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Optimization and Resistance Mitigation of Dicamba and Glufosinate in XtendFlex® Crops

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Optimization and Resistance Mitigation of Dicamba and Glufosinate in XtendFlex® Crops

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by

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Abstract

The commercial launch of XtendFlex® crops enabled the use of dicamba, glufosinate, and glyphosate in-season. Utilizing herbicides that target different sites of action within troublesome weeds has been a tactic proposed to mitigate the likelihood of target-site resistance evolving; however, if interactions of the herbicides are detrimental to control of weedy species the likelihood of metabolic resistance increases. The objective of this research was to: 1) optimize efficacy and economic benefits of dicamba, glufosinate, and glyphosate; 2) characterize any interactions that were observed; 3) understand the mechanisms responsible for the reductions in weed control; 4) attempt to overcome interactions that were detrimental to weed control; 5) identify if any Palmer amaranth populations were resistant to dicamba or glufosinate in Arkansas and identify alternative control methods. Label restrictions do not allow for mixtures of dicamba and glufosinate to be applied; therefore, evaluation of sequential application intervals and sequences were evaluated. When glufosinate was applied prior to dicamba from 6 hours to 7 days often a reduction in control was observed when compared to dicamba followed by (fb) dicamba or dicamba fb glufosinate at the 14-day interval. Utilizing ^{14}C-herbicides a reduction in dicamba translocation occurred when a prior glufosinate application was made and thus a reduction in dicamba translocation was attributed to reduction in Palmer amaranth control. When dicamba was applied prior to glufosinate a reduction in control was often observed when applications were made at intervals less than 7 days. The reduction in control was attributed to rapid reduction of Palmer amaranth groundcover following a dicamba application, thus allowing for less surface area for the later applied glufosinate to come in contact with. Generally, from field experiments, the use of dicamba fb dicamba at a 14- to 21-day interval or dicamba fb glufosinate at the 14-day interval provided the highest level of Palmer amaranth control and

highest net benefit to producers. Palmer amaranth populations in Arkansas were also found to harbor resistance to glufosinate and auxin herbicides. Alternative integrated weed management strategies (e.g. crop rotation, harvest weed seed control, cover crops, etc.) should be implemented to mitigate the spread of these biotypes as well to mitigate resistance evolving in other geographies.

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Published Papers

CHAPTER 4: Grant L Priess, Jason K Norsworthy, Andy Mauromoustakos, Thomas R Butts, Trenton L Roberts (2021) Effects of Applying Metabolic Inhibitors to Sequential Applications of Dicamba and Glufosinate. Submitted to: Agrosystems, Geosciences, Environment

CHAPTER 5: Grant L Priess, Jason K Norsworthy, Rodger B Farr, Andy Mauromoustakos, Thomas R Butts, Trenton L Roberts (2021) Impact of Auxin Herbicides on Palmer amaranth Groundcover. Weed Technol pp.1-32
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Chapter 1

Review of Literature

Troublesome weeds have evolved resistance to six herbicide sites of action in some parts of the world, allowing survival to most herbicides labeled in a particular crop (Heap 2020). For example, in the midsouthern United States, Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] has evolved resistance to acetolactate synthase (ALS) inhibitors, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, an enolpyruvyl-shikitmate-3-phosphate synthase inhibitor (glyphosate), mitosis inhibitors (dinitroanilines), photosystem II inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and very long-chain fatty acid elongase-inhibiting herbicides (Heap 2020). For some producers in the Mid-South, the only postemergence (POST) herbicide option in-crop for multiple-resistant Palmer amaranth control is glufosinate, dicamba, or 2,4-D. In the midsouthern U.S., growers commonly elect to plant genetically modified crops, engineered to have tolerance to effective POST herbicides. XtendFlex® crops enable producers to use dicamba, glyphosate, and glufosinate POST to control troublesome weed species. Broad adoption of XtendFlex® crops has exponentially increased the hectares treated with dicamba and glufosinate (USDA-NASS 2020). With a limited number of effective POST herbicides left to control multiple-resistant Palmer amaranth, an emphasis has been placed on mitigating the evolution of herbicide-resistance in this species. However, with the increase of dicamba and glufosinate use, a likewise increase in selection pressure and probability for resistance to evolve in Palmer amaranth is accrued.

To mitigate the evolution of herbicide-resistance in Palmer amaranth, it is essential to take an integrated approach. Integrated practices include, but are not limited to, cover crops, promoting canopy closure, decreasing row spacing, implementing narrow-windrow burning, zero-tolerance policies, weed seed destruction, crop rotation, inter-row cultivation, and deep tillage to reduce weed seedbank populations and to mitigate the evolution of herbicide resistance (Norsworthy et al. 2012). However, herbicides play an integral role in reducing weed seed proliferation. Optimizing efficacy of herbicides, utilizing two effective sites-of-action (SOA), and season-long herbicide programs continue to be a driving force for mitigating the evolution of herbicide resistance (Norsworthy et al. 2012). Therefore, more research is needed to optimize the efficacy of dicamba, glyphosate, and glufosinate in XtendFlex® crops. To accomplish this, a clear understanding of each herbicide SOA, symptomology, application restrictions, and how each interacts in mixture and sequential applications is needed.

