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Assessment of Control of PPO-resistant Palmer Amaranth and Salvage Options in Herbicide-resistant Cotton

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Assessment of Control of PPO-resistant Palmer Amaranth
and Salvage Options in Herbicide-resistant Cotton

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ABSTRACT

Palmer amaranth has been the most limiting weed in cotton production in the state of Arkansas for many years. Recently, resistance of Palmer amaranth to the protoporphyrinogen oxidase (PPO)-inhibiting site of action has been discovered at various locations across the cotton-producing region of the state. Cotton varieties have been developed with resistance to synthetic auxin (WSSA Group 4) herbicides. However, research to date has shown PPO-resistant Palmer amaranth to be more difficult to control with herbicides that target alternative sites of action. Herbicide efficacy is also known to vary with weed size, varying spray parameters, and environmental conditions. Preliminary research on control of PPO-resistant Palmer amaranth with preemergence cotton herbicides suggests that herbicide mixtures containing fluometuron are the most consistent option for longevity of control. Preliminary results of postemergence (POST) experiments assessing control of PPO-resistant Palmer amaranth in herbicide-resistant cotton were inconclusive. Limited rainfall impacted both POST and residual weed control. When attempting to salvage a cotton crop, weed size plays an extremely important factor in whether the weeds will be controlled. Two-pass salvage treatments were effective in dicamba-resistant cotton containing mixtures of glufosinate or glyphosate and dicamba and showed little variation in control of large (taller than 15 cm) Palmer amaranth. Interval between applications in a two-pass salvage treatment is influential on control of large weeds, although it does not ultimately affect seedcotton yield. Increasing carrier volume from 70 L ha^{-1} to 140 L ha^{-1} was a more important factor in maximizing efficacy of a dicamba application than switching from TTI to AirMix nozzles or increasing the dicamba rate from 560 to 1,120 g ae ha^{-1}. Differences in control between PPO-susceptible and PPO-resistant populations were also observed, as densities of surviving PPO-resistant Palmer amaranth were much higher than PPO-susceptible Palmer amaranth following dicamba application.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats.; cotton, *Gossypium hirsutum* L.; synthetic auxin; dicamba; fluometuron; 2,4-D; glufosinate

Key Words: PPO-resistant Palmer amaranth, herbicide-resistant cotton, dicamba-resistant cotton, synthetic auxin

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General Introduction and Review of Literature

In 2016, 3,998,294 hectares (ha) of cotton (*Gossypium hirsutum* L.) were planted in the United States. Arkansas accounted for about 3.8% of the total hectares planted to cotton, making it the fourth largest upland cotton-producing state in the country (Anonymous 2017). The value of the Arkansas cotton crop in 2016 was estimated at $275,386,000 (Anonymous 2017). Weed competition is one of the greatest yield-limiting factors of cotton. To combat this issue and more easily control weeds, many producers plant herbicide-resistant (HR) varieties. In 2017, 93% of cotton planted in Arkansas was HR, making it slightly higher than the national average of 91% (Anonymous 2017). The most commonly used HR varieties tolerate applications of glyphosate and/or glufosinate. Dicamba-resistant cotton was deregulated and approved to be grown in the US in 2015 (Anonymous 2015a) and has become another common HR trait used by growers in Arkansas and throughout the US.

Glyphosate-resistant Cotton

The introduction of HR cotton varieties greatly reduced cost and labor associated with producing a crop (Dill 2005). Farmers were able to reduce both tillage and herbicide application passes across a field. Although bromoxynil-resistant cotton was available as a transgenic HR trait before the introduction of glyphosate-resistant (GR) cotton, it wasn't widely adopted by farmers. On the other hand, within eight years of the release of GR cotton, 80% of all cotton grown in the United States was GR (Green 2012). An increase in the use of conservation-tillage practices, such as strip-till and no-till, in cotton occurred shortly after the release of GR cotton varieties, and it appears there is a strong correlation between reduced tillage and the widespread adoption of GR technologies (Young 2006). The total estimated cost savings per year of GR cotton, including reduced tillage, fewer herbicide applications, and manual labor associated with weed removal is $132 million per year (Gianessi 2005; Gianessi et al. 2002). Total income gains due to HR cotton use from 1996 to 2007 added 10.2% to the $27.5 billion value of cotton production worldwide (Brookes and Barfoot 2009). HR cotton also significantly reduced the total amount of herbicides being used. Culpepper and York (1998) found that two applications of glyphosate were as effective as four applications of other commonly used herbicide programs at the time. Herbicide use rates were cut by an estimated 2.8 million kg by the year 2000 when compared to rates applied before the introduction of GR cotton (Gianessi 2005; Gianessi et al 2002).

The widespread, year after year reliance on glyphosate by such a large number of farmers nationwide eventually yielded detrimental impacts in the form of various GR weed species. Weeds were initially not expected to develop glyphosate resistance because of glyphosate's unique mode of action, metabolism, and chemical structure. Furthermore, it was believed that since it was difficult to create GR crops by means of mutagenesis and other artificial manipulation techniques, it would be nearly impossible for plants in a wild population to evolve resistance (Bradshaw et al. 1997). It is now understood that multiple mechanisms of action (MOA) must be used in any weed control program in order to maintain herbicide efficacy and decrease the rate of selection of herbicide resistant weeds (Norsworthy et al. 2012).

Referred to by Duke and Powles (2008) as "a once-in-a-century herbicide", N-(phosphonomethyl) glycine, or glyphosate, is a broad-spectrum, systemic herbicide that can be applied for a reasonable price and poses minimal toxicological or environmental impact. The first glyphosate product came to market under the brand name Roundup in 1974. Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway, causing shikimic acid to accumulate in treated tissues (Duke and Powles

2008; Steinrucken and Amrhein 1980). The production of phenylalanine, tyrosine, and tryptophan are prevented by glyphosate, which are necessary for the synthesis of proteins, hormones, and other metabolites needed for plant growth and survival. The first GR cotton variety (trade name Roundup Ready®) was released for public use in 1996. Cotton was modified to express a gene that encodes EPSPS tolerance, called CP4 EPSPS, which occurs naturally in Agrobacterium sp. CP4. Monsanto Co. originally successfully modified two lines; 1455 and 1698, to express the CP4 EPSPS gene (Nida et al. 1996). The CP4 EPSPS gene, when expressed in plant tissue, contains an altered target site that reduces the binding potential of glyphosate, therefore allowing the plant to maintain normal enzyme activity.

Glufosinate-resistant Cotton

Glufosinate was originally limited to use in non-crop areas and burndown applications because it is a nonselective contact herbicide and provides effective control across a broad spectrum of weeds (Hass and Muller 1987). Once it is absorbed by plant tissue, glufosinate inhibits the plant's ability to transform glutamate and ammonium to the essential amino acid glutamine by restricting the activity of glutamine synthetase. The result is a sudden recession in the level of glutamine available, as well as a spike in glyoxylate and ammonia levels, ultimately causing rapid necrosis of contacted plant tissue (Coetzer and Al-Khatib 2001). In 2004, cotton varieties resistant to postemergence (POST) glufosinate applications (trade name LibertyLink®) were released for commercial use. However, LibertyLink cotton was not widely adopted by cotton producers until more recent years because of the poor yield potential associated with original LibertyLink varieties, as well as the need for control of GR weeds (UGA 2007; Dodds et al. 2015). Other lines created for tolerance to insects (trade name WideStrike™) used the phosphinothricin acetyltransferase (pat) gene as a selectable marker during plant transformation

(Anonymous 2015b). The pat gene also confers resistance to glufosinate. When WideStrike varieties were crossed with GR cotton, varieties were produced that yielded as much as, or greater than original LibertyLink varieties, while also giving growers another option for control of a broad spectrum of weeds that have evolved resistance to other herbicides (Culpepper et al. 2009).

Dicamba-resistant Cotton

Dicamba is a member of the synthetic auxin group of herbicides. It shares a similar chemical structure with the naturally occurring plant hormone indole-3-acetic acid (IAA), which is a member of the auxin class of hormones (Kirby 1980). In the plant, auxins are responsible for regulating cell division and elongation, along with a host of other processes that include floral meristem differentiation, root formation, and apical dominance. Synthetic derivatives of IAA are more stable in the plant than natural IAA and therefore evoke the same effects, but at a much more intense level, over a longer period. These effects cause disruption of growth and development processes, and ultimately result in plant death, particularly to dicotyledonous species (Grossman 2010).

Synthetic auxin herbicides have been used to control broadleaf weeds in cereal crops for more than 60 years (Green and Owen 2010). Recently, transgenic cotton resistant to glufosinate and dicamba (event MON88701) was deregulated in the United States (Brinker et al. 2014). This event was created by inserting a stacked combination of genes called dicamba monooxygenase (dmo) from *Stenotrophomonas maltophilia* and the bialaphos resistance (bar) gene from *Streptomyces hygroscopicus* (Brinker et al. 2014). The dmo gene codes for a monooxygenase enzyme that demethylates absorbed dicamba into two compounds that have no herbicidal effect on the plant (Behrens et al. 2007). Other cultivars that possess traits conferring resistance to dicamba, glyphosate, glufosinate, and insects (MON88701 by MON88913 by MON15985; brand name Bollgard II® XtendFlex™) became commercially available in 2015 (Anonymous 2015a). This gives growers the ability to apply dicamba POST for control of broadleaf weeds.

Weed Control

Weeds are a persistent threat to crop production. In the 20th century, weed control in cotton shifted from systems reliant upon hand weeding and tillage to ones that also incorporated herbicide use (Dowler and Hauser 1975; Holstun 1963). Herbicides allow growers to remove almost every unwanted plant from their fields at a fraction of the cost of physical removal systems. Even though herbicides are a highly effective tool for removing weeds from agricultural systems, weeds remain because of their ability to adapt to new environments. Throughout history, composition of weed communities has been shown to be immensely influenced by factors like tillage and herbicide use (Booth and Swanton 2002; Norsworthy et al. 2012; Reddy and Norsworthy 2010). Creating selection pressure in an agroecosystem causes a decline in the occurrence of certain species or an evolved adaptation to the factors being applied, resulting in weed species shifts (Owen 2008). Typically, the introduction of a new weed control method will initially decrease weed diversity, but eventually weeds in the system will adapt to the management practices, or adapted species will fill the niche left by the eliminated species. An example of this is how the rapid adoption and heavy use of GR crops has contributed to weed species shifts, most notably, the rise of GR weeds (Owen 2008), which will be discussed in-depth later. These shifts tend to create a new issue for producers, who must once again alter management practices. In order to break the revolving cycle of reactive weed management, more comprehensive approaches to weed control must be utilized (Booth and Swanton 2002).

Herbicide-resistant Weeds

One of the biggest issues facing field crop production systems is the ability of weeds to quickly evolve resistance to herbicides. Switzer (1957) documented the first case of herbicide resistance in wild carrot (*Daucus carota* L.) to synthetic auxin herbicides. To date, there have been 255 species of weeds worldwide with confirmed resistance to at least one herbicide, with more species documented yearly (Heap 2019).

There are various mechanisms through which weeds can develop resistance to herbicides, including gene amplification, reduced translocation of herbicides, altered target sites, and metabolic degradation of the herbicide within the plant (Burke et al. 2007; Délye et al. 2015; Gaines et al. 2011; Koger and Reddy 2005; Riar et al. 2011). In order to properly manage HR biotypes and develop new, sustainable solutions for control, identifying the specific resistance mechanism is imperative (Powles and Yu 2010). Research on herbicide resistance mechanisms is crucial in understanding herbicide interactions with target enzymes, determining how resistance genes spread throughout populations, the implications of weak resistance, and the effect of selection pressure on multiple gene mutations (Shaner et al. 2012).

Palmer amaranth

Palmer amaranth (*Amaranthus palmeri* S. Wats.), a dioecious, C4, dicotyledonous species, is one of the most problematic weeds in the Midsouth, and it continues to spread further north through the United States (Sprague 2011). In a 2016 survey of crop consultants in the Midsouthern US, Palmer amaranth was listed as the most problematic weed in soybean (*Glycine max* [L.] Merr.) production in each of the five states surveyed (Schwartz-Lazaro et al. 2017). An earlier survey of crop consultants in the Midsouth listed Palmer amaranth as the most important and problematic weed in cotton, estimating that 75% of the area scouted by all responding consultants contained GR Palmer amaranth (Riar et al. 2013). Consultants rank Palmer amaranth with such importance because one Palmer amaranth plant per 9.1 m of row can decrease cotton lint yield by 13%, and this decrease in yield follows a linear trend as Palmer amaranth density increases (Morgan et al. 2001). Furthermore, an average Palmer amaranth plant that emerges in early summer can produce 200,000 to 600,000 seeds, and upwards of 1 million seeds on some occasions (Keeley et al. 1987).

The first confirmed instance of GR Palmer amaranth was reported in Georgia in 2005 (Culpepper et al. 2006). Soon thereafter, Norsworthy et al. (2008) confirmed a GR population collected from a field in Mississippi County, Arkansas, with researchers in other states throughout the southeastern US reporting similar findings around the same time. Today, GR Palmer amaranth can be found in 27 states within the US, reaching as far north as Wisconsin and Michigan (Heap 2019).

The level of glyphosate resistance displayed in the Palmer amaranth population from Georgia was found to have a direct correlation with EPSPS gene amplification (Gaines et al. 2010). EPSPS is an enzyme found in the shikimic acid biosynthetic pathway. Its production is inhibited by glyphosate in susceptible plants. EPSPS is crucial in the production of the amino acids tryptophan, phenylalanine, and tyrosine (Steinrücken and Amrhein 1980). Plants in the Georgia population carried the EPSPS gene at a rate 40- to 100-times higher than a susceptible plant. This amplification of the EPSPS gene allows the excess enzyme produced to essentially absorb the glyphosate on a molecular level so the plant can survive as if the glyphosate application never occurred (Gaines et al. 2011).

