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Control of Protoporphyrinogen Oxidase-resistant Palmer amaranth

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Control of Protoporphyrinogen Oxidase-resistant Palmer amaranth

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Abstract

Already one of the most troublesome weeds in row crop production in the southern U.S., protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] was first documented in Arkansas in 2015. Since this confirmation, PPO-resistant Palmer amaranth has been identified throughout the Midsouth. The following research evaluated both current and future herbicide programs for controlling PPO-resistant Palmer amaranth and quantified field-level resistance to PPO-inhibiting herbicides. On-farm research, located in fields with confirmed PPO-resistant Palmer amaranth, was conducted in 2016 and 2017. In preemergence (PRE) herbicide experiments, PPO-inhibiting herbicides still proved useful when combined with herbicides such as metribuzin and/or pyroxasulfone. Interestingly, a decline in control from *S*-metolachlor (<78%) was observed in PRE experiments, suggesting heavy reliance on this herbicide alone may lead to control failures. In fact, no PRE herbicide program utilizing only one site of action (SOA) provided effective, sustained control of PPO-resistant Palmer amaranth, regardless of their chemistry. No PPO-inhibiting herbicide applied postemergence (POST) provided effective control of PPO-resistant Palmer amaranth (<40%). Postemergence experiments also highlighted the lack of achievable control in glyphosate-resistant soybean [*Glycine max* (L.) Merr.] vastly in contrast to control provided by options in glufosinate-, 2,4-D-, and dicamba-resistant soybean. Control of PPO-resistant Palmer amaranth in soybean was possible and achieved by multiple effective SOAs PRE followed by a timely POST program containing glufosinate, 2,4-D, or dicamba.

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Chapter 1

General Introduction and Review of Literature

Literature points to the confirmation of herbicide-resistant (HR) weeds and their noticeable outbreak sometime in the 1950s (Shaw 2016). Since then, HR weeds have been identified as resistant to twenty-three of the twenty-five Weed Science Society of America sites of action (SOAs) (Heap 2019). In the past 20 years, reports of HR weeds have shaped the way weed control in agriculture and the education of producers is conducted.

A group of extension specialists that identified over 300 HR weed problems released a report that 28% of the problems came from just one genus, *Amaranthus* (Scott et al. 2009). Of these 96 total reported *Amaranthus* problems, more than half of these were labeled as a critical or major issue (Scott et al. 2009). Palmer amaranth [*Amaranthus palmeri* (S.) Wats], one species of the *Amaranthus* genus, has been one of the main focuses in HR research during the past 20 years, receiving the apt label of most troublesome weed in agronomic crops in the U.S. (Wychen 2016). Palmer amaranth is well known and has confirmed resistance to various SOAs, including herbicide groups such as acetolactate synthase (ALS) inhibitors, very-long-chain fatty acid (VLFCA) inhibitors, 4-hydroxyphyenlpyruvate dioxygenase (HPPD) inhibitors, synthetic auxins, microtubule inhibitors, and the only 5-enolpyruvylshikmate 3-phospate synthase (EPSPS) inhibitor (Heap 2019; Legletier and Johnson 2013). Research is currently being conducted on other SOAs that Palmer amaranth may have developed resistance to, such as glutamine synthetase inhibitors.

Herbicide-resistant crops have been available for use in the U. S. since the mid-1990s, when glyphosate-resistant (Roundup Ready®) crops were first introduced and registered. Because of both the effectiveness and ease of weed control in herbicide-resistant crop management, more than 60 million hectares of herbicide-resistant crops are planted each year in the U. S. (USDA-ERS 2014; USDA-NASS 2018). However, because of resistance selection from over-reliance on specific herbicides in resistant crops, HR weed cases began to increase. One of the most recognized instances of this occurred in Georgia, when Culpepper et al. (2006) confirmed the presence of glyphosate-resistant Palmer amaranth. According to Heap (2014), large areas of glyphosate-treated acres on glyphosate-resistant crops caused an exponential increase in HR weed cases. Holt et al. (2013) noted that HR problems became severe in areas where only one herbicide or chemical family had been used for an extended period of time. As resistance evolved in *Amaranthus* to the EPSPS-inhibiting herbicide glyphosate, selection pressure resulted on preemergence herbicide (PRE) programs. According to Sosnoskie et al. (2013), PRE herbicide rates have to be increased to reach acceptable weed control levels when Palmer amaranth has released a large number of offspring into the soil seedbank. For example, glyphosate-resistant Palmer amaranth escapes replenished large numbers of viable seed in the soil seedbank. As a result of control failure with one herbicide, selection pressure is now present in additional SOAs, which over time has led to resistance to multiple herbicide groups. According to Tranel et al. (2010), when resistance is built up in one specific SOA, it is usually treated with a new or different herbicide SOA, which can cause multiple SOA resistance buildup. For example, multiple resistance has evolved in Arkansas to ALS-, EPSPS-, VLFCA-, PPO-, and microtubule-inhibiting herbicides (Heap 2019). With the *Amaranthus* family especially, when resistance to a new SOA is found, the plant is already commonly resistant to ALS- or EPSPS-inhibiting herbicides, as resistance to these SOAs is widespread. (Tranel et al. 2010). This widespread resistance in common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] and Palmer amaranth to ALS and EPSPS inhibitors can limit herbicide options in these species. As a consequence, understanding the different SOA resistance cases is vital to selecting effective herbicide programs and achieving weed control throughout the growing season.

Palmer amaranth is one of 75 species belonging to the genus *Amaranthus* and belongs to the family Amaranthaceae (Ward et al. 2013). It is a dioecious (separate male and female individuals), apetale, dicot plant. According to Ward et al. (2013), because it is dioecious, Palmer amaranth naturally outcrosses and has a high level of genetic diversity. Palmer amaranth has been known to cross between other *Amaranthus* species, evolving resistance genes and traits from relatives as well. This also means that it has a greater chance of producing an HR offspring when in a conducive environment. For example, Ward et al. (2013) reported that glyphosate resistance was transferred over 250 meters through pollen. This means that resistant and susceptible Palmer amaranth within that radius have the ability to transfer genetic composition through simple pollination. Palmer amaranth also has a prolific seed head, with each plant producing up to 500,000 seed per plant when not in competition with crops (Legletier and Johnson 2013). Palmer amaranth seed are small, creating ease of dispersal by multiple means (Ward et al. 2013). Palmer amaranth is also a C_4 plant and is naturally adapted to high levels of stress, such as extreme heat or lack of soil moisture. Ward et al. (2013) concluded this C_4 desert adaption also is associated with its ability to rapidly produce viable seed under extreme stress or when establishment is made within a short time frame of termination. In a Midsouth or Southeast environments, Palmer amaranth outcompeted soybeans at root penetration into high density soils, efficiency of nitrogen uptake, and seed production during drought conditions (Ward et al. 2013). Palmer amaranth, due to small seed size, has the ability to establish in minimum tillage and no-till operations (Legletier and Johnson 2013). Deeper tillage can provide relief from germinating Palmer amaranth seed and significantly reduce the soil seedbank. Another problem with control of Palmer amaranth is the offspring longevity in the soil seedbank. Although not being able to last long in the soil seedbank, it does have the ability to keep decent germination for at least a year in optimal conditions (Ward et al. 2013). At a 40-cm soil depth, Palmer amaranth viability was 61% after a full year but only 22% six months later (Sosnoskie et al. 2013). Palmer amaranth will have above 40% germination at 1- to 40-cm-depth burial after one full year (Sosnoskie et al. 2013). The implication of these data is that germination percentages decline significantly after one full growing season. However, 40% germination can result in a significant amount of weed pressure the following growing season and limit control options when paired with HR. This limitation of control, ease of genetic mutation, prolific seed production, and heightened efficiency of nutrient uptake makes Palmer amaranth one of the most challenging weeds to control.

Glyphosate-resistant Palmer amaranth was first identified in Georgia in 2006 (Culpepper et al. 2006; Gaines et al. 2011). Since then it has been identified in almost every state in the Southeast and Midsouth (Heap 2019). Even as early as 2007, Norsworthy et al. (2008a) identified that low numbers of glyphosate-resistant Palmer amaranth existed in eastern Arkansas but that overuse of glyphosate alone on cotton and soybean acreage could result in severe selection pressure for glyphosate-resistant weeds. According to a survey from Sosnoskie and Culpepper (2014), glyphosate-resistance in Georgia cotton caused a tenfold increase in use of common PPO-inhibiting herbicides, a 22 to 34% increase in *S*-metolachlor usage, and approximately 50% increase in glufosinate usage. Glyphosate usage in the same time period decreased around 15% due to lack of control of glyphosate-resistant Palmer amaranth (Sosnoskie and Culpepper 2014).

It has been determined that the mechanism of resistance in Palmer amaranth to glyphosate is gene amplification (Culpepper et al. 2006; Gaines et al. 2011). The target site of glyphosate, EPSPS, is a mutation that causes metabolism of the herbicide glyphosate (Gaines et al. 2009). According to Beckie (2011), the recommended programmatic approach for controlling Palmer amaranth in crops with glyphosate resistance was applying a PPO- or ALS-inhibiting herbicide PRE and then following it with a POST application of glyphosate and another PPO. The only difference between the before and after management of glyphosate-resistant Palmer amaranth in many cases was the addition of a residual POST. In studies from Tranel et al. (2010), it was found that some *Amaranthus* populations that tested positive for glyphosate-resistance also contained tolerance or resistance to PPO- and ALS-inhibiting herbicides. This implies that adding a PPO-inhibitor as a residual POST could add selection pressure for further resistance and cause significantly reduced yields in fields containing *Amaranthus* that have tolerance to other SOAs besides EPSPS. In an experiment conducted by Culpepper et al. (2006), glyphosate field use rates of 84 grams acid equivalent per hectare (84 g ae ha^{-1}) recommended for control of susceptible Palmer amaranth provided less than 10% control of a resistant accession at 28 days after treatment. According to Norsworthy et al. (2008b), glyphosate-resistant Palmer amaranth death probability was 100% at 10 g ha^{-1} for susceptible Palmer amaranth but barely above 0% for resistant populations. Only at almost one-hundred times the dosage of glyphosate, did the herbicide have the same probability of causing mortality in the resistant Palmer amaranth accession when compared to the susceptible (Norsworthy et al. 2008b). This lack of control at labeled rates of glyphosate paired with continual use of PPO-inhibiting herbicides as PRE and residual POST additives to glyphosate-resistant crop programs left many growers with only one effective SOA on Palmer amaranth. Ineffective herbicide rates, reliance on one SOA for weed control, and built up tolerance to ALS- and PPO-inhibiting herbicides in glyphosate-resistant Palmer amaranth created the perfect breeding ground for the future of PPO-resistant Palmer amaranth.

ALS inhibitors are some of the most used herbicides in major crops today. ALS-resistant Palmer amaranth was first reported in Arkansas in the early 1990s and now has spread to more than nine different states in the Southeast and Midsouth (Ward et al. 2013). ALS herbicides are an excellent study case for what overreliance on a single SOA will do to weeds that are excellent at building resistance (Tranel and Wright 2002). This overuse of ALS-inhibiting herbicides accompanied with some *Amaranthus* species being dioecious, creates an increased pattern for the evolution of and identifying ALS-resistant *Amaranthus* (Tranel et al. 2010). According to Franssen et al. (2001), gene transfer does occur in *Amaranthus* species and can result in the spread of ALS resistance over large areas between species such as Palmer amaranth and common waterhemp. Sprague et al. (1997) discussed biotypes of Palmer amaranth that were resistant to imazethapyr in Kansas. These resistant biotypes survived imazethapyr at 560 g ai ha^{-1} and thifensulfuron at 36 g ha^{-1}, whereas susceptible biotypes were controlled with one-eighth of those rates (Sprague et al. 1997). In a field study in Georgia, Wise et al. (2009) found that an alarming number of resistant populations were found after a 30% increased usage of ALS-inhibiting herbicides over just four years. In fact, out of the sixty-one locations, just two populations from the study had greater than 50% control with imazapic (Wise et al. 2009). Franssen et al. (2001) stated that rapid spread of ALS resistance in Palmer amaranth and other *Amaranthus* species will continue and will transfer throughout different species. The ease of resistance buildup in weeds to ALS-inhibiting herbicides has made this SOA nearly ineffective in many cases for Palmer amaranth control throughout the Midsouth.

PPO-resistant Palmer amaranth has been confirmed in at least five states: Arkansas (2011), Tennessee (2015), Illinois (2016), Mississippi (2016), and Missouri (2016) (Heap 2019). It is also believed that PPO-resistant Palmer amaranth has moved into Indiana and Kentucky along with glyphosate resistance. By the time PPO-resistant *Amaranthus* species were confirmed, some reduced efficacy had been reported with this chemistry. It is believed these problems were highlighted by both ALS and glyphosate resistance, paired with the continued ease of *Amaranthus* herbicide-resistance buildup (Tranel et al. 2010). Field-level resistance to PPO-inhibiting herbicides has taken a lot of time, being confirmed in four weed species, two of which are in the *Amaranthus* genus (Riggins and Tranel 2012).

In Arkansas, the ΔG210 deletion was determined to be the first of one of the mechanisms of resistance (Salas et al. 2016). With this mutation, the PPO-inhibiting herbicides had a reduced effect on the treated Palmer amaranth. It is established that the ΔG210 mutation is also present in the close family member common waterhemp, suggesting the same mechanism exists for both PPO resistance Palmer amaranth and common waterhemp (Salas et al. 2016). The codon deletion was identified to the PPX2 gene where the Palmer amaranth motif was identical to common waterhemp that had developed resistance to PPO-inhibiting herbicides from the ΔG210 deletion (Riggins and Tranel 2012). Another mechanism conferring PPO resistance is the substitution of Arg-98-Gly/Met (R98G/M), which is present in PPO-resistant common ragweed (*Ambrosia artemisiifolia* L.) (Giacomini et al. 2017). Similarly, Arg-128-Gly and Arg-128-Met (R128G/M), have been identified as the location of substitution for PPO resistance in Palmer amaranth (Varanasi et al. 2018). Most prevalent is the R128G mutation, which was found in 28% of total Palmer amaranth assayed accession, versus the R128M, which was found in less than 1% of all accessions tested (Varanasi et al. 2018). Control of these PPO-resistant Palmer amaranth did not differ between these mechanisms (Salas et al. 2016; Salas et al. 2017; Schwartz-Lazaro et al. 2017; Varanasi et al. 2018).

