Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas

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Abstract

BACKGROUND: The widespread occurrence of ALS inhibitor- and glyphosate-resistant Amaranthus palmeri has led to increasing use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides in cotton and soybean. Studies were conducted to confirm resistance to fomesafen (a PPO inhibitor), determine the resistance frequency, examine the resistance profile to other foliar-applied herbicides and investigate the resistance mechanism of resistant plants in a population collected in 2011 (AR11-LAW B) and its progenies from two cycles of fomesafen selection (C1 and C2).

RESULTS: The frequency of fomesafen-resistant plants increased from 5% in the original AR11-LAW-B to 17% in the C2 population. The amounts of fomesafen that caused 50% growth reduction were 6-, 13- and 21-fold greater in AR11-LAW-B, C1 and C2 populations, respectively, than in the sensitive ecotype. The AR11-LAW-B population was sensitive to atrazine, dicamba, glufosinate, glyphosate and mesotrione but resistant to ALS-inhibiting herbicides pyrithiobac and trifloxysulfuron. Fomesafen survivors from C1 and C2 populations tested positive for the PPO glycine 210 deletion previously reported in waterhemp (Amaranthus tuberculatus).

CONCLUSION: These studies confirmed that Palmer amaranth in Arkansas has evolved resistance to foliar-applied PPO-inhibiting herbicide.

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Keywords: ALS inhibitors; Amaranthus palmeri; diphenylether resistance; fomesafen; multiple resistance; resistance evolution

1 INTRODUCTION

Palmer amaranth (Amaranthus palmeri S. Watson) is one of the most common, troublesome and economically damaging agronomic weeds throughout the southern United States.1 This weed continues to emerge throughout the summer, making control critical from crop emergence to harvest. The competitive ability of Palmer amaranth is attributed to its fast growth rate,2 high fecundity,3 good light interception and high water use efficiency.4 With estimates of over 600000 seeds plant−1, it can replenish the seedbank5 in one generation. Because it is highly competitive with crops, it can reduce crop yield. Palmer amaranth densities of 8 and 9 plants m−2 can reduce soybean yield by 78%6 and corn grain yield by 91%7 respectively. Fast et al.7 reported that Palmer amaranth interference for 63 days after crop emergence caused 77% cotton yield loss. Apart from reducing yield, more severely in stripper cotton, high infestation of Palmer amaranth interferes with cotton harvest and can increase harvest time 2–4 fold.8

Palmer amaranth control has become a challenge because of its high propensity to evolve herbicide resistance, resulting in reduced herbicide options in infested crops such as cotton and soybean. To date, Palmer amaranth has been confirmed resistant to herbicides spanning five modes of action: acetolactate synthase (ALS) inhibitors, carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase) inhibitors, enolpyruvyl shikimate-3-phosphate synthase inhibitor (glyphosate), mitosis inhibitors (dinitroanilines) and photosystem II inhibitors (triazines).9 Being dioecious, Palmer amaranth is an obligate cross-pollinated species, allowing herbicide resistance to spread rapidly.10 Sosnoskie et al.11 reported that glyphosate resistance trait was transferred across a distance of at least 300 m through pollen flow.

The widespread occurrence of resistance to ALS inhibitors and glyphosate in Palmer amaranth has led to increasing use of protoporphyrinogen oxidase (PPO)-inhibitor herbicides such as fomesafen, flumioxazin, saflufenacil and sulfentrazone. Advantagesous characteristics of PPO inhibitors include a broad herbicidal spectrum, as these are active against many monocotyledon and dicotyledon weeds, have low mammalian toxicity, low effective rates, rapid onset of action and long residual activity on some
herbicides in this group. The PPO enzyme catalyzes the conversion of protoporphyrinogen IX (protogen IX) to protoporphyrin IX (proto IX), which is the last common step in the biosynthesis of heme and chlorophyll. In plants, two PPO isoforms are encoded by two different PPO nuclear genes, PPX1 and PPX2. These isoforms share little sequence identity (25%) and differ in their subcellular targeting. PP01 and PP02 are localized in plastids and mitochondria respectively; however, at least some PP02 isoforms are dual targeted to both organelles. Inhibition of PPO by herbicides results in the generation of singlet oxygen species that attack lipid and protein membranes, leading to plant death.

