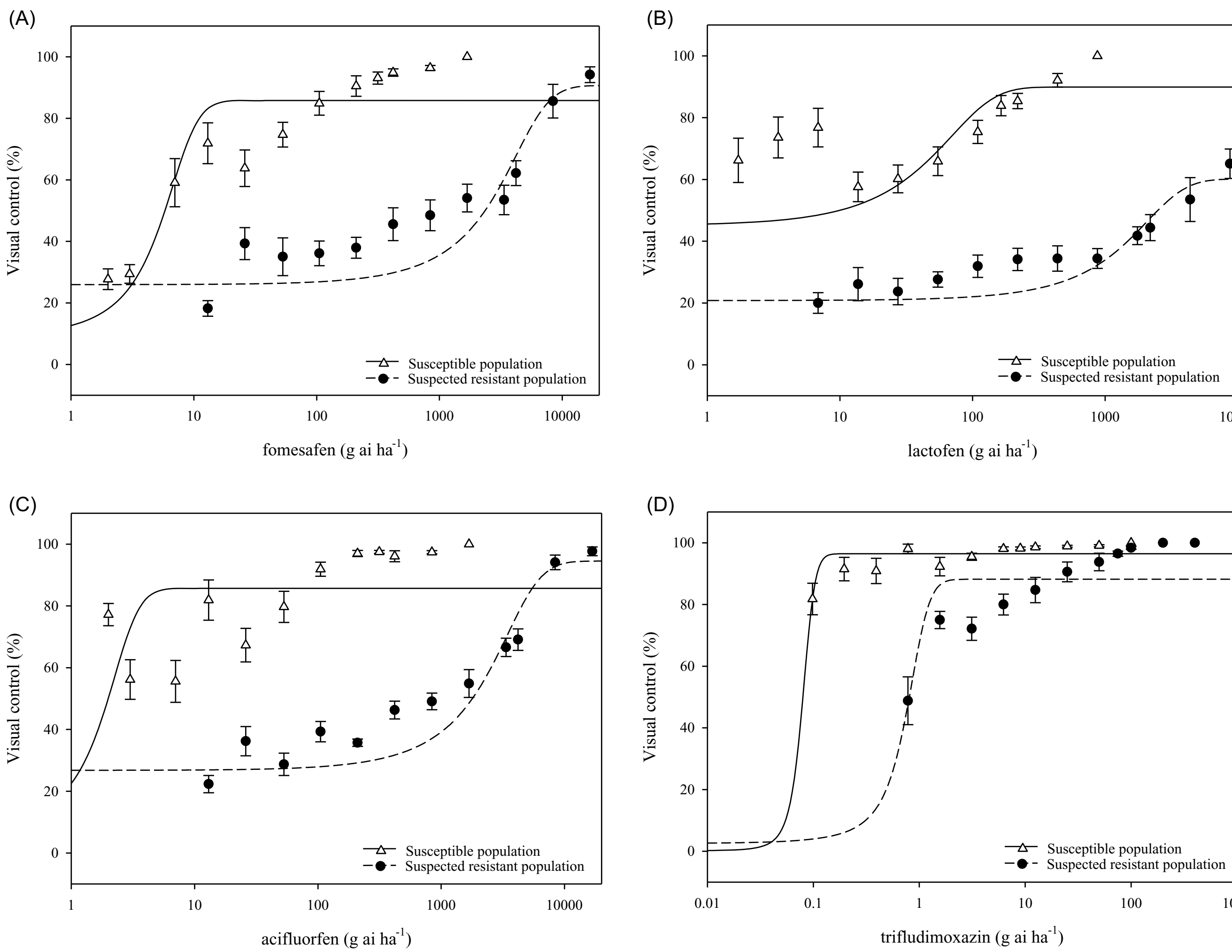

Figure 3. Palmer amaranth visual control assessments in response to herbicides that inhibit protoporphyrinogen oxidase, including fomesafen (A), lactofen (B), acifluorfen (C), and trifludimoxazin (D) applied postemergence between 2020 and 2022 in Tifton, GA. Visual control assessments, collected 8 d after treatment, were described by a three-parameter sigmoidal curve, to determine the dose required to control 50% of both the susceptible or suspected resistant population. Field rates (1×) included fomesafen at 420 g ai ha^{-1}, lactofen at 219 g ai ha^{-1}, acifluorfen at 420 g ai ha^{-1}, and trifludimoxazin at 25 g ai ha^{-1}. Fomesafen and acifluorfen applications included nonionic surfactant (0.25% v/v), lactofen included crop oil concentrate (1% v/v), and trifludimoxazin included methylated seed oil (1% v/v).

amaranth populations exhibit resistance to POST-applied PPO-inhibiting herbicides, PRE applications may still provide adequate residual weed control for an extended period. In Tennessee, emergence following applications of flumioxazin, fomesafen, and saflufenacil differed between the sensitive and resistant populations; however, effective control of both populations was still achieved at a 1× recommended field rate up to 21 DAT (Umphres et al. 2017). Schwartz-Lazaro et al. (2017) indicated