In a greenhouse environment, PPO inhibitors applied at labeled rates either POST or PRE could not provide control of confirmed Arkansas PPO-resistant accessions (Schwartz-Lazaro et al. 2017). Furthermore, other herbicides known previously to be effective, such as metribuzin and *S*-metolachlor applied alone, did not provide effective control of PPO-resistant Palmer amaranth in Arkansas (Brabham et al. 2019; Schwartz-Lazaro et al. 2017). *S*-metolachlor resistance at several Arkansas locations in Palmer amaranth were later confirmed in 2019 (Brabham et al. 2019; Heap 2019). Control of PPO-resistant *Amaranthus* has been evaluated mostly through common waterhemp because of its early discovery and available research. Since the motif and resistance mechanism are identical in common waterhemp to Palmer amaranth, the research available in waterhemp can be used for derivatives on control of PPO-resistant Palmer amaranth. In a trial in Kansas, acifluorfen was sprayed on twenty-eight common waterhemp population samples with ten showing some level of resistance (Falk et al. 2005). In this experiment, all ten resistant populations were significantly different from the susceptible populations, highlighting the spread of PPO-resistant common waterhemp in this location (Falk et al. 2005). In the resistant plants that survived, the plants showed acifluorfen symptomology, but at 14 days after treatment (DAT) the plants began to put on new growth and normal growth resumed (Falk et al. 2005). Optimal timing of herbicide application is important as well in Palmer amaranth or common waterhemp. If loss of more than 25% control has occurred, chances of regaining manageable levels of suppression and competitive yields is unlikely. For example, acifluorfen and fomesafen had over 5% greater control of common waterhemp when sprayed at the 2- to 3-leaf stage versus the 4- to 6-leaf stage, herbicide rates being identical (Falk et al.

2006). Since PPO-inhibiting herbicides are commonly used as PRE herbicides, the issue with obtaining season-long control starts with *Amaranthus* suppression. However, POST programs can include PPO-inhibiting herbicides as tank-mix combination partners with POST herbicides such as glyphosate or glufosinate. In a study from Falk et al. (2006), field studies suggested that PPO-inhibiting herbicides actually had a greater effect on PPO-resistant common waterhemp as a PRE treatment than they did as a POST application by an average of 30% just 14 DAT. In the PRE treatments, control exceeded 80% throughout 14 DAT on PPO-resistant common waterhemp with acifluorfen, azafenidin, flumioxazin, fomesafen, lactofen, oxyfluorfen, and sulfentrazone (Falk et al. 2006). Therefore, PPO-resistant *Amaranthus* species can be controlled up to 80% for 14 to 21 DAT with a variety of PPO-inhibiting herbicides applied PRE. The issue with *Amaranthus* species that are resistant to PPO-inhibiting herbicides is the steep decline in control after 28 DAT and the poor performance as a POST or POST additive. PPO-resistant *Amaranthus* control with PPO-inhibiting herbicides is better PRE, suggesting PPO-inhibiting herbicides could still be of use along with tank mixes of other herbicides with effective SOAs. The ineffectiveness of POST applications of PPO-inhibiting herbicides in *Amaranthus rudis* infers that the same should be expected in Palmer amaranth and other weed species with the R98/128 and ΔG210 mutations.

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Chapter 2

Evaluation of Preemergence Herbicide Programs for Control of Protoporphyrinogen Oxidase-resistant Palmer amaranth (*Amaranthus palmeri*)

Abstract

The presence of protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth in Arkansas was confirmed in 2015. On-farm field trials were conducted in 2016 and 2017 to assess PPO-resistant Palmer amaranth control options in soybean. Experiments were established in Crawfordsville, Gregory, and Marion, Arkansas, in 2016 and in Crawfordsville and Marion the following year. Twelve trials consisted of twenty-six preemergence (PRE) treatments which were evaluated for visible Palmer amaranth control at 28 days after treatment (DAT) and for Palmer amaranth density. Results indicate that treatments which rely solely on PPO- or acetolactate synthase (ALS)-inhibiting herbicides such as flumioxazin (72 g ai ha^{-1}) or sulfentrazone + cloransulam (195 g ha^{-1} + 25 g ha^{-1}) had average control ratings less than 60%. At 28 DAT, treatments that included mixtures of a very-long-chain fatty acid (VLFCA) plus the photosystem II (PSII)-inhibiting herbicide metribuzin provided increased control over single herbicide sites of action (SOA) or herbicides mixtures to which Palmer amaranth exhibited resistance to included SOA. Pyroxasulfone + metribuzin (149 g ha^{-1} + 314 g ha^{-1}) provided an average of 91% control across these twelve trials at 28 DAT. *S*-metolachlor alone, regardless of year, did not provide consistent or acceptable control of PPO-resistant Palmer amaranth (55 to 77%), and there has since been documented resistance some of the sites. Therefore, it is recommended that a minimum of two effective herbicides be included in soybean PRE programs, when possible.

Nomenclature: Cloransulam-methyl; flumioxazin; metribuzin; pyroxasulfone; *S*-metolachlor; sulfentrazone; Palmer amaranth, *Amaranthus palmeri* (S.) Wats.

Key words: PPO-resistant Palmer amaranth, residual herbicide, multi-resistant Palmer amaranth

Introduction

Over-reliance on a single herbicide or site of action (SOA) perpetuates the evolution and spread of herbicide-resistant biotypes (Heap 2019; Norsworthy et al. 2012; Tranel et al. 2011). In Palmer amaranth, the continual use of acetolactate synthase (ALS)-inhibiting herbicides and glyphosate rapidly selected for biotypes with resistance to both chemistries (Bond et al. 2006; Burgos et al. 2001; Norsworthy et al. 2008). Because of the loss of effective herbicides, glyphosate-resistant *Amaranthus* species caused a major concern for longevity of herbicide chemistries in soybean [*Glycine max* (L.) Merr.] production in the mid-2000s (Legleiter et al. 2009). Subsequently, growers shifted towards PRE followed by residual postemergence (POST) weed management programs (USDA-NASS 2005; USDA-NASS 2015).

After the identification of glyphosate-resistant Palmer amaranth, PPO-inhibiting herbicides became a popular option, offering both residual and foliar control of glyphosate-resistant Palmer amaranth (Krausz et al. 1998; Wuerffel et al. 2015a). Shown to provide excellent Palmer amaranth control, PPO inhibitors offered both versatility and reliability (Niekamp et al. 1999; Whitaker et al. 2011). Fomesafen, a PPO-inhibiting herbicide in the diphenylether family, became so popular that usage spiked from 2 to 16% of all U.S. soybean acreage after confirmation of glyphosate-resistant Palmer amaranth (USDA-NASS 2005; USDA-NASS 2015), with the heaviest reliance on this herbicide in the Midsouth. Only two herbicides were used on more acreage in the U.S., sulfentrazone (17%), which is another PPO-inhibiting herbicide, and glyphosate (85%) (USDA-NASS 2015).

Season-long *Amaranthus* control is usually greater when PRE herbicide applications are included. Hoffner et al. (2012) reported that *S*-metolachlor and fomesafen PRE followed by glufosinate POST controlled Palmer amaranth better than sequential applications of glufosinate alone. In a similar experiment, at-harvest waterhemp [*Amaranthus rudis* (Moq.) Sauer] control with one glufosinate application was improved 22% when PRE flumioxazin was added (Aulakh and Jhala 2015). These findings lead to the conclusion that inclusion of PRE and residual POST herbicides increase season-long *Amaranthus* control, even when utilizing an effective, non-residual POST herbicide. Interestingly, modeled resistance risk in Palmer amaranth was found to be significantly reduced when effective PRE herbicides were included with POST programs as well (Neve et al. 2011). Reduced resistance risk, improved season-long control, and confirmed ALS- and glyphosate-resistant Palmer amaranth made residual herbicides, especially PPO-inhibitors, a necessary component of successful weed control programs in soybean (Neve et al. 2011; Norsworthy et al 2008).

Palmer amaranth resistance to PPO inhibitors was first reported in 2015 (Heap 2019; Salas et al. 2016). Previously, waterhemp had been the focus of PPO resistance research, with seven states having confirmed the resistant biotype (Heap 2019). Susceptible Palmer amaranth sequences were identical to susceptible waterhemp before the ΔG210 deletion occurred. Palmer amaranth, like waterhemp, remained absent of the nucleotide polymorphism seen in the *Amaranthus acanthochiton* at the replacement codon (Riggins and Tranel 2012). Riggins and Tranel (2012) identified that the repeat motif in Palmer amaranth was identical to waterhemp for the PPX2 gene, which suggested that PPO-resistant Palmer amaranth was likely to develop through the ΔG210 deletion. Known to cross-breed, it is possible that the dioecious *Amaranthus* species crossed with one another and led to a complex offspring, or essentially a transfer of the ΔG210 deletion to Palmer amaranth (Salas et al. 2016; Sauer 1950; Steckel 2007). Two additional PPO resistance-conferring mutations, Arg-128-Gly and Arg-128-Met, in Palmer amaranth were reported as amino acid substitutions (Giacomini et al. 2017; Salas et al. 2017).

Each of these mutations have since been determined to confer cross-resistance to other PPO-inhibiting herbicides (Schwartz-Lazaro et al. 2017; Varanasi et al. 2018; Wuerffel et al. 2015b).

Field trials conducted in several locations showed that PRE applications of PPO-inhibiting herbicides remained an effective control option for PPO-resistant waterhemp (Falk et al. 2006; Wuerffel et al. 2015b). In these field trials, PPO-resistant waterhemp was confirmed resistant not only to the diphenylether chemistry, but to all tested PPO-inhibiting herbicides (Falk et al. 2006 Wuerffel et al. 2015a). Wuerffel et al. (2015a) and Tranel et al. (2011) noted the potential dangers to further select for PPO-resistant waterhemp if PRE use continued on this biotype. As shown in a study by Wuerffel et al. (2015a), adding an effective herbicide such as *S*-metolachlor can help prevent resistance selection by decreasing the number of resistant plants that survive. Preemergence applications of PPO-inhibiting herbicides provided some control of resistant waterhemp, but the addition of another effective SOA with residual activity often resulted in improved and more consistent control across environments (Falk et al. 2006; Wuerffel et al. 2015a; Wuerffel et al. 2015b).

As early as 1998, sulfentrazone and flumioxazin were noted as having higher overall control of waterhemp when in mixture with an effective residual herbicide such as metribuzin (Krausz et al. 1998; Niekamp et al. 1999). Evidence supporting the benefit of PPO inhibitors, even after the confirmation of PPO-resistant *Amaranthus*, could lead to continued utilization of this chemistry if they are applied along with other effective herbicides.

With widespread ALS resistance and the confirmation of PPO-resistant Palmer amaranth now in seven states, Palmer amaranth control using PRE herbicide programs once deemed effective need to be reevaluated (Bond et al. 2006; Burgos et al. 2001; Salas et al. 2016; Varanasi et al. 2018). The objective of this research was to evaluate the effectiveness of PRE-applied herbicide programs typically used in soybean for on-farm Palmer amaranth populations confirmed to harbor PPO resistance.

Materials and Methods

Field experiments were conducted at on-farm locations near Marion, AR, on a Dubbs silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) in 2016 and 2017; Gregory, AR, on a Wiville sandy loam (fine-loamy, siliceous, active, thermic Utic Hapludalfs) in 2016; and Crawfordsville, AR, on both a Forestdale silty clay loam (fine, smectitic, thermic Typic Endoaqualfs) in 2016 and a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) in 2016 and 2017. The soil near Marion had a pH of 5.8 with 1.6% organic matter and the soil for both fields near Crawfordsville had a pH of 5.28 and 5.34 with an organic matter contents of 1.8 and 1.95%, respectively. Trials were planted in four single-row 97-cm-wide plots at a density of 370,500 seeds ha^{-1} in Marion, twelve single-row 19-cm-wide plots at 449,540 seeds ha^{-1} in Gregory, and four twin-row 97-cm-wide plots at 345,800 seeds ha^{-1} in Crawfordsville (Table 1). Trials were set up with 9.1 m long by 3.9 m wide plots in Marion, 6.1 m long by 2.3 m wide plots in Gregory, and 7.6 m long by 3.9 m wide plots in Crawfordsville. Group IV soybean varieties were used for this experiment and are listed along with planting dates in Table 1. Herbicide applications were made at 140 L ha^{-1} with a four-nozzle boom attached to a CO_2-pressurized backpack sprayer in Crawfordsville and 112 L ha^{-1} using a Bowman Mudmaster™ multi-boom system in Marion and Gregory. All herbicides were applied with 110015 TeeJet® air induction extended range nozzles at a speed of 4.8 kph. All locations were absent of irrigation, requiring precipitation for PRE treatment activation. Listed in Table 2, precipitation gathered from on-site weather stations for each site-year shows at least 1.72 cm of rainfall within the first 14 DAT.