The PPO enzyme is inhibited by several herbicide chemical classes (e.g. diphenyl ethers, heterocyclic phenyl ethers, oxadiazoles, phenyl imides, triazolinones and pyrazoles). Herbicides that inhibit PPO have been in the market since the 1960s and were primarily used in soybean. One of these is fomesafen, a diphenyl ether herbicide, which can be applied preplant, pre-emergence or post-emergence for control or suppression of broadleaf weeds, grasses and sedges in soybean. Resistance to PPO herbicides has been slow to evolve (about four decades from first commercialization), and to date has been confirmed only in seven weed species. The first weed to evolve resistance to PPO herbicides was waterhemp (Amaranthus tuberculatus) in 2001. Resistance to PPO herbicides in weedy species has been attributed to target-site mutation in the PPX2 gene. A unique target-site amino acid deletion (Gly110) and Arg98Leu substitution confer PPO resistance in waterhemp and common ragweed (Ambrosia artemisiifolia) respectively.

PPO herbicides are widely utilized for controlling glyphosate-resistant Palmer amaranth in conventional and RoundupReady® soybeans and in cotton. Intensive use of herbicides exerts high selection pressure leading to the evolution of herbicide-resistant weed populations. This paper describes fomesafen resistance in Palmer amaranth populations from Arkansas.

2 MATERIALS AND METHODS

2.1 Plant materials

In the late summer of 2011, Palmer amaranth samples escaping from either glufosinate or PPO-inhibiting herbicide applications were collected from several fields in Arkansas. Inflorescences from ten female Palmer amaranth plants per field were collected, dried, threshed and cleaned for bioassay in the greenhouse. One such field in Lawrence County has been planted with LibertyLink® soybean and RoundupReady® corn in alternate years since 2011 and treated with either fomesafen and glufosinate or glyphosate. Fomesafen was applied during the soybean production year. A quantity of 500 mg of seeds from each of the ten plants per field were mixed to make a composite, which was used for subsequent experiments. Susceptible Palmer amaranth seeds (SS) were collected from a vegetable field in Crawford County, Arkansas. This vegetable field was not exposed to glyphosate or PPO-inhibiting herbicides.

Plants were grown in a greenhouse maintained at 32±25°C day/night temperature with a 16h photoperiod. Plants were watered daily and fertilized with MiracleGro®, a water-soluble all-purpose plant food containing 15-30-15 NPK, every 2 weeks.

2.2 Fomesafen resistance bioassay in the greenhouse

Palmer amaranth seeds were planted in 28×54 cm cellular trays (Redwayfeed Garden and Pet Supply, Redway, CA) using Sunshine® premix soil (Sunshine premix No. 1®, Sun Gro Horticulture, Bellevue, WA). The experiment was conducted in a randomized complete block design with five replications. Each replication consisted of one cellular tray with 50 seedlings, grown at one seedling per cell. The test was repeated. Thus, from each composite seed sample, a total of 500 plants were sprayed with the recommended dose of fomesafen at 264 g ha⁻¹ (Flexstar 1.88 EC; Syngenta, Greensboro, NC) when seedlings were 7.5–9 cm tall. The herbicide was applied with 0.5% by volume nonionic surfactant (NIS), using a laboratory sprayer equipped with a flat fan spray nozzle (TeJet spray nozzles; Spraying Systems Co., Wheaton, IL) delivering 187 L ha⁻¹ at 269 kPa. After 21 days, the overall effects of fomesafen (stunting, chlorosis, necrosis and desiccation) were assessed visually relative to the non-treated control using a scale of 0 to 100, where 0 = no visible injury and 100 = complete death.