that in a population of Palmer amaranth from Arkansas, resistance to PPO-inhibitors applied PRE was evident following emergence assessments at 10 DAT, however, their PRE applications were followed by POST applications (DAT timing unknown) of the same herbicide. Therefore, it is not possible to clearly differentiate the effects of the PRE and POST applications. Similar to the work conducted in Arkansas, Lillie et al. (2020) recorded resistance to PRE-applied PPO-inhibiting herbicides following assessments

Table 2. Herbicide dose required for 50% Palmer amaranth control, mortality, and biomass reduction in the susceptible and suspected resistant populations.[a,b]

	Control			Mortality			Biomass		
	Susceptible	Suspect		Susceptible	Suspect		Susceptible	Suspect	
	EC_{50}	EC_{50}	RFF	EC_{50}	EC_{50}	RFF	EC_{50}	EC_{50}	RFF
Fomesafen	5.92	15.85	3	2.64	12.4	5	2.58	7.32	3
Flumioxazin	0.45	13.76	31	0.27	5.85	21	0.34	9.26	27
Oxyflurofen	0.003	0.017	6	0.0012	0.0165	14	0.0007	0.0152	22
Trifludimoxazin	0.17	1.37	8	0.17	6.42	38	0.2	1.5	8

[a]Abbreviations: EC_{50}, effective concentration for 50% inhibition; PPO, protoporphyrinogen oxidase; PRE, preemergence; RRF, relative resistance factor
[b]The RRF for each assessment has been calculated based on the response of the respective population following PRE applications of PPO-inhibiting herbicides.

