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Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action

Lauren M. Schwartz-Lazaro [a, *], Jason K. Norsworthy [a], Robert C. Scott [b], L. Tom Barber [b]

[a] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72704, USA
[b] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Lonoke, AR 72086, USA

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ABSTRACT

Herbicide resistance may evolve at a rapid rate with the lack of proper adoption of best management practices (BMPs). This wide-spread resistance problem is particularly impactful for fields with problematic weeds such as Palmer amaranth (*Amaranthus palmeri*). Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have been one of the remaining effective sites of action (SOAs) for the control of Palmer amaranth, but even these herbicides have begun to fail in many soybean fields across Arkansas, USA. The objective of this research was to determine which of the most commonly used PPO-inhibiting herbicides have the greatest effect on two putative PPO-resistant Palmer amaranth populations, compared to a susceptible standard, when applied preemergence (PRE) and postemergence (POST); as well as to test for multiple resistance to other commonly used herbicides. A dose-response study was conducted under greenhouse conditions that examined five PRE herbicides and four POST herbicides on one PPO-susceptible and two PPO-resistant populations. Complete control was not achieved at the 8X rate with any PPO-inhibiting herbicide at the PRE application; whereas for the POST application, complete control was not achieved until after the 32X rate for all herbicides. Furthermore, twenty-one different herbicides, representing various SOAs, were used to test various application timings (preplant incorporated (PPI), PRE and POST) on these populations at a 1X field rate. Both of the PPI herbicides (trifluralin and pendimethalin) provided 9.7% mortality of Palmer amaranth. Only two PRE herbicides (in 2 SOAs) showed greater than 85% mortality and three POST application herbicides (3 SOAs) all had greater than 95% mortality of both Palmer amaranth populations. Thus, it is likely that PPO-inhibiting herbicides or the other commonly used herbicides, which were tested, cannot be solely relied upon in the field. Thus, the use of multiple effective sites of action along with other integrated weed management tactics need to be a focus for management of this species.

1. Introductionintroduction

Palmer amaranth control has become a challenge because of its ability to evolve multiple herbicide resistance, continual flushes of germination throughout the growing season, rapid growth, high fecundity, and high resource use (Keeley et al., 1987; Jha et al., 2008; Ward et al., 2013). With upwards of 600,000 seeds per female plant, this species can easily replenish the soil seedbank in one growing season (Keeley et al., 1987). Furthermore, Palmer amaranth is highly competitive with crops and has been shown to reduce soybean (*Glycine max* L. Merr.) and corn (*Zea mays* L.) yield by 78 and 91%, respectively, at densities of less than 9 plants m^{-2} (Bensch et al., 2003; Massinga et al., 2001). To date, Palmer amaranth has been confirmed resistant to six herbicide sites of action: acetolactate synthase inhibitors, 4-hydroxyphenylpyruvate dioxygenase inhibitors, 5-enolpyruvyl shikimate-3-phosphate synthase inhibitor, mitosis inhibitors, photosystem II inhibitors, and protoporphyrinogen oxidase inhibitors (Heap, 2016).

The overreliance on one mode of action, such as glyphosate, in the past decade has led to a high selection rate of resistance. This has altered the success of weed management strategies and effectiveness over the past decade (Hager et al., 2003; Riggins and Tranel, 2012). Modified weed management strategies are becoming more reliant on soil-residual herbicides, especially in row crops, such as soybean and cotton (*Gossypium hirsutum* L.), where herbicide-resistance has limited POST herbicide options

* Corresponding author.
E-mail address: lmschwar@uark.edu (L.M. Schwartz-Lazaro).

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(Norsworthy et al., 2012). The continual evolution of resistance to highly used and effective sites of action (SOAs) has led to increasing use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides for Palmer amaranth control. This rapid evolution to multiple herbicide SOAs is partially caused by Palmer amaranth being an obligate cross-pollinated species (Steckel, 2007; Sosnoskie et al., 2012) as well as by the overuse of a single SOA.

In Arkansas, PPO resistance by Palmer amaranth was first discovered in 2011 (Heap, 2016). Since then, there has been little research conducted on the level of resistance or the level of preemergence (PRE) and postemergence (POST) control of Palmer amaranth that can be expected across PPO-inhibiting herbicides from differing classes (Salas et al., 2016). However, similar work has been conducted on waterhemp (*Amaranthus tuberculatus*) that has showed populations have resistance to PPO-inhibiting herbicides applied PRE and POST (Shoup et al., 2003; Patzoldt et al., 2005; Wuerffel et al., 2015). Thus, the objective of this study was to determine which of the most commonly used PPO-inhibiting herbicides have the greatest effect on two putative PPO-resistant Palmer amaranth populations and a PPO-susceptible population when applied PRE and POST; as well as to test for multiple resistance to other commonly used herbicides.

2. Methods

Several greenhouse experiments were conducted at Fayetteville, Arkansas in the greenhouse at the Altheimer Laboratory at the University of Arkansas. The experiments examined three populations of Palmer amaranth which included one known susceptible biotype from a 1986 population, that has been used routinely for resistance screening experiments, due to its limited exposure to herbicides (Norsworthy et al., 2008), and two putative PPO-resistant populations (hereafter referred to as Crittenden and Gregory - the two cities closest to where these populations were collected). The Crittenden and Gregory populations seed were collected in 2015. Little is known about the field histories, except for the growers sprayed PPO-inhibiting herbicides that year and there were Palmer amaranth plants that escaped the management programs. All of the populations were subjected to a PRE and POST dose response to various PPO-inhibiting herbicides as well as to several herbicides in other SOAs.