Twenty-five PRE programs were evaluated in soybean for Palmer amaranth control, not including the nontreated. PRE programs consisted of single active ingredients and herbicide mixtures with more than one SOA. Preemergence treatments focused on four distinct SOAs: ALS-, PPO-, very-long-chain fatty acid (VLCFA)-, and PSII-inhibiting herbicides. Table 3 is a comprehensive list of treatment composition, herbicide group, and rate of each herbicide for this experiment. Visible estimates of Palmer amaranth control and Palmer amaranth density in each plot were recorded 28 DAT, targeting evaluations before a typical POST application timing. Ratings were taken on a 0 to 100% scale, with 0% providing no control and 100% indicating complete weed mortality (Frans and Talbert 1977). To determine Palmer amaranth density, two separate counts per 0.5 m^2 were recorded when the density exceeded 25 plants m^{-2}, otherwise all plants were counted in the center two rows of the plots. Palmer amaranth density on a m^2 basis was then converted to a percentage relative to counts in the nontreated plots. Palmer amaranth densities were not recorded, as this measurement was failed to be taken at the Gregory location, hence the exclusion of this site in Table 3.

Data were analyzed using JMP Genomics 8 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513). This experiment was set up as a randomized complete block design with four replications, utilizing data from 10 trials in 2016, and 2 in 2017. Experiments were analyzed across location and separately by year using a fit model and beta distribution. Replication was considered a random effect in each individual model, and treatment was designed as a fixed effect in each model. Box and whisker plots were also included to provide treatment variation, which demonstrate both outliers and the range of control of individual treatments (Figures 1 and 2). Means and separation are included, which were derived from a Tukey's HSD of visible control ratings. As explained in each figure, a sample size of 32 points for each treatment was included in 2016, and 8 in 2017. Data from the nontreated were excluded and not used in analyses. Where appropriate, data were separated using Tukey's HSD (P=0.05).

Results and Discussion

For each location and year, there appeared to be adequate rainfall in a timely manner for PRE herbicide activation, as each location received at least 1.72 cm of precipitation in the first 14 DAT (Table 2). Differences in rainfall can be observed in Table 2, listed by location and year. Experiments at each location were conducted using common producer methods, with a considerable difference in seeding rate and row-spacing only in Gregory (Table 1). Gregory, Marion, and Crawfordsville Palmer amaranth populations were all previously confirmed to be PPO-resistant, in addition to known ALS and glyphosate resistance (Heap 2019; Salas et al. 2016; Schwartz-Lazaro et al. 2017; Varanasi et al. 2018).

2016. The nontreated plots at Marion and Crawfordsville in 2016 averaged 221 Palmer amaranth plants m^{-2} at 28 DAT (data not shown). For all herbicide treatments at 28 DAT, Palmer amaranth density reduction ranged from 30 to 95% (Table 3). Sulfentrazone + cloransulam (196 + 25 g ha^{-1}) provided the lowest end of this range, and saflufenacil + dimethenamid-p + pyroxasulfone + metribuzin (25 + 219 + 149 + 314 g ha^{-1}) provided the highest density reduction. The lack of Palmer amaranth control in treatments relying solely on PPO-inhibiting herbicides was apparent. For example, flumioxazin (72 g ha^{-1}) alone provided only 35% density reduction of Palmer amaranth at 28 DAT. Furthermore, because of the additional confirmed ALS resistance at these sites, PRE treatments that consisted only of a PPO or ALS inhibitor averaged only 55% density reduction (Table 3). The only treatment without the presence of an effective SOA that provided above 80% reduction of Palmer amaranth density was flumioxazin + cloransulam (106 + 35 g ha^{-1}) at 84%. Although reliance of these SOAs alone would lead to poor control, the combination of PPO and ALS inhibitors with other effective herbicides can still be a viable option. This is demonstrated by treatments such as flumioxazin + pyroxasulfone (88 + 111 g ha^{-1}), which reduced Palmer amaranth density 93% at 28 DAT.

Preemergence treatments that include both group 5 and 15 herbicides reduced density 90% on average (Table 3). Excellent control, 87% average across all mixtures over both years, was achieved when mixing these two herbicide groups is also shown in Figure 1 in box and whisker plots of the control data. Comparison of means for the box and whisker plots can be derived from an ANOVA mentioned previously ($p < 0.0001$). When closely inspecting these visible estimates of control, herbicide treatments that utilized only one herbicide, regardless of SOA, did not provide more than 68% control. This result was not surprising because in previous greenhouse research on some of these populations, the single SOA herbicide treatments had low efficacy on these multi-resistant Palmer amaranth (Schwartz-Lazaro et al. 2017). Surprisingly, *S*-metolachlor (1064 g ha^{-1}), which averaged only 63%, had an extremely wide range of control at 28 DAT (Figure 1). Poor control with *S*-metolachlor is also reflected by the 55% density reduction (Table 3). Just recently, *S*-metolachlor resistance has been confirmed at the Marion and Crawfordsville sites (Brabham et al. 2019; Heap 2019), but no resistance testing or confirmation has occurred for Gregory.

2017. The two trials conducted in 2017 focused exclusively on the Marion and Crawfordsville sites. The nontreated plots across Marion and Crawfordsville in 2017 averaged 75 Palmer amaranth per m^{-2} at 28 DAT (data not shown). The likely reason for the lower density in 2017 is that Palmer amaranth seed production was not allowed at these on-farm sites in 2016. As a result of the lower density in 2017, overall herbicides tended to perform better in the second year, albeit trends in treatment performance seemed consistent between years. Palmer amaranth density reduction ranged from 31 to 98% in 2017 (Table 3). In 2017, two PRE treatments having confirmed resistance to each herbicide SOA applied provided above 80% Palmer amaranth control with these being flumioxazin + cloransulam (106 + 35 g ha^{-1}) at 88% and flumioxazin + chlorimuron-ethyl + thifensulfuron (72 + 23 + 7 g ha^{-1}) at 87%. In 2017, density reduction at 28 DAT shows that reliance on PPO or ALS inhibitors alone would often result in poor control of these Palmer amaranth populations, especially without the presence of a group 5 or 15 herbicide. Box and whisker plots, with corresponding means and separation, for 28 DAT in 2017 show less variability in the majority of treatments versus 2016 (Figure 2). The more consistent response of herbicides among replications and the two locations in 2017 is in part why years were analyzed separately for visible estimates of control, as the p-value for the year effect was 0.0016 when included in the ANOVA model. At 28 DAT, metribuzin provided 71% control of PPO-resistant Palmer amaranth, confirming that even herbicides without known resistance would not be highly effective when applied alone. Visible estimates of Palmer amaranth control for *S*-metolachlor, while higher than in 2016 averaged only 77%, with a relatively high amount of variation across plots and locations (Figure 2). This finding leads to the speculation that attempted control of these Palmer amaranth populations with *S*-metolachlor alone could be extremely variable depending upon location within a field, in turn further selecting for group 15 resistance. However, including this herbicide inside an effective PRE program still has benefit in that it provides some Palmer amaranth control as well as other weeds such as barnyardgrass (*Echinochloa crus-galli* L. Beauv.), the most common grass weed of soybean in this region.

Practical Implications

Reliance on PPO- and ALS-inhibiting herbicides for PRE control of glyphosate-, ALS-, and PPO-resistant Palmer amaranth provided less than acceptable control at 28 DAT and will put added pressure on POST applications to be successful. It was evident that PRE programs including metribuzin and a group 15 herbicide can successfully control PPO-resistant Palmer amaranth for the first four weeks after soybean planting. However, reliance on metribuzin alone failed to provide acceptable control; hence, the need for two or more PRE herbicides. For both 2016 and 2017, treatments containing pyroxasulfone never provided less than 80% control and averaged 89% control across all site-years. This indicates pyroxasulfone remains one of the few proven, effective herbicides for control of PPO-resistant Palmer amaranth. *S*-metolachlor-resistant Palmer amaranth, now confirmed in Crawfordsville and Marion, Arkansas, has further limited potential weed control options in soybean. Although *S*-metolachlor can be used in combination with other effective herbicides, it should be noted that reduced efficacy is likely with the confirmation of *S*-metolachlor-resistant Palmer amaranth in 2019 (Brabham et al. 2019). Also, it should be noted that there is a slight reduction in sensitivity of the Marion and Crawfordsville accessions to pyroxasulfone (Brabham et al. 2019) and continued reliance on this group 15 herbicide could continue selection for group 15 resistance in these fields, even specifically this herbicide family. While flumioxazin was not deemed effective alone (67 to 81%), the data suggested it could have value in a tank-mix PRE application for control of PPO-resistant Palmer amaranth. Regardless, if it is an effective SOA, no herbicide should be used alone PRE for Palmer amaranth control.

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Table 1. Locations, soybean variety, planting dates, row spacing, and seeding rates for the PRE herbicide program soybean trial.

Location	Variety[a]	Technology	Planting date	Row spacing	Seeding rate
				cm	1,000 seed ha^{-1}
Crawfordsville	AG 47x6	Roundup Ready	May 11, 2016	97	346
	AG 47x6	Roundup Ready 2 Xtend	May 11, 2016	97	346
	HBK 4950 LL	LibertyLink	May 11, 2016	97	346
	43R15Y9	Enlist	June 10, 2016	97	346
	P49T09 BR	LibertyLink	May 22, 2017	97	346
Gregory	AG 4633	Roundup Ready	May 5, 2016	19	450
	DG 4957 LL	LibertyLink	May 5, 2016	19	450
	43R15Y9	Enlist	May 5, 2016	19	450
Marion	AG 4632	Roundup Ready	May 12, 2016	97	370
	AG 47x6	Roundup Ready 2 Xtend	May 12, 2016	97	370
	P49T31 LL	LibertyLink	May 12, 2016	97	370
	HBK 4953 LL	LibertyLink	May 10, 2017	97	370

[a]Abbreviations: AG, Asgrow, Bayer CropScience, Research Triangle Park, NC 27709; DG, Delta Grow, Delta Grow Seed, England, AR 72046; HBK, Hornbeck, Hornbeck Seed Company, De Witt, AR 72042; P, Pioneer, DowDupont Midland, MI 48674; 43R15Y9, Enlist soybean variety designation, DowDupont Midland, MI 48674.

Table 2. Rainfall during preemergence experiment in Crawfordsville, Gregory, and Marion, Arkansas, in 2016 and 2017. [a]

	Rainfall[b]					
	2016				2017	
DAT	Crawfordsville[c]		Gregory	Marion	Crawfordsville	Marion
	cm					
7	0.28	3.91	1.47	0.58	3.15	1.57
14	3.63	0	0.25	3.53	1.57	1.98
21	4.90	3.68	3.96	1.80	0	3.45
28	0.28	2.59	4.75	0.18	3.15	1.19
35	3.91	2.06	0.25	3.91	6.80	0
42	0	0.18	0	0	0.23	3.18

[a]Abbreviation: DAT, days after treatment.
[b]Values represent cumulative precipitation from previous timing.
[c]Two columns are presented for Crawfordsville in 2016 as three experiments were established on May 12th, 2016, represented by the first column, and one was established on June 10th, 2016, represented by the second column.

Table 3. Preemergence Palmer amaranth density reduction averaged over Crawfordsville and Marion, Arkansas, in 2016 and 2017.[a,b,c]

			28 DAT	
			Density[d]	
Herbicide	Rate	WSSA Group	2016	2017
	g ai ha^{-1}		%	
Saflufenacil	25	14	95 a	98 a
+ dimethenamid-p	219	15		
+ pyroxasulfone	149	15		
+ metribuzin	314	5		
Flumioxazin	88	14	93 a	98 a
+ pyroxasulfone	111	15		
S-metolachlor	1,105	15	92 a	85 ab
+ metribuzin	242	5		
Flumioxazin	70	14	91 a	97 a
+ metribuzin	250	5		
+ chlorimuron-ethyl	22	2		
+ pyroxasulfone	90	15		
Pyroxasulfone	149	15	90 a	98 a
+ metribuzin	314	5		
Metribuzin	343	5	90 a	96 a
+ chlorimuron-ethyl	17	2		
+ *S*-metolachlor	1,105	15		
Metribuzin	269	5	88 ab	67 cd
+ chlorimuron-ethyl	45	2		
+ *S*-metolachlor	1,070	15		
Flumioxazin	78	14	86 ab	98 a
+ pyroxasulfone	98	15		
+ chlorimuron-ethyl	21	2		
S-metolachlor	1,389	15	86 ab	94 ab
+ metribuzin	420	5		

Table 3. Preemergence Palmer amaranth density reduction averaged over Crawfordsville and Marion, Arkansas, in 2016 and 2017.[a,b,c]

			28 DAT	
			Density[d]	
Herbicide	Rate	WSSA Group	2016	2017
	g ai ha^{-1}		%	
Metribuzin	269	5	85 ab	92 ab
+ chlorimuron-ethyl	45	2		
+ pyroxasulfone	90	15		
Flumioxazin	106	14	84 a-c	88 ab
+ cloransulam	35	2		
Flumioxazin	70	14	80 a-c	92 ab
+ metribuzin	250	5		
+ chlorimuron-ethyl	22	2		
Sulfentrazone	134	14	78 a-c	86 ab
+ *S*-metolachlor	1,210	15		
Flumioxazin	71	14	74 a-c	79 a-c
+ cloransulam	24	2		
Flumioxazin	76	14	73 a-c	93 ab
+ chlorimuron-ethyl	13	2		
+ thifensulfuron	4	2		
+ pyroxasulfone	90	15		
Metribuzin	420	5	73 a-c	74 a-c
Flumioxazin	72	14	67 a-c	94 ab
+ chlorimuron-ethyl	23	2		
+ thifensulfuron	7	2		
+ pyroxasulfone	90	15		
Flumioxazin	76	14	61 a-c	73 a-c
+ chlorimuron-ethyl	13	2		
+ thifensulfuron	4	2		
Flumioxazin	63	14	56 a-c	77 a-c
+ chlorimuron-ethyl	22	2		

Table 3. Preemergence Palmer amaranth density reduction averaged over Crawfordsville and Marion, Arkansas, in 2016 and 2017.[a,b,c]

Herbicide	Rate	WSSA Group	28 DAT Density[d] 2016	28 DAT Density[d] 2017
S-metolachlor	1,064	15	55 a-c	79 a-c
Flumioxazin	72	14	53 a-c	87 ab
+ chlorimuron-ethyl	23	2		
+ thifensulfuron	7	2		
Sulfentrazone	188	14	51 a-c	77 a-c
+ metribuzin	282	5		
Sulfentrazone	130	14	47 a-c	31 d
+ cloransulam	17	2		
Flumioxazin	72	14	35 bc	77 a-c
Sulfentrazone	196	14	30 c	54 cd
+ cloransulam	25	2		
Herbicide			<0.0001	<0.0001

[a]Abbreviations: DAT, days after treatment; WSSA, Weed Science Society of America.
[b]Means within a column followed by the same lowercase letter are not different based on Tukey's HSD (P=0.05).
[c]Average Palmer amaranth density for the nontreated control was 217 per m^2 in 2016 and 37 per m^2 in 2017.
[d]Gregory not included as density data are not available.