Survivors from fomesafen treatment were grown, and allowed to interbreed, to produce C1 and C2 populations of progenies. A subset of nine survivors (which eventually consisted of three females and six males) from AR11-LAW-B produced the C1 population. C1 plants were sprayed with fomesafen, following the same procedure described previously. A subset of six survivors (which eventually consisted of one female and five males) from C1 plants were grown for seed production to produce the C2 population.

2.3 Progression of resistance from the original AR11-LAW-B to the C2 Palmer amaranth population

Seeds from the C1 and C2 Palmer amaranth populations were planted in cellular trays to determine the frequency of fomesafen-resistant plants and the progression of resistance from the original population to the C2 population. The experiment was conducted twice in a randomized complete block design with five replications. In each cellular tray or replication, 50 plants were grown separately and sprayed with 264 g ha⁻¹ of fomesafen using the application method described previously. Mortality and plant injury were recorded at 21 DAT.

2.4 Fomesafen dose—response bioassay

Palmer amaranth seeds of the susceptible, original AR11-LAW-B, C1 and C2 populations were planted in 11×11 cm square pots filled with Sunshine Mix LC1 potting soil (Sun Gro Horticulture Canada Ltd, Vancouver, Canada). Seedlings were thinned to five per pot. Seedlings, 7.5–9 cm tall, were sprayed with eight doses of fomesafen from 0 to 2109 g ha⁻¹, which corresponds to 0–8 times the recommended field dose. The SS population was sprayed with seven doses from 4 to 264 g ha⁻¹, corresponding to 1/64 to 1x the recommended dose, with a non-treated check. The herbicide was applied with a 0.5% NIS as described in Section 2.2. The experiment was conducted twice in a randomized complete block design with five replications. At 21 DAT, visible injury and the number of survivors were recorded. The aboveground plant tissue was harvested, placed in a brown paper bag and dried at 60°C for 48 h, and dry weights were recorded. Data were expressed as percentage of biomass reduction relative to the non-treated control. The biomass data generated from two runs were pooled, as the test for homogeneity of variance showed that the variance across runs was similar. Regression analysis was conducted using SigmaPlot v.13 (Systat Software, Inc., San Jose, CA, USA). The percentage biomass reduction and mortality were fitted to a...
non-linear, sigmoid, three-parameter Gompertz regression model defined by

\[ y = a^* \exp \left\{ -\exp \left[ -b^* (x - c) \right] \right\} \tag{1} \]

where \( Y \) is the biomass reduction expressed as a percentage of the non-treated control or mortality percentage, \( a \) is the asymptote, \( b \) is the growth rate, \( c \) is the inflection point and \( x \) is the fomesafen dose. The dose needed to kill 50% (LD50) of the population, or cause 50% biomass reduction (GR50), was calculated from the above equation.

### 2.5 Response of Palmer amaranth population to other foliar-applied herbicides

Palmer amaranth seeds from the susceptible and original AR11-LAW-B populations were planted in cellular trays in the greenhouse. Uniform-sized plants (7.5–9 cm tall) were treated with atrazine at 2244 g ha\(^{-1}\), dicamba at 280 g ha\(^{-1}\), glufosinate at 547 g ha\(^{-1}\), glyphosate at 870 g ha\(^{-1}\), mesotrin at 105 g ha\(^{-1}\) and ALS inhibitors. Glufosinate and mesotrin treatments included 3366 g ammonium sulfate (AMS) ha\(^{-1}\) and 1% crop oil concentrate (COC), respectively. The ALS inhibitors and their respective rates included pyrithiobac at 73 g ha\(^{-1}\) and trifloxysulfuron at 8 g ha\(^{-1}\), applied with 0.25% NIS by volume. Herbicide treatments were applied as described in Section 2.2. Following application, plants were placed on greenhouse benches in a randomized complete block design. Each treatment was replicated twice, with each replication consisting of 50 plants. Mortality was assessed at 21 DAT.