The resistance of Palmer amaranth to glyphosate and ALS-inhibiting herbicides has led to growers searching for new solutions to control Palmer amaranth. The most common solution that has been utilized in soybean is in the form of protoporphyrinogen oxidase (PPO)-inhibiting herbicides because of their ability to control a broad spectrum of weeds with rapid effectiveness when applied POST. In addition, some herbicides in this group provide sustained residual activity when applied to the soil preemergence (PRE) (Hao et al. 2011). PPO-inhibiting herbicides affect the plant by preventing the PPO enzyme from catalyzing the conversion of protoporphyrinogen IX to protoporphyrin IX, which is the last step in biosynthesizing heme and chlorophyll (Deybach et al. 1985). The inhibition of PPO ultimately leads to the generation of singlet (highly reactive) oxygen species that decompose lipid and protein membranes, causing plant death (Sherman et al. 1991).

The first instance of PPO-resistant Palmer amaranth was confirmed by Salas et al. (2016) in Arkansas. Progeny of a sample collected from a field in Lawrence County, Arkansas, showed resistance to the PPO-inhibiting herbicide fomesafen applied POST in a greenhouse experiment. Subsequent samples from resistant progeny showed a mutation in the PPO gene which no longer coded for ΔG210, causing a target site resistance. The ΔG210 deletion is the same mutation that confers resistance to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus* [Moq.] Sauer) (Thinglum et al. 2011). Target-site mutations were thought to be the only resistance mechanism to PPO-inhibiting herbicides in Palmer amaranth until very recently, when metabolic resistance to fomesafen was discovered (Varanasi et al. 2018a) Presently, PPO-resistant Palmer amaranth is a widespread issue throughout eastern Arkansas that is becoming increasingly prevalent (Varanasi et al. 2018b). To date, little research has been conducted on the control of PPO-resistant Palmer amaranth in cotton.

Barnyardgrass

Barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.) is another problematic C4 weed common in a variety of Midsouth crop production systems. Barnyardgrass is originally native to Europe and Asia and is currently a problem weed in 36 crops across 61 countries (Holm et al. 1991). In a recently published survey of Arkansas crop consultants, respondents ranked barnyardgrass as the fifth most economically important weed (Riar et al. 2013), in part because it can emerge from mid-spring until 7 weeks after cotton emergence (JK Norsworthy, unpublished data). When barnyardgrass competes with cotton for 6, 9, 12, and 25 weeks, it can diminish cotton yields by 21, 59, 90, and 97%, respectively (Keeley and Thullen 1991). Barnyardgrass also has the ability to produce vast amounts of seed. Bagavathiannan et al. (2012) found that barnyardgrass allowed to emerge with a cotton crop can produce 35,500 seeds plant^{-1}.

Over time, barnyardgrass has evolved resistance to nine herbicide modes of action worldwide (Heap 2019). Barnyardgrass was ranked the most problematic weed in rice by Arkansas and Mississippi crop consultants in a 2012 survey (Norsworthy et al. 2013). Most barnyardgrass resistance issues have developed from the repeated use of certain herbicides in rice. In a survey of barnyardgrass accessions from around the state of Arkansas, populations resistant to six different herbicides, encompassing five different modes of action were identified, with some populations containing multiple resistance to as many as four herbicide modes of action (Rouse et al. 2018). None of the herbicides included by Rouse et al. in the herbicide resistance screening are commonly recommended in Arkansas cotton, but these findings indicate that barnyardgrass has the ability to develop resistance quickly if weed management practices are not diversified (Scott et al. 2018). In Tennessee, barnyardgrass has evolved resistance to glyphosate. This is perhaps the most concerning instance of herbicide-resistant barnyardgrass to cotton producers because tank mixtures of glyphosate and dicamba, which are commonly applied in dicamba-resistant crops, provide little to no control of this GR biotype (Steckel 2018).

Salvage Situations

A variety of factors can hinder a grower's ability to control weeds at an optimum time. Previously discussed herbicide resistance issues can sometimes combine with other factors such as unfavorable weather conditions and rapid growth rate of weeds, creating a situation where timely weed control is impossible. Throughout the US, Palmer amaranth has been documented as resistant to eight different herbicide sites of action (Heap 2019), three of which contain herbicides that have traditionally been used as residual herbicides applied PRE in cotton. Although new HR traits provide options for effective weed control POST, residual PRE herbicide options are becoming more limited, increasing the chances for weeds to emerge with the crop. Moreover, label restrictions on herbicides such as dicamba limit POST applications to very specific environmental conditions.

When weeds grow past heights for consistent control listed on the herbicide label, the crop can either be replanted or an attempt can be made to salvage it. Vann et al. (2017a) observed 92 to 97% control of large Palmer amaranth (about 20 cm) when dicamba and high rates of glufosinate were applied twice, 10 days apart in a dicamba-resistant cotton crop. When the first POST application is delayed 28 days after weeds reach heights for consistent control listed on the herbicide label, Palmer amaranth can still be controlled 87% using two applications of dicamba plus a high rate of glufosinate applied 14 days apart (Vann et al. 2017b). Although high levels of control of large Palmer amaranth can be achieved, weed interference with the young crop may still result in decreased nodes and bolls per plant, and ultimately decreased lint yield (Burke et al. 2005; Vann et al. 2017b). Although not permitted for use, mixtures of dicamba and glufosinate to dicamba-resistant cotton have been shown to cause minor transient necrosis, but the crop rapidly recovers (Cahoon et al. 2015; Dixon et al. 2014; Vann et al. 2017a).

Spray Parameters

A variety of factors can manipulate spray solution droplet sizes including, but not limited to, application pressure, orifice size, nozzle design, and solution characteristics. Droplet sizes within the spray pattern exist in great variation. Droplet sizes produced by a particular nozzle can be classified by the volume median diameter (VMD) of spray droplets, which is the value of the median size of spray droplets produced (i.e. 50% of droplets are larger and 50% are smaller than this value). Increasing VMD can contribute to decreased particle drift when herbicides are applied, but it can also decrease the efficacy of some herbicides (Meyer et al. 2016). The herbicide formulation or mixture being applied can also cause variation in droplet size. When comparing the VMD of applications of glyphosate, glufosinate, and paraquat, Etheridge et al. (1999) determined that a smaller VMD was generated by glufosinate than the other two chemicals. Chemical mixes can also play a role in altering the VMD of a spray solution. When glufosinate was applied alone with a Turbo TeeJet Induction (TTI) nozzle, a VMD of 617 was produced, but when glufosinate was mixed with glyphosate and dicamba and applied with the same nozzle type, a VMD of 877 was produced (Meyer et al. 2015).

The spray nozzle is an applicator's last chance to influence the droplet size and spray pattern of a herbicide solution before it leaves the closed system of the application equipment. Nozzles are designed to control spray angle, spray pattern, droplet size, and solution flow rate as precisely as possible. Nozzles are available that produce a variety of spray patterns, and a variety of orifice sizes are available for each spray pattern. Increased droplet size can be obtained by increasing the orifice size for any given nozzle (Nuyttens et al. 2007). In order to increase droplet size without altering orifice size or spray pressure, nozzles with an inlet above the orifice are produced. These are typically referred to as air induction (AI) or venturi-type nozzles. These nozzles essentially impregnate spray droplets with air, making them larger and less likely to drift (Etheridge et al. 1999; Etheridge et al. 2001).

Although not as important for the control of horizontally structured broadleaf weeds, smaller droplets adhere better to upright grasses and therefore provide better control (Etheridge et al. 2001; McKinlay et al. 1974). Droplet size also plays a vital role in control levels provided by contact herbicides. When glufosinate and paraquat were applied to broadleaf signalgrass (*Urochloa platyphylla* [Munro ex C. Wright] R.D. Webster) and common cocklebur (*Xanthium strumarium* L.) with AI nozzles (coarser droplets) and flat fan nozzles (finer droplets), decreased control was noted in treatments where AI nozzles were used (Etheridge et al. 2001). McKinlay et al. (1974) observed decreased paraquat efficacy on common sunflower (*Helianthus annuus* L.) as VMD increased. Meyer et al. (2015) also observed a decrease in control of Palmer amaranth, hemp sesbania (*Sesbania herbacea* [Mill.] McVaugh), velvetleaf (*Abutilon theophrasti* Medik.), and barnyardgrass with glufosinate as droplet size increased. Conflicting conclusions exist on the effect of droplet size and synthectic auxin efficacy. 2,4-D efficacy has been shown to decrease dramatically with increases in VMD (McKinlay et al. 1972). These are similar findings to Way (1969) and Ennis and Williamson (1963), who observed that synthetic auxin efficacy increased as droplet size decreased. Meyer et al. (2015), however, noted no difference in efficacy of dicamba on Palmer amaranth, hemp sesbania, velvetleaf, and prickly sida (*Sida spinosa* L.) across VMD values ranging from 340 to 756 µm.

Another factor that can influence efficacy of a foliar-applied herbicide is the carrier volume, or amount of herbicide solution being applied per acre (Knoche 1994). Creech et al. (2015) observed no difference in control of *Amaranthus* spp. when glyphosate was applied at 70, 94, 140, and 187 L ha^{-1}. However, in the same study, efficacy of 2,4-D on amaranth and soybean increased with increases in carrier volume (Creech et al. 2015), which is similar to findings by Smith (1946). Butts et al. (2018) observed a negative correlation for weed mortality between carrier volume and droplet size at 47 L ha^{-1}, but a positive correlation at 187 L ha^{-1} when dicamba was applied postemergence to actively growing weeds, suggesting that greater carrier volume and larger droplets provide better coverage of the leaf surface than a lower carrier volume with the same droplet size.

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CHAPTER 2

Control of PPO-resistant Palmer amaranth in Herbicide-resistant Cotton

Palmer amaranth throughout northeastern Arkansas is now resistant to protoporphyrinogen oxidase (PPO)-inhibiting herbicides. Although the option to control Palmer amaranth with PPO-inhibiting herbicides at burndown and preplant has been eliminated, it is the reduced sensitivity of PPO-resistant populations to herbicides that target alternative sites of action that is of greater concern for cotton production. To assess the efficacy of herbicides commonly used in cotton for controlling PPO-resistant Palmer amaranth, field experiments were conducted at two on-farm locations in 2018. All experiments were organized as a randomized complete block design and included a weed-free check for comparison. Preemergence (PRE) herbicides commonly applied in cotton were evaluated to determine efficacy on two PPO-resistant Palmer amaranth populations. Another experiment evaluated the efficacy of the postemergence (POST) herbicides 2,4-D, dicamba, and glufosinate, alone or in combination with one of three chloroacetamide herbicides. Environmental differences between locations played a factor in PRE weed control; however, similar trends were observed for both locations. Treatments containing herbicide mixtures controlled Palmer amaranth at higher levels than treatments of single herbicides 4 weeks after application (WAA). Furthermore, herbicide mixtures containing fluometuron provided superior control over all other herbicide mixtures 4 WAA. POST experiments also differed between locations due to rainfall and light intensity, as well as differences in weed size at application. At Crawfordsville 4 WAA, treatments containing dicamba had lower Palmer amaranth densities than treatments containing 2,4-D or glufosinate. At Marion, however, treatments containing glufosinate resulted in the lowest Palmer amaranth densities 4 WAA. Herbicide mixtures are recommended for PRE control of PPO-resistant Palmer amaranth, especially those containing fluometuron. For POST control of PPO-resistant Palmer amaranth, it is important to make multiple timely applications.

Nomenclature: 2,4-D; dicamba; glufosinate; Palmer amaranth, *Amaranthus palmeri* S. Wats., cotton, *Gossypium hirsutum* L.

Key Words: Herbicide resistance, herbicide-resistant cotton, chloroacetamide herbicides

INTRODUCTION

Palmer amaranth is one of the most economically important weeds in cotton. The interference and competition of Palmer amaranth with the crop can cause a variety of problems including slower canopy closure, reduced crop biomass, harvest difficulties, and decreased lint yield (Morgan et al. 2001; Rowland et al. 1999; Smith et al. 2000). Palmer amaranth can severely impact cotton growth and development because it grows at rates much higher than cotton due to its C_4 photosynthetic pathway (Ehleringer 1983). Morgan et al. (2001) determined that 10 Palmer amaranth plants in 9.1 m of row of cotton can reduce lint yield by 54%. A typical female Palmer amaranth plant will produce between 200,000 and 600,000 seeds, but on some occasions seed production can total over 1 million from a single female plant (Keeley et al. 1987). Steckel et al. (2004) reported a Palmer amaranth germination rate of 83% in a growth chamber that simulated a seed burial depth of 2 cm in bare soil, with an average temperature of 30 C. With such abundant seed production and germination, it is important to control Palmer amaranth before it produces seed. The escape of one Palmer amaranth plant in a growing season can result in 1,020 Palmer amaranth escapes in two years, even with 99.9% control of germinated seedlings (Barber et al. 2015).

In Arkansas, Palmer amaranth has been confirmed resistant to multiple herbicide sites of action. Currently, Palmer amaranth can be found throughout the state with resistance to WSSA Groups 2 (acetolactate synthase [ALS] inhibitors), 9 (glyphosate), and 14 (protoporphyrinogen oxidase-inhibitors) (Heap 2018). Following the development of glyphosate and ALS resistance, PPO-inhibitors became a leading choice for Palmer amaranth control in soybean [*Glycine max* (L.) Merr.] because of the excellent control they provided of glyphosate-resistant (GR) Palmer amaranth (Norsworthy et al. 2008). PPO-inhibiting herbicides affect the plant by preventing the

PPO enzyme from catalyzing the conversion of protoporphyrinogen IX to protoporphyrin IX, which is the last step in biosynthesizing heme and chlorophyll (Deybach et al. 1985). The inhibition of PPO ultimately leads to the generation of singlet (highly reactive) oxygen species that decompose lipid and protein membranes, resulting in plant death (Sherman et al. 1991).

