Weed Science 2017 65:718–731

Frequency of Gly-210 Deletion Mutation among Protoporphyrinogen Oxidase Inhibitor–Resistant Palmer Amaranth (*Amaranthus palmeri*) Populations

Reiofeli A. Salas-Perez, Nilda R. Burgos, Gulab Rangani, Shilpa Singh, Joao Paulo Refatti, Leonard Piveta, Patrick J. Tranel, Andy Mauromoustakos, and Robert C. Scott*

The widespread occurrence of Palmer amaranth resistant to acetolactate synthase inhibitors and/or glyphosate led to the increased use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides. This research aimed to: (1) evaluate the efficacy of foliar-applied fomesafen to Palmer amaranth, (2) evaluate cross-resistance to foliar PPO inhibitors and efficacy of foliar herbicides with different mechanisms of action, (3) survey the occurrence of the *PPO* Gly-210 deletion mutation among PPO inhibitor–resistant Palmer amaranth, (4) identify other *PPO* target-site mutations in resistant individuals, and (5) determine the resistance level in resistant accessions with or without the *PPO* Gly-210 deletion. Seedlings were sprayed with fomesafen (263 g ai ha^{-1}), dicamba (280 g ai ha^{-1}), glyphosate (870 g ai ha^{-1}), glufosinate (549 g ai ha^{-1}), and trifloxysulfuron (7.84 g ai ha^{-1}). Selected fomesafen-resistant accessions were sprayed with other foliar-applied PPO herbicides. Mortality and injury were evaluated 21 d after treatment (DAT). The *PPX2L* gene of resistant and susceptible plants from a selected accession was sequenced. The majority (70%) of samples from putative PPO-resistant populations in 2015 were confirmed resistant to foliar-applied fomesafen. The efficacy of other foliar PPO herbicides on fomesafen-resistant accessions was saflufenacil > acifluorfen = flumioxazin > carfentrazone = lactofen > pyraflufen-ethyl > fomesafen > fluthiacet-methyl. With small seedlings, cross-resistance occurred with all foliar-applied PPO herbicides except saflufenacil (i.e., 25% with acifluorfen, 42% with flumioxazin). Thirty-two percent of PPO-resistant accessions were multiple resistant to glyphosate and trifloxysulfuron. Resistance to PPO herbicides in Palmer amaranth occurred in at least 13 counties in Arkansas. Of 316 fomesafen survivors tested, 55% carried the *PPO* Gly-210 deletion reported previously in common waterhemp. The *PPO* gene (*PPX2L*) in one accession (15CRI-B), which did not encode the Gly-210 deletion, encoded an Arg-128-Gly substitution. The 50% growth reduction values for fomesafen in accessions with Gly-210 deletion were 8- to 15-fold higher than that of a susceptible population, and 3- to 10-fold higher in accessions without the Gly-210 deletion.

Nomenclature: Acifluorfen; carfentrazone; dicamba; flumioxazin; fluthiacet-methyl; fomesafen; lactofen; glufosinate; glyphosate; pyraflufen-ethyl; saflufenacil; trifloxysulfuron; common waterhemp, *Amaranthus rudis* Sauer, AMATA; Palmer amaranth, *Amaranthus palmeri* S. Wats, AMAPA.

Key words: ALS resistance, cross-resistance, glyphosate resistance, multiple resistance, *PPO* gene, *PPO* Gly-210 deletion, *PPO* Arg-128-Gly mutation, target-site resistance.

Palmer amaranth is one of the most troublesome and economically damaging agronomic weeds in the southern United States. It is able to adapt to diverse climatic and agricultural conditions. The photosynthetic rate of Palmer amaranth (81 μmol m^{-2} s^{-1}) is three to four times that of corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.] (Ehleringer 1983). This high photosynthetic rate translates into its rapid growth rate of 5 cm day^{-1} under optimum growing conditions (Horak and Loughin 2000). Consequently, this rapid growth rate results in a narrow window of opportunity for effective POST herbicide application. Palmer amaranth control becomes difficult when it is >10-cm tall (Riar et al. 2013). A female Palmer amaranth can produce up to 1 million seeds; however, its seeds are relatively short-lived in the soil, with about 80% mortality in 3 yr (Sosnoskie et al. 2014). With its fast growth rate, high fecundity, season-long emergence, high

DOI: 10.1017/wsc.2017.41

* First, second, third, fourth, fifth, sixth and ninth authors: Graduate Student, Professor, Postdoctoral Fellow, Graduate Student, Graduate Student, Graduate Student, and Professor–Weed Extension Specialist, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701; seventh author: Professor, Department of Crop Sciences, University of Illinois, Urbana, IL 61801; eighth author, Professor, Department of Agricultural Statistics, University of Arkansas, Fayetteville, AR 72701. Current addresses of fourth author: Department of Crop and Soil Sciences, Texas A&M University, College Station, TX 77843; fifth and sixth authors: Department of Crop Protection, Universidade Federal do Pelotas (UFPEL), Rio Grande do Sul, Brazil 96010-900. Corresponding author's E-mail: nburgos@uark.edu

photosynthetic rate, and high propensity to evolve herbicide resistance, Palmer amaranth has become a serious weed in row crops and vegetables (Ehleringer et al.1997; Guo and Al-Khatib 2003; Jha and Norsworthy 2012; Steckel 2007). Palmer amaranth infestation can reduce corn, cotton, and soybean yield 91%, 77%, and 78%, respectively (Bensch et al. 2003; Fast et al. 2009; Massinga et al. 2001). In the past decade, reports abound of Palmer amaranth evolving resistance to glyphosate and other herbicides (Culpepper et al. 2006; Jha et al. 2008; Norsworthy et al. 2008). To date, Palmer amaranth has been confirmed resistant to acetolactate synthase (ALS) inhibitors (Burgos et al. 2001; Horak and Peterson 1995), dinitroanilines (Gossett et al. 1992), 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Jhala et al. 2014), glyphosate (Culpepper et al. 2006; Norsworthy et al. 2008), photosystem II herbicides (Vencill et al. 2011), and most recently, protoporphyrinogen oxidase (PPO) inhibitors (Salas et al. 2016).

Several chemical families inhibit PPO activity, including diphenylethers (e.g., acifluorfen, fomesafen, lactofen, oxyfluorfen), *N*-phenylphthalimide (e. g., flumioxazin, flumiclorac), phenylpyrazoles (e.g., fluazolate, pyraflufen-ethyl), oxadiazole (e.g., oxadiazon), oxazolidinones (e.g., pentoxazone), pyrimidinediones (e.g., saflufenacil), thiadiazole (e.g., fluthiacet-methyl), and triazolinone (e.g., carfentrazone, sulfentrazone) (Heap 2017). Diphenylether herbicides are used PRE or POST. Some triazolinones and *N*-phenylphthalimide have high soil activity and are phytotoxic to the crop when applied foliar, hence most are commonly used PRE (Dayan and Duke 2010). There are two known nuclear *PPO* genes in plants, *PPX1* and *PPX2*, which encode plastid- and mitochondrial-targeted PPO isoforms, respectively; however, some *PPX*2 isoforms are dual targeted to both organelles (Lermontova and Grimm 2000; Lermontova et al. 1997; Watanabe et al. 2001). The inhibition of PPO causes accumulation of protophorphyrinogen IX, which leaks from the plastid to the cytoplasm, where it is oxidized rapidly into photosensitive protoporphyrin IX (Becerril and Duke 1989; Jacobs et al. 1991; Lee et al. 1993). Upon exposure to light, protoporphyrin IX generates singlet oxygen molecules that cause lipid peroxidation, membrane destruction, and ultimately cell death (Becerril and Duke 1989; Jacobs et al. 1991).

The first weed species to evolve resistance to PPO-inhibiting herbicides was common waterhemp in 2001 (Shoup et al. 2003). Resistance to PPO-inhibiting herbicides in Palmer amaranth was first reported in Arkansas and is confirmed also in Tennessee and Illinois (Heap 2017; Salas et al. 2016; L Steckel and K Gage, personal communication). When this research was conducted, resistance to PPO herbicides in *Amaranthus* spp. was attributed to a target-site mutation in the *PPX2* gene only (Patzoldt et al. 2006; Salas et al. 2016). The Gly-210 deletion mutation was present in all PPO-resistant waterhemp populations in Illinois, Kansas, and Missouri (Thinglum et al. 2011). However, a novel PPO mutation, Arg-98-Leu, was detected in PPO-resistant common ragweed (*Ambrosia artemisiifolia* L.) (Rousonelos et al. 2012).