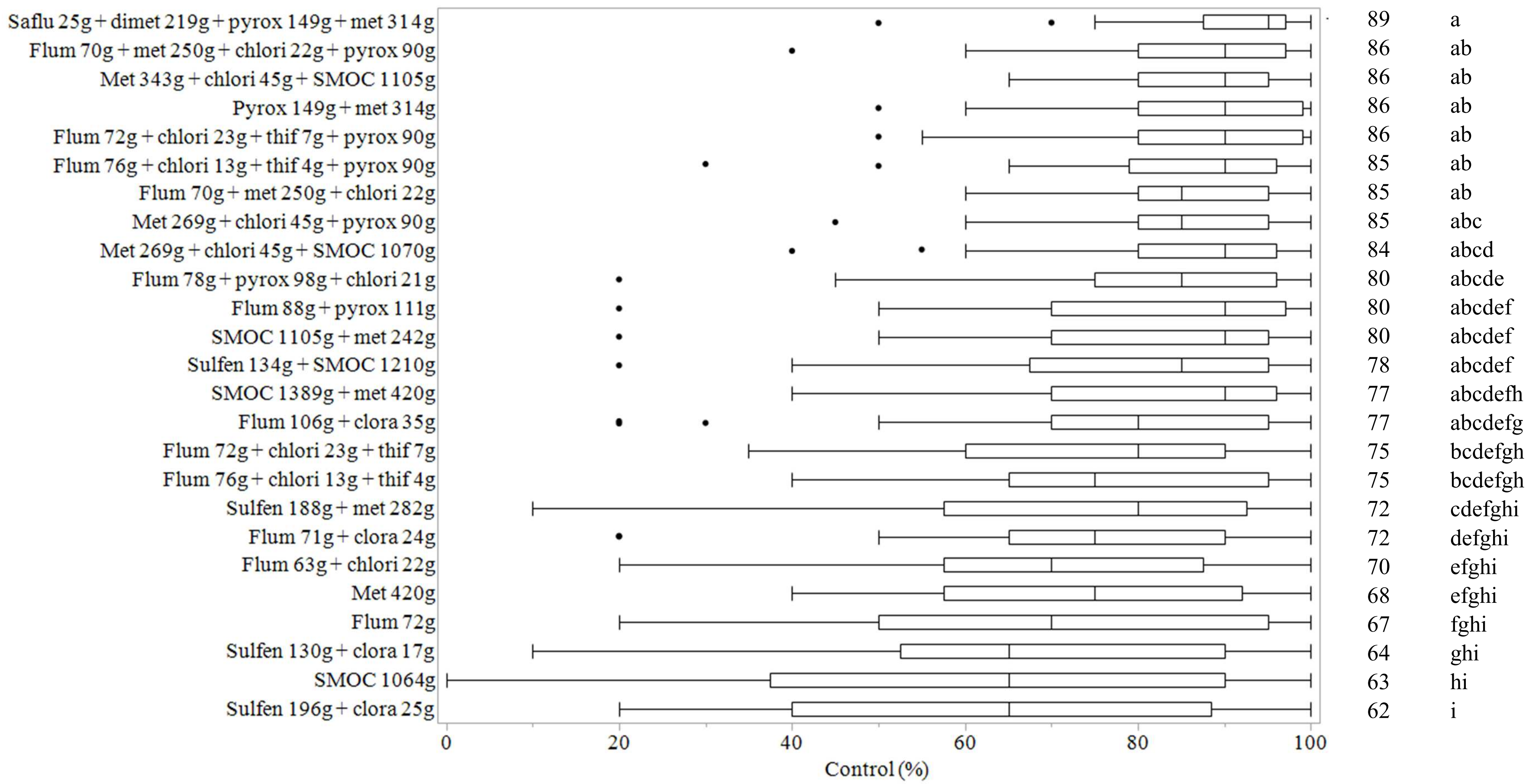

Figure 1. Box and whisker plots for Palmer amaranth control at 28 days after treatment (DAT) over locations in 2016. Statistical means and separation are shown with the corresponding treatment in descending order, derived from a Tukey's HSD. Each treatment listed included a sample size of 32 data points. Use rates are provided to the right of each herbicides in g ai ha^{-1}.[a]

[a]Abbreviations: chlori, chlorimuron-ethyl; clora, cloransulam; dimet, dimethenamid-p; flum, flumioxazin; met, metribuzin; pyrox, pyroxasulfone; saflu, saflufenacil; SMOC, *S*-metolachlor; sulfen, sulfentrazone; thif, thifensulfuron.

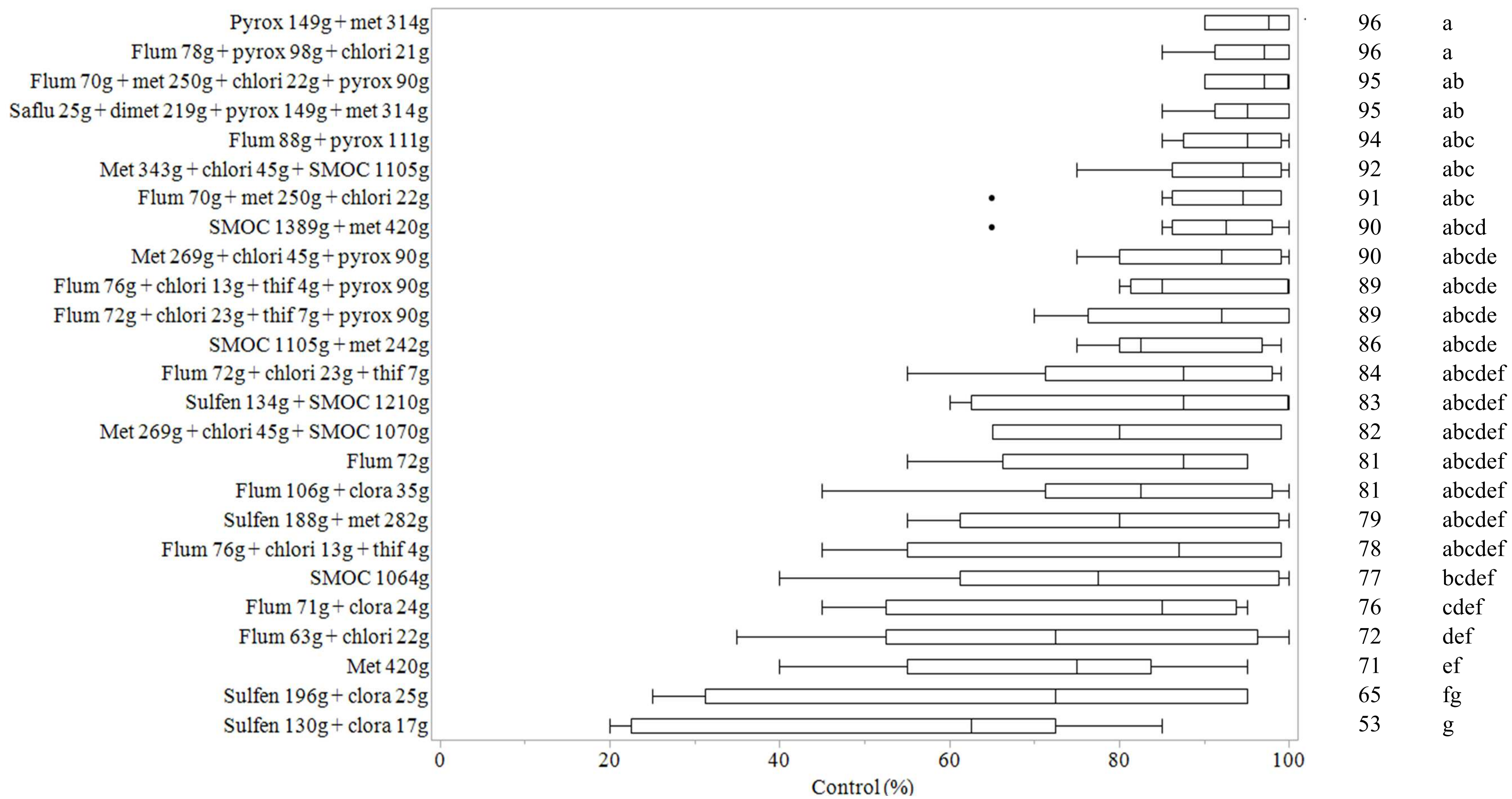

Figure 2. Box and whisker plots for Palmer amaranth control at 28 days after treatment (DAT) over locations in 2017. Statistical means and separation are shown with the corresponding treatment in descending order, derived from a Tukey's HSD. Each treatment listed included a sample size of 8 data points. Use rates are provided to the right of each herbicides in g ai ha^{-1}.[a]

[a]Abbreviations: chlori, chlorimuron-ethyl; clora, cloransulam; dimet, dimethenamid-p; flum, flumioxazin; met, metribuzin; pyrox, pyroxasulfone; saflu, saflufenacil; SMOC, *S*-metolachlor; sulfen, sulfentrazone; thif, thifensulfuron.

Chapter 3

Comparison of Weed Control Technologies for Protoporphyrinogen Oxidase-Resistant Palmer amaranth (*Amaranthus palmeri*)

Abstract

Confirmation of protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth in 2015 limited future weed control options for soybean producers. With the already prevalent existence of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS)-, acetolactate synthase (ALS)-, and beta-tubulin-resistant Palmer amaranth, there are today few remaining effective preemergence (PRE) and postemergence (POST) herbicides labeled in soybean. The recent commercialization of soybean varieties resistant to dicamba and 2,4-D will give producers more effective POST options, in addition to glufosinate, to control multi-resistant Palmer amaranth. To evaluate new and existing seed technologies and their associated POST herbicide options, two experiments were conducted in 2017. These experiments were conducted in Marion and Crawfordsville, Arkansas, two locations with confirmed PPO-, EPSPS-, beta-tubulin-, and ALS-resistant Palmer amaranth. Each technology was tested over three PRE programs that established distinct levels of residual control (74%, 88%, and 95%) prior to the first POST (POST 1) application. At 28 days after (DA) PRE, POST applications were made for each technology when Palmer amaranth plants were approximately 10 cm in height. Fourteen days later, POST 1 weed control ratings were taken, at which point glufosinate-, 2,4-D-, and dicamba-resistant soybean programs provided at least 95% control of Palmer amaranth across all PRE treatments, which was greater than the 83% control provided by the glyphosate-resistant soybean program. Visible control ratings taken at 14 DA second post (POST 2) also showed clear separation between glufosinate- (97%), 2,4-D- (97%), and dicamba-resistant (96%) soybean programs from the glyphosate-resistant soybean program (59%). These results indicate effective PRE programs followed by a glufosinate-, 2,4-D-, or dicamba-containing POST program will provide season-long control of PPO-resistant Palmer amaranth, but adequate control of this Palmer amaranth population in a glyphosate-resistant soybean system may not be feasible.

Nomenclature: 2,4-D; dicamba; glufosinate; glyphosate; Palmer amaranth, *Amaranthus palmeri* S. Wats.; soybean, *Glycine max* (L.) Merr.

Key words: weed control program, herbicide-resistant soybean, herbicide-resistant weeds, residual herbicides, foliar herbicides

Introduction

Palmer amaranth is the most troublesome weed of agronomic crops in the U. S., partly because of its competitiveness, widespread occurrence, and resistance to several herbicide sites of action (SOAs), of which glyphosate resistance is likely the most important (Van Wychen 2016; Heap 2018). In addition to glyphosate, Palmer amaranth in the U. S. has documented resistance to SOA comprised of the WSSA Groups 2, 3, 7, 27, and most recently Group 14 (Heap 2018). In Arkansas, Palmer amaranth accessions have been confirmed resistant to all of the above SOAs, except Group 7, severely limiting weed control options in soybean (Heap 2018; Varanasi et al. 2018). As the number of herbicide-resistant (HR) weed cases increase, new HR soybean traits have been released to provide growers increased herbicide options. In addition to available glufosinate-, dicamba-, and glyphosate-resistant soybean varieties, 2,4-D and certain 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides may become commercially available in some soybean traits soon. New technologies would be vital to producers as glyphosate-resistant Palmer amaranth has caused a heavy reliance on limited available residual herbicides.

In multi-resistant Palmer amaranth accessions tested by Schwartz-Lazaro et al. (2017), residual herbicides such as metribuzin, *S*-metolachlor, and isoxaflutole provided 60% less control when compared to a susceptible standard accession. Reliance on preemergence (PRE) programs paired with declining control from available residual options has created a dynamic need for soybean herbicide-resistant traits that provide the option to apply an effective postemergence (POST) herbicide. Glufosinate-, dicamba-, and 2,4-D-resistant soybean varieties all increase herbicide options for control of multi-resistant Palmer amaranth both PRE and POST due to their effectiveness on broadleaf weeds (Chahal and Johnson 2012; Johnson et al. 2010;

Merchant et al. 2013). Utilizing a wider range of herbicides not only provides more options for weed control, but also embodies better management practices designed to reduce risk of new HR cases (Norsworthy et al. 2012).