Because AR11-LAW-B was found to be resistant to pyrithiobac and trifloxysulfuron, dose–response assays were conducted to determine the level of resistance to these herbicides. Seeds were planted in 13 cm round pots filled with commercial potting soil, and seedlings were thinned to five per pot. The SS population was sprayed with eight herbicide doses from 1/16 to 4× the recommended dose of pyrithiobac (1× = 73 g ha\(^{-1}\)) and trifloxysulfuron (1× = 8 g ha\(^{-1}\)), including a non-treated check. The AR11-LAW-B population was treated with eight doses of pyrithiobac (0–1166 g ha\(^{-1}\)) and eight doses of trifloxysulfuron (0–31 g ha\(^{-1}\)), which correspond to 0–16× the recommended herbicide dose. Herbicides were applied following the procedure described previously. The experiment was conducted in a completely randomized design with five replications. At 28 DAT, plants were cut at the soil surface, dried for 2 days, and the dry weights were recorded. Percentage biomass reduction relative to the non-treated control was fitted to the non-linear, sigmoid, three-parameter Gompertz regression model. The dose needed to reduce the aboveground biomass by 50% was obtained from regression equation using SigmaPlot v.13.

### 2.6 Mechanism of resistance in PPO-resistant Palmer amaranth

Fomesafen survivors from the C1 and C2 populations were tested for the presence of the PPO glycinine210 deletion (\(\Delta G_{210} \)). This deletion confers resistance to PPO herbicides in waterhemp, a relative weed species of Palmer amaranth.19 Young leaf tissues from 81 C1 and 13 C2 plants that survived the application of 264 g fomesafen ha\(^{-1}\) were collected and stored at –80 °C. Tissues from three sensitive plants (SS) were also collected. Genomic DNA from 100 mg of leaf tissue was extracted using the hexadecytrimethylammonium bromide (CTAB) method21 following the modification of Sales et al.22 The extracted genomic DNA was quantified using a NanoDrop spectrophotometer model ND-1000 (Thermo Scientific, Wilmington, DE).

The \(\Delta G_{210} \) codon deletion was detected using an allele-specific PCR assay as previously described for waterhemp.23 The same assay was predicted to work for Palmer amaranth, based on the PPOX2 sequence data previously generated from this species.24

### 3 RESULTS AND DISCUSSION

#### 3.1 Progression of PPO resistance in the PA-AR11-LAW-B population

The frequency of fomesafen-resistant plants increased from 5% in the original AR11-LAW-B to 17% in the C2 population, in response to the 264 g ha\(^{-1}\) dose of fomesafen. Of the 500 plants treated, 25 survived in the original population. The number of survivors increased in the C1 (\(n = 56\)) and C2 (\(n = 86\)) populations. By the practical description of a resistant population,25 the field population in 2011 (5% resistant individuals) would still be considered a susceptible population. The few remnant plants in the field did not cause any economic loss, nor were they noticed by the farmer. Until the number of resistant plants reaches a level that presents a problem for management and yield loss, the population would not be considered resistant.25 The present work showed that, once a few individuals carrying the resistance trait are selected, sustained herbicide selection pressure on a prolific species such as Palmer amaranth could produce a resistant population in 2 years. This would be when the farmer would call for assistance from crop advisors or extension service (personal experience). By the time a population-level resistance is noticed, it is already too late for that field; by then the resistant plants would have already deposited a large amount of seeds in the soil and the resistant allele(s) would remain in the population.