This study was conducted to investigate the response of Palmer amaranth from Arkansas to various foliar-applied PPO-inhibiting herbicides (acifluorfen, carfentrazone, flumioxazin, fluthiacet-methyl, fomesafen, pyraflufen-ethyl, saflufenacil) and to foliar-applied herbicides with different modes of action (dicamba, glyphosate, glufosinate, trifloxysulfuron). The study also aimed to determine the frequency of Gly-210 deletion among the PPO-resistant biotypes, identify other PPO target-site mutations that endow resistance to PPO-inhibiting herbicides, and compare the resistance level to fomesafen in accessions that contained or lacked the Gly-210 deletion.

Materials and Methods

Plant Materials. A total of 124 Palmer amaranth accessions were collected in the summer between 2008 and 2015 from 23 counties in Arkansas, primarily from the eastern half of the state. Sampling sites were identified by county extension agents and crop consultants. Samples in 2008 and 2009 were collected from 18 counties (17 from eastern Arkansas and 1 from central Arkansas) to survey resistance to glyphosate. Seven counties were sampled in 2011 from fields that were planted with LibertyLink® crops to verify differential response to glufosinate. Fifty-two fields from 16 counties were sampled in 2014. Samples in 2015 (23 accessions) were collected specifically to survey fields with remnant Palmer amaranth after having been sprayed with PPO herbicides. Inflorescences from at least 10 mature female plants^{-1} field were collected, dried, and threshed. Equal amounts of seeds from each plant were mixed to make a composite seed sample to represent the field. A sensitive standard population (SS) was included in each experiment for comparison. Plants were grown in a greenhouse maintained at 32/25 ± 3 C day/night temperature

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

with a 16:8-h day:night regime and photon flux density of 0.0005 mol m^{-2} s^{-1}. Plants were planted using commercial potting soil (Sunshine® Premix No. 1; Sun Gro Horticulture, Bellevue, WA), watered daily, and fertilized with Miracle-Gro® (Scotts Miracle-Gro, Marysville, OH) every 2 wk.

Palmer Amaranth Response to Foliar-applied Fomesafen. One hundred-twenty four Palmer amaranth accessions (Table 1) were tested in the greenhouse for resistance to fomesafen. Composite seed samples were planted in 24 by 54 cm cellular trays. Seedlings were thinned to 1 plant $cell^{-1}$ and sprayed with 263 g ai ha^{-1} fomesafen (Flexstar®, Syngenta Crop Protection, Greensboro, NC 27419) when seedlings were 7- to 8-cm tall. The herbicide was applied with 0.5% by volume nonionic surfactant (NIS) (Induce®, Helena Chemical, Collierville, TN 38017) using a laboratory sprayer equipped with a flat-fan spray nozzle delivering 187 L ha^{-1} at 221 kPa. The experiment was conducted in a randomized complete block design with two replications and repeated. Each replication consisted of 50 seedlings grown in a cellular tray at 1 seedling $cell^{-1}$. The plants were assessed visually relative to nontreated plants at 21 d after treatment (DAT) using an injury scale of 0 to 100, where 0% = no visible injury and 100% = complete desiccation. Survivors with 0% to 10%, 11% to 30%, 31% to 60%, and 61% to 89% injury were classified as highly resistant, resistant, moderately resistant, and slightly resistant, respectively. Individuals with 90% injury or higher were considered sensitive. In this experiment, an accession with >10% survivors that were at least slightly resistant was considered to be PPO resistant. Data were analyzed using hierarchal clustering in JMP Pro v. 13 (SAS Institute, Cary, NC.).

Response to Other Foliar-applied PPO Herbicides. Twelve accessions that had low mortality (<83%) from foliar-applied fomesafen were bioassayed in the greenhouse for their response to other foliar-applied PPO herbicides. A sensitive standard accession was also included. Seedlings (100 per accession, 10-cm tall) were treated with the recommended doses of acifluorfen, carfentrazone, flumioxazin, fluthiacet-methyl, lactofen, pyraflufen-ethyl, or saflufenacil (Table 2). Carfentrazone, pyraflufen-ethyl, flumioxazin, and fluthiacet-methyl treatments included 0.25% NIS (v/v), whereas acifluorfen included 0.125% NIS (v/v). Lactofen was sprayed with 0.5% crop oil concentrate (v/v). Saflufenacil was sprayed with 1% methylated seed oil and 1% ammonium sulfate (w/v). Following herbicide applications, the plants were placed in the greenhouse, grouped by herbicide, and the accessions were randomized within each herbicide group. Mortality and injury of survivors were evaluated at 21 DAT. In the second run of the experiment, herbicides were sprayed when seedlings were 5- to 8-cm tall. Data were analyzed, by herbicide, using JMP Pro v. 13 (SAS Institute, Cary, NC).

Table 1. Number of Palmer amaranth accessions in Arkansas tested with foliar-applied fomesafen at 263 g ha^{-1}.

Year collected[a]	Accessions tested
2008	25
2009	10
2011	16
2014	50
2015	23
Total	124

[a] Palmer amaranth samples were collected from fields with a history of glyphosate, glufosinate, or protoporphyrinogen oxidase (PPO)-inhibiting herbicide use. Sampling in 2015 was done specifically to survey fields with remnant Palmer amaranth population after having been sprayed with PPO herbicides.

Response to Foliar-applied Non-PPO Herbicides. Seventy-three accessions collected in 2014 and 2015 were tested in the greenhouse for response to dicamba, glufosinate, glyphosate, and trifloxysulfuron (Table 2). The SS accession was also included for comparison. The experiment was conducted in a completely randomized design with two replications and two runs. Each replication consisted of 50 plants grown in a cellular tray. Composite seed samples were planted as described earlier. The foliar herbicides were applied to uniform-sized plants (7.6-cm tall). Glufosinate and trifloxysulfuron were sprayed with 3,366 g AMS ha^{-1} and 0.25% NIS (v/v), respectively. Herbicide applications were made as described earlier. Plants were evaluated for injury and mortality at 21 DAT. Data were analyzed by herbicide using JMP Pro v. 13 (SAS Institute, Cary, NC). Response to glyphosate was analyzed using hierarchal clustering, collectively considering mortality and injury of survivors.

Mechanism of Resistance to PPO Herbicides. A total of 316 survivors from 47 accessions treated with foliar-applied fomesafen were analyzed for the Gly-210 deletion mutation (Patzoldt et al. 2006; Salas et al. 2016). Young leaf tissues were collected from up to 10 survivors each of 35 accessions sprayed

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

Table 2. Common name, trade name, and manufacturer of herbicides used in the study.

Site of action[a]	Common name	Trade	Rate	Company	Company website
			g ai ha^{-1}		
PPO	Acifluorfen	Ultra Blazer® 2SL	560	United Phosphorus, Inc., King of Prussia, PA 19406	www.upi-usa.com
PPO	Carfentrazone	Aim® 2EC	280	FMC Corporation, Philadelphia, PA 19103	www.fmc.com
PPO	Flumioxazin	Valor® SX 51 WDG	70.6	Valent USA Corporation, Walnut Creek, CA	www.valent.com
PPO	Fluthiacet-methyl	Cadet® 0.91EC	0.672	FMC Corporation, Philadelphia, PA 19103	www.fmc.com
PPO	Fomesafen	Flexstar 1.88 SL	263	Syngenta Crop Protection, LLC, Greensboro, NC 27419	www.syngenta-us.com
PPO	Lactofen	Cobra® 2EC	224	Valent USA Corporation, Walnut Creek, CA	www.valent.com
PPO	Pyraflufen-ethyl	ET® 0.2EC	3.64	Nichino America, Wilmington, DE 19808	www.nichino.net
PPO	Saflufenacil	Sharpen® 4F	24.6	FMC Corporation, Philadelphia, PA 19103	www.fmc.com
Auxin	Dicamba	Clarity® 4SL	280[b]	BASF Corporation, Research Triangle Park, NC 27709	https://www.basf.com
Glutamine synthetase	Glufosinate	Liberty® 280SL	549	Bayer CropScience LP, Research Triangle Park, NC 27709	www.bayer.com
EPSPS	Glyphosate	Roundup PowerMax® 4.5SL	870[b]	Monsanto Company, St Louis, MO 63167	www.monsanto.com
ALS	Trifloxysulfuron	Envoke® 75	7.84	Syngenta Crop Protection, LLC, Greensboro, NC 27419	www.syngenta-us.com

[a] Abbreviations: ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; PPO, protoporphyrinogen oxidase.
[b] Rate in g ae ha^{-1}.

with the recommended dose of foliar-applied fomesafen in the greenhouse bioassays. In addition, leaf tissues were collected from 82 plants in 14 fields with high population density of Palmer amaranth after having been sprayed with PPO inhibitors, among other herbicides. DNA was extracted using a modified Cetyltrimethylammonium bromide (CTAB) protocol (Sales et al. 2008) and quantified using a NanoDrop spectrophotometer (ND-1000, Thermo Scientific, Wilmington, DE). An allele-specific PCR assay was used to detect the codon Gly-210 following the protocol described for common waterhemp, which also worked for Palmer amaranth (Lee et al. 2008; Salas et al. 2016).