Glufosinate. Glufosinate is a nonselective broad-spectrum POST herbicide traditionally used as a burndown option before crop planting (Coetzer et al. 2000). The utility and efficacy of glufosinate to control troublesome weed species such as glyphosate-resistant Palmer amaranth has made it a popular herbicide for incorporation into herbicide-resistant crops. The LibertyLink® cropping system utilizes glufosinate as an over-the-top POST herbicide option in canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton (*Gossipium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.]. Glufosinate use increased more than 5-fold in soybean from 2012 to 2017 (USDA-NASS 2020) because of the spread of glyphosate-resistant weeds (Culpepper et al. 2010; Reddy and Norsworthy 2010). However, with the escalation of glufosinate use, there is a potential for poor management that may result in the evolution of weeds with resistance to this herbicide.

Glufosinate is active in many plant species through competitive inhibition of glutamine synthetase, the enzyme that converts glutamate and ammonia to glutamine (Lea 1984). One theory is that the inhibition of glutamine synthetase results in an accumulation of ammonia in the plant (Tachibana et al. 1986), consequently inhibiting photosystem I and photosystem II reactions and destroying cell membranes through the production of reactive oxygen species (Sauer 1987). An alternative theory, proposed by Hess (2000), is that the inhibition of glutamine synthetase results in a circuitous route for disrupting photosynthesis, which is the primary reason for phytotoxicity. Seelye (1995) reported that ammonia accumulation was not the primary cause of phytotoxicity because when glutamine was added to glufosinate-treated plants, phytotoxicity decreased although ammonia levels remained high. Hess (2000) suggested that the reduction in amino donors (i.e., glutamate) for the glycolate pathway (glycolate » glyoxylate » glycine) leads to the breakdown of the transamination reaction of glyoxylate to glycine in the photorespiration cycle. The ultimate result is accumulation of phosphoglycolate, glycolate, and glyoxylate, which has been shown to inhibit ribulose-1,5 bisphosphate carboxylase/oxygenase (rubisco) and the light-dependent reaction in photosynthesis (González-Moro et al. 1997; Wendler et al. 1992; Wild and Wendler 1993). Although the mode-of-action of glufosinate is controversial, the chain of events following the inhibition of glutamine synthetase is not; end results observed are membrane disruption, cell leakage, tissue necrosis, and eventual plant death. Currently, no glufosinate-resistant weeds have been discovered in row-crop agriculture systems (Heap 2020).

Many factors can contribute to the efficacy of glufosinate. To optimize glufosinate efficacy, environmental conditions and application techniques should be considered. Glufosinate efficacy is dependent on environmental conditions at application. Glufosinate performs better when used in high light intensity environments (Ahrens 1994), on actively growing weeds with available water (Anderson et al. 1996), and in a humid climate (Coetzer et al. 2000). Glufosinate efficacy is also dependent on application techniques. Glufosinate is a contact herbicide; therefore, selecting a Medium to Very Coarse droplet producing nozzle and increasing the amount of spray volume per acre likewise increases efficacy (Etheridge et al. 2001; Meyer et al. 2015). It is unclear how the aforementioned factors will affect glufosinate efficacy when applied in mixture or sequence with dicamba.

Dicamba. Dicamba is a synthetic auxin in the benzoic acid family (Weed Science Society of America Group 4). Dicamba was primarily used as a preplant burndown herbicide or early POST in corn and grain sorghum (*Sorghum bicolor* L. Moench.) (Anonymous 2014). The deregulation of dicamba-tolerant cotton and soybean in 2015 and 2016, respectively, eventually led to the registration of XtendiMax® (Monsanto Corporation, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) for in-crop applications beginning in 2017. XtendiMax® and Engenia®, both commercial formulations of dicamba, led to increased use of the herbicide in the U.S. (Anonymous 2018; USDA-NASS 2020). With an increase in dicamba use nationwide, a likewise increase in selection pressure on weed populations is expected.