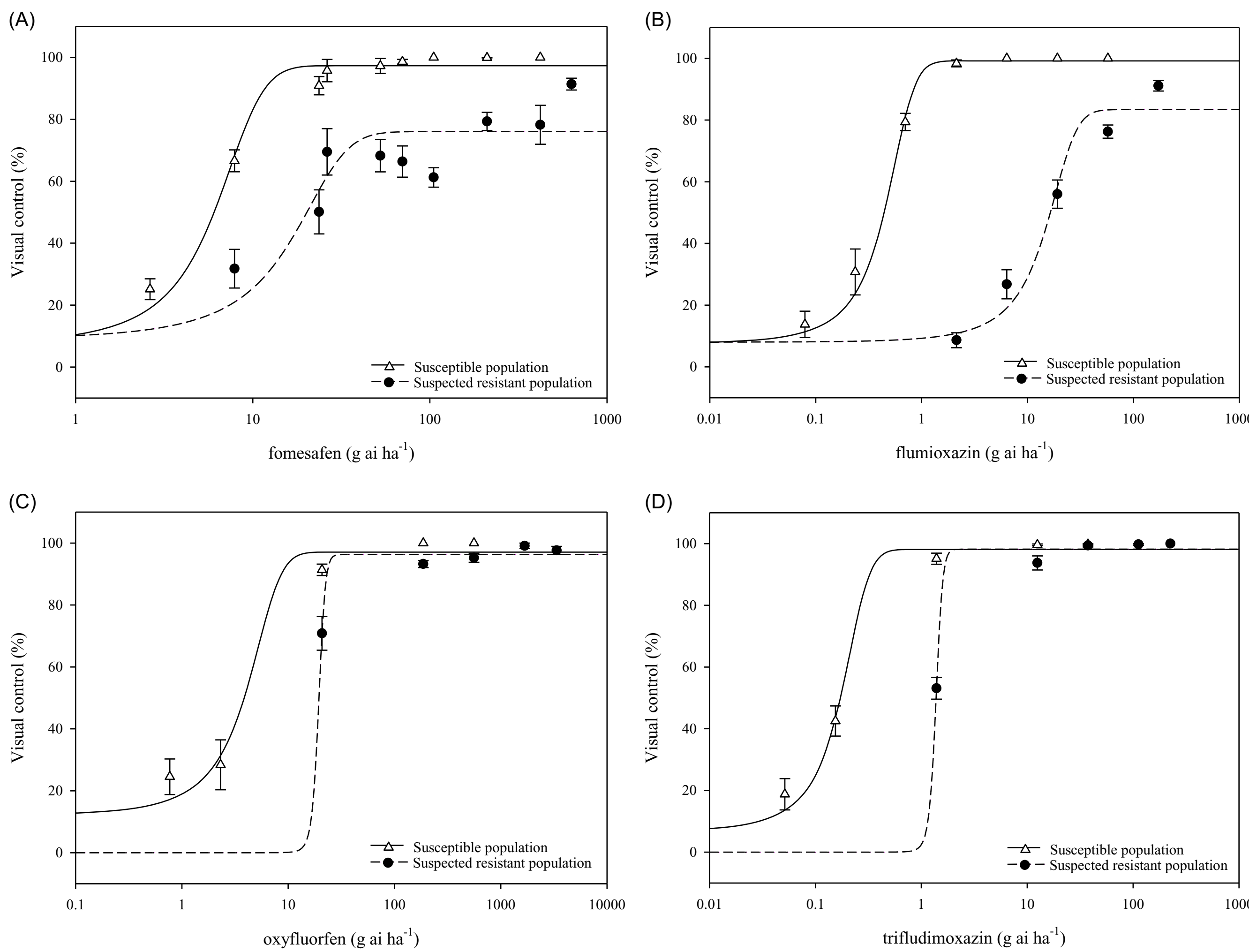

Figure 4. Palmer amaranth visual control assessments in response to herbicides that inhibit protoporphyrinogen oxidase, including fomesafen (A), flumioxazin (B), oxyfluorfen (C), and trifludimoxazin (D) applied preemergence between 2020 and 2022 in Tifton, GA. Visual control assessments, collected 21 d after treatment, were described by a three-parameter sigmoidal curve, to determine the dose required to control 50% of both the susceptible or suspected resistant population. Field rates (1×) included fomesafen at 210 g ai ha^{-1}, flumioxazin at 57 g ai ha^{-1}, oxyfluorfen at 561 g ai ha^{-1}, and trifludimoxazin at 38 g ai ha^{-1}.

collected 10 DAT; however, due differing rate structures, applications rates higher than 1× were used in those studies. Contradictory to the information currently available in the literature on PPO resistance in Palmer amaranth, the Georgia population evaluated in this paper was not effectively controlled with PRE applications of PPO inhibitors as early as 1 wk after treatment. When emergence was assessed at 7 DAT, 61, 51, 10, and 31 times more Palmer amaranth plants had emerged in the suspect population compared with the sensitive population with fomesafen ($P < 0.0001$), flumioxazin ($P < 0.0001$), oxyfluorfen ($P = 0.0349$), and trifludimoxazin ($P < 0.0001$), respectively (data not shown). At this timing, no differences were observed when comparing emergence of the two populations when herbicides were not applied. To ensure that results were a result of herbicide sensitivity, and not differences in emergence, as discussed in research by Umphres et al. (2017), these assessments were continued weekly through 21 DAT. For all herbicides evaluated, results remained consistent with higher emergence recorded in the suspect versus susceptible populations at all assessment timings.