Not all progenies of fomesafen-resistant survivors carried the PPO mutation, as shown by the resistance frequencies. This suggests that the resistance trait is segregating and the progenies are heterogeneous. Palmer amaranth has wide genetic variability owing to its cross-pollinating behavior. However, for every round of selection, the population becomes more homogeneous. The C2 population had more frequency of resistant plants than C1 or its parent population AR11-LAW-B. After two cycles of selection, the frequency of resistant plants increased about threefold. There are examples of rapid evolution of herbicide resistance in response to intense and sustained selection pressure.26 The Weed Science Society of America (WSSA) defines herbicide resistance as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide that is normally lethal to the wild type.27 In herbicide resistance there is a change in the weed population response (i.e. reduced efficacy of the herbicide) with time, as observed with AR11-LAW-B. Thus, the field-collected sample in 2011 was in the early state of resistance evolution to PPO herbicides. The large-scale testing of our Palmer amaranth collection (2008–2011) for differential tolerance to fomesafen was conducted in 2013. The tests for heritability and confirmation of \(G_{210} \) deletion among fomesafen survivors were completed in 2015. Thus, the detection and confirmation of resistance to PPO herbicides in Palmer amaranth happened a few years after collecting the resistance-bearing population.

The farmer of the AR11-LAW-B field adopted a corn–soybean crop rotation system. In 2015, the field was clean, except for a sparse remnant Palmer amaranth (about 1 ha\(^{-1}\)). Three plants were sampled and tested for resistance to fomesafen; one of these tested positive for the \(G_{210} \) deletion mutation. This demonstrates...
that once the resistant allele has been selected for, it could remain in the population unless the seedbank is depleted.

3.2 Resistance level to fomesafen

Increasing the dose of fomesafen reduced the dry weight of SS, AR11-LAW-B, C1 and C2 populations. The fomesafen dose that caused 50% growth reduction (GR50 ± 1 standard error) was 13 ± 0.86, 82 ± 6.1, 168 ± 11.9 and 265 ± 20.4 g fomesafen ha\(^{-1}\) for the SS, AR11-LAW-B, C1 and C2 populations respectively (Fig. 1 and Table 1). Based on these GR50 values, the level of resistance to fomesafen in the AR11-LAW-B, C1 and C2 populations was 6-, 13- and 21-fold, respectively, relative to the sensitive population (SS). The GR50 increased from 82 g fomesafen ha\(^{-1}\) in the original AR11-LAW-B to 265 g fomesafen ha\(^{-1}\) in the C2 population. The commercial field dose of fomesafen (264 g ha\(^{-1}\)) is required to reduce aboveground biomass by 50% in the C2 population, indicating that the normal field dose would no longer be effective. After two more cycles of selection from the point where 5% resistant individuals were detected, the GR50 increased threefold, reflecting the observed increase in the frequency of resistant plants.

The herbicide dose that caused 50% mortality (LD50) was 16 ± 1.3, 45 ± 8.8, 181 ± 16.8 and 262 ± 30.1 g fomesafen ha\(^{-1}\) for the SS, AR11-LAW-B, C1 and C2 populations, respectively (Fig. 2 and Table 1). On the basis of LD50 values, the AR11-LAW-B, C1 and C2 populations had 3-, 11- and 16-fold resistance relative to the SS population. The LD50 values of the C1 and C2 populations were relatively similar to the GR50 values. The 0.7× and 1× commercial field doses of fomesafen would kill 50% of the C1 and C2 populations respectively. Thus, the normal field dose would not control all plants in the C1 and C2 populations, allowing the resistant plants to proliferate progressively in the next growing season. The PPO-resistant common waterhemp in Kansas had about the same GR50 value (270 g fomesafen ha\(^{-1}\)) as the C2 population.\(^{18}\) Although AR11-LAW-B had higher GR50 and LD50 than SS, the commercial dose of fomesafen could still kill >90% of the AR11-LAW-B population. However, continued selection had shifted the response of progenies in just two cycles. If each cycle of selection represents one cropping season, we can predict that resistance to PPO herbicides in the source field (in 2011) would be apparent in 2013. Unaware of the impending resistance problem in the field, the farmer happened to have adopted a RoundupReady® corn–LibertyLink® soybean cropping system, and kept the field clean, except for a few remnant Palmer amaranth in 2015 (1/3 of which still carried the resistance trait). Thus, prevention of seed production of survivors cannot be overstated. With good management practices, the evolution of resistant populations can be slowed down.