Extending the use of dicamba into soybean and cotton also increases the selection pressure on weed populations that emerge later in the growing season. Currently, three weed species have evolved resistance to dicamba in the United States, including kochia [*Bassia scoparia* (L.) A. J. Scott], waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), and prickly lettuce [*Latuca serriola* L.] (Heap 2020). The mechanism for dicamba resistance in kochia has been attributed to a 2-nucleotide base change, which results in a glycine to asparagine amino acid substitution in the highly conserved region of a AUX/idone-3acetic acid (IAA) protein, KsIAA16 (LeClere et al. 2018). The KsIAA16 mutation allows for a 30-fold increase in dicamba tolerance. The KsIAA16 mutation leads to cross-resistance of multiple auxin herbicides: dicamba, 2,4-dichlorophenoxyacetic acid (2,4-D), and fluroxypyr (LeClere et al. 2018). With a limited number of effective herbicides to control troublesome weeds like *Amaranthus* ssp., mitigating the evolution of synthetic auxin resistance is of the utmost importance.

Mitigating dicamba resistance in Palmer amaranth will be challenging. Tehranchian et al. (2017) found that after three generations of low-dose dicamba selection, the third generation of Palmer amaranth had nearly a 3-fold increase in tolerance to the herbicide. Further, a common waterhemp population was found to have a 3-fold increase in tolerance to dicamba (Bernards et al. 2012). With known hybridization between waterhemp and Palmer amaranth, gene flow between the two species could likely result in dicamba-resistant Palmer amaranth biotypes (Trucco et al. 2007). The mechanism of resistance in waterhemp is not well understood. However, Dellaferrera et al. (2018) found that pretreatment of a cytochrome P-450 monooxygenase enzyme inhibitor, piperonyl butoxide (PBO), reversed the dicamba and 2,4-D resistance found in smooth pigweed (*Amaranthus hybridus* L.) populations in Argentina. Therefore, if dicamba-resistant populations of Palmer amaranth emerge, the likelihood of metabolic resistance is high.

Glyphosate. Glyphosate-resistant soybean was commercially launched in 1996. The adoption of glyphosate-resistant crops was rapid. Herbicide programs quickly transformed from multiple SOA to utilizing only glyphosate. In 2004, the first glyphosate-resistant (GR) Palmer amaranth biotype was identified in Georgia (Culpepper et al. 2006). Currently, 26 states in the continental U.S. have reported finding GR Palmer amaranth (Heap 2020).

Glyphosate is an *N*-phosphonomethyl-modified derivative of glycine. Glyphosate binds to 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS) with a higher affinity than glycine, thus inhibiting the shikimate pathway in the chloroplast (Anderson et al. 1990; Dill et al. 2010; Duke and Powles 2008). The inhibition of the shikimate pathway is lethal to a broad spectrum of weed species. Glyphosate has a high efficacy on many weed species due to the detrimental downstream effects of inhibiting the shikimate pathway and the ability of the herbicide to translocate to the meristematic regions of growing plants (Tardif and Leroux 1991). Glyphosate is commonly used to control troublesome monocot species in the southern United States (Johnson et al. 2009). In the XtendFlex® system, glyphosate is recommended to be applied in mixture with dicamba for control of monocot species (Anonymous 2018).

Herbicide interactions in the XtendFlex® system. The process by which a single POST herbicide enters a plant is intricate and dependent on various physical, chemical, and plant-related factors. These processes quickly become convoluted when herbicides are applied in mixture or sequentially. Up-coming technologies that include stacked herbicide resistance, like XtendFlex® crops, require additional research to understand how to optimize the use of dicamba, glyphosate, and glufosinate in a single system.

There have been reports of interactions of glyphosate and glufosinate (Bethke et al. 2013, dicamba and glyphosate (Devkota and Johnson 2019, Hedges et al. 2018; Spaunhorst and Bradley 2013, and glufosinate and dicamba (Chahal and Johnson 2012; Vann et al. 2017). Results in the literature mentioned above are variable and exclusive to individual weed species. The aforementioned interactions evaluated can be influenced by a multitude of variables. Label restrictions do not allow for dicamba and glufosinate to be applied in mixture due to a decrease in spray solution pH and a likewise increase in the potential for dicamba volatility (Anonymous 2018). Therefore, additional research is needed to understand how to optimize the efficacy of dicamba, glyphosate, and glufosinate when applied sequentially.

Dicamba + glyphosate mixture. The addition of glyphosate to dicamba is commonly recommended for increased control of grass weeds (Anonymous 2018). The addition of glyphosate to dicamba can also increase efficacy on GR waterhemp. When glyphosate was added to dicamba, GR waterhemp control increased by 16- to 36-percentage points (Spaunhorst and Bradley 2013). Two hypotheses were formed to try to interpret why an increase in GR waterhemp efficacy was observed: first, the addition of two sites-of-action increased the stress placed on the weed; second, the addition of glyphosate acted as an adjuvant and increased dicamba absorption into the plant.