The first instance of PPO-resistant Palmer amaranth was confirmed by Salas et al. (2016) in Arkansas. Progeny of a seed sample collected from a field in Lawrence County in Arkansas showed resistance to the PPO-inhibiting herbicide fomesafen applied POST in a greenhouse experiment. Subsequent samples from resistant progeny showed a mutation in the PPO gene that no longer coded for ΔG210, causing a target site resistance. This is the same mutation that confers resistance to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus* [Moq.] Sauer) and, at the time of discovery, was thought to be the only mechanism of resistance to this site of action (Thinglum et al. 2011). However, non-target site (metabolic) resistance of Palmer amaranth to PPO-inhibitors was recently discovered in Arkansas, raising concern that this resistance pattern may confer resistance to other herbicide sites of action (Varanasi et al. 2018). To date, very little research has been conducted on the control of PPO-resistant Palmer amaranth in cotton.

Growers in the Midsouth currently rely on a combination of preplant burndown, PRE, POST, and postemergence-directed (PDIR) herbicide applications to control weeds in cotton, utilizing a residual herbicide at each timing (Barber, personal communication). Cotton technologies resistant to multiple herbicides are currently on the market. In 2004, cotton varieties resistant to POST glufosinate applications (tradename LibertyLink®) were released for commercial use (Gardner et al. 2006). However, LibertyLink cotton was not widely adopted by cotton producers until later, when growers needed a solution to control GR Palmer amaranth

(Dodds et al. 2015). In 2015, two new herbicide-resistant (HR) cotton lines were released: one that was resistant to dicamba, glyphosate, glufosinate, and lepidopteran insects (tradename Bollgard II® XtendFlex™), and another that was resistant to 2,4-D, glyphosate, glufosinate, and lepidopteran insects (tradename Enlist™ Cotton) (Anonymous 2015a; Anonymous 2015b). This gives growers in locations outside of Arkansas the ability to apply dicamba POST at any time during the growing season for control of broadleaf weeds (Anonymous 2018), and growers in any region the option to apply 2,4-D POST for control of broadleaf weeds. Because limited research has been conducted on controlling PPO-resistant Palmer amaranth with common cotton herbicides, experiments were conducted to determine the efficacy of standalone herbicides and herbicide mixtures applied PRE and POST in cotton. It was hypothesized that herbicide mixtures would provide greater control of PPO-resistant Palmer amaranth than standalone herbicides.

MATERIALS AND METHODS

Two field experiments were conducted in 2018 at two on-farm locations at Marion, AR, and near Crawfordsville, AR, to assess the potential control of PPO-resistant Palmer amaranth PRE and POST in cotton. At Marion, the experiments were conducted on a Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs) with 27.1% sand, 62.9% silt, 10% clay, 1.64% organic matter (OM), and a pH of 6.1. At Crawfordsville, experiments were conducted on a Dundee silt loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs) with 10.7% sand, 76.9% silt, 12.4% clay, 1.95% OM, and a pH of 5.5. Each location was a site where naturally occurring populations of PPO-resistant Palmer amaranth had previously been identified. Rainfall data (Table 1) were collected from a weather station in the field at Crawfordsville and from a nearby (8.4 km) National Oceanic and Atmospheric Administration (NOAA) weather station for Marion (NOAA 2018). All experiments were organized in a randomized complete block design with four replications, where the factor of herbicide treatment was examined. Plots for all experiments were two rows wide (1.93 m) by 9.1 m long.

PRE Application Experiments. The first set of experiments evaluated herbicides applied PRE in cotton. The herbicides acetochlor (Warrant, Bayer CropScience, St. Louis, MO), dicamba (Xtendimax, Bayer CropScience, St. Louis, MO), fluridone (Brake, SePro Corporation, Carmel, IN), fluometuron (Cotoran, Syngenta, Greensboro, NC), diuron (Direx 4L, Adama USA, Raleigh, NC), and prometryn (Caparol, Syngenta, Greensboro, NC) were evaluated for control of Palmer amaranth alone, and in various combinations. A treatment of the PPO-inhibiting herbicide fomesafen (Reflex, Syngenta, Greensboro, NC) was included as a comparative baseline for PPO-resistant Palmer amaranth control. Both sites were weed-free prior to herbicide application. At the Crawfordsville location, DP1518B2XF cotton (Bayer Crop Science, St. Louis, MO) was planted on May 16 at 118,560 seeds ha^{-1}. No cotton was planted at the Marion location; therefore, treatments were applied to freshly tilled soil. At both locations, applications were made with a CO_2-pressurized backpack sprayer attached to a handheld boom containing four 110015 AirMix® nozzles (Greenleaf Technologies, Covingtion, LA) with 48 cm spacing, calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa. Herbicide applications were made on May 16 at Crawfordsville and July 25 at Marion.

Visible weed control ratings were collected weekly from 1 to 4 WAA on a scale of 0 to 100% control, relative to the nontreated check, with 0% being no control and 100% being death of all Palmer amaranth (Frans and Talbert 1977). Palmer amaranth densities (plants m^{-2}) were recorded at the 4 WAA rating by counting the number of plants in two 0.5-m^{2} quadrats in each plot.

POST Application Experiments. A second set of experiments was conducted to determine control levels of PPO-resistant Palmer amaranth with combinations of residual herbicides applied POST. Treatments included glufosinate (Liberty, BASF Corporation, Florham Park, NJ), dicamba (Xtendimax, Bayer CropScience), and 2,4-D (Enlist One, Corteva AgriScience, Indianapolis, IN) alone, and in combination with *S*-metolachlor (Dual II Magnum, Syngenta, Greensboro, NC), acetochlor (Warrant, Bayer CropScience, St. Louis, MO), and dimethenamid-P (Outlook, BASF Corporation, Florham Park, NJ). Experiments at both Crawfordsville and Marion were established in non-crop areas where Palmer amaranth was already emerged, and applications were made when weeds reached 7.5 to 10 cm in height at Marion and 15 to 20 cm in height at Crawfordsville. At Crawfordsville, treatments were applied with a CO_2-pressurized backpack sprayer attached to a handheld boom containing four 110015 AirMix® nozzles (Greenleaf Technologies, Covington, LA) with 48-cm spacing, calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa. At Marion, treatments were applied with a Bowman Mudmaster (Bowman Manufacturing, Newport, AR) using the same nozzles, nozzle spacing, carrier volume, and pressure as at Crawfordsville. Treatments were applied May 16, at Crawfordsville and May 31, at Marion.

Visible control ratings for Palmer amaranth were recorded weekly from 1 to 6 WAT on the same scale of 0 to 100% used in the PRE experiments. Additionally, Palmer amaranth densities (plants m^{-2}) were recorded at the same time as the 4 WAA rating by counting the number of plants in two 0.5-m^2 quadrats in each plot.

Statistical Analysis. All data were subject to analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc. Cary, NC). A beta distribution was assumed for Palmer amaranth control and a gamma distribution was assumed for Palmer amaranth density for both experiments (Gbur et al. 2012). Because environmental differences affected the behavior of PRE herbicides applied at the two sites, data were analyzed separately by location, with block considered a random effect in each model. Contrasts analyses were also conducted for PRE Palmer amaranth control to test for differences between herbicide mixtures and herbicides applied alone (fomesafen was excluded from analysis) as well as differences between herbicide mixtures containing fluometuron and mixtures containing no fluometuron. Means were separated using Fisher's protected LSD (P=0.05).

RESULTS AND DISCUSSION

Location Description and Environmental Conditions. Several soil properties influence the control provided by soil-applied herbicides, including soil texture, soil chemical properties, and soil moisture (Curran 2001; Eberline et al. 1984; Hartzler 2002). Differences in soil texture were minimal between locations and therefore had minimal effect on the results of this experiment. The availability of some herbicides is affected by soil pH. For example, fluridone is more active as pH increases; therefore, fluridone was likely more available for plant uptake at Marion (pH 6.1) than at Crawfordsville (pH 5.5) (Shea and Weber 1983). Fomesafen and photosystem II-inhibiting herbicides such as prometryn have also been shown to increase in availability as soil pH increases (Cobucci et al. 1998; Ladlie et al. 1976; Weber et al. 1968).

Rainfall amounts received after application of soil-applied herbicides were the main difference between the two experimental locations. At Crawfordsville, PRE applications were made to dry soil and a cumulative total of 3.75 cm of rainfall was received over the next 9 days (Table 1). For the Marion location, applications were also made to dry soil, but a cumulative total of 4.73 cm of rainfall was received over the next 5 days, with no measurable rainfall occurring for the remainder of the experiment (Table 1).

The efficacy of postemergence herbicides is influenced by several factors including weed size, relative humidity, temperature, and light intensity at and shortly after application (Anderson et al. 1993; Gerber et al. 1983; Hess 2000; Martinson et al. 2005). As relative humidity, temperature, and light intensity increase weed control with glufosinate also increases (Coetzer et al. 2001; Petersen and Hurle 2001). Also, if glufosinate is applied shortly before dark, a decrease in efficacy is observed (Hess 2000; Sellers et al. 2003). POST treatments were applied at Crawfordsville late in the day to 15- to 20-cm Palmer amaranth, which decreased control provided by all treatments (Table 5). Furthermore, the high temperature the day following the application was 25 C with heavy cloud cover, which severely impacted the efficacy of all treatments containing glufosinate for that location. New weed emergence after POST applications at Marion was reduced due to dry conditions (Table 1).

PRE Application Experiments. Overall, lower levels of control of PPO-resistant Palmer amaranth were observed at Crawfordsville for both ratings. Control levels ranged from 57 to 89% 2 WAA (Table 2). For this location, control decreased 27 percentage points, averaged over all treatments, between 2 WAA and 4 WAA. At 4 WAA, only one treatment provided greater than 75% control of Palmer amaranth. Fomesafen controlled PPO-resistant Palmer amaranth 79% 2 WAA, but control decreased drastically by 4 WAA to 36% (Table 2). Fluridone + fluometuron provided 76% control 4 WAA; however, this was not different from fluometuron + prometryn or fluometuron + acetochlor, which both controlled PPO-resistant Palmer amaranth 68% at Crawfordsville (Table 2).

For the Marion location, control levels 2 WAA were from 77% to 99% (Table 3). At this location, control decreased 17 percentage points, averaged over all treatments, between 2 WAA and 4 WAA. Overall control was higher at Marion where nine treatments still controlled PPO-resistant Palmer amaranth at least 75% 4 WAA (Table 3). The PPO-inhibiting herbicide fomesafen provided 86% control 2 WAA, but only 59% control 4 WAA, which was the lowest level of control by any treatment at that timing for the Marion location. Fluridone + dicamba controlled PPO-resistant Palmer amaranth 95% 4 WAA, which was the highest level of control observed at this location (Table 3).

Overall differences in control levels and decreases in control levels over time between the two experimental sites are likely due to weed population and rainfall differences (Table 1). Weed population dynamics affect efficacy of preemergence herbicides, as well as the amount of herbicide absorbed by each seed (Hartzler and Roth 1993; Winkle et al. 1981). Because no irrigation was available at either location, activation of the herbicides and moisture availability for germination of new Palmer amaranth were solely dependent on rainfall occurrences. The Crawfordsville location continued to receive rainfall throughout the duration of the trial, allowing for new Palmer amaranth to emerge between ratings, whereas the Marion location received no more rainfall after the initial rainfall displayed in Table 1 (data not shown).

Contrast analysis indicated no differences in control of Palmer amaranth between a single herbicide and mixtures 2 WAA at Crawfordsville ($P=0.6449$) (Table 4). This was not the case, however, 4 WAA where mean control of Palmer amaranth was 11 percentage points higher with a herbicide mixture than with a single herbicide ($P<0.0001$) (Table 4). The same analysis at Marion revealed a significant difference in Palmer amaranth control with herbicide mixtures 2 WAA ($P=0.02235$) (Table 4). The same trend was observed 4 WAA for Marion, where control was 7 percentage points higher with herbicide mixtures ($P=0.0002$) (Table 4). Similar longevity of control of PPO-resistant Palmer amaranth with herbicide mixtures was observed by Houston et al. (2019).

A trend in PPO-resistant Palmer amaranth control was also observed between herbicide mixtures containing fluometuron and mixtures containing no fluometuron at Crawfordsville. Palmer amaranth control with treatments containing fluometuron was greater both 2 and 4 WAA than with treatments not containing fluometuron ($P<0.0001$, <0.0001) (Table 4). At 4 WAA, control with mixtures containing fluometuron was 22 percentage points higher than with mixtures lacking fluometuron. The same trend was not observed at 2 or 4 WAA at Marion ($P=0.1101$, $P=0.0542$) (Table 4). Although not significant, mixtures containing fluometuron provided a 5-percentage point advantage in Palmer amaranth control over treatments containing no fluometuron (Table 4). Weed densities and lack of rainfall for germination of new Palmer amaranth likely influenced the lack of significance at Marion.

Weed densities at Crawfordsville did not correspond with visible control ratings because densities were counted several days after the 4 WAA rating; therefore, weed densities for this location are not shown. Weed densities were more evenly distributed throughout the experiment at Marion and more accurately correlated to weed control. The treatment with the greatest control 4 WAA (fluridone + dicamba) also reduced density to only 3 plants m^{-2}, compared to 76 plants m^{-2} in the nontreated (Table 3). Whitaker et al. (2011) found fomesafen to be an effective PRE herbicide for controlling Palmer amaranth; however, with the current state of PPO-resistance in Arkansas, fomesafen alone is not a viable option for PPO-resistant populations, indicated by a density of 23 Palmer amaranth m^{-2} (Table 3).