Some PPO-resistant plants that did not have the Gly-210 deletion mutation were selected for sequencing of the *PPX2L* gene. Total RNA was extracted using the RNeasy extraction kit (Qiagen 74903, Valencia, CA) and converted to cDNA using the Reverse Transcription kit (Promega A3500, Madison, WI). Initially a partial sequence of the *PPX2L* gene was amplified using the primer pair ppx2-1F (5′-GTAATTCAATCCATTACCCACCTT-3′) and ppx2-3R (5′-TTACGCGGTCTTCTCATCCAT-3′) and sequenced using the internal primers: ppx2-1R (5′-TTCCATACGTCGGGAAATGT-3′), ppx2-2F (5′-TGTTGGAACCATTTCTCTGG-3′), ppx2-2R (5′-GGGGATAAGAACTCCGAAGC-3′) and ppx2-3F (5′-GATGCTGTGGTTGTCACTGC-3′). Eventually, the full-length sequences of *PPX2L* in PPO-sensitive and PPO-resistant plants were obtained (GenBank accession nos. MF583744 and MF583746) by designing primers based on the upstream and downstream gene sequence of Prince-of-Wales feather (*Amaranthus hypochondriacus* L.) (GenBank accession no. EU024569.1). These additional primers were: ppx2-5′UTR (5′-TGGCAGATTGAGACAAAATT GG-3′) and ppx2-3′UTR (5′-GGCAGAAAAGTCAC TGCACA-3′).

Fomesafen Dose–Response Bioassay. Seven PPO-resistant accessions, including the SS, were used in whole-plant bioassays to determine the resistance level to fomesafen. Three accessions that contained the Gly-210 deletion (14MIS-H, 15MIS-C, 15MIS-E) and four accessions that lacked the Gly-210 deletion mutation (14CRI-C, 15CRI-B, 15PHI-A) were selected. Seedlings were grown in 15-cm-diameter pots and thinned to 5 plants pot^{-1}. Seedlings, 5- to 7-cm tall, were sprayed with 10 doses of fomesafen from 0 to 2,107 g ai ha^{-1}. The SS was sprayed with 9 doses from 2 to 263 g ha^{-1}, corresponding to 1/128 to 1X the recommended dose. A nontreated check was included.

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

The herbicide was applied with 0.5% NIS. The experiment was conducted in a randomized complete block design with four replications. At 21 DAT, plants were cut at the soil surface, shoots were dried for 48 h at 60°C, and the dry weights were recorded.

Data were analyzed using SAS JMP Pro v.13 (SAS Institute, Cary, NC) in conjunction with SigmaPlot v.13 (Systat Software, San Jose, CA) for regression analysis. The percentage biomass reduction was fit to a nonlinear, sigmoid, four-parameter logistic regression model defined by

$$y = c + [(d - c)/(1 + e\{-a(x - b)\})] \qquad [1]$$

where y is the biomass reduction expressed as percentage relative to the untreated control, a is the growth rate, b is the inflection point, c is the lower asymptote, d is the upper asymptote, and x is the fomesafen dose. Estimates of herbicide dose that cause 50% growth reduction (GR_{50}) were determined using the fitted regression equation.

Results and Discussion

Palmer Amaranth Response to Foliar-applied Fomesafen. The frequency distribution of mortality from fomesafen treatment was highly skewed, with a greater proportion of high mortality; therefore, the median values were used to describe the data, except for the 2015 accessions (Figure 1). The 2008 accessions generally incurred 97% mortality with the field dose of fomesafen, except for three accessions with <90% mortality. One rare accession from Phillips County had only 74% mortality; however, the survivors incurred >75% injury and did not produce seeds. Thus, the early samples had individuals that were relatively more tolerant to fomesafen than others. The accessions in 2009 and 2011 were all susceptible, but one of two accessions with 95% mortality, 11LAW-B, contained rare individuals carrying the Gly-210 deletion mutation (Salas et al. 2016). Eleven accessions (22%) collected in 2014 and 16 accessions (70%) in 2015 were classified resistant. The 2015 samples were from fields with suspected resistance to PPO herbicides, and the results confirmed most growers' suspicions. The mortality data with fomesafen treatment was normally distributed from 23% to 100%, with an average of 71%. Accessions classified as PPO resistant in 2015 consisted of individuals with variable response to fomesafen (Figure 2). For example, 15CLA-A had 50% survivors and almost all showed ≤10% injury. On the other hand, 15GRE-A had 75% survivors, and only about one-half showed

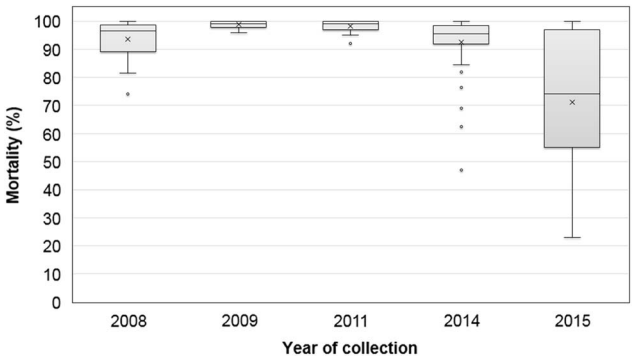

Figure 1. Variability in response to foliar-applied fomesafen (263 g ha^{-1}) among Palmer amaranth accessions collected between 2008 and 2015. Box plot shows median values (horizontal line inside the box), mean values (marked X), first and third quartile values (box outlines), minimum and maximum values (whiskers), and outlier values (open circles).

≤10% injury. Overall in 2015, 16 accessions from 10 counties showed mostly <90% mortality with fomesafen and were classified resistant based on further evaluations discussed in succeeding sections. This shows that resistance to PPO herbicides among Palmer amaranth has evolved quasi-simultaneously across counties in Arkansas.

Prior to 2015, there were fields with a few Palmer amaranth remaining after PPO herbicide application, but such low numbers did not catch the growers' attention because of the expected variability in plant response under various environmental conditions. In some cases, any escapes would have been blamed on various factors. Continued selection with different herbicides, but with the same PPO-inhibitor mode of action, has increased the frequency of surviving individuals to a field-scale, observable level. PPO-inhibiting herbicides are used extensively to control glyphosate- and ALS-resistant Palmer amaranth in soybean and cotton (Salas et al. 2016). Prior to the widespread glyphosate-resistance problem in *Amaranthus*, fomesafen had been used primarily as PRE or POST herbicide for broadleaf weed control in soybean. Since fomesafen commercialization in the 1960s, several other herbicides targeting the PPO enzyme have been commercialized. With the expansion of PPO-herbicide use pattern to cotton and PPO-herbicide use in preplant applications, the selection pressure from this mode-of-action group has intensified greatly. Considering that rare PPO-resistant Palmer amaranth individuals were detected retroactively in a population collected in 2011, it took 4 yr before several reports arose of field-level escapes from PPO herbicide application in 2015.