3.3 Response of Palmer amaranth population to other foliar-applied herbicides

The AR11-LAW-B population was found to be susceptible to atrazine, dicamba, glufosinate, glyphosate and mesotrione but resistant to ALS inhibitors pyrithiobac and trifloxysulfuron (Table 2). AR11-LAW-B was controlled 17 and 44% by commercial field doses of pyrithiobac and trifloxysulfuron, respectively. The GR50 for pyrithiobac was 3.2 ± 1.2 and 44.5 ± 6.2 g ha\(^{-1}\), respectively, for SS and AR11-LAW-B (Table 3). Similarly, the GR50 for trifloxysulfuron for SS and AR11-LAW-B was 0.9 ± 0.7 and 5.1 ± 1.1 g ha\(^{-1}\), respectively. About 0.6× the recommended doses of pyrithiobac and trifloxysulfuron were needed to reduce the aboveground biomass of AR11-LAW-B by 50%. The AR11-LAW-B

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Table 1. GR50 and LD50 values of original, C1 and C2 AR11-LAW-B populations in Arkansas

| Population | GR50 (g ha\(^{-1}\)) ± SE | R/S | LD50 (g ha\(^{-1}\)) ± SE | R/S |
|------------|--------------------------|-----|--------------------------|-----|
| AR11-LAW-B | 81.8 ± 5.1c              | 6   | 44.8 ± 8.8c              | 3   |
| C1         | 167.8 ± 11.9             | 13  | 180.8 ± 16.8             | 11  |
| C2         | 265.0 ± 20.4             | 21  | 262.5 ± 30.1             | 16  |
| SS\(^d\)   | 12.9 ± 0.8               | 163.3                     |     |

\(^a\) Resistance levels (R/S) calculated using the GR50 of the resistant population relative to the susceptible standard.

\(^b\) Resistance levels (R/S) calculated using the LD50 of the resistant population relative to the susceptible standard.

\(^c\) Standard error.

\(^d\) Herbicide-susceptible standard population.
Table 2. Response of *Amaranthus palmeri* AR11-LAW-B population to foliar-applied herbicides other than protoporphyrinogen oxidase inhibitors, Arkansas

| Herbicide    | Mortality (%)a | Resistance classification |
|--------------|----------------|---------------------------|
| Atrazine     | 100            | Susceptible               |
| Dicamba      | 100            | Susceptible               |
| Glufosinate  | 100            | Susceptible               |
| Glyphosate   | 84             | Susceptibleb              |
| Mesotrione   | 94             | Susceptible               |
| Pyrithiobac  | 17             | Resistant                 |
| Triflorylsulfuron | 44  | Resistant                 |

a Uniform-sized plants (7.5–9 cm tall) were sprayed with atrazine at 2244 g ha⁻¹, dicamba at 280 g ha⁻¹, glufosinate at 547 g ha⁻¹, glyphosate at 870 g ha⁻¹, mesotrione at 105 g ha⁻¹, pyrithiobac at 73 g ha⁻¹ and triflorylsulfuron at 8 g ha⁻¹. Glufosinate and mesotrione treatments included 3366 g AMS ha⁻¹ and 1% COC, respectively. Pyrithiobac and triflorylsulfuron were applied with 0.25% NIS by volume. Mortality was recorded 21 days after herbicide application.
b Plants were not dead at evaluation time, but survivors incurred high injury and did not grow to maturity.