POST Application Experiments. Overall, PPO-resistant Palmer amaranth control was greater at Marion than Crawfordsville due to differences in weed size, weed density, and environment shortly after application. Treatments of dicamba, dicamba + *S*-metolachlor, 2,4-D + *S*-metolachlor, and dicamba + acetochlor provided 65% or greater control of PPO-resistant Palmer amaranth 2 WAA at Crawfordsville (Table 5). Glufosinate alone provided comparable control to treatments containing 2,4-D alone and 2,4-D + dimethenamid-P 2 WAA, but glufosinate-containing treatments provided less control than dicamba-containing treatments (Table 5). Control averaged over all treatments 2 WAA was only 60% at Crawfordsville (Table 5). At 4 WAA, only treatments of dicamba + dimethenamid-P and dicamba + acetochlor increased control over the 2 WAA rating time and, then, only minimally (Table 5). All treatments containing glufosinate provided lower control levels than any treatment containing 2,4-D or dicamba 4 WAA (Table 5). Dicamba alone resulted in the lowest density of Palmer amaranth at 27 m^{-2}, but this was not different from dicamba + *S*-metolachlor or dicamba + acetochlor (Table 5). Remaining densities indicate a need for a second application to achieve adequate control of Palmer amaranth, regardless of herbicide applied.

At Marion, all glufosinate-containing treatments provided the highest control of PPO-resistant Palmer amaranth 2 WAA (Table 6). 2,4-D + dimethenamid-P provided greater control than all other treatments containing 2,4-D or dicamba 2 WAA (Table 6). Control averaged over all treatments 2 WAA at Marion was 82% (Table 6). At 4 WAA, Palmer amaranth control with treatments containing 2,4-D or dicamba was higher than control at the 2 WAA rating (Table 6). PPO-resistant Palmer amaranth control at Marion averaged over all treatments 4 WAA was 90% (Table 6). Palmer amaranth density 4 WAA was relatively low, and less than 22 plants m^{-2} for all treatments (Table 6). The treatment of 2,4-D + acetochlor had the highest number of Palmer amaranth at 21 plants m^{-2}, but this was not different from treatments of dicamba + dimethenamid-P, dicamba + *S*-metolachlor, or 2,4-D + *S*-metolachlor (Table 6). However, it should be noted that soil conditions were dry at the time of application and no rainfall was received at Marion after the rainfall event listed in Table 1, resulting in conditions that were too dry for new Palmer amaranth to emerge (data not shown). Residual herbicides, therefore, had little to no effect on weed densities 4 WAA.

Practical Implications. Environmental factors and weed densities influenced each experimental site differently, which highlights the need to plan PRE herbicide applications near a predicted rainfall event of enough significance to activate the specific herbicide applied if no irrigation is available. PPO-resistant Palmer amaranth can be controlled in cotton with PRE herbicides available for use today. Using two effective sites of action is needed to control PPO-resistant Palmer amaranth PRE. Selecting a mixture containing fluometuron is a good option for controlling PPO-resistant Palmer amaranth (Tables 2, 3, 4). Growers should rely on the best management practices outlined by Norsworthy et al. (2012) to manage PPO-resistant Palmer amaranth, particularly understanding the biology of this weed, planting into weed-free fields, and using multiple herbicide sites of action to keep fields weed free.

It is critical that growers are aware of weed densities and size in order to properly time POST applications. Waiting 28 to 35 days after planting to make a POST application is too long when weed densities are high, as demonstrated by control levels at Crawfordsville. In herbicide-resistant cotton, it is important to make applications before weeds reach 10 cm in height, as demonstrated by the poor control achieved by all treatments at Crawfordsville, where weeds were 15 to 20 cm in height at application. When POST applications are made at the optimum time, greater than 85% control can be achieved in 2,4-D-, glufosinate-, and dicamba-resistant cotton (Lawrence et al. 2018; Reed et al. 2014; Meyer et al. 2016). These data demonstrate that multiple applications of effective POST herbicides will likely be required to control PPO-resistant Palmer amaranth, similar to findings by Steckel (2018). Although there was not sufficient rainfall to activate the residual herbicides applied POST at Marion, overlapping residuals are critical in limiting Palmer amaranth emergence between POST applications (Barber, personal communication).

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TABLES

Table 1. Rainfall amounts received within 10 days of herbicide application for all experiments in 2018.[a,b,c]

Location	Experiment	Date	Amount (cm)
Crawfordsville	PRE and POST	May 17	0.97
Crawfordsville	PRE and POST	May 19	0.81
Crawfordsville	PRE and POST	May 20	0.81
Crawfordsville	PRE and POST	May 21	0.33
Crawfordsville	PRE and POST	May 22	0.28
Crawfordsville	PRE and POST	May 24	0.4
Crawfordsville	PRE and POST	May 25	0.15
Total			3.75
Marion	PRE	July 27	1.78
Marion	PRE	July 29	2.87
Marion	PRE	July 30	0.08
Total			4.73
Marion	POST	June 1	0.03
Total			0.03

[a] Abbreviations: PRE, experiment evaluating protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control with preemergence herbicides; POST, experiment evaluating protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control with postemergence herbicides

[b] PRE experiment application dates: May 16 at Crawfordsville, July 25 at Marion. POST experiment application dates: May 16 at Crawfordsville, May 31 at Marion.

[c] Dates not listed are days when no rainfall was received

Table 2. Protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control at Crawfordsville, AR, experimental site using preemergence herbicides in 2018.[a,b,c]

Herbicide	Rate	Control 2 WAA		Control 4 WAA	
	g ai ha^{-1}	%			
Nontreated	---	---		---	
Fomesafen	280	80	bcd	36	ghi
Fluridone	170	70	de	26	j
Prometryn	1120	71	cd	55	cde
Fluometuron	1120	81	abc	48	def
Diuron	560	85	ab	61	bc
Acetochlor	1260	88	ab	49	def
Dicamba	560	59	ef	43	fgh
Dicamba	1120	89	a	46	efg
Fluridone + prometryn	170 + 1120	59	ef	31	ij
Fluridone + fluometuron	170 + 1120	86	ab	76	a
Fluridone + diuron	170 + 560	86	ab	58	bcd
Fluridone + acetochlor	170 + 1260	58	f	34	hij
Fluridone + dicamba	170 + 560	85	ab	64	bc
Fluometuron + prometryn	1120 + 1120	88	ab	68	ab
Fluometuron + acetochlor	1120 + 1260	86	ab	68	ab
Acetochlor + dicamba	1260 + 560	87	ab	65	bc
Herbicide treatment		<0.0001		<0.0001	

[a] Rates of dicamba are listed in g ae ha^{-1}
[b] Abbreviations: WAA, weeks after application
[c] Means within a column followed by the same letter are not different based on Fisher’s protected LSD (P=0.05)

Table 3. Protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control and densities at Marion, AR, experimental site using preemergence herbicides in 2018.[a,b,c]

Herbicide	Rate	Control 2 WAA		Control 4 WAA		Density 4 WAA	
	g ai ha^{-1}	%		%		plants m^{-2}	
Nontreated	---	---		---		76	a
Fomesafen	280	87	def	60	g	23	bcd
Fluridone	170	85	ef	70	efg	24	bc
Prometryn	1120	77	f	66	fg	22	bcde
Fluometuron	1120	96	abc	75	def	15	cdef
Diuron	560	91	cde	72	ef	31	b
Acetochlor	1260	98	ab	88	b	3	i
Dicamba	560	98	abc	77	cde	12	fg
Dicamba	1120	99	a	81	bcd	7	gh
Fluridone + prometryn	170 + 1120	99	a	67	fg	13	def
Fluridone + fluometuron	170 + 1120	96	abc	85	bc	7	gh
Fluridone + diuron	170 + 560	92	bcde	70	efg	29	b
Fluridone + acetochlor	170 + 1260	98	abc	72	ef	20	bcdef
Fluridone + dicamba	170 + 560	99	a	95	a	6	ghi
Fluometuron + prometryn	1120 + 1120	94	abcd	82	bcd	12	fg
Fluometuron + acetochlor	1120 + 1260	99	a	87	b	5	hi
Acetochlor + dicamba	1260 + 560	98	ab	83	bcd	6	ghi
Herbicide treatment		<0.0001		<0.0001		<0.0001	

[a] Rates of dicamba are listed in g ae ha^{-1}

[b] Abbreviations: WAA, weeks after application

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

Table 4. Significance of contrast statements between standalone herbicides and herbicide mixtures, as well as mixtures containing fluometuron and mixtures containing no fluometuron.[a,b,c]

	Palmer amaranth control 2 WAA			
Contrast	Crawfordsville	Means	Marion	Means
Single herbicide vs herbicide mixture	0.6449	77 vs 78	0.02235*	95 vs 98
Mixtures including fluometuron vs mixtures with no fluometuron	<0.0001*	87 vs 74	0.1101	97 vs 98
	Palmer amaranth control 4 WAA			
Contrast	Crawfordsville	Means	Marion	Means
Single herbicide vs herbicide mixture	<0.0001*	44 vs 55	0.0002*	75 vs 82
Mixtures including fluometuron vs mixtures with no fluometuron	<0.0001*	70 vs 48	0.0542	85 vs 80

[a] Abbreviations: WAA, weeks after application; Crawfordsville, on-farm location near Crawfordsville, AR; Marion, on-farm location in Marion, AR
[b] Significant P-values (P=0.05) are indicated by (*)
[c] Fomesafen was not included in contrast for standalone herbicide

Table 5. Protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control and densities at Crawfordsville, AR, experimental site using postemergence herbicides in 2018.[a,b,c]

Herbicide	Rate	Control 2 WAA	Control 4 WAA	Density 4 WAA
	g ai ha^{-1}	%	%	plants m^{-2}
Nontreated		---	---	93 a
2,4-D	1060	61 cde	55 de	58 ab
2,4-D + *S*-metolachlor	1060 + 1075	66 abc	61 cd	74 a
2,4-D + dimethenamid-P	1060 + 670	59 def	53 e	87 a
2,4-D + acetochlor	1060 + 1260	64 bcd	64 bc	66 a
Dicamba	560	71 a	69 ab	27 c
Dicamba + *S*-metolachlor	560 + 1075	69 ab	68 ab	33 c
Dicamba + dimethenamid-P	560 + 670	64 bcd	65 bc	57 ab
Dicamba + acetochlor	560 + 1260	65 abcd	71 a	34 bc
Glufosinate	595	56 efg	28 f	68 a
Glufosinate + *S*-metolachlor	595 + 1075	44 h	29 f	84 a
Glufosinate + dimethenamid-P	595 + 670	57 g	33 f	83 a
Glufosinate + acetochlor	595 + 1260	54 fg	33 f	73 a
Herbicide treatment		<0.0001	<0.0001	<0.0001

[a] Abbreviations: WAA, weeks after application

[b] Rates of dicamba and 2,4-D are listed in g ae ha^{-1}

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

Table 6. Protoporphyrinogen oxidase-inhibiting herbicide-resistant Palmer amaranth control and densities at Marion, AR, experimental site using postemergence herbicides in 2018.[a,b]

Herbicide	Rate	Control 2 WAA	Control 4 WAA	Density 4 WAA
	g ai ha^{-1}	%	%	plants m^{-2}
Nontreated	---	---	---	74 a
2,4-D	1060	76 bc	88 ab	9 cd
2,4-D + *S*-metolachlor	1060 + 1075	70 c	90 a	10 bcd
2,4-D + dimethenamid-P	1060 + 670	82 b	91 a	6 de
2,4-D + acetochlor	1060 + 1260	72 c	80 b	21 b
Dicamba	560	75 c	94 a	4 e
Dicamba + *S*-metolachlor	560 + 1075	74 c	91 a	11 bcd
Dicamba + dimethenamid-P	560 + 670	74 c	93 a	17 bc
Dicamba + acetochlor	560 + 1260	71 c	94 a	6 de
Glufosinate	595	96 a	93 a	6 de
Glufosinate + *S*-metolachlor	595 + 1075	96 a	90 a	6 de
Glufosinate + dimethenamid-P	595 + 670	96 a	92 a	5 de
Glufosinate + acetochlor	595 + 1260	96 a	89 ab	4 e
Herbicide treatment		<0.0001	<0.0001	<0.0001

[a] Abbreviations: WAA, weeks after application

[b] Rates of dicamba and 2,4-D are listed in g ae ha^{-1}

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

CHAPTER 3

Salvage Weed Control Options in Dicamba-resistant Cotton

Timely weed control can sometimes be delayed by unforeseen circumstances such as poor weather conditions, equipment malfunctions, and label restrictions. When weeds grow past optimum size for control, they become harder to kill, and multiple herbicide applications may be needed to salvage the crop. Current trait packages in dicamba-resistant cotton give growers another site of action to control weeds postemergence. Two field experiments were conducted in 2017 and 2018 to determine the best treatments for controlling large (≥15 cm) Palmer amaranth and barnyardgrass in dicamba-resistant cotton. Studies were organized in a randomized complete block and included a non-treated at all locations. The location where seedcotton yield was collected included a weed-free check for comparison. Treatments included glyphosate, glufosinate, and dicamba alone or in various two-pass combinations. In 2017, when treatments were applied to 28- to 35-cm weeds, poor control was observed for all treatments. In 2018, treatments were applied to 15- to 23-cm weeds and all but one two-pass treatment controlled Palmer amaranth >91%. Contrast analysis between 7-day and 14-day application intervals revealed differences in weed control for both 2018 locations. Applying the second application of a two-pass treatment 7 days after the first controlled Palmer amaranth 96%, whereas control was 92% when the second treatment was applied 14 days after the first. An inverse relationship was observed for barnyardgrass control in 2018. When two-pass treatments were applied 7 days apart, barnyardgrass control was 92%, but when applied 14 days apart, barnyardgrass was controlled 98%. No trend in seedcotton yield were observed based on salvage treatments. Contrast analysis indicated no difference in Palmer amaranth control between applying dicamba first or second in split applications, indicating that dicamba can possibly be applied before cutoff dates as specified by the herbicide label for certain areas.

Nomenclature: dicamba; glyphosate; glufosinate; barnyardgrass, *Echinochloa crus-galli* (L.) P. Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; cotton, *Gossypium hirsutum* L.