Similar to POST applications, plant mortality and fresh weight biomass response 21 to 28 DAT differed between populations based on the herbicide applied. For plants suspected of being resistant to PPO-inhibiting herbicides, applications of the 1× recommended field dose resulted in 100% mortality and a complete reduction of biomass (100%) compared with the nontreated control (Supplementary Tables S3 and S4). With suspect plants, the dose required to reach 50% mortality and reduction in fresh weight biomass was significantly greater. Calculated RRF values for mortality indicate resistance of 5×, 21×, 14×, and 38× following PRE applications of fomesafen, flumioxazin, oxyfluorfen, and trifludimoxazin, respectively, while RRF values for biomass reduction were 3×, 27×, 22×, and 8× for the same herbicides (Table 2).

In conclusion, our research results indicate that a population of Palmer amaranth from Georgia exhibits resistance to both PRE- and POST-applied PPO inhibitors, with the level of resistance dependent on herbicide applied and application method. While response following POST application is similar to that observed in other states, the population appears to be unique in its lack of response to PRE applications. Due to widespread, intensive use of PPO-inhibiting herbicides, it is highly unlikely that this is the only resistant Palmer amaranth present in Georgia, therefore, growers must have a plan to diversify their weed management practices and reduce selection pressure being placed on this species by the overuse of PPO-inhibiting herbicides. Furthermore, previous research has identified both target-site and non-target-site resistance mechanisms when describing PPO-inhibitor resistance in Palmer amaranth throughout the literature (Copeland et al. 2018; Lillie et al. 2020; Salas et al. 2016; Varanasi et al. 2018). While not a focus of this research, understanding the mechanism of resistance provides an opportunity to better understand the potential of a population to develop cross-resistance to multiple PPO-inhibiting herbicide chemical families, or multiple resistance to different herbicide mechanisms of action. This information is essential to ensuring that practical, integrated weed management practices are implemented at the field level; therefore, future research efforts should focus on understanding the mechanism that allowed this biotype to develop resistance within 200 m of Palmer amaranth that remains susceptible.

Practical Implications

Palmer amaranth continues to challenge Georgia agriculture and threaten farm sustainability each year. This weed has a tremendous ability to compete with crops and subsequently reduce yields, and with dynamic growth and prolific reproduction, its control must constantly be on the forefront of production management decisions. Following the widespread distribution of glyphosate-resistant Palmer amaranth in Georgia, many agronomic growers began to rely heavily on PPO-inhibiting herbicides for PRE, POST, and row-middle weed control. For example, in Georgia's most valuable agronomic crop, cotton, PRE applications of PPO-inhibiting herbicides form the foundation of residual weed control in which sound weed management practices are built upon for the remainder of the growing season.

In 2017, University of Georgia Extension personnel identified a population of Palmer amaranth exhibiting a reduced sensitivity to the PPO-inhibiting class of herbicide chemistry. Initial field bioassays, greenhouse bioassays, and dose-response assessments were conducted to investigate the response of this population to numerous PPO herbicides applied PRE or POST. Through these quantitative assessments, it was confirmed that this Palmer amaranth population was resistant to the PPO-inhibiting herbicide chemistry applied either PRE or POST.

These results further confirm that growers must implement dynamic, diversified weed management programs and to limit their overreliance on any single management tactic, including relying too heavily on the PPO inhibitors. Furthermore, due to the widespread use of these herbicides around the state, it is highly unlikely that this is the only resistant population, thereby highlighting the importance of limiting selection pressure to protect the utility of these herbicides across Georgia for the future.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2024.12

Acknowledgments. We thank Tim Richards for his technical assistance with this research. We also thank the Georgia Cotton Commission for their financial support. No conflicts of interest have been declared.

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