Control of multi-resistant Palmer amaranth with glyphosate alone has become perilous in Midsouth soybean production. In many cases, single applications of glyphosate-containing mixtures do not provide greater than 50% Palmer amaranth control, even when following an effective PRE program (Spaunhorst et al. 2014). Fomesafen, one of the most common herbicides to be mixed with glyphosate, has seen an increase in usage since the confirmation of glyphosate-resistant Palmer amaranth (Sosnoskie and Culpepper 2014). The ability of fomesafen to control Palmer amaranth has been diminished in recent years, however, following identification of PPO-resistant Palmer amaranth (Schwartz-Lazaro et al. 2017). With no effective POST herbicide available in glyphosate-resistant soybean, PRE programs became the only option for control of this multi-resistant Palmer amaranth. Even when using effective PRE options, control of multi-resistant Palmer amaranth may not be sustainable throughout the growing season in glyphosate-resistant soybean. This is due to the high likelihood of Palmer amaranth escapes from residual herbicide applications, which would cause yield loss due to competition with the crop.

The 2,4-D-, glufosinate-, and dicamba-containing weed control programs have been shown to provide similar control of multi-resistant *Amaranthus* species, as long as POST programs are initiated within four weeks after a PRE herbicide (Meyer et al. 2015). While the level of *Amaranthus* control is similar among these POST herbicides when applications are timely, some research indicates that both 2,4-D and dicamba can achieve better control of Palmer amaranth compared to glufosinate as application is delayed and weed size exceeds 10 cm (Cahoon et al. 2015; Chahal and Johnson 2012). Control of *Amaranthus* species with POST applications of glufosinate alone is possible, but control will vary based on weed size, air temperature, time of application, and humidity (Anderson et al. 1993; Coetzer et al. 2002; Hoffner et al. 2012).

Coetzer et al. (2002) reported that 80% Palmer amaranth control could be achieved, but only with sequential applications of glufosinate, when absent a residual POST to suppress new emergence. The variability of weed control with glufosinate alone leads to the addition of herbicides such as fomesafen (PPO inhibitor) in POST programs to ensure Palmer amaranth mortality (Hoffner et al. 2012). In Palmer amaranth populations with confirmed PPO and ALS resistance, additional herbicide options like imazethapyr and fomesafen, are ineffective. Gardner et al. (2006) found that when no additional POST herbicides were added to glufosinate, control was lower on both grasses and *Amaranthus* species. In the same trial, Gardner et al. (2006) concluded that with an effective PRE program followed by glufosinate, only annual grass control declined when absent another POST herbicide. Hence, in the absence of an effective PRE program, Palmer amaranth control in a glufosinate-based program can become challenging.

Dicamba and 2,4-D, while becoming new POST options in soybean, have been an effective option for control of broadleaf weed species in crops such as corn (*Zea mays* L.) (Johnson et al. 2010). For control of monocot weed species, POST mixtures may be necessary with dicamba and 2,4-D. Dicamba, in combination with glyphosate, controls a larger assortment of weed species, in addition to providing options for glyphosate-resistant *Amaranthus* and giant ragweed (*Ambrosia trifida* L.) (Spaunhorst et al. 2014). In the experiment conducted by Spaunhorst et al. (2014), the addition of POST dicamba to glyphosate at one timing provided 40% higher control of glyphosate-resistant *Amaranthus* and a 60% increase in control when dicamba is added to multiple POST timings. High levels of control were achieved with the addition of dicamba, suggesting that dicamba-resistant soybean can provide growers with an effective POST tool for control of multi-resistant Palmer amaranth, unlike glyphosate-resistant soybean, even when glyphosate is applied mixed with fomesafen.

The objective of this research was to evaluate soybean technologies for control of Palmer amaranth with multiple resistance to glyphosate, ALS inhibitor, PPO inhibitor, and beta-tubulin inhibitor herbicides when PRE programs differ in levels of efficacy.

Materials and Methods

A field experiment was conducted in 2017 at an on-farm location near Crawfordsville, AR, on a Dundee silt loam soil (fine-silty, mixed, active, thermic Typic Endoaqualfs), and another on-farm site near Marion, AR, on a Dubbs silt loam soil (fine-silty, mixed, active, thermic Typic Hapludalfs). Experiments were set up as a split-plot design with four replications, using the POST herbicide specific for a soybean technology as the whole-plot factor and the PRE program as the sub-plot factor. At each location, plots of 3.9 m wide by 7.6 m long were established on 97-cm-wide rows. Plots were established in a weed-free location prepared by preplant burndown herbicide and tillage. A preplant burndown was applied as paraquat (700 g ai ha^{-1}) at least 2 weeks prior to planting. Preemergence applications were made at planting, the first POST treatments were applied at 28 days after planting (DAP), and the second post application was made at 42 DAP. All herbicide applications in Crawfordsville were made at 140 L ha^{-1} with a four-nozzle boom attached to a CO_2-pressurized backpack sprayer, and applications in Marion were made at 112 L ha^{-1} using a four-nozzle multi-boom attached to a Bowman Mudmaster™ sprayer, each at 4.8 km hr^{-1}. Planting dates, variety, and seeding rates for each location are shown in Table 1. The soil near Marion had a pH of 5.8 with 1.6% organic matter and the soil near Crawfordsville had a pH of 5.3 with an organic matter content of 1.8%. The soil at Marion has a soil texture of 26% sand, 64% silt, and 10% clay, whereas the soil at Crawfordsville was 11% sand, 77% silt, and 12% clay. Both locations were dry-land sites, making precipitation necessary to activate PRE treatments. Rainfall data from a combination of in-field and Plant Board weather stations at both sites are shown in Figure 1.

Residual. Preemergence programs consisted of three options, which were expected to provide distinctly different levels of Palmer amaranth control (excellent, fair, poor) based on previous research at these sites. The PRE programs consisted of saflufenacil (25 g ai ha^{-1}) + dimethenamid-p (219 g ha^{-1}) + pyroxasulfone (149 g ha^{-1}) + metribuzin (314 g ha^{-1}), *S*-metolachlor (1,105 g ha^{-1}) + metribuzin (242 g ha^{-1}), and flumioxazin (72 g ha^{-1}) alone (Table 2). Preemergence treatments were applied with 110015 TeeJet® air induction extended range (AIXR) nozzles. Effectiveness of PRE programs were assessed at 28 DA PRE with ratings based on a 0 to 100% scale of control, with 0% being complete loss of control and 100% being complete weed mortality, each relative to the nontreated control. A nontreated control was established inside each soybean herbicide-resistant trait to provide accurate assessments for PRE programs. Weed densities, which were taken as two separate 0.5 m^{-2} counts in each plot, were recorded at 28 DA PRE as well.

Foliar. Seed technologies included were: glyphosate-, glufosinate-, dicamba-, and 2,4-D-resistant cultivars (Table 1). Each seed technology had at least one POST treatment, with all but the 2,4-D- and glyphosate-resistant soybean programs receiving a second POST application. Postemergence 1 treatments were applied 28 DA PRE, and POST 2 treatments were applied 14 DA POST 1. Postemergence treatments included for this experiment were based on currently labeled, recommended programs for their respective soybean herbicide-resistant trait. The dicamba-containing POST program did not include additional herbicides, such as glyphosate, due to label restrictions in Arkansas at the time of trial initiation (Anonymous 2018). For POST 1, treatments consisted of glyphosate (706 g ae ha^{-1}) + fomesafen (266 g ha^{-1}) + *S*-metolachlor (1,212 g ha^{-1}), glufosinate (594 g ha^{-1}) + fomesafen (266 g ha^{-1}) + *S*-metolachlor (1,212 g ha^{-1}), dicamba (560 g ae ha^{-1}), and 2,4-D (1,064 g ae ha^{-1}) + glyphosate (1,009 g ha^{-1}) + *S*-metolachlor (1,064 g ha^{-1}) (Table 3). The POST 2 treatments were applied to the glufosinate- (glufosinate at 594 g ha^{-1}) and dicamba-resistant (dicamba at 560 g ha^{-1}) soybean technologies (Table 3). Dicamba- and 2,4-D-containing treatments were applied with 110015 turbo Teejet® induction (TTI) nozzles, to avoid particle drift and subsequent damage to neighboring plots of soybean that were not resistant to dicamba or 2,4-D (Anonymous 2018). Glufosinate- and glyphosate-containing treatments were applied with 110015 AIXR nozzles. Postemergence applications were made to the middle two rows of the four-row plot, as to provide relative control provided by the POST treatments to the PRE programs. Visible estimates of weed control for the POST applications were taken at 14 DA POST 1 and 14 DA POST 2. Each rating was based on a 0 to 100% scale, relative to the nontreated control, with 0% being no control and 100% being complete weed mortality (Frans and Talbert 1977). Weed densities were collected as two separate 0.5 m^{-2} counts at 14 DA POST 2. Palmer amaranth densities from Crawfordsville were not recorded 28 DA PRE.

All data were analyzed in SAS 9.4 (SAS Institute Inc., SAS Campus Drive, Car, North Carolina 27513) using the GLIMMIX procedure to evaluate each timing as proportions utilizing a beta distribution. Locations, replication (nested within locations), and all interactions containing these effects were considered random effects in the model. The whole-plot (POST), sub-plot (PRE), and interaction (POST*PRE) between the two were considered fixed effects in this model. Palmer amaranth densities were analyzed as a percent of the nontreated. Nontreated weed control rating data were removed from analysis because of 0% control in all replications at both locations. All means were separated using Fisher's protected LSD (P=0.05).

Results and Discussion

From the analysis previously mentioned, a POST by PRE interaction was not observed at any point throughout the experiment (Table 3). The absence of an interaction between POST and PRE was not surprising, as POST herbicides applied to the glufosinate-, 2,4-D-, and dicamba-resistant soybean provided similar control across all PRE treatments at both 14 DA POST 1 and 2. The following results are focused on each split-plot factor separately.

Residual. Visible estimates of control for Palmer amaranth at 28 DA PRE show that PRE programs differed in effectiveness as expected (Table 2). Flumioxazin, a PPO-inhibiting herbicide, provided less than 75% control of PPO-resistant Palmer amaranth. Flumioxazin, as noted in previous research, can still provide control of some Palmer amaranth due to segregating PPO-resistant *Amaranthus* populations (Schwartz-Lazaro et al. 2017). *S*-metolachlor + metribuzin provided 88% control, significantly better than flumioxazin. The best PRE program for control of PPO-resistant Palmer amaranth was saflufenacil + dimethenamid-p + pyroxasulfone + metribuzin at 95%, significantly higher than both other options (Table 2). Densities, which are shown as percent of the nontreated, showed similar numerical trends but treatments were not statistically different (Table 2). Density reductions do not account for the factors of weed size and are taken as a sample from the plot instead of the whole plot, likely attributing to the densities failing to separate like the visible control ratings at 28 DA PRE.

Residual control improved as number of applied SOAs increased, suggesting a PPO-inhibiting herbicide alone PRE will not effectively control these Palmer amaranth populations as reported previously (Schwartz-Lazaro et al. 2017). At 14 DA POST 1, visible Palmer amaranth control improved for each PRE program (Table 2). Visible weed control for 14 DA POST 2 declined slightly across all PRE programs, each down 3 to 6% percentage points, but maintained significance trend (Table 2). At each rating, PRE treatments with metribuzin in the mixture increased control of PPO-resistant Palmer amaranth over the single flumioxazin application; control was increased even more when metribuzin was combined with an efficacious very long-chain fatty acid inhibitor herbicide. Even though *S*-metolachlor proved to be an effective tank-mix partner with metribuzin throughout the PRE evaluations, recent research have found *S*-metolachlor-resistant Palmer amaranth populations at both of these locations, putting an emphasis on finding an efficacious very long-chain fatty acid inhibitor herbicide if considered for use (Brabham et al. 2019). Densities statistically separated as well, with flumioxazin (89%) providing less control than the other PRE programs (both 97%).

Foliar. Glufosinate + fomesafen + *S*-metolachlor (96%), 2,4-D + glyphosate + *S*-metolachlor (95%), and dicamba (95%) provided better Palmer amaranth control than glyphosate + fomesafen + *S*-metolachlor (83%) at 14 DA POST 1 (Table 3). The glufosinate-, 2,4-D-, and dicamba-resistant soybean programs did not differ for Palmer amaranth control at 14 DA POST 1. In the glyphosate + fomesafen + *S*-metolachlor POST program, there is no effective foliar herbicide because of the multi-resistant Palmer amaranth population. Because this program has no effective POST, control ratings are mainly a product of the residual herbicides applied. At 14 DA POST 2, visible control ratings for the glufosinate-, 2,4-D, and dicamba-resistant soybean programs increased to 97, 96, and 97%, respectively. Visible control ratings in the glyphosate-resistant soybean technology declined to 59%, as both foliar and residual herbicides failed to control PPO-resistant Palmer amaranth (Table 3). Significant differences in densities occurred for the glyphosate- (87%) vs 2,4-D- (98%) technologies and the glufosinate-resistant (98%) soybean program at 14 DA POST 2. Expectedly, the glyphosate-resistant soybean technology did not provide an effective POST option for control of these multi-resistant Palmer amaranth populations (Meyer et al. 2015; Norsworthy et al. 2008). Also to be noted, densities from the dicamba-resistant soybean program (94%) did not differ from any other system (Table 3).