Key Words: Palmer amaranth, barnyardgrass

INTRODUCTION

Palmer amaranth is a dioecious, C4, dicotyledonous species which is spreading further north through the United States (Sprague 2011). Crop consultants in the midsouthern US ranked Palmer amaranth as the most problematic weed in soybean (*Glycine max* [L.] Merr.) production in each of the five states surveyed in 2016 (Schwartz-Lazaro et al. 2017). An earlier survey of crop consultants in the Midsouth listed Palmer amaranth as the most important and problematic weed in cotton (*Gossypium hirsutum* L.), as well, estimating that 75% of the area scouted by all responding consultants contained glyphosate-resistant (GR) Palmer amaranth (Riar et al. 2013). One Palmer amaranth plant per 9.1 m of row can decrease cotton lint yield by 13%, and this decrease in yield follows a linear trend as Palmer amaranth density increases, which is why consultants rank this weed with such importance (Morgan et al. 2001). Furthermore, an average Palmer amaranth plant that emerges in early summer can produce 200,000-600,000 seeds, and upwards of 1 million seeds on some occasions (Keeley et al. 1987).

Glyphosate suppresses the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway, causing shikimic acid to accumulate in treated tissues, resulting in plant death (Duke and Powles 2008; Steinrucken and Amrhein 1980). The first case of GR Palmer amaranth was confirmed in Georgia in 2005 (Culpepper et al. 2006). Norsworthy et al. (2008) confirmed a GR population collected from a field in Mississippi County, Arkansas, with researchers in other states throughout the southeastern US reporting similar findings soon thereafter. Glyphosate-resistant Palmer amaranth is currently found in 28 states within the US, as far north as Wisconsin and Michigan (Heap 2019).

Because of its broad-spectrum efficacy, including GR Palmer amaranth, glufosinate was originally limited to use in noncrop areas and burndown applications (Hass and Muller 1987).

Glufosinate obstructs the transformation of glutamate and ammonium to the essential amino acid glutamine by inhibiting the enzyme glutamine synthetase, resulting in limited glutamine availability, and an increase in glyoxylate and ammonia levels, ultimately causing rapid necrosis of contacted plant tissue (Coetzer and Al-Khatib 2001). In 2004, cotton varieties resistant to postemergence (POST) glufosinate applications (trade-name LibertyLink®) were released for commercial use. LibertyLink cotton was not widely adopted by cotton producers until more recent years because of the poor yield potential associated with original LibertyLink varieties, as well as the need for control of GR weeds (Dodds et al. 2015; UGA 2007). Other cultivars were created using the phosphinothricin acetyltransferase (pat) gene as a selectable marker during plant transformation to confer tolerance to insects (trade-name WideStrike™), that also conferred resistance to glufosinate (Anonymous 2015a). When WideStrike varieties were crossed with GR cotton, varieties were produced that yielded as much as, or greater than original LibertyLink varieties, while also giving growers another option for broad-spectrum control of weeds that had evolved resistance to other herbicides (Culpepper et al. 2009).

Many factors can affect herbicide application timing, sometimes preventing weed control at an optimum time. Unfavorable weather conditions and rapid growth rate of weeds can sometimes create a situation where timely weed control is impossible, especially when compounded with herbicide resistance issues. Throughout the US, Palmer amaranth has been documented as resistant to eight different herbicide sites of action (Heap 2019), including three that have traditionally been applied PRE in cotton. Although new herbicide-resistant cotton varieties provide options for effective weed control POST, residual PRE herbicide options are becoming more limited, increasing the chances for weeds to emerge with the crop. Label restrictions on herbicides such as dicamba also limit POST applications to very specific environmental conditions, creating more difficulty in making a legal, timely application (Anonymous 2018).

A salvage situation occurs when weeds grow past heights for consistent control listed on the herbicide label. When dicamba and high rates of glufosinate were applied twice, 10 days apart in a dicamba-resistant cotton crop, Vann et al. (2017a) observed 92 to 97% control of large Palmer amaranth (about 20 cm). When the first POST application is delayed 28 days after weeds reach heights for consistent control listed on the herbicide label, Palmer amaranth can still be controlled 87% using two applications of dicamba plus a high rate of glufosinate applied 14 days apart (Vann et al. 2017b). Although very high levels of control of large Palmer amaranth can be achieved, weed interference with the young crop may still result in decreased nodes and bolls per plant, and ultimately decreased lint yield (Burke et al. 2005; Vann et al. 2017b). Applications of dicamba and glufosinate to dicamba-resistant cotton have been shown to cause minor transient necrosis, but the crop rapidly recovers (Cahoon et al. 2015; Dixon et al. 2014; Vann et al. 2017a). However, mixtures of dicamba and glufosinate are not approved for use (Anonymous 2018).

Another problematic C4 weed common in a variety of Midsouth crop production systems is barnyardgrass. Barnyardgrass is originally native to Europe and Asia and has been recognized as a problem weed in 36 crops across 61 countries (Holm et al. 1991). Riar et al. (2013) reported from a survey of Arkansas crop consultants that respondents ranked barnyardgrass as the fifth most economically important weed, in part because it can emerge from mid-spring until 7 weeks after cotton emergence. When barnyardgrass is allowed to compete with cotton for 6, 9, 12, and 25 weeks, it can diminish cotton yields by 21, 59, 90, and 97%, respectively (Keeley and Thullen

1991). Bagavathiannan et al. (2012) reported barnyardgrass seed production of 35,500 seeds plant^{-1} when it was allowed to emerge with a cotton crop.

Dicamba shares a similar chemical structure with the naturally occurring plant hormone indole-3-acetic acid (IAA), which is a member of the auxin class of hormones (Kirby 1980). Auxins are responsible for regulating cell division and elongation, along with a host of other processes that include floral meristem differentiation, root formation, and apical dominance when naturally occurring in the plant. Synthetic derivatives of IAA evoke the same effects as natural IAA, but at a much more intense level, over a longer period because they are more stable in the plant than natural IAA. Effects of synthetic IAA are particularly effective in dicotyledonous species, causing disruption of growth and development processes, and ultimately resulting in plant death (Grossman 2010).

For more than 60 years, synthetic auxin herbicides have been used to control broadleaf weeds in cereal crops (Green and Owen 2011). Transgenic cotton resistant to glufosinate and dicamba (event MON88701) was recently deregulated in the United States (Brinker et al. 2014). Resistance to glufosinate and dicamba was created by inserting a stacked combination of genes called dicamba monooxygenase (*dmo*) from *Stenotrophomonas maltophilia* and the bialaphos resistance (*bar*) gene from *Streptomyces hygroscopicus* (Brinker et al. 2014). The *dmo* gene codes for a monooxygenase enzyme that demethylates absorbed dicamba into two compounds that have no herbicidal effect on the plant (Behrens et al. 2007). Bollgard II® XtendFlex™ cotton (MON88701 by MON88913 by MON15985) that possesses traits conferring resistance to dicamba, glyphosate, glufosinate, and lepidopteran insects became commercially available in 2015 (Anonymous 2015b). This gives growers the ability to apply dicamba, glufosinate, and glyphosate POST for control of broadleaf weeds.

Minimal research has been published on salvage options with label-approved herbicide mixtures in dicamba-resistant cotton, to date. Therefore, one objective of this research was to determine the best timing for a sequential application in order to effectively control larger weeds. A second objective was to determine the best combination of glyphosate, glufosinate, and dicamba for control of the problematic weeds Palmer amaranth and barnyardgrass in dicamba-resistant cotton. It was hypothesized that a 7-day interval between applications would provide greater control of problematic weeds than a 14-day interval.

MATERIALS AND METHODS

A salvage weed control experiment was conducted in 2017 at the Rohwer Research Station (RRS) near Watson, AR, on an Hebert silt loam (Fine-silty, mixed, active, thermic Aeric Epiaqualfs). A crop tolerance experiment was also conducted in 2017 at the Lon Mann Cotton Research Station (LMCRS) near Marianna, AR, on a Memphis silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs) to assess the tolerance of dicamba-resistant cotton to the salvage treatments tested in the weed control experiment. The weed control experiment was repeated in 2018 at LMCRS near Marianna, AR, on a Zachary soil (Fine-silty, mixed, active, thermic Typic Albaqualfs), as well as on-farm at Marion, AR, on a Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs). Cotton was planted at each location in 2018 so weed control and crop injury could be assessed simultaneously.

For all trials, the experimental design was a single-factor randomized complete block with four replications at RRS and LMCRS (2017 and 2018) and three replications at Marion. The factor of herbicide treatment was evaluated. Treatments consisted of a single-pass salvage application or sequential salvage applications made 7 or 14 days apart. The first application was at the same time for all treatments, with the second application 7 or 14 days later, for respective treatments. In all experiments, a nontreated was included for comparison. In the 2018 experiment at LMCRS, a weed-free control was included as a comparative baseline for cotton height and yield. Treatments included dicamba (Engenia® herbicide, BASF Corporation, Florham Park, NJ), glufosinate (Liberty, BASF Corporation, Florham Park, NJ), and glyphosate (Roundup PowerMAX® II herbicide, Bayer CropScience, St. Louis, MO) in various combinations in 2017. In 2018, single application treatments were added to evaluate control compared to sequential application treatments. A list of treatments is displayed in Table 1.

At RRS, no cotton was planted, but Deltapine® 1518B2XF (Bayer CropScience, St. Louis, MO) dicamba-resistant cotton was planted in both years at LMCRS and at Marion at 9.8 seeds m^{-1} of row. Plots at RRS and LMCRS were 3.9 by 9.1 m, with the center 1.9 m of each plot receiving treatments, creating a weedy check between all plots. Plots at Marion were 1.9 by 9.1 m and the entire area was treated. At RRS, the first salvage application was made when Palmer amaranth was 28 to 33 cm (30 plants m^{-2}) and barnyardgrass was 30 to 35 cm (20 plants m^{-2}). At LMCRS 2017, cotton was at the five-leaf growth stage at application. At LMCRS 2018 and Marion, the first salvage application was made when Palmer amaranth was 15 to 20 cm (35 and 40 plants m^{-2}, respectively), and barnyardgrass was 18 to 23 cm (only present at LMCRS, 40 plants m^{-2}). For 2018 locations, cotton was at the two-leaf growth stage at Marion and the three-leaf growth stage at LMCRS at the time of the first application.

The experiment at the Marion location was terminated after weed control and crop injury ratings were collected due to drought and no irrigation being available. In 2017 at LMCRS, the experiment was terminated after crop injury data collection because late planting would not allow for crop maturity. Soil test reports at LMCRS indicated concentrations of 146 kg ha^{-1} of phosphorous and 278 kg ha^{-1} of potassium were present in the experimental area. Soil fertility programs at LMCRS and RRS and crop management practices at LMCRS were followed according to recommendations prescribed by University of Arkansas Extension (Robertson et al. 2018). Following the final application for each treatment at LMCRS 2018, no more weed control practices were attempted. For the weed-free check, standard herbicide recommendations for the state of Arkansas were followed (Scott et al. 2018). The final POST application of dicamba (560 g ae ha^{-1}) + acetochlor (Warrant, Bayer CropScience, St. Louis, MO) (1260 g ai ha^{-1}) was made to the weed-free check at the same time as the second application to the 14-day-interval salvage treatments.

All herbicide applications were made using a Bowman Mudmaster (Bowman Manufacturing, Newport, AR) with 110015 AirMix® nozzles (Greenleaf Technologies, Covingtion, LA) for treatments without dicamba and 110015 TTI (TeeJet Technologies, Wheaton, IL) nozzles for treatments containing dicamba. Nozzles were spaced at 48 cm and calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa. Salvage application, planting, and harvest dates for each experimental site are displayed in Table 2.

Crop injury was assessed after each treatment at locations where cotton was planted on a scale of 0 to 100, with 0 being no crop injury and 100 being crop death (Frans and Talbert 1977). Weed control ratings were collected separately for Palmer amaranth and barnyardgrass 21 days after the final treatment (21 DAFT) was applied for each respective treatment. Ratings were on a scale of 0 to 100, with 0 being no weed control and 100 being complete death of the species being evaluated. At 42 days after the final treatment (42 DAFT), heights of five random cotton plants were measured at LMCRS in 2018. Heights of five random cotton plants were measured in nontreated and weed-free plots at the same time as treatments where a single application was made (i.e. 42 days after the initial salvage application). Seedcotton yields were collected for LMCRS 2018 using a small-plot cotton picker to harvest the treated rows of each plot.

Statistical Analysis. A single factor analysis of variance was used to evaluate data using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, Cary, NC). A beta distribution was assumed for weed control and a gamma distribution for plant height and seedcotton yield (Gbur et al. 2012). Data were analyzed jointly for Palmer amaranth control at LMCRS 2018 and Marion, with replication nested within experimental location and treated as random effects in the model. Weed control at RRS and barnyardgrass control at LMCRS 2018 were analyzed alone, with replication considered a random effect due to differences in weed size at the time of application between 2017 and 2018 locations. Contrast analyses were conducted to determine differences in weed control, cotton height, and seedcotton yield based on interval between salvage applications and herbicides used in salvage treatments. Means were separated based on Fisher's protected LSD (P=0.05).

RESULTS AND DISCUSSION

Weed control levels at RRS in 2017 were lower than desirable (Table 3) because of the large weed size at application (28- to 35-cm). To create a more realistic salvage situation in 2018, cotton was planted at both locations, and all plots remained untreated until Palmer amaranth reached 15- to 20-cm in height. Since weeds were smaller at application than in 2017, single application treatments were added to evaluate the importance of sequential applications, even with smaller weeds.

Palmer amaranth control. Because of differences in weed size, Palmer amaranth control data for RRS were analyzed separately from Marion and LMCRS, where data were analyzed jointly. As previously mentioned, control levels at RRS in 2017 were low. Only glyphosate + glufosinate followed by (fb) the same herbicides 7 days later and glyphosate + dicamba fb the same herbicides 7 days later provided >80% control of Palmer amaranth. Although glyphosate + glufosinate fb the same mixture 7 days later showed 88% control 21 DAFT (Table 3), this is not an acceptable level as noted by Barber et al. (2015), and further treatments would be needed to control escapes.