Fields sampled in 2015 were those with remnant infestations of Palmer amaranth after a weed

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

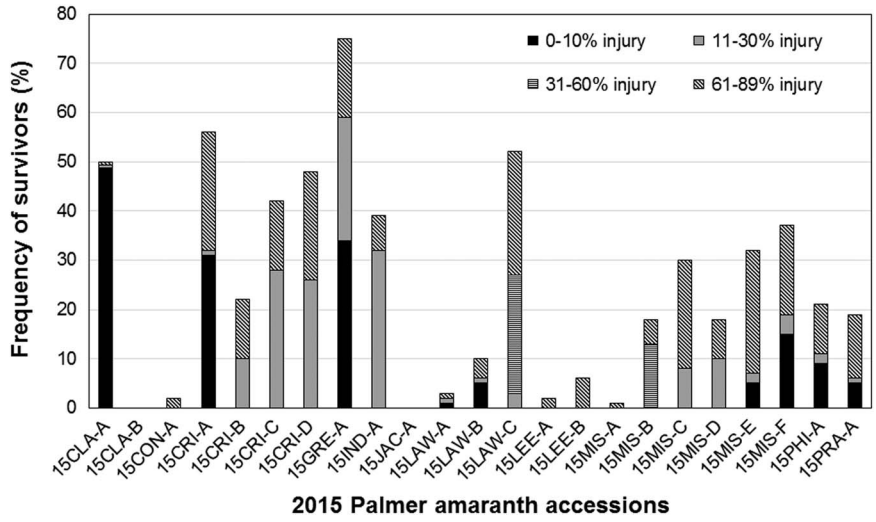

Figure 2. Frequency of fomesafen-resistant plants in Palmer amaranth accessions collected in 2015 from Arkansas. Fomesafen at 263 g ha^{-1} was applied with 0.5% nonionic surfactant to 7.6-cm-tall seedlings. Survivors were categorized based on visible injury.

management program that included multiple applications of a PPO herbicide. Most of those fields had plants resistant to 263 g ha^{-1} fomesafen. The frequency of resistance reported here pertained mostly to fields with putative PPO herbicide–resistance problem. A random survey of fields infested with Palmer amaranth across the state, irrespective of cropping history, is expected to produce a lower frequency of resistance to PPO herbicides. Of the 23 fields verified for resistance in 2015, only two had 100% mortality with a field dose of fomesafen. The rest had resistant individuals showing different levels of injury of survivors (Figure 2). The majority of survivors from three accessions (15CLA-A and 15CRI-A) incurred minimal injury (0% to 10%). High frequency of PPO-resistant individuals among the 2015 accessions threatens the continued use of PPO herbicides.

Multivariate cluster analysis was conducted to group the 2014 and 2015 accessions based on their response to the field use rate of fomesafen, taking into account both mortality data and injury of survivors. The 2014 and 2015 accessions differentiated into four clusters (Figure 3). The 50 accessions belonging to the first cluster were the most sensitive, the majority of which were from the quasi-random collection in 2014. The second cluster is composed of 19 resistant accessions with the

Cluster	No. of accessions	Mortality (%)			Mean frequency of plants per accession, at different levels of injury (%)				
		Mean	Min	Max	0-10% injury	11-30% injury	31-60% injury	61-89% injury	91-100% injury
1	50	96	88	100	0	0	1	3	97
2	19	68	43	87	2	0	11	15	73
3	2	63	46	80	0	19	2	15	65
4	3	42	23	62	38	0	9	14	40

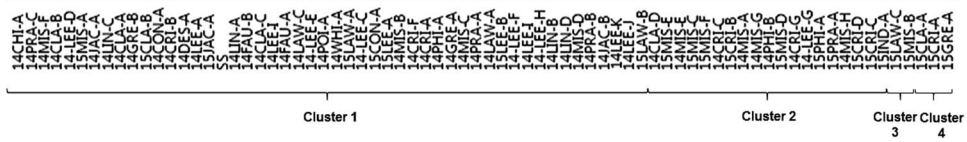

Figure 3. Hierarchal clustering of 2014 and 2015 Palmer amaranth accessions treated with foliar-applied fomesafen at 263 g ha^{-1}. The herbicide was applied with 0.5% nonionic surfactant to 7.6-cm-tall seedlings. Cluster 1, most sensitive to fomesafen (50 accessions); Cluster 2, resistant to fomesafen, the majority of survivors incurred >60% injury (19 accessions); Cluster 3, resistant to fomesafen, majority of survivors sustained 11% to 30% injury (2 accessions); Cluster 4, resistant to fomesafen, the majority of survivors incurred 0% to 10% injury (3 accessions).

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

majority of survivors showing >60% injury, from the 2014 and 2015 collection. The third cluster comprises two resistant accessions in which most survivors incurred low (11% to 30%) injury. The fourth cluster contains the three resistant accessions in which a large number of survivors incurred the lowest (<11%) injury. This indicates that a large number of PPO-resistant plants incurred low to moderate levels of injury from field use rate of fomesafen, allowing them to mature and reproduce. The evolution of observable, population-level resistance to PPO herbicides in multiple fields in 2015 demonstrated the quasi-simultaneous evolution of resistance to PPO herbicides in Palmer amaranth. This occurred after several decades of use primarily in soybean, then more recently in cotton. The proportion of resistant populations that evolved via gene flow (pollen or seed) or via independent selection is yet to be determined.

Response to Other Foliar-applied PPO-inhibiting Herbicides. The response of Palmer amaranth to other foliar-applied PPO herbicides and to other non-PPO foliar-applied herbicides was evaluated, because PPO herbicides are used heavily to control ALS- and glyphosate-resistant Palmer amaranth. Twelve of the most fomesafen-resistant accessions were tested with other PPO-inhibiting herbicides (Table 3). Mortality with pyraflufen-ethyl and fluthiacet-methyl was similar across runs; data were therefore combined. These herbicides controlled the SS 100%. The mortality of all accessions, excluding the SS, was <72% with fluthiacet-methyl. Palmer amaranth generally has variable sensitivity to this PPO herbicide and is therefore not listed on the label; whereas this herbicide is expected to provide only partial control of common waterhemp (Anonymous 2011). The mortality rating of all PPO-resistant accessions was at least 17% lower than the SS when treated with pyraflufen-ethyl, with the exception of 14CRI-C (>90% mortality). Pyraflufen-ethyl also is naturally weak on Palmer amaranth, although it killed the SS in these experiments, as did the other herbicides.

Mortality was different between runs for acifluorfen, carfentrazone, flumioxazin, lactofen, and saflufencil;

Table 3. Response of fomesafen-resistant Palmer amaranth accessions to the recommended rate of various foliar-applied protoporphyrinogen oxidase herbicides.

	Mortality[b]						
Accession[a]	Acifluorfen	Carfentrazone	Flumioxazin	Fluthiacet-methyl	Lactofen	Pyraflufen-ethyl	Saflufenacil
	%						
14CRI-C	100[c] (100)[d]	84 (99)	100 (100)	71[f]	93 (95)	94[f]	98 (100)
14MIS-H	55 (97)	67 (81)	66 (75)	18	42 (73)	37	79 (100)
15CLA-A	38 (64)	48 (45)	55 (44)	13	23 (66)	29	50 (98)
15CRI-A	68 (94)	54 (55)	74 (90)	39	49 (72)	53	73 (100)
15CRI-C	91 (100)	74 (87)	100 (100)	48	67 (92)	73	92 (100)
15CRI-D	72 (98)	60 (66)	91 (98)	19	44 (75)	60	93 (100)
15GRE-A	31 (78)	66 (72)	48 (81)	21	21 (29)	35	38 (99)
15IND-A	70 (94)	63 (74)	87 (90)	44	70 (79)	64	78 (100)
15LAW-C	73 (93)	58 (84)	81 (98)	33	49 (80)	57	91 (99)
15MIS-D	83 (99)	63 (80)	80 (60)	31	45 (77)	34	89 (100)
15MIS-E	73 (86)	69 (66)	79 (91)	30	54 (78)	54	95 (100)
15MIS-F	60 (93)	67 (67)	68 (89)	21	39 (69)	43	84 (100)
SS[f]	100 (100)	100 (100)	100 (100)	100	100 (100)	100	100 (100)
$LSD_{0.05}$[g]	14 (15)	19 (19)	15 (19)	23	16 (23)	22	12

[a] Accessions were collected between 2014 and 2015 in Arkansas. Accessions were confirmed resistant to foliar-applied fomesafen (263 g ha^{-1}), except for the susceptible standard (SS).