Practical Implications. Preemergence programs that do not include multiple, effective SOAs did not provide above 80% control of PPO-resistant Palmer amaranth. Although POST programs in glufosinate-, 2,4-D, and dicamba-resistant soybean did regain some lost control in the least effective PRE program, ineffective PRE programs like flumioxazin alone is not recommended due to an increase in selection for resistance to POST herbicides and possible failure of a POST program because of weed sizes greater than 10 cm upon application and density when environmental conditions are not conducive for a timely application. Residual herbicides are also recommended to be included in early POST applications to suppress emerging Palmer amaranth until canopy formation is achieved. Glufosinate, 2,4-D, and dicamba all have excellent activity on PPO-resistant Palmer amaranth, and these programs were shown here to provide an extended period of weed control. There is no effective POST herbicide option available in glyphosate-resistant soybean when the Palmer amaranth population exhibits resistance to glyphosate as well as ALS- and PPO-inhibiting herbicides. Regardless of PRE program, extended Palmer amaranth control was not possible and suggests the lack of viability from the glyphosate-resistant technology. Producers should consider glufosinate-, 2,4-D-, and dicamba-resistant soybean as the only technologies with viable POST options in areas where Palmer amaranth with the above mentioned resistance portfolio has been confirmed or confirmation is likely.

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Table 1. Soybean variety, planting dates, and seeding rate for Crawfordsville and Marion locations. [a]

Location	Variety	POST technology	Planting date	Seeding rate
				seed ha^{-1}
Crawfordsville	Pr 4909	Roundup Ready	May 23, 2017	345,800
	P48T67 LL	LibertyLink		
	AG 46x6	Xtend		
	51E16H2	Enlist		
Marion	AG 47x6	Roundup Ready	May 10, 2017	370,500
	HBK 4953LL	LibertyLink		
	AG 47x6	Xtend		
	51E16H2	Enlist		

[a]Abbreviations: AG, Asgrow, Bayer CropScience, Research Triangle Park, NC 27709; HBK, Hornbeck, Hornbeck Seed Company, De Witt, AR 72042; P, Pioneer, DowDupont Midland, MI 48674; POST, postemergence; Pr, Progeny, Progeny Ag Products, Wynne, AR 72396; Xtend, Roundup Ready 2 Xtend; 51E16H2, Enlist soybean variety, DowDupont Midland, MI 48674.

Table 2. Preemergence Palmer amaranth control and density reduction across POST technologies averaged over experiments conducted at Crawfordsville and Marion, Arkansas, in 2017. [a,b,c]

Herbicide	Rate	Control			Density reduction[d]	
		28 DA PRE	14 DA POST 1	14 DA POST 2	28 DA PRE	14 DA POST 2
	g ai ha^{-1}	%				
Flumioxazin	72	74 b	86 b	83 b	72	89 b
S-metolachlor	1105 +	88 a	94 a	88 a	82	97 a
+ metribuzin	242					
Saflufenacil	25 +	95 a	96 a	91 a	87	97 a
+ dimethenamid-p	219 +					
+ pyroxasulfone	149 +					
+ metribuzin	314					
PRE (p-value)		<0.0001	0.0060	0.0071	0.2572	0.0238

[a]Abbreviations: PRE, preemergence; DA, days after; POST, postemergence.

[b]Means within a column followed by the same lowercase letter are not different based on Fisher's protected LSD (P=0.05).

[c]Average Palmer amaranth density for the nontreated was 34 per m^2 at 28 DA PRE and 23 per m^2 at 14 DA POST2.

[d]Density reduction ratings for 28 DA PRE include only Marion, AR.

Table 3. Postemergence program Palmer amaranth control and density reduction across PRE averaged over experiments conducted at Marion and Crawfordsville, Arkansas in 2017. [a,b,c]

	Herbicide			Control		Denisty reduction
POST program	POST 1	POST 2	Rate	14 DA POST 1	14 DA POST 2	
			g ai or g ae ha^{-1}	——————%	——————	
Roundup Ready	Glyphosate + fomesafen + S-metolachlor	-	706 + 266 + 1212	83 b	59 b	87 B
Enlist	2,4-D + glyphosate + *S*-metolachlor	-	1064 + 1009 + 1064	95 a	96 a	98 A
Xtend	Dicamba	Dicamba	560 fb 560	95 a	97 a	94 Ab
LibertyLink	Glufosinate + fomesafen + *S*-metolachlor	Glufosinate	594 + 266 + 1212 fb 594	96 a	97 a	98 A
POST	(p-value)			<0.0001	<0.0001	0.0095
POST*PRE	(p-value)			0.8936	0.7959	0.1140

[a]Abbreviations: POST, postemergence application; DA, days after; Xtend, Roundup Ready 2 Xtend.
[b]Means within a column followed by the same lowercase letter are not different based on Fisher's protected LSD (P=0.05).
[c]Average Palmer amaranth density for the nontreated was 23 per m^2 at 14 DA POST2.

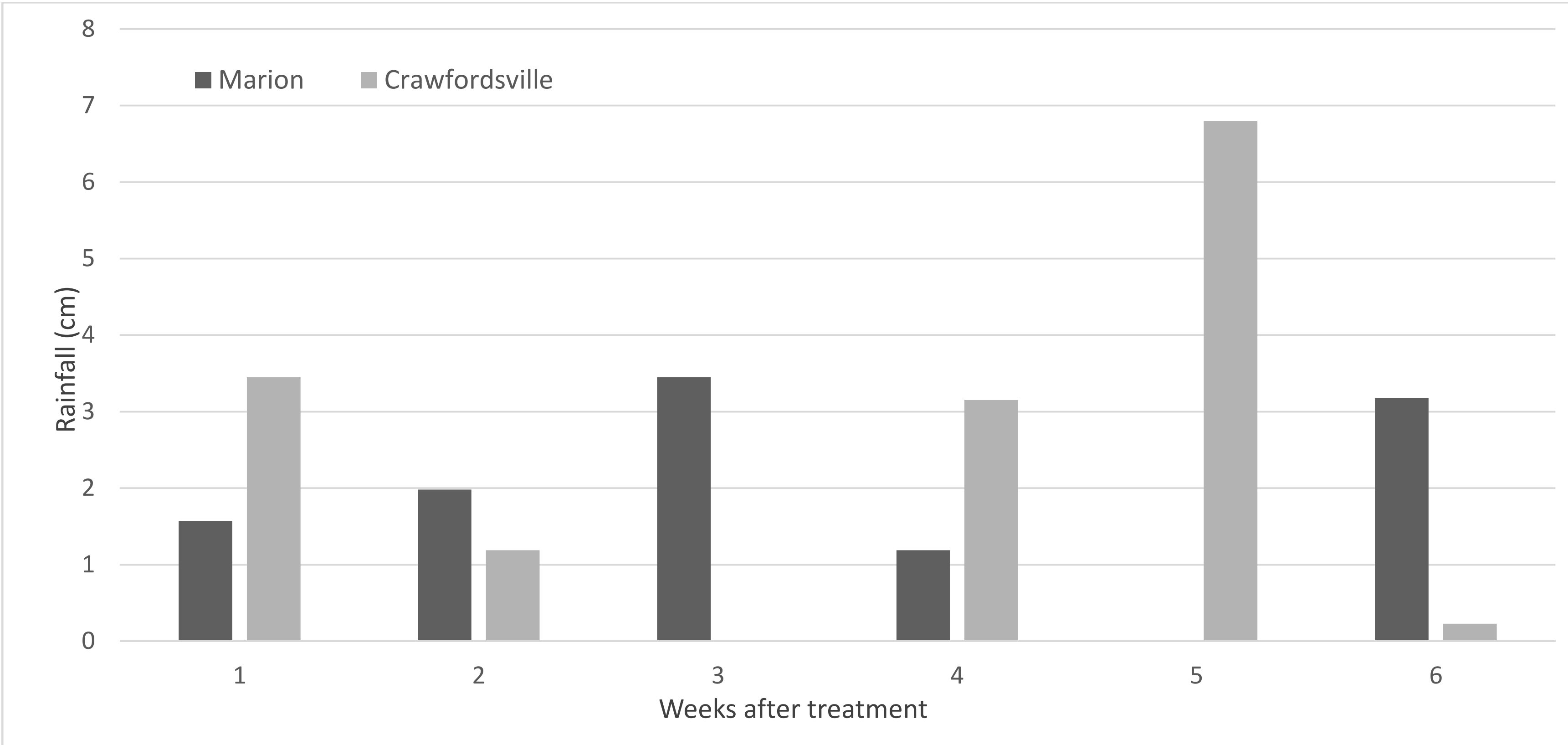

Figure 1. Rainfall data for Marion and Crawfordsville, Arkansas each week after planting in 2017.

Chapter 4

Field Evaluation of Preemergence and Postemergence Herbicides for Control of Protoporphyrinogen Oxidase-Resistant Palmer amaranth (*Amaranthus palmeri*)

Abstract

Palmer amaranth accessions resistant to protoporphyrinogen oxidase (PPO), 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), and acetolactate synthase (ALS) inhibitor herbicides are widespread in the Midsouth, making control difficult. Field experiments were conducted in Marion and Crawfordsville, AR in 2016 and 2017 to assess preemergence (PRE) and postemergence (POST) herbicides labeled for use in corn, cotton, or soybean for control of multi-resistant Palmer amaranth. Accessions at both locations were resistant to glyphosate and ALS inhibitors and segregating for both the R128 and ΔG210 PPO resistance mechanisms. Of the 15 herbicide treatments tested, only atrazine (1,120 g ai ha^{-1}), pyroxasulfone (149 g ha^{-1}), and flumioxazin (144 g ha^{-1}) provided 85% or greater Palmer amaranth control 14 days after treatment (DAT). Visible control ratings at 35 DAT declined sharply with no treatment providing more than 84% control, suggesting POST applications should be made no later than 28 DAT. Glufosinate (594 and 818 g ha^{-1}), dicamba (560 g ae ha^{-1}), 2,4-D + glyphosate (784 g ae ha^{-1} + 834 g ae ha^{-1}), and paraquat (700 g ha^{-1}) applied POST to 7 to 10 cm plants reduced Palmer amaranth density 83% or more 14 DAT. Both glyphosate (1,266 g ha^{-1}) and pyrithiobac sodium (73 g ha^{-1}) provided less than 7% Palmer amaranth control. Although flumioxazin alone at a labeled rate controlled Palmer amaranth 82% in the PRE experiment, PPO inhibitors by themselves applied POST provided no more than 37% control at 14 DAT. Effective foliar herbicides applied POST, including residual herbicides, should be made when Palmer amaranth are less than 10 cm in size for optimal control of these multi-resistant Palmer amaranth accessions.

Nomenclature: atrazine; dicamba; flumioxazin; glufosinate; glyphosate; paraquat; pyrithiobac sodium; pyroxasulfone; 2,4-D; Palmer amaranth, *Amaranthus palmeri* S. Wats.; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.

Key words: Multi-resistance, selection pressure

Introduction

Multiple herbicide-resistant Palmer amaranth in corn, cotton, and soybean fields is difficult to control and can drastically reduce yields if not managed effectively (Culpepper et al. 2010; Fast et al. 2009; Forseth et al. 1984; Klingaman and Oliver 1994; Massinga et al. 2001; Ward et al. 2013). In the U. S. alone, Palmer amaranth has evolved resistance to herbicides from eight site-of-action (SOA) groups: inhibitors of EPSPS, ALS, 4-hydroxyphenylpyruvate dioxygenase (HPPD), photosystem II (PSII), PPO, synthetic auxins, very-long chain fatty acid, and microtubule assembly, and many of these accessions exhibit multiple resistance mechanisms (Heap 2019). In Arkansas and surrounding states, Palmer amaranth accessions resistant to both EPSPS- and ALS-inhibiting herbicides are common (Burgos et al. 2001; Norsworthy et al. 2008) and this widespread infestation has caused a dynamic shift in weed control programs (Hoffner et al. 2012; Neve et al. 2011). For example, after the spread of multi-resistant Palmer amaranth in Georgia cotton fields, Sosnoskie and Culpepper (2014) reported a ten-fold usage increase in PPO-inhibiting herbicides, such as flumioxazin and fomesafen.

Consequently, the increased reliance on PPO inhibitors for Palmer amaranth control selected for PPO resistance. Fomesafen resistance in Palmer amaranth was initially identified in an accession collected in 2011 (Salas et al. 2016). Since then PPO-resistant Palmer amaranth accessions have been positively identified in 18 of the 29 agriculture counties in Arkansas and in three states (Arkansas, Tennessee, Illinois) (Heap 2019; Varanasi et al. 2018). The major resistant mechanisms are the ΔG210 glycine amino acid deletion, and the Arg-128-Gly/Met (R128) amino acid substitution, which are both target-site mechanisms and confer broad-spectrum resistance to PPO-inhibiting herbicides (Salas et al. 2016; Salas et al. 2017; Varanasi et al. 2018). These mechanisms are well known, with the ΔG210 deletion also identified in waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], and the R128/R98 mutation present in common ragweed (*Ambrosia artemisiifolia* L.). Both mechanisms are widespread in Palmer amaranth in Arkansas and have even been found in the same locations together (Varanasi et al. 2018). In fact, Varanasi et al. (2018) reported that of the 167 accessions screened in Arkansas in 2017, 28% harbored the R128 mutation and 49% harbored the ΔG210 deletion.

Preemergence control of PPO-resistant Palmer amaranth can still be achieved in part with specific PPO-inhibiting herbicides. Umphres et al. (2017) concluded that flumioxazin and sulfentrazone would still provide some residual control of PPO-resistant Palmer amaranth as seen previously for waterhemp (Wuerffel et al. 2015). Even so, reliance on these two specific herbicides could lead to control failures and difficulty in achieving zero-tolerance weed control programs (Norsworthy et al. 2012; Norsworthy et al. 2014). Unlike residual PRE activity, effective control of PPO-resistant Palmer amaranth with PPO inhibitors POST is only achievable at much higher than labeled rates (Schwartz-Lazaro et al. 2017). Fomesafen, a commonly used POST herbicide in soybean, did not control Palmer amaranth progeny originating from Crittenden County, Arkansas when applied at a higher than labeled rate (420 g ha^{-1}) (Schwartz-Lazaro et al. 2017).