With the exception of glyphosate applied alone and the single application of glufosinate at the lower rate (595 g ai ha^{-1}), all treatments in 2018 provided ≥89% Palmer amaranth control 21 DAFT (Table 4). Glyphosate provided only 10% control because both locations have populations of glyphosate-resistant Palmer amaranth, although it appears that about 10% of plants are still susceptible to glyphosate at these locations (personal observation). Numerically, the greatest control was provided by glyphosate + glufosinate fb dicamba 7 days later (98%). All but two, two-pass treatments provided ≥91% control 21 DAFT, and five two-pass treatments resulted in ≥95% control. No single application provided equal control to sequential applications of glufosinate, glyphosate + dicamba, or glyphosate + glufosinate fb dicamba, with the exception of dicamba applied once at 1120 g ae ha^{-1} (Table 4). Vann et al. (2017a) achieved similar levels of control of large Palmer amaranth with sequential applications of glufosinate and dicamba in various mixtures in dicamba-resistant cotton. Merchant et al. (2014) also controlled large Palmer amaranth at high levels with sequential applications of herbicide mixtures in 2,4-D-resistant cotton.

Contrast analysis for 7-day interval and 14-day interval treatments was not significant for RRS (P=0.5818) (Table 5). For 2018 locations, where weeds were smaller at the time of treatment, however, the same analysis revealed an advantage in Palmer amaranth control with the 7-day interval treatments (P=0.0043) (Table 5). The difference in Palmer amaranth control due to sequential application interval suggests that regardless of salvage treatment used, a 7-day interval between applications will provide greater control of 15- to 20-cm Palmer amaranth than a 14-day interval.

Glyphosate + dicamba fb glufosinate and glyphosate + glufosinate fb dicamba, averaged over application intervals controlled Palmer amaranth at similar levels at RRS and the 2018 locations (P=0.5517, 0.4347) (Table 5), indicating there is no weed control penalty for applying dicamba in the first pass of a salvage treatment in cases where the Arkansas dicamba application cutoff date is approaching (ASPB 2019). Dicamba treatments and glufosinate treatments also were not different at both RRS and the 2018 locations (P=0.08317, 0.0819) (Table 5), suggesting equal effectiveness of the herbicides and that if a salvage situation occurs in dicamba-resistant cotton after the dicamba application cutoff date, or in glufosinate-resistant cotton, the crop can still be salvaged.

Barnyardgrass control. There was no barnyardgrass present at Marion, and due to differences in weed size, barnyardgrass control was analyzed separately between RRS and LMCRS. Barnyardgrass control at RRS was lower overall than Palmer amaranth control at that location (Table 3). This response was expected because treatments for this experiment were designed with a focus on controlling Palmer amaranth. For RRS, the greatest barnyardgrass control resulted from glyphosate + dicamba fb the same combination 7 days later (84%) (Table 3). Sequential applications of glyphosate + glufosinate provided 76% control, regardless of interval between applications. Barnyardgrass control averaged over all treatments was 61% (Table 3). A lack of herbicide coverage caused by a thick weed canopy and the upright leaf angle of barnyardgrass, compounded with large weed size at application likely contributed to poor control.

Barnyardgrass control at LMCRS was much higher than RRS. All two-pass treatments provided ≥90% control, except glyphosate + glufosinate fb dicamba at either second application interval (Table 4). Glyphosate + dicamba fb the same treatment 14 days later and glyphosate + dicamba fb glufosinate 14 days later controlled barnyardgrass 99%. The only single-pass treatment that provided exceptional control of barnyardgrass was glyphosate at 1550 g ae ha^{-1} (98%), which was expected (Table 4). Werth et al. (2008) observed glyphosate to be highly efficacious on reducing barnyardgrass densities in glyphosate-resistant cotton. While this may be a viable single-pass treatment for controlling larger barnyardgrass in cotton, it is likely that glyphosate-resistant weed species will also be present, requiring the use of a herbicide with an alternative site of action.

Contrast analysis between second application interval timings for barnyardgrass control revealed no differences for RRS (P=0.9538) (Table 5). For LMCRS, where barnyardgrass was 10 to 12 cm shorter at application, barnyardgrass was controlled at higher levels at the 14-day second application interval compared to the 7-day application interval (P=0.0005). Mean control for the 14-day interval was 6 percentage points higher than the 7-day interval (98 vs 92%) (Table 5).

Cotton response. Injury to cotton was variable and never greater than 10% for any location; therefore, statistical analysis was not conducted (data not shown). Symptoms of phytotoxicity were observed; however, these symptoms lessened within 5 to 7 days for all treatments where cotton was injured. Comparable levels of injury and similar patterns in cotton recovery have been observed when combinations of dicamba and glufosinate or glyphosate were applied to dicamba-resistant cotton (Vann et al. 2017a; Cahoon et al. 2015).

Cotton height was measured 42 DAFT in 2018. All cotton receiving a two-pass treatment was taller than the weed-free treatment likely due to etiolation (Table 6). Single pass treatment heights were generally shorter than the two-pass treatments and likely correspond to poor weed control (Table 6). Two-pass treatments differed in height between 7- and 14-day second POST intervals (P=0.0354) (Table 7). Height increased as the interval between applications increased likely because cotton was etiolating over the period that weeds were present, attempting to compete for light. Similar changes in cotton heights with weed competition were noted by Buchanan et al. (1977).

Seedcotton yield. With the exception of glufosinate (1190 g ai ha^{-1}), no other one-pass treatment yielded as high as the weed-free check (Table 6). Although it did not differ from the weed-free treatment, cotton treated with glyphosate + dicamba fb the same treatment 14 days later (3830 kg ha^{-1}) yielded slightly higher than the weed-free treatment (3730 kg ha^{-1}). Cotton receiving the same treatment at the 7-day interval between applications yielded nearly the same (3720 kg ha^{-1}) as the weed-free check (Table 6). Cotton receiving these two treatments likely produced comparable yields to the weed-free check because they both provided total weed control of 97%, averaged over both weed species 21DAFT, which is higher than any other treatment (Table 3).

Contrast analysis showed no difference in yield between application intervals for the two-pass treatments (P=0.5976). Mean yields for the 7- and 14-day interval treatments did not differ from the weed-free check, either (P=0.8396, 0.6122). Yield from cotton treated with two-pass treatments containing dicamba did not differ from treatments that lacked dicamba (P=0.5369) (Table 7). No differences between treatments with and without dicamba suggests that growers can control Palmer amaranth and barnyardgrass in a salvage situation without using dicamba to recover a large portion of potential yield.

Practical Implications. Weed size is critical in controlling weeds in any situation, especially a salvage situation because weed control with herbicides decreases as weed size increases (Jordan et al. 1997; Mellendorf et al. 2013). These data suggest that salvage treatments become less effective as weed sizes increase.

Overall, the best treatment for controlling both Palmer amaranth and barnyardgrass was glyphosate + dicamba fb the same treatment 7 days later. This was consistent regardless of site year (Tables 3 and 4). Results of this experiment also indicate that growers have the flexibility to choose a treatment that best fits a specific situation. For the 2018 locations, Palmer amaranth was controlled at higher rates when the second application of a two-pass system was delayed 7 days (96%) compared to 14 days (92%) (Table 5). However, for barnyardgrass, allowing 14 days between applications provided 98% control, whereas a 7-day period between treatments provided 92% control (Table 5).

Current laws in Arkansas state that dicamba can legally be applied until May 25 each spring, but not in mixture with glyphosate (ASPB 2019). Since salvage situations typically occur early in the season, it is possible that a salvage treatment may be initiated within the legal use period of dicamba, and the subsequent application, 7 or 14 days later, would be outside the legal use period. Treatments of glyphosate + dicamba fb glufosinate controlled Palmer amaranth 69 and 94% in 2017 and 2018, respectively, compared to treatments of glyphosate + glufosinate fb dicamba, which controlled Palmer amaranth 65 and 95% in the same respective years (Table 5). Control was not different between the two herbicide combinations in either year, indicating growers would likely face no weed control penalty for applying dicamba in the first pass of a salvage treatment instead of the second.

Should a salvage situation occur after the dicamba cutoff date or in a glufosinate-resistant cultivar, there are also options for salvaging the crop, as long as weeds are no taller than 20- to 25-cm. For example, the single application of glufosinate at 1190 g ai ha^{-1} and all two-pass treatments that did not contain dicamba controlled Palmer amaranth an average of 93% in 2018 (Table 5). Seedcotton yield when comparing all two-pass treatments with and without dicamba differed by 260 kg ha^{-1}, which was not significant (Table 7).

Overall, there was an apparent relationship between treatments that controlled both weed species at the highest levels and seedcotton yield. Glyphosate + dicamba fb the same treatment at either application interval averaged 97% total weed control but did not differ from two applications of glufosinate (Table 4). Yields comparable to the weed-free check were not expected; however, results indicate yield potential can be recovered with an effective salvage treatment. These results should in no way discourage the use of residual herbicides and timely POST applications for weed control in cotton.

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TABLES

Table 1. Treatments for salvage and crop tolerance experiments at all locations.[a,b,c]

Program	Rate	Second app.
	g ae ha^{-1}	days
Dicamba	560	-
Dicamba	1120	-
Glufosinate	595	-
Glufosinate	1190	-
Glyphosate	1550	-
Glufosinate fb same	595 fb 595	7
Glufosinate fb same	595 fb 595	14
Glyphosate + glufosinate fb same	1065 + 595 fb same	7
Glyphosate + glufosinate fb same	1065 + 595 fb same	14
Glyphosate + dicamba fb same	1065 + 560 fb same	7
Glyphosate + dicamba fb same	1065 + 560 fb same	14
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	7
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	14
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	7
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	14

[a] Single-pass treatments were not evaluated in 2017.
[b] Abbreviations: app., application; fb, followed by; same, same herbicide(s) and rate(s) used in the second application that were used in the first application
[c] Glufosinate rates are listed in g ai ha^{-1}

Table 2. Planting, salvage herbicide application, and harvest dates for trials in 2017 and 2018.[a]

	Dates of importance				
Location	Planting	Initial application	7-day interval	14-day interval	Harvest
LMCRS 2017	June 26	July 17	July 24	July 31	
RRS 2017		June 15	June 22	June 29	
LMCRS 2018	May 24	June 12	June 19	June 26	Oct 8
Marion 2018	May 16	June 6	June 13	June 20	

[a] Abbreviations: LMCRS, Lon Mann Cotton Research Station near Marianna, AR; RRS, Rohwer Research Station near Rohwer, AR; Marion, on-farm location in Marion, AR

Table 3. Mean control of Palmer amaranth and barnyardgrass salvage treatments 21 DAFT at Rohwer Research Station near Rohwer, AR in 2017.[a,b,c]

Program	Rate	Second app.	Control 21 DAFT: Palmer amaranth	Control 21 DAFT: Barnyardgrass
	g ae ha^{-1}		%	%
Glufosinate fb same	595 fb 595	7	44 e	50 ef
Glufosinate fb same	595 fb 595	14	54 de	65 c
Glyphosate + glufosinate fb same	1065 + 595 fb same	7	88 a	76 b
Glyphosate + glufosinate fb same	1065 + 595 fb same	14	73 c	76 b
Glyphosate + dicamba fb same	1065 + 560 fb same	7	81 ab	84 a
Glyphosate + dicamba fb same	1065 + 560 fb same	14	75 bc	56 de
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	7	70 c	61 cd
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	14	69 c	66 c
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	7	56 d	32 g
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	14	73 c	45 f
Herbicide program			<0.0001	<0.0001

[a] Abbreviations: DAFT, days after final treatment; app., application; fb, followed by; same, same herbicide(s) and rate(s) used in the second application that were used in the first application

[b] Glufosinate rates are listed in g ai ha^{-1}

[c] Means within a column followed by the same letter within a column are not different according to Fisher's protected LSD (P=0.05)

Table 4. Mean control of Palmer amaranth and barnyardgrass salvage treatments at Lon Mann Cotton Research Station near Marianna, AR and on-farm in Marion, AR.[a,b,c,d]

			Control 21 DAFT	
Program	Rate	Second app.	Palmer amaranth	Barnyardgrass
	g ae ha^{-1}		%	
Dicamba	560	-	89 bc	0 i
Dicamba	1120	-	96 ab	0 i
Glufosinate	595	-	80 c	58 h
Glufosinate	1190	-	89 bc	74 g
Glyphosate	1550	-	10 d	98 a
Glufosinate fb same	595 fb 595	7	97 a	90 de
Glufosinate fb same	595 fb 595	14	94 ab	96 abc
Glyphosate + glufosinate fb same	1065 + 595 fb same	7	93 ab	92 bcd
Glyphosate + glufosinate fb same	1065 + 595 fb same	14	89 bc	98 a
Glyphosate + dicamba fb same	1065 + 560 fb same	7	97 a	97 ab
Glyphosate + dicamba fb same	1065 + 560 fb same	14	95 ab	99 a
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	7	97 a	91 cde
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	14	89 bc	99 a
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	7	98 a	85 ef
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	14	91 ab	79 fg
Herbicide program			<0.0001	<0.0001

[a] Abbreviations: DAFT, days after final treatment; Second app., interval between initial application and second application; fb, followed by; same, same herbicide(s) and rate(s) used in the second application that were used in the first application

[b] Treatments that consisted of only one application are denoted by (-) in the “Second app.” column

[c] Glufosinate rates are listed in g ai ha^{-1}

[d] Means within a column followed by the same letter within a column are not different according to Fisher’s protected LSD (P=0.05)

Table 5. Significance of contrast statements between 7-day and 14-day interval salvage treatments, glyphosate + dicamba fb glufosinate and glyphosate + glufosinate fb dicamba, and treatments containing dicamba and treatments containing no dicamba.[a,b,c,d]

	Palmer amaranth control 21 DAFT			
Contrast	RRS	Means	2018	Means
7-day vs 14-day application interval	0.5818	70 vs 69	0.0043*	96 vs 92
Dicamba applied first vs dicamba applied last	0.5517	69 vs 65	0.4347	94 vs 95
Dicamba treatments vs glufosinate treatments	0.08317	71 vs 67	0.0819	95 vs 93
	Barnyardgrass control 21 DAFT			
Contrast	RRS	Means	LMCRS	Means
7-day vs 14-day application interval	0.9538	62 vs 62	0.0005*	92 vs 98

[a] Abbreviations: DAFT, days after final treatment; RRS, Rohwer Research Station near Rowher, AR; 2018, Marion, AR on-farm location and Lon Mann Cotton Research Station near Marianna, AR

[b] Significant P values (P=0.05) are indicated by (*)

[c] Dicamba applied at 560 g ae ha^{-1}, glufosinate applied at 595 g ai ha^{-1}, and glyphosate applied alone were excluded from contrast comparing dicamba and glufosinate treatments.