[b] Mortality ratings from acifluorfen, carfentrazone, flumioxazin, lactofen, and saflufenacil treatments were different across runs; data were therefore analyzed separately. Carfentrazone (280 g ha^{-1}), pyraflufen-ethyl (3.64 g ha^{-1}), and flumioxazin (70.6 g ha^{-1}) treatments included 0.25% v/v nonionic surfactant (NIS). Acifluorfen at 560 g ha^{-1} included 0.125% v/v NIS. Lactofen (224 g ha^{-1}) was sprayed with 0.5% v/v crop oil concentrate. Saflufenacil (24.6 g ha^{-1}) was applied with 1% methylated seed oil and 1% ammonium sulfate.

[c] Values obtained from the first run of experiment. Herbicides were applied to 10-cm-tall seedlings.

[d] Numbers in parentheses were data from the second run of the experiment. Herbicides were applied to 5- to 8-cm-tall seedlings.

[e] Average mortality across two runs. Mortality ratings across runs were similar in pyraflufen-ethyl and fluthiacet-methyl treatments; data were therefore combined.

[f] SS, susceptible standard population.

[g] Fisher's protected LSD was used to compare accessions within each herbicide.

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

data were therefore analyzed separately. In general, mortality was higher in the second run than in the first run due to smaller plant size at the time of herbicide treatment. With acifluorfen, only 2 of 12 fomesafen-resistant accessions tested (14CRI-C and 15CRI-C) were effectively controlled (>90% mortality) in the first run; however, 75% of fomesafen-R accessions were susceptible to acifluorfen when smaller plants were sprayed in the second run. With carfentrazone, all 12 fomesafen-resistant accessions showed only 48% to 83% mortality in the first run. Similar results were observed in the second run. With flumioxazin, eight accessions that had ≥74% mortality in the first run were verified to be sensitive in the second run. About 42% of fomesafen-resistant accessions tested were cross-resistant to foliar-applied flumioxazin. The mortality of all accessions was <70% with lactofen in the first run, with the exception of 15CRI-C (93% mortality). In the second run, all accessions were still poorly to moderately controlled, except for 14CRI-C and 15CRI-C (>90% mortality). Saflufenacil was effective only on five accessions (>90% mortality) with larger seedlings. With smaller seedlings, all 12 fomesafen-resistant accessions showed >97% mortality with saflufenacil. Across all chemistries, regardless of herbicidal strength, application on small seedlings is necessary for maximum possible efficacy. Application on bigger seedlings is risky. Previous reports indicated that resistance of common waterhemp to foliar-applied PPO herbicides becomes prevalent at the 4- to 6-leaf stage (Falk et al. 2006). Plant size is a critical factor in the efficacy and consistency of performance of most foliar-applied herbicides. When growers miss the application window, selection for resistance is expected to be stronger. If survival in greenhouse bioassays is seen to be consistent across replications and repetitions, then the risk of having survivors in the field populations is high.

Consistent in both runs, fluthiacet-methyl was the least effective and saflufenacil was the most effective of all foliar-applied PPO herbicides. The response to PPO herbicides, in order of decreasing efficacy, was as follows: saflufenacil > acifluorfen = flumioxazin > carfentrazone = lactofen > pyraflufen-ethyl > fluthiacet-methyl. In this series, fomesafen would fall between the last two herbicides. The fomesafen-resistant accessions were generally cross-resistant to other foliar-applied PPO herbicides, with the exception of saflufenacil. However, saflufenacil is not an in-crop option for soybean or cotton. Although saflufenacil showed the greatest efficacy, some accessions already showed reduced sensitivity to the field use rate of saflufenacil when applied to 10-cm-tall seedlings. Previous studies on diphenylether-resistant tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] also showed cross-resistance to other foliar-applied PPO herbicide families (Patzoldt et al. 2005; Shoup et al. 2003; Wuerffel et al. 2015a).

Response to Foliar-applied Non–PPO Inhibiting Herbicides. Palmer amaranth accessions collected between 2014 and 2015 were tested with dicamba, glufosinate, glyphosate, and trifloxysulfuron. The 73 accessions, including the SS accession, differentiated into four clusters in response to glyphosate (Figure 4A). The first cluster was composed of 13 resistant

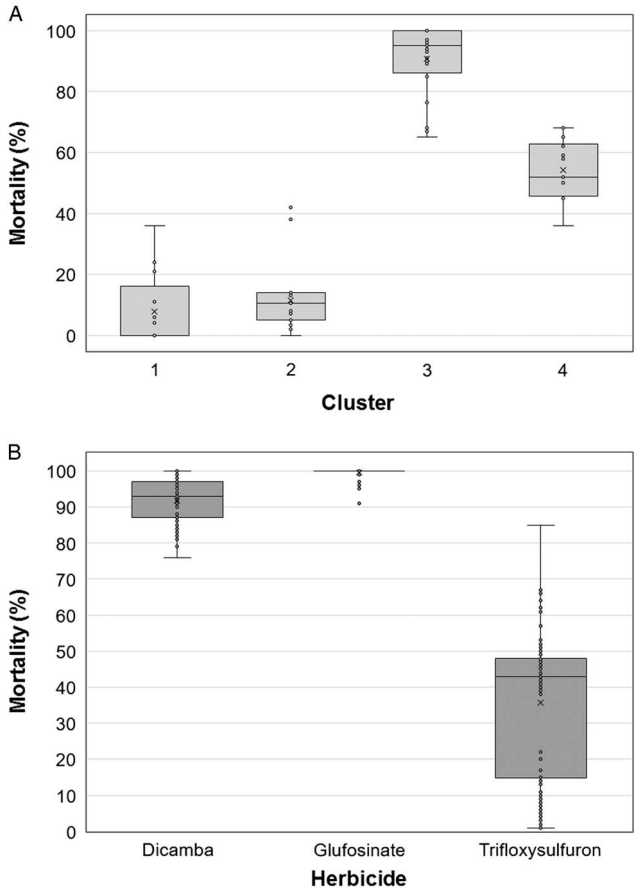

Figure 4. (A) Cluster analysis of Palmer amaranth accessions collected in 2014 and 2015 treated with 870 g ha^{-1} glyphosate. Cluster 1 (n = 13 accessions; resistant to glyphosate, the majority of survivors incurred 31% to 60% injury), Cluster 2 (n = 23 accessions; resistant to glyphosate, the majority of survivors incurred <11% injury), Cluster 3 (n = 23 accessions; most resistant to glyphosate), and Cluster 4 (n = 14 accessions; resistant to glyphosate, the majority of survivors incurred 61% to 89% injury). Glyphosate was applied to 7.6-cm-tall seedlings. (B) Variability in response to dicamba (280 g ae ha^{-1}), glufosinate (549 g ha^{-1}), and trifloxysulfuron (7.84 g ha^{-1}) among Palmer amaranth accessions collected in 2014 and 2015 from Arkansas. Box plot shows median values (horizontal line inside the box), mean values (marked with X), first and third quartile values (box outlines), minimum and maximum values (whiskers), and outlier values (open circles).

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

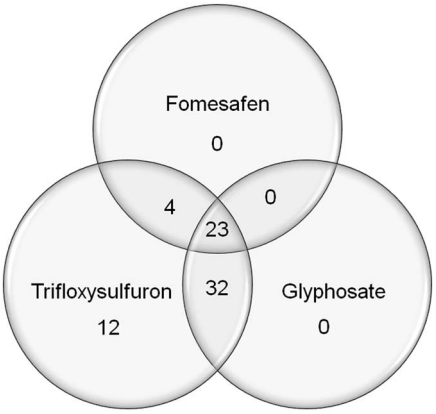

Figure 5. Herbicide resistance profiles of Palmer amaranth populations from Arkansas sampled in 2014 and 2015.

accessions that had 0% to 36% mortality with glyphosate, with the majority of survivors incurring 31% to 60% injury. Twenty-three accessions constituted the second cluster, with resistance to glyphosate in which a large number of the survivors incurred < 11% injury. The third cluster was composed of 24 accessions with ≥65% mortality, 17 of which were classified as sensitive (at least 90% mortality). The fourth cluster included 14 resistant accessions (36% to 68% mortality), in which most of the survivors sustained >60% injury from glyphosate treatment. Overall, about 50% of accessions (Clusters 1 and 2) were resistant to glyphosate, with mortality ranging from 0% to 42%. In addition, all accessions were also resistant to trifloxysulfuron, showing <86% mortality (Figure 4B). The widespread occurrence of ALS- and glyphosate-resistant Palmer amaranth in Arkansas was reported previously (Burgos et al. 2009). The ALS-inhibiting herbicides and glyphosate had been used extensively in the past. Overall, the 27 PPO-resistant accessions were also resistant to either ALS inhibitor or glyphosate, or both (Figure 5; Table 4). The majority of PPO-resistant accessions (85%) exhibited resistance to both glyphosate and trifloxysulfuron.