Table 3. GR₉₀ values and resistance levels to ALS inhibitors in AR11-LAW-B Palmer amaranth population in Arkansas

| Population | Pyrithiobac GR₉₀ (g ha⁻¹) | R/S³ | Triflorylsulfuron GR₉₀ (g ha⁻¹) | R/S |
|------------|---------------------------|------|-------------------------------|-----|
| AR11-LAW-B | 44.5 ± 6.2b               | 14   | 5.1 ± 1.1                     | 5   |
| SS³        | 3.2 ± 1.2                 | –    | 0.9 ± 0.7                     | –   |

a Resistance levels (R/S) calculated using the GR₉₀ of the resistant population relative to the susceptible standard.
b Standard error of estimate.
c Herbicide-susceptible standard population.

As s-metolachlor + metribuzin applied pre-emergence, followed by pyroxasulfone or s-metolachlor applied early post-emergence. Glufosinate is still an effective option in LibertyLink® soybeans; however, it should be coupled with a pre-emergence program, followed by glufosinate tank mixed with s-metolachlor or pyroxasulfone early post-emergence. In addition, integration of cultural and mechanical control practices would be helpful in managing PPO-resistant Palmer amaranth populations.

3.4 Resistance mechanism in PPO-resistant Palmer amaranth

Results revealed that 74 out of 81 C1 plants and all 13 C2 samples tested positive for the ΔG₂₁₀ mutation, whereas all the sensitive plants tested negative for the said mutation (data not shown). This indicated that these survivors were resistant to fomesafen owing to target-site mutation in the PPO gene. Thinglum et al. reported that ΔG₂₁₀ mutation confers resistance to PPO herbicides in water-hemp populations in Illinois, Kansas and Missouri, suggesting that the ΔG₂₁₀ mutation is likely the only known mechanism of resistance to PPO inhibitors in waterhemp. Palmer amaranth and waterhemp belong to the *Amaranthus* genus and may therefore have some common morphological, biological and physiological characteristics and genomic tendencies. In fact, based on previous DNA sequence comparisons, it was predicted that the ΔG₂₁₀ mutation would evolve in Palmer amaranth. The loss of a glycine residue in the PPO gene alters the architecture of the substrate-binding domain of the PPO enzyme. As a result, the mutated PPO enzyme has reduced affinity for several PPO-inhibiting herbicides.

Some survivors that did not show the PPO mutation may harbor other resistance mechanisms such as non-target-site-based resistance mechanisms. Diphenyl ether herbicides are detoxified in soybeans by homoglutathione conjugation. Similarly, tolerance to the diphenyl ether fluorodifen in peas is due to rapid conjugation with glutathione. Alternatively, the lack of detection of the ΔG₂₁₀ mutation in some resistant plants could be due to sequence polymorphisms at the primer binding sites, resulting in false negatives.

3.5 Implications and future research

PPO inhibitors have been used heavily in past years to combat herbicide-resistant Palmer amaranth. Before this, PPO herbicides were among the most widely used herbicides for soybean. History has shown that intensive use of any herbicide often results in the selection for genes conferring herbicide resistance in weed populations. This research confirmed the occurrence of the first Palmer amaranth population to have evolved resistance to a PPO-inhibiting herbicide. Recurrent selection with the same herbicide significantly increased the frequency of resistant plants, and population-level resistance is expected to evolve within 2–3 years of having selected rare, resistant individuals if use of the same selector is continued. Because resistance to glyphosate is also rampant, it is expected that populations with multiple resistance to PPO and ALS inhibitors as well as to PPO inhibitors and glyphosate will evolve. With Palmer amaranth resistant to these modes of action, limited herbicide options remain for Palmer amaranth control. The evolution of resistance to PPO inhibitors in Palmer amaranth is a recent phenomenon in the southern United States; thus, best management practices (including diversification of herbicide modes of action) are vital to manage the spread of resistance, especially in soybean and cotton acres. As PPO resistance may still be localized, best management practices should be employed on a broader scale immediately.
Future research will investigate the genetics and mechanism of inheritance to PPO inhibitors in selected resistant populations, the efficacy of other PPO herbicides (soil or foliar) on PPO-resistant populations, the distribution and population genetics of PPO-resistant Palmer amaranth populations and the fitness of multiple-resistant plants.

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