Table 6. Cotton height and seedcotton yield at Lon Mann Cotton Research Station near Marianna, AR in 2018.[a,b,c,d]

Program	Rate	Second app.	Cotton: Height 42 DAFT	Cotton: Yield
	g ae ha^{-1}		cm	kg ha^{-1}
Nontreated			53 e	
Weed-free			100 b	3730 a
Dicamba	560	-	69 d	750 d
Dicamba	1120	-	74 cd	1330 c
Glufosinate	595	-	99 b	1910 bc
Glufosinate	1190	-	100 b	2570 ab
Glyphosate	1550	-	80 c	590 d
Glufosinate fb same	595 fb 595	7	102 ab	3330 a
Glufosinate fb same	595 fb 595	14	114 a	3240 ab
Glyphosate + glufosinate fb same	1065 + 595 fb same	7	109 ab	3130 ab
Glyphosate + glufosinate fb same	1065 + 595 fb same	14	112 ab	3540 a
Glyphosate + dicamba fb same	1065 + 560 fb same	7	109 ab	3720 a
Glyphosate + dicamba fb same	1065 + 560 fb same	14	116 a	3830 a
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	7	105 ab	3270 ab
Glyphosate + dicamba fb glufosinate	1065 + 560 fb 595	14	116 a	3640 a
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	7	109 ab	3340 a
Glyphosate + glufosinate fb dicamba	1065 + 595 fb 560	14	112 ab	3660 a
Herbicide program			<0.0001	<0.0001

[a] Abbreviations: DAFT, days after final treatment; Second app., interval between initial application and second application; fb, followed by; same, same herbicide(s) and rate(s) used in the second application that were used in the first application

[b] Treatments that consisted of only one application are denoted by (-) in the “Second app.” Column

[c] Glufosinate rates are listed in g ai ha^{-1}

[d] Means within a column followed by the same letter within a column are not different according to Fisher’s protected LSD (P=0.05)

Table 7. Significance of contrast statements between salvage treatments with 7-day and 14-day application intervals and weed-free check against each interval.

	Cotton height 42 DAFT (cm)	
Contrast	LMCRS	Means
7-day vs 14-day application interval	0.0354*	107 vs 114
Weed-free vs 7-day interval	0.1872	100 vs 107
Weed-free vs 14-day interval	0.0128	100 vs 114
	Seedcotton yield (kg ha^{-1})	
Contrast	LMCRS	Means
7-day vs 14-day application interval	0.5976	3350 vs 3570
Weed-free vs 7-day interval	0.8396	3730 vs 3350
Weed-free vs 14-day interval	0.6122	3730 vs 3570
Dicamba vs non-dicamba	0.5369	3570 vs 3310

[a] Abbreviations: DAFT, days after final treatment; LMCRS, Lon Mann Cotton Research Station near Marianna, AR

[b] Significant P values (P=0.05) are indicated by (*)

[c] Dicamba applied at 560 g ae ha^{-1}, glufosinate applied at 595 g ai ha^{-1}, and glyphosate applied alone were excluded from contrast comparing dicamba and glufosinate treatments.

CHAPTER 4

Effect of Application Parameters and Dicamba Rate on Two Palmer amaranth Populations

Throughout eastern Arkansas, Palmer amaranth resistant to protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Group 14) has become widespread. Although most PPO-resistant Palmer amaranth populations possess a target-site mutation conferring resistance at this site of action, some populations now contain a metabolic resistance mechanism to fomesafen (Group 14). Once metabolic resistance manifests, plants may also be tolerant to other herbicides and sites of action. Dicamba can now be applied postemergence in many areas of the country due to the recent release of dicamba-resistant cotton and soybean varieties. To evaluate whether varying spray parameters affected control of PPO-resistant Palmer amaranth, field trials were conducted in 2017 and 2018 at the Lon Mann Cotton Research Station near Marianna, AR, and on-farm in Marion, AR. The experiment was designed as a four-factor split plot organized as a randomized complete block. Split plot factors included dicamba rate, nozzle type, and carrier volume, with a whole plot factor of Palmer amaranth population. Dicamba was applied at 560 or 1120 g ae ha^{-1} through 110015 TTI or AirMix nozzles at 70 or 140 L ha^{-1} to PPO-resistant or PPO-susceptible Palmer amaranth. Palmer amaranth control 14 days after treatment (DAT) was influenced by an interaction between population and carrier volume. PPO-resistant Palmer amaranth control at 14 DAT was 81% regardless of carrier volume, compared to 90% control at 70 L ha^{-1} and 95% control at 140 L ha^{-1} of the PPO-susceptible population. An interaction between nozzle type and carrier volume influenced Palmer amaranth control 21 DAT, where AirMix nozzles at 140 L ha^{-1} controlled Palmer amaranth at a greater level (94%) than any other nozzle and carrier volume combination (≤90%). An interaction between population and dicamba rate influenced Palmer amaranth relative density 21 DAT. PPO-resistant Palmer amaranth showed a higher rate of

survival than PPO-susceptible Palmer amaranth at both dicamba rates, relative to the nontreated check. Results concur with those of other research that suggest PPO-resistant Palmer amaranth is harder to control with dicamba. Otherwise, increasing carrier volume affected overall Palmer amaranth control to a greater degree than any other factor.

Nomenclature: Dicamba; Palmer amaranth, *Amaranthus palmeri* S. Wats.; cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.

Key Words: Herbicide resistance, dicamba, PPO-resistance, nozzle type, carrier volume

INTRODUCTION

First confirmed in Arkansas in 2011, protoporphyrinogen oxidase (PPO)-inhibiting herbicide-resistant Palmer amaranth is now widespread throughout the crop-producing region of the state (Salas et al. 2016; Varanasi et al. 2018). The resistant populations in this area mostly possess a target-site resistance to all PPO-inhibiting herbicides, as well as resistance to other common herbicides like glyphosate and acetolactate synthase (ALS)-inhibiting chemistries (Varanasi et al. 2018; Heap 2019). Some populations of PPO-resistant Palmer amaranth have been noted as harder to control with other herbicides that are effective on PPO-susceptible Palmer amaranth (Schwartz-Lazaro et al. 2017; Steckel 2018).

In 2018, metabolic resistance of Palmer amaranth to fomesafen was confirmed in Arkansas (Varanasi et al. 2018a). A short time thereafter, metabolic resistance to the very long chain fatty acid inhibitor *S*-metolachlor was also identified in Arkansas (Brabham et al. 2019). While metabolic resistance to dicamba has not been identified in Arkansas Palmer amaranth, the discovery of metabolic resistance mechanisms in Arkansas suggests that resistance to other herbicide sites of action could be building (Yu and Powles 2014).

Dicamba-resistant cotton was released for commercial use in 2015 and dicamba-resistant soybean was released shortly thereafter. With the release of this new technology, certain label restrictions were required for the products approved for use in these cropping systems to limit the off-target movement of dicamba to sensitive crops. These limitations include nozzle type and spray volume specifications, among others (Anonymous 2018a; Anonymous 2018b).

Herbicide application is influenced by application pressure, orifice size, nozzle design, and characteristics of the spray solution. The droplet sizes a nozzle produces are commonly classified by the volume median diameter (VMD), or D_{v50} of spray droplets, which is the value of the median size of spray droplets produced (i.e. 50% of droplets are larger and 50% are smaller than this value). Increasing VMD can contribute to decreased particle drift when herbicides are applied, but in turn it can decrease the efficacy of some herbicides (Meyer et al. 2016). Another way to classify droplets produced by a nozzle is by examining the relative span (RS) of the droplet spectrum. The RS is a unitless measurement that represents the total variation in droplet sizes produced by a nozzle, where a smaller number indicates less variation in droplet size. Herbicide droplet size can also be affected by the product being applied in the spray solution (Mueller and Womac 1997). When comparing the VMD of applications of glyphosate, glufosinate, and paraquat, Etheridge et al. (1999) determined that a smaller VMD was generated by glufosinate than the other two chemicals. Chemical mixtures can also play a role in altering the VMD of a spray solution. When glufosinate was applied alone with a Turbo TeeJet Induction (TTI) 11004 nozzle, a VMD of 617 µm was produced, but when glufosinate was mixed with glyphosate and dicamba and applied with the same nozzle type, a VMD of 877 µm was produced (Meyer et al. 2015).

Nozzles are designed to control spray angle, spray pattern, droplet size, and solution flow rate as precisely as possible. Nozzles are available that produce a variety of spray patterns, in a variety of orifice sizes (Anonymous 2014). Increased droplet size can be obtained by increasing the orifice size for any given nozzle (Nuyttens et al. 2007). In order to increase droplet size without altering orifice size or spray pressure, nozzles with an inlet above the orifice are produced. These are typically referred to as air induction or venturi-type nozzles and work by essentially impregnating spray droplets with air, making them larger and less likely to drift (Etheridge et al. 1999; Etheridge et al. 2001).

When water is sprayed through an AirMix 110015 nozzle at 276 kPa, a droplet size classification of medium (VMD 236-340 µm) is produced. At the same pressure with a TTI 110015 nozzle, a droplet size classification of ultra-coarse (VMD >665 µm) is produced. Daggupati (2007) found that AirMix 11003 nozzles covered 2.8, 4.6, and 6.9 percentage points more total ground area than TTI 11003 nozzles at 207, 276, and 344 kPa, respectively. In an experiment, Meyer et al. (2015) demonstrated that mixtures of dicamba and glyphosate do not vary from droplet size classifications obtained with water for two Venturi-type nozzles, one specifically being the TTI nozzle. Meyer et al. (2015) also found that increasing carrier volume from 94 to 187 L ha^{-1} increased spray coverage of a dicamba + glyphosate solution by 7% when averaged over three nozzle types.

Although not as important for the control of horizontally structured broadleaf weeds, smaller droplets adhere better to upright grasses, and therefore provide better control (McKinlay et al. 1974; Etheridge et al. 2001). Droplet size also plays a vital role in control levels provided by contact herbicides. When glufosinate and paraquat were applied to broadleaf signalgrass (*Urochloa platyphylla* [Munro ex C. Wright] R.D. Webster) and common cocklebur (*Xanthium strumarium* L.) with air induction (AI) nozzles (coarser droplets) and flat fan nozzles (finer droplets), decreased control was noted in treatments where AI nozzles were used (Etheridge et al. 2001). McKinlay et al. (1974) observed decreased paraquat efficacy on common sunflower (*Helianthus annuus* L.) as VMD increased. Meyer et al. (2015) also observed a decrease in control of Palmer amaranth, hemp sesbania (*Sesbania herbacea* [Mill.] McVaugh), velvetleaf (*Abutilon theophrasti* Medik.), and barnyardgrass (*Echinochloa crus-galli* [L.] P. Beauv.) with glufosinate as droplet size increased. Conflicting conclusions exist on the effect of droplet size and synthectic auxin efficacy. 2,4-D efficacy has been shown to decrease with increases in VMD

(McKinlay et al. 1972). These are similar findings to Way (1969) and Ennis and Williamson (1963), who observed that synthetic auxin efficacy increased as droplet size decreased. Meyer et al. (2015), however, noted no difference in efficacy of dicamba on Palmer amaranth, hemp sesbania, velvetleaf, and prickly sida (*Sida spinosa* L.) across VMD values ranging from 340 to 756 µm.

Another factor that can influence efficacy of a foliar-applied herbicide is the carrier volume, or amount of herbicide solution being applied per hectare (Knoche 1994). Creech et al. (2015) observed no difference in control of *Amaranthus* spp. when glyphosate was applied at 70, 94, 140, and 187 L ha^{-1}. However, in the same study, efficacy of 2,4-D on Amaranth and soybean increased with increases in carrier volume (Creech et al. 2015), which is similar to findings by Smith (1946). When dicamba was applied postemergence to actively growing weeds, Butts et al. (2018) observed a greater effect of droplet size on weed mortality with a carrier volume of 47 L ha^{-1} than when dicamba was applied at a carrier volume of 187 L ha^{-1}. Because weed control can be affected by a variety of application factors and a metabolic resistance mechanism has been discovered in PPO-resistant Palmer amaranth, the objective of this research was to determine whether or not there were differences in control of Palmer amaranth between two populations when spray parameters were varied.

MATERIALS AND METHODS

Field experiments were conducted on-farm in Marion, AR, on a Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs) with a PPO-resistant population of Palmer amaranth and at the Lon Mann Cotton Research Station (LMCRS) near Marianna, AR, on a Zachary soil (Fine-silty, mixed, active, thermic Typic Albaqualfs) with a PPO-susceptible population of Palmer amaranth in 2017 and 2018. The objective was to compare the efficacy of dicamba on two populations of Palmer amaranth when it is applied according to varying spray parameters. No crop was planted at either location in 2017, and in 2018 Deltapine® 1518B2XF (Bayer CropScience, St. Louis, MO) was planted at both locations at 9.8 seeds m^{-1} of row with 96-cm row spacing to provide a crop canopy. Plots for all experiments were 3.9 m wide by 9.1 m long, with only the center 1.95 m receiving herbicide applications, creating a weedy check between plots.