Table 4. Herbicide resistance profile of all protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth accessions to other foliar-applied non-PPO herbicides.

Accession	Mortality[a]				
	Fomesafen	Dicamba	Glyphosate	Glufosinate	Trifloxysulfuron
	%				
14CLA-D	62	97	91	100	85
14CRI-C	82	86	6	100	1
14CRI-G	77	93	85	100	2
14JAC-B	88	97	100	100	61
14LEE-G	86	85	95	100	49
14LEE-J	85	100	0	100	46
14MIS-A	85	97	24	100	44
14MIS-E	69	91	52	100	64
14MIS-G	85	100	89	100	38
14MIS-H	47	98	0	100	67
14PHI-B	83	100	52	100	42
15CLA-A	62	86	14	100	17
15CRI-A	40	87	5	95	7
15CRI-B	77	87	61	91	3
15CRI-C	55	93	5	100	6
15CRI-D	44	76	11	100	4
15GRE-A	23	79	93	99	10
15IND-A	43	83	14	100	22
15LAW-B	88	85	46	96	2
15LAW-C	46	84	13	97	13
15MIS-B	80	87	14	100	1
15MIS-C	64	88	9	99	5
15MIS-D	58	81	14	100	1
15MIS-E	65	84	14	100	8
15MIS-F	56	87	7	100	5
15PHI-A	74	83	2	100	11
15PRA-A	79	79	18	99	11

[a] Fomesafen (263 g ai ha^{-1}), dicamba (280 g ae ha^{-1}), glufosinate (549 g ai ha^{-1}), glyphosate (870 g ae ha^{-1}), and trifloxysulfuron (7.84 g ha^{-1}) were applied to 7.6-cm-tall seedlings.

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

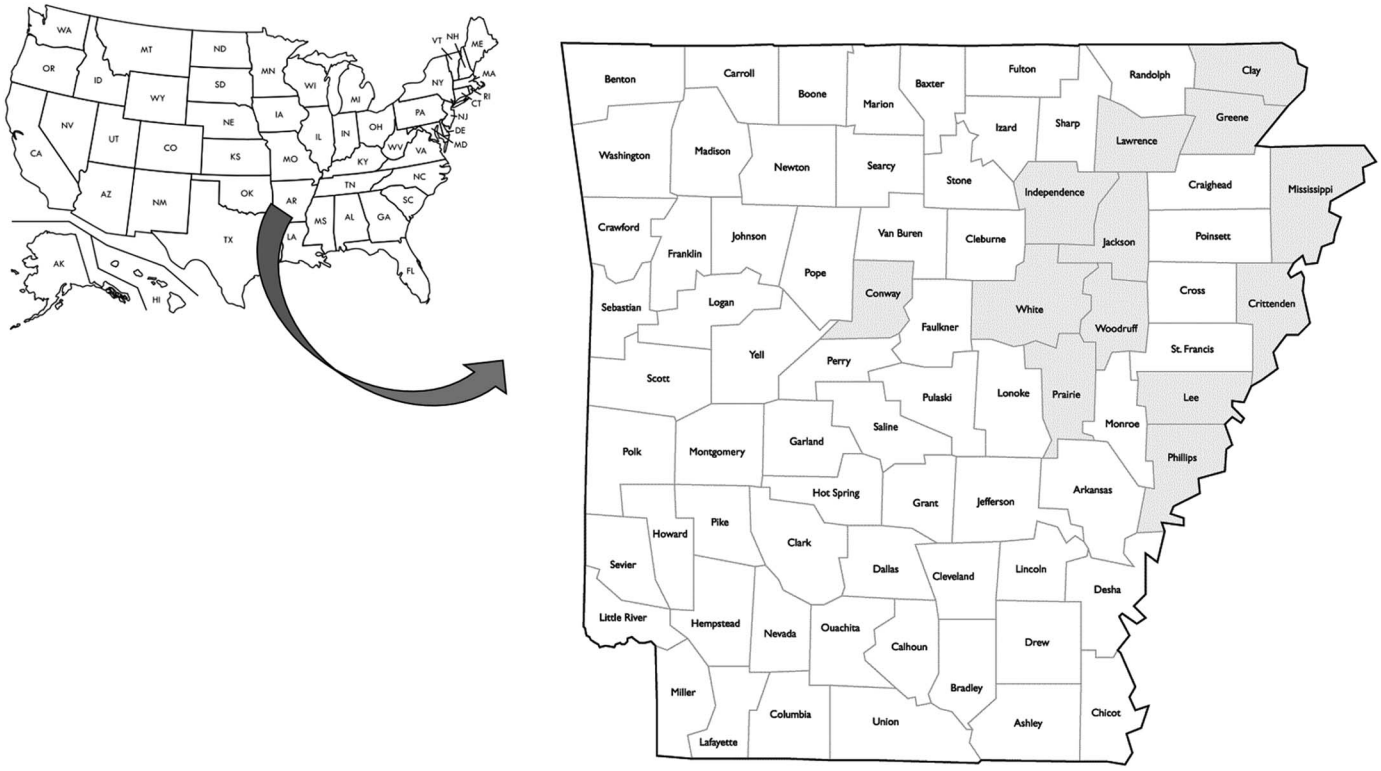

Figure 6. Distribution of fields with confirmed protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth in Arkansas. Counties that are shaded had at least one field with a PPO-resistant Palmer amaranth population carrying the Gly-210 deletion in the PPO gene.

The 2014 and 2015 accessions were all sensitive to the 549 g ai ha^{-1} glufosinate (>90% mortality) (Figure 4B). All accessions incurred >95% mortality, except for 15CRI-B (91%). In the same manner, dicamba controlled all accessions (Figure 4B). Dicamba caused >90% mortality in 61% of the accessions. Mortality of the remaining accessions was <89%; however, the survivors incurred >75% injury. Anecdotal reports, however, indicated poor performance of dicamba in fields infested with PPO-resistant Palmer amaranth.

Prevalence of the *PPO*-Gly-210 Deletion Mutation in Resistant Palmer Amaranth. The Gly-210 deletion in the *PPX2*L gene confers resistance to PPO-inhibiting herbicides in PPO-resistant common waterhemp and Palmer amaranth (Patzoldt et al. 2006; Salas et al. 2016; Wuerffel et al. 2015b). A point mutation in *PPX2* of common ragweed also confers resistance to PPO herbicides; however, whereas a plastid-targeting signal was not found to be encoded by *PPX2* in common ragweed (Rousonelos et al. 2012), *PPX2L* in common waterhemp encodes plastidic and mitochondrial isoforms of PPO (Patzoldt et al. 2006). This dual-targeting phenomenon has been reported previously for spinach (*Spinacia oleracea* L.) and corn (Watanabe et al. 2001). The Gly-210 deletion in *PPX2L* imparts herbicide resistance to the dual-targeted protein by altering the architecture of the substrate binding domain (Dayan et al. 2010).