The widespread distribution of EPSPS-, ALS-, and PPO-resistant Palmer amaranth has severely limited PRE and POST options for Palmer amaranth control in soybean, corn, and cotton. Therefore, PRE and POST fallow experiments were conducted to determine how to control multi-resistant Palmer amaranth accessions harboring both the ΔG210 and R128 PPO resistance mechanisms.

Materials and Methods

Field experiments were conducted in 2016 and 2017 near Crawfordsville and Marion, AR (Crittenden County), at on-farm sites on a Dundee silt loam soil (fine-silty, mixed, active, thermic Typic Endoaqualfs) and a Dubbs silt loam soil (fine-silty, mixed, active, thermic Typic Hapludalfs), respectively. The soil near Marion had a pH of 5.8 with 1.6% organic matter and the soil near Crawfordsville had a pH of 5.3 with an organic matter content of 1.8%. Each site-year contained a PRE-only and a POST-only experiment to determine herbicide efficacy on multi-resistant Palmer amaranth. All experiments were established into a crop-free environment. Herbicides labeled for use in soybean, cotton, and corn production were applied at the typical field use rates listed in the herbicide index, Table 1. Visible control ratings and density reduction data were gathered in both experiments, each on a 0 to 100% scale relative to the nontreated, with 0% being no control and 100% being complete weed mortality. Nontreated controls were included in each replication to assess relative control.

PRE. Preemergence trials were conducted in a randomized complete block design with four replications. Plots were 3.9 m wide by 7.6 m long on 97-cm-wide beds. Herbicide treatments were applied to weed-free ground that had been sprayed for winter annual weed control (paraquat at 700 g ha^{-1}) and tilled using standard production practices. Fifteen different herbicides were applied at standard in-crop labeled use rates (Table 2). Additionally, the four PPO-inhibiting herbicides were applied at two times the recommended rate (2x) to evaluate the effect of increased dosage. A nontreated control was included for comparison. At Marion, herbicides were applied at 112 L ha^{-1} spray solution with a Bowman Mudmaster™ sprayer. At Crawfordsville, herbicides were applied at 140 L ha^{-1} spray solution using a CO_2-pressurized backpack sprayer. All treatments were applied with a four-nozzle boom equipped with 110015

TeeJet® air induction extended range nozzles at an application speed of 4.8 km h^{-1}. Density data was not available in Crawfordville in 2016.

Because irrigation was not available at either of these sites, rainfall was the only source for incorporation of herbicides into soil solution. Rainfall data for all experiments were recorded from in-field and local weather stations and are shown in Figures 1 and 2. At each location, a 2 cm or more rainfall event occurred within one to two weeks after herbicide treatments were applied in both years.

POST. Postemergence trials were conducted in a randomized complete block design with 2 m wide by 7.6 m long plots on flat, bed-absent ground. Trials at Marion contained three replications, while trials in Crawfordsville contained four replications each year. POST herbicide treatments were applied when Palmer amaranth reached 7 to 10 cm in height. *S*-metolachlor was applied at 1,064 g ha^{-1} across each trial at the time of POST application to prevent emerging Palmer amaranth from becoming a factor in density reduction and visible control ratings. All POST herbicides were applied at labeled crop use rates and included crop oil concentrate at 1% v/v, except for treatments containing glufosinate and glyphosate (Table 3). Postemergence applications were made at 140 L ha^{-1} spray volume with a four-nozzle boom at 4.8 km h^{-1} attached to a pressurized backpack. Treatments containing dicamba or 2,4-D were made with 110015 turbo Teejet® induction nozzles, while all other treatments were applied with 110015 TeeJet® air induction extended range nozzles. Density data were not available in Marion in 2016 and Crawfordsville in 2017.

Data from both experiments were separately subjected to an analysis of variance using JMP Genomics 8 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513). Data from both experiments were analyzed with a randomized site-year effect and randomized replications. Treatment was the only fixed factor for each experiment. Densities measured from each experiment were converted to a percent of the nontreated control for the corresponding replication. Data from the nontreated control for each experiment were not included in analyses. Prior to experimentation, particular interest was in comparing the effects of a labeled (1x) and 2x rate of PPO inhibitors and to determine the effectiveness of labeled PPO-inhibiting herbicides vs other SOAs. Additionally, interest was in comparing glufosinate, 2,4-D, and dicamba POST for potential efficacy differences when used for control of multi-resistant Palmer amaranth. Thus, contrasts were used to test these specific interactions in both experiments. Where appropriate, data were separated using Tukey's HSD at an alpha level value of 0.05.

Results and Discussion

PRE. Timely and adequate rainfall occurred for incorporation of PRE herbicides into soil solution in both years at both locations. Precipitation was comparable across both years and locations in the first two weeks, averaging between to 2.5 to 3 cm. Both locations also shared similar soil textures, field history, and management practices (soybean production prepared by conventional tillage). Both Marion and Crawfordsville locations were silt loam soil textures, previously in continuous soybean production, and were prepared by conventional tillage each year. The most notable difference in soil characteristics between the two locations was soil pH, and even then, the difference was only 0.5 units. At both locations, PPO-resistant Palmer amaranth with target-site resistance mechanisms was confirmed previously (Varanasi et al. 2018).

Herbicide activity was evaluated at both 28 and 35 DAT because POST herbicides typically need to be applied by 4 weeks after emergence of corn, cotton, or soybean to protect crop yields (Halford et al. 2001; Mulugeta and Boerboom 2000). Control of Palmer amaranth with the labeled rates of imazaquin, pendimethalin, fomesafen, saflufenacil, and sulfentrazone was less than 66% at 28 DAT and no more than 50% at 35 DAT (Table 2). Palmer amaranth has confirmed resistance to the SOAs of these herbicides at these locations (Heap 2019; Varanasi et al. 2018). Interestingly, flumioxazin, another PPO-inhibiting herbicide, controlled Palmer amaranth 82% at 72 g ha^{-1} and 85% at 144 g ha^{-1}. Of the 19 herbicide treatments evaluated, only atrazine at 1,120 g ha^{-1} (91%) and pyroxasulfone at 149 g ha^{-1} (88%) provided greater than 85% control at 28 DAT (Table 2). In cropping systems without the presence of an effective POST option for multi-resistant Palmer amaranth, such as conventional soybean, these PRE herbicide treatments would not be enough to ensure extended control alone. In this experiment, emphasis was placed on comparing the efficacy of multiple PRE PPO-inhibiting herbicides at a labeled rate vs a 2x rate and compare with activity of other SOAs (Table 2).

Regardless of the variation in response between individual herbicides within a group, contrasts for control at 28 and 35 DAT identified separation in only three group pairings (Table 2). Contrasts indicated that the 2X rate of Group 14 herbicides was more effective than the 1X rate based on density reduction and visible weed control ratings at 28 DAT and control at 35 DAT (Table 2). When comparing Palmer amaranth densities and control at 28 DAT and control at 35 DAT, Group 2, 3, and 4 herbicides were never more effective than Group 14 herbicides at a labeled rate based on contrasts. Conversely, Group 5 & 7 collectively along with Group 15 and Group 27 herbicides were more effective in controlling multi-resistant Palmer amaranth at 28 DAT than the labeled rate of Group 14 herbicides. The ineffectiveness of the Group 2 and 3 SOAs is attributed to herbicide resistance within these populations.

POST. Visible control ratings for 7- to 10-cm-tall Palmer amaranth at application ranged from 18 to 91% at 7 DAT (Table 3). Control with paraquat (91%) was statistically similar to control with diuron at 841 g ha^{-1} (80%) and both rates of glufosinate (70% and 82%). Conversely, control with glyphosate, pyrithiobac sodium, tembotrione, or carfentrazone did not exceed 32%. As expected, contrasts between auxin herbicides and glufosinate at 7 DAT indicate that glufosinate provided higher initial control. More rapid control of Palmer amaranth with glufosinate than with dicamba and 2,4-D at 7 DAT was not surprising because glufosinate is a contact herbicide and the other two are systemic (Coetzer and Al-Khatib 2001; Grossman 2010).

At the 14 DAT timing, no treatment provided above 75% control (Table 3). Glyphosate and pyrithiobac sodium at 14 DAT provided less than 7% Palmer amaranth control. The lack of control from treatments evaluated infer that reliance on POST herbicides alone for control of these resistant Palmer amaranth accessions is not an acceptable weed control program. No single application of a herbicide, including dicamba, glufosinate, and 2,4-D, provided 85% control, demonstrating their lack of viability as stand-alone options. Density reduction data at 14 DAT followed similar trends as visible control data (Table 3). Contrast at 14 DAT between auxin herbicides and glufosinate revealed the options were comparable for controlling these Palmer amaranth accessions ($p= 0.11947$). Although efficacy of dicamba and 2,4-D at 14 DAT was evident, Palmer amaranth control with these herbicides would likely have increased with continued ratings at 21 and 28 DAT.

Practical Implications. Suppression of multi-resistant Palmer amaranth with Group 5, 7, 15, and 27 herbicides is possible at 28 DAT, but not as stand-alone options. As for flumioxazin, although still providing good control of PPO-resistant Palmer amaranth accessions, applying flumioxazin alone could result in escapes and high selection pressure for further PPO inhibitor resistance. Due to the lack of control of multi-resistant Palmer amaranth with ALS- and PPO-inhibiting herbicides, earlier POST applications may be necessary to ensure that herbicides are applied in a timely manner to small weeds. Even when using the most effective PRE herbicide tested, it was not possible to maintain a high level of Palmer amaranth control through 35 DAT. For this reason, POST herbicides should be applied no later than 28 days after PRE application and residuals should be overlapped to provide season-long control (Aulakh and Jhala 2015; Halford et al. 2001). Overlapping effective residuals is key for Palmer amaranth control, demonstrated by the lack of control options in the POST experiment. Acetolactate synthase-, EPSPS-, and PPO-inhibiting herbicides should not be relied on POST in areas with confirmed or suspected Palmer amaranth resistance to these SOAs as poor weed control will result. Even though *S*-metolachlor was applied PRE to all plots in the POST experiment, one application of any POST herbicide was not found to be efficacious in controlling these Palmer amaranth accessions. Based on these data an optimum herbicide program will likely require multiple effective residuals at planting followed by two applications of an effective herbicide POST. In addition to using multiple SOA for weed control, it will also delay the onset of further herbicide resistance. Control of multi-resistant Palmer amaranth is not possible with ALS-, EPSPS-, or PPO-inhibiting herbicides alone.

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Tables of Figures

Table 1. Herbicides, rates, timing, and manufacturer details for PRE and POST experiments. [a]

Herbicide	Trade name	Rate	Manufacturer	Location	Application timing
		g ai or ae ha^{-1}			
Imazaquin	Scepter® 70 DG	138	BASF Corporation	Research Triangle Park, NC	PRE
Pendimethalin	Prowl® H20	1,603	BASF Corporation	Research Triangle Park, NC	PRE
Dicamba	Xtedimax®	560	Monsanto Company, Inc.	St. Louis, MO	PRE
Atrazine	Aatrex® 4L	1,120	Syngenta Crop Protection, LLC	Greensboro, NC	PRE
Metribuzin	Tricore® DF	420	UPL NA Inc.	King of Prussia, PA	PRE
Diuron	Diuron 4L	840	Loveland Products, Inc.	Loveland, CO	PRE
Flumioxazin	Valor® SX	72 and 144	Valent U.S.A. Corporation	Walnut Creek, CA	PRE
Fomesafen	Reflex®	280 and 560	Syngenta Crop Protection, LLC	Greensboro, NC	PRE
Saflufenacil	Sharpen®	50 and 100	BASF Corporation	Research Triangle Park, NC	PRE
Sulfentrazone	Spartan® 4F	280 and 560	FMC Corporation	Philadelphia, PA	PRE
Acetochlor	Warrant®	1,261	Monsanto Company, Inc.	St. Louis, MO	PRE
S-metolachlor	Dual Magnum®	1,389	Syngenta Crop Protection, LLC	Greensboro, NC	PRE
Pyroxasulfone	Zidua®	149	BASF Corporation	Research Triangle Park, NC	PRE
Isoxaflutole	Balance Flexx®	88	Bayer CropScience	Research Triangle Park, NC	PRE
Mesotrione	Callisto® 480 SC	211	Syngenta Crop Protection, LLC	Greensboro, NC	PRE
Pyrithiobac sodium	Staple® LX	73	E.I. du Pont de Nemours and Co.	Wilmington, DE	POST
Dicamba	Engenia®	280 and 560	BASF Corporation	Research Triangle Park, NC	POST
Atrazine	Aatrex® 4L	1,120	Syngenta Crop Protection, LLC	Greensboro, NC	POST
Diuron	Diuron 4L	840	Loveland Products, Inc.	Loveland, CO	POST
Glyphosate	Roundup PowerMAX®	1,266	Monsanto Company, Inc.	St. Louis, MO	POST
Glufosinate	Liberty® 280 SL	594 and 818	Bayer CropScience	Research Triangle Park, NC	POST
Carfentrazone	Aim® EC	22	FMC Corporation	Philadelphia, PA	POST
Flumioxazin	Valor® SX	72	Valent U.S.A. Corporation	Walnut Creek, CA	POST
Fomesafen	Flexstar®	263 and 396	Syngenta Crop Protection, LLC	Greensboro, NC	POST
Saflufenacil	Sharpen®	25	BASF Corporation	Research Triangle Park, NC	POST
Paraquat	Gramoxone® SL 2.0	700	Syngenta Crop Protection, LLC	Greensboro, NC	POST
Mesotrione	Callisto® 480 SC	105	Syngenta Crop Protection, LLC	Greensboro, NC	POST
Tembo	Laudis®	92	Bayer CropScience	Research Triangle Park, NC	POST
TCM + tembo	Capreno®	15 + 76	Bayer CropScience	Research Triangle Park, NC	POST
2,4-D + glyphosate	Enlist Duo®	784 + 834	BASF Corporation	Research Triangle Park, NC	POST

[a]Abbreviations: TCM, thiencarbazone-methyl; tembo, tembotrione; PRE, preemergence; POST, postemergence.