Experiments were designed as a split plot, four-factor factorial and included a nontreated as a basis for comparison. Split plot factors evaluated were nozzle type, carrier volume, and dicamba rate, with a whole plot factor of PPO-susceptible (LMCRS) or PPO-resistant (Marion) Palmer amaranth population. All herbicide treatments were applied to 15- to 20-cm tall Palmer amaranth using a Bowman Mudmaster (Bowman Manufacturing, Newport, AR) calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1} or 70 L ha^{-1} at 9.6 km h^{-1} with 276 kPa of pressure. Nozzle types evaluated were AirMix® 110015 (Greenleaf Technologies, Covingtion, LA) and TTI 110015 (TeeJet Technologies, Wheaton, IL), all at 48-cm nozzle spacing. It should be noted that neither nozzle used is approved for use on current dicamba labels (Anonymous 2018a; Anonymous 2018b). The dicamba herbicide Engenia® (BASF Corporation, Florham Park, NJ) was applied at 560 g ae ha^{-1} or 1120 g ae ha^{-1} in combination with glyphosate (Roundup PowerMAX® II herbicide, Bayer CropScience, St. Louis, MO) at 870 g ae ha^{-1}. 80 to 90% of Palmer amaranth at both sites was GR (data not shown). Spray characteristics for each nozzle, herbicide, and carrier volume combination are displayed in Table 1. Plots were rated 21 days after application (DAA) for Palmer amaranth control on a scale of 0 to 100, with 0 being no Palmer amaranth injury and 100 being death of all Palmer amaranth. Densities of surviving Palmer amaranth m^{-2} were also estimated at 21 DAA by counting the number of living Palmer amaranth in two 0.5-m^{-2} quadrats placed randomly in each plot.

Droplet size spectra for each nozzle, carrier volume, and herbicide combination were analyzed in a lowspeed wind tunnel at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE. Laser diffraction was used to detect particle size distribution with a Sympatec Helos Vario KR particle size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) equipped with a R7 lens. In order to analyze the width of the nozzle plume, a 121 linear actuator was used to move the nozzle across the laser. The laser was positioned 30 cm from the tip of the nozzle in a low speed wind tunnel with speeds of 24 km hr^{-1} during testing. The same spray solutions were evaluated through the same nozzles in the wind tunnel that were evaluated in field experiments. Each treatment was replicated three times in accordance with American Society of Agricultural and Biological Engineers S572.1.

Statistical Analysis. Means were separated using analysis of variance via the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). A beta distribution was assumed for Palmer amaranth control and relative density of Palmer amaranth (Gbur et al. 2012). Palmer amaranth densities were measured relative to the nontreated control to account for differences in natural weed density between experimental locations. Mean separation was based on Fisher's protected LSD (P=0.05).

RESULTS AND DISCUSSION

The effect of year was not significant for this experiment (P=0.4653); therefore, years were analyzed together. Both Palmer amaranth control at 14 and 21 DAT and Palmer amaranth density 21 DAT were influenced by several two-way interactions and main effects (Table 2). No three- or four-way interactions were significant for any parameter.

Palmer amaranth Control. Palmer amaranth control 14 DAT was influenced by main effects of population and carrier volume, as well as an interaction between population and carrier volume (Table 2). There was no interaction between nozzle type and carrier volume 14 DAT at P=0.05, but differences between means for this combination were nearly significant (P=0.0517) (Tables 2 and 3). Control of PPO-resistant Palmer amaranth with dicamba at Marion 14 DAT was 81% regardless of carrier volume, whereas at LMCRS, 90 and 95% control of PPO-susceptible Palmer amaranth was observed at 70 and 140 L ha^{-1}, respectively (Table 3).

By 21 DAT, similar control was observed for the interaction of population x carrier volume, but control levels were not significantly different (Tables 2 and 3). The interaction between nozzle type and carrier volume was not significant at P=0.05 14 DAT. There was a tendency (P=0.0517), however, for Palmer amaranth control to be 5 to 6 percentage points higher with AirMix nozzles at 140 L ha^{-1} than with other nozzle type and carrier volume combinations 14 DAT (Table 3). Greater control was likely observed with AirMix nozzles at 140 L ha^{-1} because they produced smaller droplets (VMD=360 µm) than TTI nozzles at the same carrier volume (VMD=727 µm), and therefore provided greater coverage of the leaf surface (Table 1).

A main effect of carrier volume and an interaction between nozzle type and carrier volume were significant 21 DAT (Table 2). At this timing, applications made with AirMix nozzles at 140 L ha^{-1} (VMD=360 µm) controlled Palmer amaranth 94%, whereas applications made with TTI nozzles controlled Palmer amaranth 90% and 89% at 70 L ha^{-1} (VMD=688 µm) and 140 L ha^{-1} (VMD=727 µm), respectively (Tables 1 and 3). These results indicate that carrier volume was more important for Palmer amaranth control in this experiment when smaller droplets were being produced. Meyer et al. (2016) observed greater control of glyphosate-resistant Palmer amaranth with dicamba + glyphosate at 94 L ha^{-1} (VMD=385 µm) than at 187 L ha^{-1} (VMD=487 µm), but his droplet size for the TTI nozzles was smaller than that used here.

Palmer amaranth density. Relative densities of Palmer amaranth 21 DAT were influenced by main effects of population, nozzle type, carrier volume, and an interaction between population and dicamba rate (Table 2). Averaged over all other factors, densities of Palmer amaranth relative to the nontreated were 2 percentage points lower when dicamba was applied with AirMix nozzles (9%) than TTI nozzles (11%), suggesting the smaller droplets produced by AirMix nozzles (VMD=336 to 362 µm), compared to TTI nozzles (VMD=683 to 734 µm), probably increased dicamba absorption by the plants (Tables 1 and 4).

For the main effect of carrier volume, treatments applied at 140 L ha^{-1} reduced Palmer amaranth densities to 9% relative to the nontreated, whereas treatments applied at 70 L ha^{-1} reduced densities to 11% relative to the nontreated (Table 4). The influence of carrier volume suggests that applying dicamba at 140 L ha^{-1} allows for greater coverage of the treated area than a carrier volume of 70 L ha^{-1}, again placing more dicamba on the leaf surface. The significant effects of nozzle type and carrier volume for relative Palmer amaranth density reflect the significant interaction between nozzle type and carrier volume for weed control 21 DAT, where AirMix nozzles at 140 L ha^{-1} provided greater control than all other nozzle type and carrier volume combinations due to greater coverage and smaller droplet sizes being produced (Tables 1, 3, and 4).

For the interaction between population and dicamba rate, the PPO-susceptible population at LMCRS was unaffected by dicamba rate. At this location, only 6 and 7% of treated Palmer amaranth, relative to the nontreated, survived dicamba application at 560 g ae ha^{-1} and 1120 g ae ha^{-1}, respectively (Table 4). Dicamb at 560 g ha^{-1} was likely so effective at LMCRS that no differences in density could be observed between the two rates. However, at Marion, with PPO-resistant Palmer amaranth, 19% of treated Palmer amaranth survived a dicamba application at 560 g ae ha^{-1}, and 14% of treated Palmer amaranth survived dicamba applied at 1120 g ae ha^{-1} (Table 4). Although the Palmer amaranth population at Marion appeared to be controlled at comparable levels to the population at LMCRS 21 DAT based on visible control ratings, relative density data indicate that the Marion population was more difficult to kill. Differences in weed densities between the two locations suggest that the Palmer amaranth population at Marion is more tolerant to dicamba than at LMCRS. Schwartz-Lazaro et al. (2017) found that PPO-resistant populations were less sensitive to dicamba in the greenhouse than PPO-susceptible populations. These findings are not unlike other research which suggests that multiple postemergence applications of dicamba may be required to control PPO-resistant Palmer amaranth (Steckel 2018).

Practical Implications. In this experiment, carrier volume was the most important factor in Palmer amaranth control with dicamba. In general, treatments applied at a carrier volume of 140 L ha^{-1} provided better control of Palmer amaranth than treatments applied at 70 L ha^{-1}, regardless of other factors. AirMix nozzles provided higher levels of Palmer amaranth mortality than did TTI nozzles, likely due to the smaller droplet size produced. However, current dicamba labels approved for postemergence use in cotton state that dicamba must be applied through nozzles that produce extremely coarse or larger droplets for Engenia and ultra-coarse droplets for Xtendimax (Anonymous 2018a; Anonymous 2018b). By increasing carrier volume, applicators can mitigate reduced levels of weed control caused by using a nozzle producing coarser droplets by increasing carrier volume.

Special attention should also be paid to Palmer amaranth control with dicamba in fields where PPO-resistance is suspected. Metabolic resistance to *S*-metolachlor was recently confirmed in the Marion population and it is possible that this metabolic resistance could potentially be the cause of reduced mortality of Palmer amaranth treated with dicamba at this location (Brabham et al. 2019). Because PPO-resistant populations have proven to be harder to control with dicamba, other weed control methods may need to be employed. Following best management practices to mitigate resistance is recommended to control PPO-resistant Palmer amaranth (Norsworthy et al. 2012).

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TABLES

Table 1. Mean spray characteristics as influenced by dicamba rate, nozzle type, and carrier volume.[a,b,c,d]

Dicamba rate	Nozzle	Carrier volume	Droplet spectra parameters: D_{v10}		D_{v50}		D_{v90}		Relative span	
g ae ha^{-1}		L ha^{-1}	µm	SE	µm	SE	µm	SE	-	SE
560	TTI	70	350	0.7	683	1.9	984	2.1	0.93	0.000
560	AirMix	70	156	0.7	342	1.8	565	2.0	1.20	0.006
560	TTI	140	383	2.4	720	0.9	1048	4.2	0.92	0.012
560	AirMix	140	170	0.5	362	0.8	579	0.8	1.13	0.000
1120	TTI	70	357	2.1	692	1.0	994	4.3	0.92	0.000
1120	AirMix	70	151	0.4	336	0.2	553	3.7	1.20	0.009
1120	TTI	140	381	0.7	734	3.9	1076	9.7	0.95	0.009
1120	AirMix	140	166	1.2	358	1.4	570	4.1	1.12	0.003

[a] Data are reported as means followed by the standard error (SE) of the mean

[b] All treatments contained glyphosate at a rate of 870 g ae ha^{-1}

[c] Abbreviations: D_{v10}, 10% of droplets are smaller than this value; D_{v50}, 50% of droplets are smaller than this value; D_{v90}, 90% of droplets are smaller than this value; TTI, Turbo TeeJet Induction

[d] All nozzles used were 110015 orifice size

Table 2. Significance of P-values for factor main effects and interactions for Palmer amaranth control and density averaged over site years.[a,b]

Source	Control		
	14 DAT	21 DAT	Density 21 DAT
Population	0.0003*	0.1311	<0.0001*
Nozzle type	0.0909	0.1380	0.0389*
Carrier volume	0.0199*	0.0159*	0.0177*
Dicamba rate	0.5198	0.0766	0.4247
Population x Nozzle type	0.4719	0.4594	0.3300
Population x Carrier volume	0.0117*	0.2701	0.2097
Population x Dicamba rate	0.8502	0.1232	0.0207*
Nozzle type x Carrier volume	0.0517	0.0022*	0.0928
Nozzle type x Dicamba rate	0.3325	0.2235	0.4799
Carrier volume x Dicamba rate	0.7780	0.3588	0.3886
Population x Nozzle type x Carrier volume	0.0772	0.3388	0.4307
Population x Nozzle type x Dicamba rate	0.7956	0.5760	0.7177
Population x Carrier volume x Dicamba rate	0.6810	0.1678	0.0760
Nozzle Type x Carrier volume x Dicamba rate	0.8522	0.6857	0.1628
Population x Nozzle type x Carrier volume x Dicamba rate	0.5082	0.5406	0.2870

[a]Abbreviation: DAT, days after treatment
[b]Asterisks (*) indicate significant treatment effects

Table 3. Palmer amaranth control as influenced by significant interactions of population x carrier volume and nozzle type x carrier volume.[a,b]

Factor	Control 14 DAT		Control 21 DAT	
	-------------- % -------------			
Population x Carrier volume				
Marion x 70 L ha^{-1}	81	c	88	
Marion x 140 L ha^{-1}	81	c	90	
LMCRS x 70 L ha^{-1}	90	b	89	
LMCRS x 140 L ha^{-1}	95	a	93	
Nozzle type x Carrier volume				
AirMix x 70 L ha^{-1}	86		88	b
AirMix x 140 L ha^{-1}	92		94	a
TTI x 70 L ha^{-1}	87		90	b
TTI x 140 L ha^{-1}	87		89	b

[a] Abbreviations: DAT, days after treatment; LMCRS, Lon Mann Cotton Research Station near Marianna, AR

[b] Means within a column followed by the same letter are not different according to Fisher's protected LSD at (P=0.05). Means for non-significant interactions of Population x Carrier volume 21 DAT and Nozzle type x Carrier volume 14 DAT presented for informational purposes.

Table 4. Palmer amaranth relative density 21 days after treatment as influenced by main effects of nozzle type and carrier volume, as well as the interaction of population x dicamba rate.[a,b]

Factor	Density	
	% of nontreated	
Nozzle type		
AirMix	9	b
TTI	11	a
Carrier volume		
70 L ha^{-1}	11	a
140 L ha^{-1}	9	b
Population x Dicamba rate		
Marion x 560 g ae ha^{-1}	19	a
Marion x 1120 g ae ha^{-1}	14	b
LMCRS x 560 g ae ha^{-1}	6	c
LMCRS x 1120 g ae ha^{-1}	7	c

[a] Densities of Palmer amaranth in nontreated plots were as follows, Marion 2017: 31 plants m^{-2}; LMCRS 2017: 40 plants m^{-2}; Marion 2018: 51 plants m^{-2}; LMCRS 2018: 36 plants m^{-2}

[b] Abbreviations: TTI, Turbo TeeJet Induction; LMCRS, Lon Mann Cotton Research Station near Marianna, AR

[c] Means within a column followed by the same letter are not different according to Fisher's protected LSD at (P=0.05).