2.1. Cross resistance to PPO-Inhibiting herbicides

The PRE experiment was conducted by filling 10- by 15-cm flats with sieved silt loam field soil which had a pH of 5.7 and consisted of 16% sand, 73% silt, and 11% clay in the top 10 cm (NRCS, 2015). One hundred seeds of each population were placed into individual flats. The experiment was conducted as a randomized complete block design with four replications and two temporal replications. There was no significant difference between temporal replications for any of the experiments; therefore, data were pooled. Each population was subjected to eight different doses per herbicide; however, the rate differed between the resistant and susceptible populations. The resistant populations, consisted of eight doses of fomesafen (Reflex 2 LC) applied at 17.5–2240 g ai ha^{-1}, flumioxazin (Valor 51 WDG) applied at 4–565 g ai ha^{-1}, sulfentrazone (Spartan 4 F) applied at 17.5–2240 g ai ha^{-1}, saflufenacil (Sharpen 2.85 SC) applied at 3–394 g ai ha^{-1}, and oxadizon (Ronstar 50 SP) applied at 28–3587 g ai ha^{-1}. These rates are equivalent to 1/16X to 8X field use rates, and the rates were doubled each time beginning with the lower rate until achieving the 8X rate. The susceptible population was sprayed with the same herbicides ranging from 1/128X to 1X rates, again with rates increased two-fold from the lowest rate. Herbicide applications were made at the time of seeding using a laboratory sprayer equipped with two flat fan spray nozzles (TeeJet spray nozzles; Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L ha^{-1} at 269 kPa. Seedling counts were taken 10 days after treatment (DAT-PRE). The PRE application was followed by a POST application of fomesafen (Flexstar 1.88 EC) at 426 g ai ha^{-1} when the largest plants were at the 3-leaf stage to confirm resistance and to determine the effectiveness of one SOA. Follow-up counts were taken 14 DAT-POST.

The POST experiment was set up similar to the PRE experiment, where there was a total of eight replications and 160 plants per herbicide per dose. Twenty individual plants were transplanted into celled trays which were sprayed at the 3-leaf stage with fomesafen at 2.2 to 143,360 g ai ha^{-1}, flumioxazin at 0.5 to 36,160 g ai ha^{-1}, saflufenacil at 0.375 to 25,216 g ai ha^{-1}, and carfentrazone (Aim 2 EC) at 1.125 to 9216 g ai ha^{-1}. The resistant populations were sprayed with ten doses ranging from a 1/2X to a 512X rate; whereas the susceptible population was sprayed with seven doses ranging from 1/16X to 4X, totaling to 864 experimental plants. Herbicide applications were done the same way as the PRE experiment. Live/dead counts were taken 14 and 21 DAT. Fresh aboveground biomass was collected for both the PRE, after the final fomesafen application, and POST experiments and dried at 60 °C for 48 h, after which dry weights were recorded.

2.2. Multiple resistance screening

In addition to the PPO-inhibiting herbicide cross resistance study, these populations were further tested against ten additional SOAs through preplant incorporation (PPI), PRE-, and POST-applications. The multiple resistance screening tests were set up and sprayed in the same manner as the cross-resistance studies. Each of the 24 herbicides tested were used at the 1X field rate for soybean at medium soil textures (Scott et al., 2016). The PPI herbicides tested were trifluralin (Treflan 4 EC) and pendimethalin (Prowl 3.3 EC). The PPI herbicides were incorporated by placing the soil into a paper bag and manually shaking the contents then placing the soil back into the trays prior to planting. The PRE-applied herbicides were acetochlor (Warrant 8 EC), *S*-metolachlor (Dual Magnum 7.62 EC), metribuzin (Sencor 75 DF), atrazine (Aatrex 4 L), isoxaflutole (Balance Flexx 2.5 L), mesotrione (Callisto 4 L), imazaquin (Scepter 70 DG), dicamba (Clarity 4 SL), and pyroxasulfone (Zidua 0.85 WG). The POST herbicides included imazethapyr (Pursuit 2 L), chlorimuron (Classic 25 DF), 2,4-D (2,4-D 3.8 L), glyphosate (Roundup PowerMax 4.5 L), glufosinate (Liberty 2.34 SL), diuron (Direx 4 L), paraquat (Gramoxone 2 SL), dicamba, atrazine, and mesotrione. The imazethapyr, 2,4-D, chlorimuron, diuron, paraquat, and dicamba herbicide treatments all included 0.25% v/v nonionic surfactant (NIS); whereas the mesotrione treatment had 1% v/v crop oil concentrate (COC) added. Data collections were the same as in the PPO-inhibiting cross resistance study.

2.3. Statistical analysis

A two-way mixed model ANOVA was used to determine the effects of herbicide and dose or application timing on Palmer amaranth control. Additionally, a regression analysis was conducted using SAS. The percentage biomass reduction and mortality were fitted to a non-linear, sigmoid, three-parameter Gompertz regression model defined by

$$y = a*\exp\{-\exp[-b*(x-c)]\} \quad (1)$$

where y is the biomass reduction expressed as a percentage of the non-treated control or mortality percentage, a is the asymptote, b is