Table 2. Preemergence Palmer amaranth control and density reduction averaged over experiments in Crawfordsville and Marion, Arkansas in 2016 and 2017. [a,b,c]

Herbicide	Rate	WSSA Group	Density reduction 28 DAT		Control 28 DAT		Control 35 DAT	
	g ai or ae ha^{-1}		%					
Imazaquin	138	2	51	c	53	d	38	ef
Pendimethalin	1,603	3	57	bc	61	cd	43	d-f
Dicamba	560	4	67	a-c	71	a-d	50	c-f
Atrazine	1,121	5	96	a	91	a	84	a
Metribuzin	420	5	88	ab	78	a-c	60	a-f
Diuron	841	7	83	a-c	76	a-c	59	a-f
Flumioxazin	72	14	89	ab	82	a-c	61	a-f
Flumioxazin	144	14	91	ab	85	ab	70	a-c
Fomesafen	280	14	68	a-c	65	b-d	43	c-f
Fomesafen	560	14	81	a-c	76	a-c	59	a-f
Saflufenacil	50	14	65	a-c	54	d	34	f
Saflufenacil	100	14	83	a-c	73	a-d	57	a-f
Sulfentrazone	280	14	78	a-c	64	b-d	50	c-f
Sulfentrazone	560	14	84	a-c	77	a-c	63	a-e
Acetochlor	1,261	15	77	a-c	73	a-d	56	b-f
S-metolachlor	1,389	15	80	a-c	73	a-d	65	a-e
Pyroxasulfone	149	15	92	ab	88	a	79	ab
Isoxaflutole	88	27	83	a-c	82	a-c	70	a-d
Mesotrione	211	27	80	a-c	71	a-d	62	a-e
Treatment			<0.0001		<0.0001		<0.0001	
Contrasts[d]								
Group 2 vs. Group 14 (1x)			0.0029 (51 vs 75)[e]		0.0102 (53 vs 66)		0.1430 (38 vs 47)	
Group 3 vs. Group 14 (1x)			0.0255 (57 vs 75)		0.3014 (61 vs 66)		0.4712 (43 vs 47)	
Group 4 vs. Group 14 (1x)			0.347 (67 vs 75)		0.2600 (71 vs 66)		0.6470 (50 vs 47)	
Group 5 & 7 vs Group 14 (1x)			0.0075 (89 vs 75)		<0.0001 (82 vs 66)		<0.0001 (68 vs 47)	
Group 15 vs Group 14 (1x)			0.1759 (83 vs 75)		0.0055 (78 vs 66)		<0.0001 (67 vs 47)	
Group 27 vs Group 14 (1x)			0.2798 (82 vs 75)		0.0054 (77 vs 66)		<0.0001 (66 vs 47)	
Group 14 (1x vs 2x)			0.0422 (75 vs 85)		<0.0001 (66 vs 78)		<0.0001 (47 vs 62)	

[a]Abbreviations: DAT, days after treatment; WSSA, Weed Science Society of America.
[b]Means within a column followed by the same lowercase letter are not different based on Tukey's HSD (P=0.05).
[c]Density ratings were not available for Crawfordsville, AR in 2016.
[d]For contrasts, P $\leq$ 0.05 was considered significant.
[e]Means for contrasts are shown in parentheses.

Table 3. Postemergence Palmer amaranth control and density reduction averaged over experiments in Crawfordsville and Marion, Arkansas in 2016 and 2017. [a,b]

Herbicide[d]	Rate	WSSA Group	Control 7 DAT		Control 14 DAT		Density reduction[c] 14 DAT	
	g ai or ae ha^{-1}		%					
Pyrithiobac sodium	73	2	18	i	6	gh	50	b-d
Dicamba	280	4	56	d-g	62	a-c	83	ab
Dicamba	560	4	56	c-g	66	ab	86	ab
Atrazine	1,121	5	66	b-e	46	b-e	76	a-c
Diuron	841	7	80	a-c	63	a-c	92	a
Glyphosate	1,266	9	24	hi	1	h	33	cd
Glufosinate	594	10	70	a-d	48	b-e	89	ab
Glufosinate	818	10	82	ab	57	a-d	91	a
Carfentrazone	22	14	32	e-i	13	f-h	28	d
Flumioxazin	72	14	62	b-f	37	c-f	54	a-d
Fomesafen	263	14	42	f-i	19	e-h	36	cd
Fomesafen	396	14	37	e-i	15	f-h	36	cd
Saflufenacil	25	14	42	e-i	12	f-h	40	cd
Paraquat	700	22	91	a	75	a	85	a
Mesotrione	105	27	39	f-i	30	d-g	46	b-d
Tembotrione	92	27	33	e-i	17	f-h	34	d
Thiencarbazone-methyl +	15	2	49	d-h	42	b-f	78	a-c
tembotrione	76	27						
2,4-D +	784	4	59	b-f	58	a-c	83	ab
glyphosate	834	9						
Treatment			<0.0001		<0.0001		<0.0001	
Contrast[e]								
Group 4 vs Group 10			<0.0001		0.1195		0.7795	
			(58 vs 76)[f]		(62 vs 53)		(85 vs 90)	

[a]Abbreviations: DAT, days after treatment; WSSA, Weed Science Society of America.
[b]Means within a column followed by the same lowercase letter are not different based on Tukey's HSD (P=0.05).
[c]Crawfordsville, AR in 2017 and Marion, AR in 2016 density reduction data not available.
[d]All treatments, excluding those containing glyphosate or glufosinate, were applied with a crop oil concentrate at 1% volume per volume (v/v).
[e]For contrasts, $P \leq 0.05$ was considered significant.
[f]Means for contrasts are shown in parentheses.

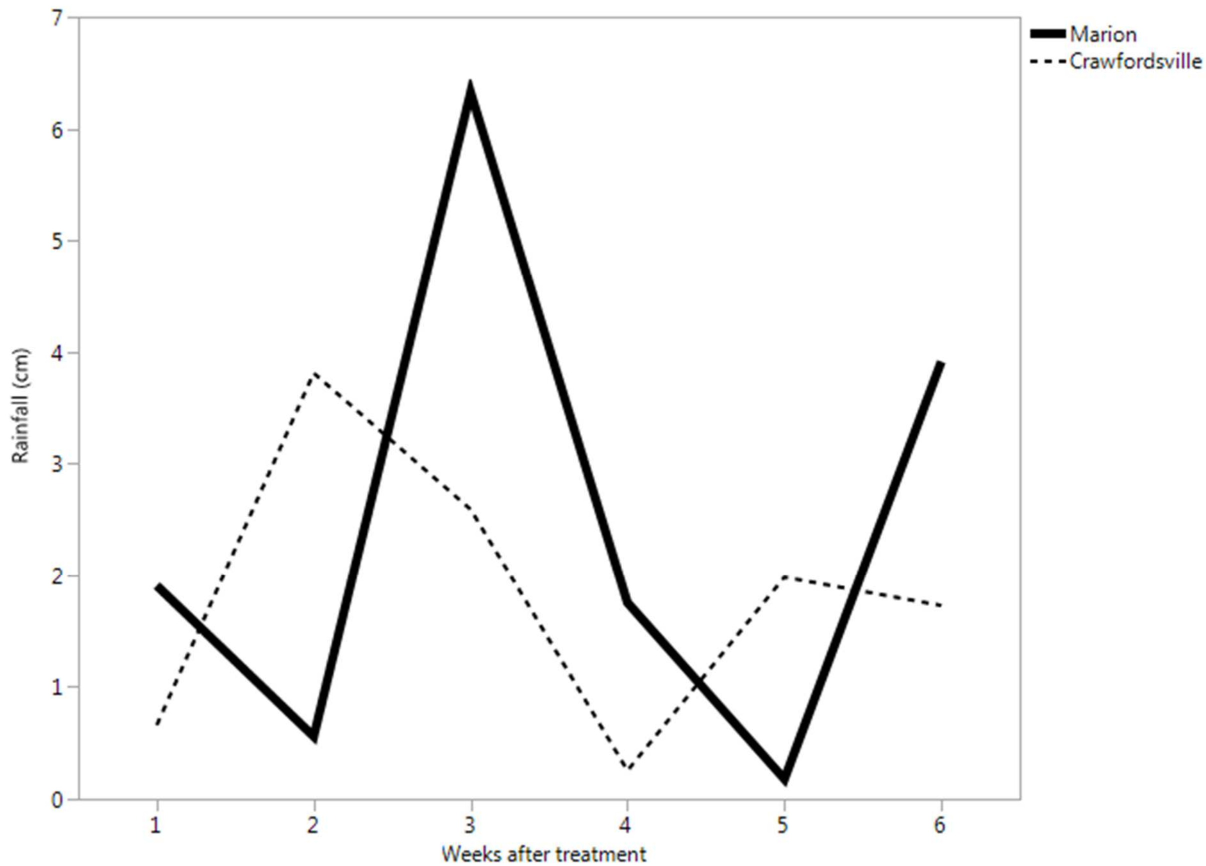

Figure 1. Rainfall data for Marion and Crawfordsville, Arkansas, each week after PRE treatment in 2016.

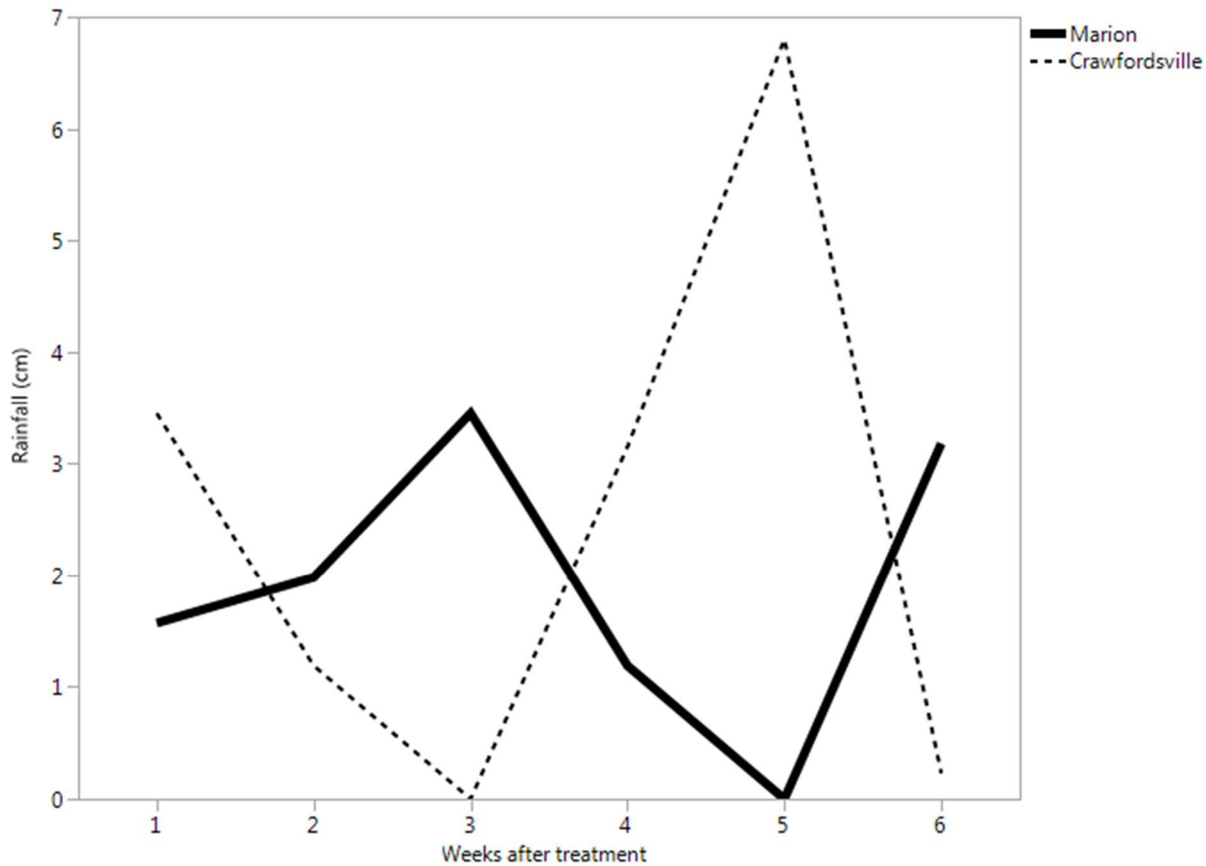

Figure 2. Rainfall data for Marion and Crawfordsville, Arkansas, each week after PRE treatment in 2017.

General Conclusions

The repeated usage of PPO-inhibiting herbicides provided relief in glyphosate-resistant Palmer amaranth control, but at a cost. After the confirmation of PPO-resistant Palmer amaranth, other sites of action (SOA) that once provided effective control at these locations have failed to do so, such as *S*-metolachlor. Although control of multi-resistant Palmer amaranth is a challenge, several effective SOA still exist. The availability of effective preemergence (PRE) herbicide programs and herbicide-resistant crop technologies such as LibertyLink, Xtend, and Enlist continue to provide producers with options for control of multi-resistant Palmer amaranth. As important as the chemistries themselves, proper postemergence herbicide application timing has shown to be key in control of multi-resistant Palmer amaranth as well. With effective tools for multi-resistant Palmer amaranth control on the market, producers should utilize available resources to avoid potential catastrophic yield loss.