The molecular survey was carried out on 316 fomesafen-resistant plants from 38 accessions representing 13 counties in Arkansas including Clay, Conway, Crittenden, Greene, Independence, Jackson, Lawrence, Lee, Independence, Mississippi, Phillips, White, and Woodruff (Figure 6). The Gly-210 codon deletion mutation was found in 46% and 60% of the survivors from 2014 and 2015 accessions, respectively (unpublished data). The PPO Gly-210 was prevalent among the PPO-resistant accessions; however, a substantial number of the resistant plants did not carry this mutation (Figure 7, A and B). In most of the PPO-resistant accessions (68%), resistant individuals were mixtures of carriers and noncarriers of the Gly-210 deletion. Only 13% of the PPO-resistant accessions ($n = 6$) contained individuals that were all carriers of the Gly-210 deletion. In nine accessions, none of the resistant individuals carried the Gly-210 deletion. This indicates that either an alternative target-site mutation is present or another resistance mechanism is occurring within and among resistant populations in the field. To verify the occurrence of alternative

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

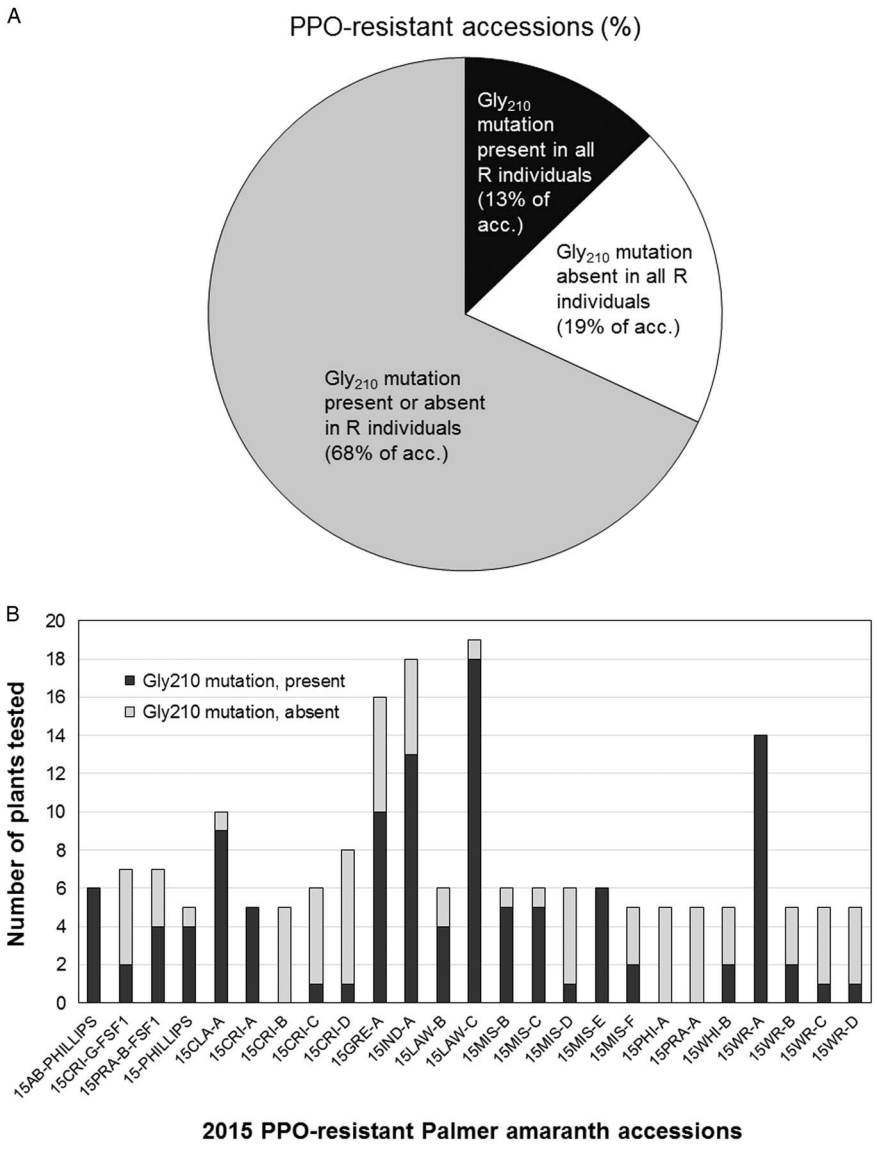

Figure 7. Prevalence of protoporphyrinogen oxidase (PPO) Gly-210 deletion in PPO-resistant Palmer amaranth from Arkansas. (A) The proportion of PPO-resistant accessions that contained, or lacked, the *PPO* Gly-210 deletion. Black, all fomesafen survivors in these accessions contained the Gly-210 deletion; white, all survivors in these accessions lacked the Gly-210 deletion; gray, survivors in these accessions are a mixture of carriers and noncarriers of the Gly-210 deletion mutation. (B) Proportion of Gly-210 deletion carriers in PPO-resistant accessions from 2015. 15CRI-B, 15PHI-A, and 15PRA-A were noncarriers of the Gly-210 deletion mutation, but had 77%, 74%, and 79% mortality, respectively, and estimated resistance factors of 51- to 125-fold.

target-site mutations, we generated a 1,542-bp sequence of *PPX2L* in selected resistant individuals without the Gly-210 deletion (GenBank accession no. MF583745) from the noncarrier accession 15CRI-B. Resistant plants in this accession contained a different amino acid mutation, Arg-128-Gly. This mutation was reported recently in parallel investigations of PPO-resistant Palmer amaranth, along with an Arg-128-Met mutation (Giacomini et al. 2017). A mutation at the homologous site was identified previously in PPO-resistant common ragweed in the form of an Arg-98-Leu substitution (Rousonelos et al. 2012).

Our survey of more than 300 PPO inhibitor–resistant individuals representing 38 field populations showed that a resistant population may contain a mixture of plants carrying either one of these mutations. Henceforth, testing for these mutations should be done simultaneously on suspected PPO inhibitor–resistant plants using available tools (Giacomini et al. 2017). Sequencing of Palmer amaranth with different resistance levels to PPO inhibitors, with or without the Gly-210 mutation, is ongoing.

The occurrence of other resistance-conferring mutations in other loci of the PPO gene is rare, as indicated by previous research. Many single or

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

Table 5. Resistance levels of protoporphyrinogen oxidase–resistant Palmer amaranth accessions in Arkansas.

Accession	Gly-210 deletion	GR_{50}[a]	R/S
		g ai ha^{-1}	
14MIS-H	Yes	232 (148–315)	15
15MIS-C	Yes	116 (80–153)	8
15MIS-E	Yes	141 (94–188)	9
14CRI-C	No	70 (43–97)	4
14CRI-G	No	153 (112–195)	10
15CRI-B	No	125 (85–164)	8
15PHI-A	No	51 (32–70)	3
SS	No	15 (10–21)	1

[a] GR_{50}, dose of herbicide required to cause 50% biomass reduction. Values in parentheses are 95% confidence intervals.

double point mutations were reported in mutant PPO genes in an attempt to obtain PPO herbicide–resistant *Arabidopsis* (Li and Nicholl 2005). Those authors' data showed that single point mutations either provided low resistance or resulted in substantial fitness penalty.

Resistance Level to Fomesafen. The fomesafen dose that caused 50% growth reduction (GR_{50}) ranged from 116 to 232 g ha^{-1} in accessions that contained the Gly-210 deletion, whereas GR_{50} ranged from 51 to 153 g ha^{-1} in accessions that lacked the Gly-210 deletion (Table 5). Based on these GR_{50} values, the level of resistance to fomesafen relative to the SS ranged from 8- to 15-fold in accessions that contained the Gly-210 and from 3- to 10-fold in accessions that lacked the Gly-210 deletion.The dose–response bioassay indicated high resistance level in accessions carrying the Gly-210 deletion mutation. However, some accessions (14CRI-G and 15CRI-B) that lacked the Gly-210 deletion had GR_{50} values comparable to those of accessions that contained the Gly-210 deletion. Accession 15CRI-B, which contained the Arg-128-Gly mutation, had similar GR_{50} values to those of the resistant accessions carrying the Gly-210 deletion. This indicates that Gly-210 and Arg-128-Gly mutations result in comparable levels of resistance to fomesafen. Accessions 14CRI-C and 15PHI-A, which lacked the Gly-210 deletion, had lower GR_{50} values than the other PPO-resistant accessions but higher GR_{50} values than the SS. These accessions have not yet been studied for the occurrence of other *PPO* mutations or other resistance mechanisms. Although our results indicated that the Arg-128-Gly mutation may confer a resistance level similar to that of the Gly-210 deletion, additional data are needed to quantify the impact of this mutation on resistance to PPO herbicides. In addition, we have not ruled out the possibility that other resistance mechanism (s) exist in some PPO inhibitor–resistant accessions, particularly those exhibiting low-level resistance to PPO inhibitors. It is also possible also the populations showing high-level resistance harbor multiple resistance mechanisms, as in the case of ALS-resistant turnipweed [*Rapistrum rugosum* (L.) All.] (Hatami et al. 2016).

The increasing number of Palmer amaranth populations with resistance to PPO herbicides is a great concern, because this limits herbicide options for cotton and soybean. Most PPO-resistant populations were also resistant to glyphosate and trifloxysulfuron, which leaves almost no herbicides for POST Palmer amaranth control. The remaining POST herbicide options for PPO-resistant Palmer amaranth include glufosinate in LibertyLink® crops, dicamba in Roundup Xtend® or Engenia®, or 2,4-D in Enlist Duo® crops. However, overdependence on glufosinate and phenoxy herbicides must be avoided, as some Palmer amaranth populations show high tolerance to dicamba (unpublished data). If we lose glufosinate and dicamba, there will be zero POST options for weed control in soybean and cotton, unless HPPD and 2,4-D traits are commercialized soon.

Overall, the majority of the PPO-resistant individuals carried the *PPO* Gly-210 deletion. An alternative target-site mutation, Arg-128-Gly, was identified in resistant plants of one accession analyzed that did not carry the Gly-210 mutation. The occurrence of other resistance-conferring mutations or other resistance mechanisms is being investigated. The combination of these mutations in one plant may be lethal, but the presence of these mutations in different plants in one field, or different mutations in proximal fields, may accelerate the evolution and spread of resistance to PPO inhibitors in Palmer amaranth.

Acknowledgments

The authors thank the Arkansas Soybean Promotion Board, Syngenta Crop Protection, and Cotton Inc. for funding this research. The authors also thank Seth

https://doi.org/10.1017/wsc.2017.41 Published online by Cambridge University Press

Bernard Abugho, Claudia Oliveira, Josiane Argenta, Leopoldo Estorninos, Vijay Singh, Teal Penka, and Christopher Rouse for assistance in research activities and several Arkansas County Extension Agents for facilitating the plant sampling.

Literature Cited

Anonymous (2011) Cadet herbicide product label. Philadelphia, PA: FMC. 4 p

Becerril JM, Duke SO (1989) Protoporphyrin IX content correlates with activity of photobleaching herbicides. Plant Physiol 90:1175–1181

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

Burgos NR, Alcober EAL, Lawton-Rauh A, Estorninos L, Tseng TM, Smith KL (2009) The Spread and Population Genetics of Glyphosate-Resistant Palmer Amaranth in Arkansas. Fayetteville, AR: University of Arkansas. Pp 94–104

Burgos NR, Kuk YI, Talbert RE (2001) *Amaranthus palmeri* resistance and differential tolerance of *Amaranthus palmeri* and *Amaranthus hybridus* to ALS-inhibitor herbicides. Pest Manag Sci 57:449–457

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620–626

Dayan FE, Daga PR, Duke SO, Lee RM, Tranel PJ, Doerksen RJ (2010) Biochemical and structural consequences of a glycine deletion in the α-8 helix of protoporphyrinogen oxidase. Biochim Biophy Acta 1804:1548–1556

Dayan FE, Duke SO (2010) Protoporphyrinogen oxidase-inhibiting herbicides. Pages 1722–1751 *in* Krieger R, Doull J, Hodgson E, Maibach H, Reiter L, Ritter L, Ross J, Slikker WJ, Van Hemmen J, eds. Haye's Handbook of Pesticide Toxicology. San Diego: Academic

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert summer annual. Oecologia 57:107–112

Ehleringer JR, Cerling TE, Helliker BR (1997) C-4 photosynthesis, atmospheric CO_2 and climate. Oecologia 112:285–299

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

Fast BJ, Murdoch SW, Farris RL, Willis JB, Murray DS (2009) Critical timing of Palmer amaranth (*Amaranthus palmeri*) removal in second-generation glyphosate-resistant cotton. J Cotton Sci 13:32–36

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

Gossett BJ, Murdock EC, Toler JE (1992) Resistance of Palmer amaranth (*Amaranthus palmeri*) to the dinitroaniline herbicides. Weed Technol 6:587–591

Guo PG, Al-Khatib K (2003) Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). Weed Sci 51:869–875

Hatami ZM, Gherekhloo J, Rojano-Delgado AM, Osuna MD, Alcantara R, Fernandez P, Sadeghipor HR, de Prado R (2016) Multiple mechanisms increase levels of resistance in *Rapistrum rugosum* to ALS herbicides. Front Plant Sci 7:169

Heap I (2017) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed January 15, 2017

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. Weed Sci 48:347–355

Horak MJ, Peterson DE (1995) Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazathapyr and thifensulfuron. Weed Technol 9:192–195

Jacobs JM, Jacobs NJ, Sherman TD, Duke SO (1991) Effect of diphenyl ether herbicides on oxidation of protoporphyrinogen to protoporphyrin in organellar and plasma membrane enriched fractions of barley. Plant Physiol 97:197–203

Jha P, Norsworthy JK (2012) Influence of late-season herbicide applications on control, fecundity, and progeny fitness of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri)* biotypes from Arkansas. Weed Technol 26:807–812

Jha P, Norsworthy JK, Riley MB, Bielenberg DG, Bridges W (2008) Acclimation of Palmer amaranth (*Amaranthus palmeri*) to shading. Weed Sci 56:729–734

Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of traizine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38

Lee HJ, Duke MV, Duke SO (1993) Cellular localization of protoporphyrinogen oxidizing activities of etiolated barley (*Hordeum vulgare* L) leaves—relationship to mechanism of action of protoporphyrinogen oxidase-inhibiting herbicides. Plant Physiol 102:881–889

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci 56:371–375

Lermontova I, Grimm B (2000) Overexpression of plastidic protoporphyrinogen IX oxidase leads to resistance to the diphenyl-ether herbicide acifluorfen. Plant Physiol 122:75–83

Lermontova I, Kruse E, Mock HP, Grimm B (1997) Cloning and characterization of a plastidal and a mitochondrial isoform of tobacco protoporphyrinogen IX oxidase. Proc Natl Acad Sci USA 94:8895–8900

Li X, Nicholl D (2005) Development of PPO inhibitor-resistant cultures and crops. Pest Manag Sci 61:277–285

Massinga RA, Currie RS, Horak MJ, Boyer J (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208

Norsworthy JK, Scott RC, Smith KL, Oliver LR (2008) Response of northeastern Arkansas Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate. Weed Technol 22:408–413

Patzoldt WL, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30–36

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

Riar DS, Norsworthy JK, Steckel LE, Stephenson DO, Eubank TW, Scott RC (2013) Assessment of weed management practices and problem weeds in the midsouth Unites States—soybean: a consultant's perspective. Weed Technol 27: 612–622

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS- and PPO-inhibiting herbicides. Weed Sci 60:335–344

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

Sales MA, Shivrain VK, Burgos NR, Kuk YI (2008) Amino acid substitutions in the acetolactate synthase gene red rice (*Oryza sativ*a) confer resistance to imazethapyr. Weed Sci 56:485–489

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

Sosnoskie LM, Webster TM, Culpepper SA, Kichler J (2014) The Biology and Ecology of Palmer Amaranth: Implications for Control. Athens, GA: University of Georgia Cooperative Extension. 2 p. http://extension.uga.edu/publications/files/pdf/C%201000_2.PDF. Accessed December 15, 2016

Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567–570

Thinglum KA, Riggins CW, Davis AS, Bradley KW, Al-Khatib K, Tranel PJ (2011) Wide distribution of the waterhemp (*Amaranthus tuberculatus*) ΔG_{210} PPX2 mutation, which confers resistance to PPO-inhibiting herbicides. Weed Sci 59:22–27

Vencill W, Grey W, Culpepper S (2011) Resistance of weeds to herbicides. Pages 585–594 *in* Kortekamp A, ed. Herbicides and Environment. Rijeka, Croatia: InTech

Watanabe N, Che FS, Iwano M, Takayama S, Yoshida S, Isogai A (2001) Dual targeting of spinach protoporphyrinogen oxidase II to mitochondria and chloroplasts by alternative use of two in-frame initiation codons. J Biol Chem 276:20474–20481

Wuerffel RJ, Young JM, Lee RM, Tranel PJ, Lightfoot DA, Young BG (2015b) Distribution of the ΔG_{210} 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 (2015a) Characterization of PPO-inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting herbicides. Weed Sci 63:511–521

Received February 14, 2017, and approved June 9, 2017.

Associate Editor for this paper: Prashant Jha, Montana State University.