Contrary to the aforementioned increase in broadleaf control from the mixture of dicamba and glyphosate, dicamba can antagonize graminicides when applied in mixture. Hart and Wax (1996) observed a decrease in imazethapyr efficacy on shattercane [*Sorghum bicolor* (L.) Moench], giant foxtail (*Setaria faberi* Hermm), and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] when applied in mixture with dicamba. The decrease in efficacy was attributed to a reduction in imazethapyr absorption into the plant. The severity of antagonism was reduced when non-ionic surfactant was added or the imazethapyr rate was increased.

Dicamba antagonizes glyphosate efficacy on some grasses. Dicamba has been observed to reduce glyphosate efficacy on, but not limited to johnsongrass [*Sorghum halepense* (L.) Pers.] (Flint and Barrett 1989), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), wild oats (*Avena fatua* L.) (O'Sullivan and O'Donovan 1980), and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (Meyer and Norsworthy 2019). Flint and Barrett (1989) determined that the reduction in glyphosate efficacy when mixed with dicamba contributed to a decrease in glyphosate absorption through the johnsongrass cuticle. To avoid increasing the likelihood of herbicide resistance, antagonistic mixtures should be avoided, or rates should be optimized such that high levels of weed control are achieved.

Dicamba + glufosinate mixture. Even though the mixture of dicamba and glufosinate is not labeled for use over-the-top of XtendFlex® crops (Anonymous 2018), the mixture has been evaluated over various weed species (Joseph et al. 2018). Merchant et al. (2013) found that the mixture of dicamba and glufosinate increased the control of 10 weed species when compared to glufosinate or dicamba alone. However, Meyer et al. (2015) observed a reduction in barnyardgrass control when dicamba and glufosinate were applied in mixture compared to glufosinate alone. The interaction between dicamba and glufosinate is species-specific and can be impacted by the nozzle used to apply the mixture (Meyer et al. 2015). In general, an application of dicamba and glufosinate made with a Very Coarse nozzle provided an increase in efficacy of multiple weed species when compared to applications made from an Ultra Coarse nozzle (Meyer et al. 2015).

Sequential applications of dicamba and glufosinate. The potential for antagonism between dicamba and glufosinate when applied sequentially may be high. When contact and systemic herbicides are applied in mixture, it is common to observe antagonism due to the contact herbicide reducing translocation of the systemic herbicide. Burke et al. (2005) found a 52% and 50% reduction in clethodim efficacy on goosegrass (*Eleusine indica* spp.) when clethodim and glufosinate were applied in mixture and when glufosinate was applied 7 to 14 days prior to clethodim, respectively. The reduction in clethodim efficacy was attributed to a reduction in absorption and translocation of the herbicide. It is unlikely that applying glufosinate before dicamba in the XtendFlex® system would optimize efficacy as a reduction in absorption and systemic translocation of dicamba could be expected.

Conversely, growth-regulating herbicides have the potential to antagonize contact herbicides. O'Donovan and O'Sullivan (1982) found that dicamba caused a reduction in paraquat phytotoxicity to barley [*Hordeum vulgare* (L.) 'Summit']. This type of interaction is less understood because absorption and translocation were not affected. Thus, other mechanisms of antagonism may include the upregulation of detoxification enzymes. The upregulation of detoxification enzymes may be mitigated with the use of selective inhibitors; therefore, it is possible to overcome this form of antagonism.

Classifying herbicide interactions. The ultimate goal of understanding herbicide interactions is to determine if any interaction will optimize efficacy and mitigate the evolution of herbicide resistance. Many complicated techniques have been designed to analyze herbicide interactions in mixture (Hatzios and Penner 1985). Colby's method (Colby 1967) is a common and straightforward analysis to understand the interaction of a herbicide mixture (Besançon et al. 2018; Kohrt and Sprague 2017). However, Colby's method cannot be used to determine the interaction between two herbicides applied sequentially. Therefore, an interaction between two herbicides applied sequentially (i.e. dicamba and glufosinate) cannot be determined as antagonistic, synergistic, or additive. The efficacy of the herbicide sequence should be compared to sequential applications of the herbicide alone.

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Chapter 2

Response of Palmer amaranth to sequential applications of dicamba and glufosinate for the XtendFlex® system

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Abstract. The evolution of herbicide resistance in Palmer amaranth has left producers with only auxin herbicides and glufosinate as effective postemergence herbicide options in soybean and cotton in some geographies. An experiment was conducted in 2019 and 2020 including a total of six site-years and across four locations in Arkansas. The objective of the experiment was to determine the best sequence and timing interval of sequential applications of dicamba and glufosinate and to compare the sequential use of two sites of action (SOA) to single and sequential applications of dicamba and glufosinate alone. Data were analyzed by Palmer amaranth size: labeled (<10-cm height) and non-labeled (13- to 20-cm height) at the time of application. Single applications of dicamba, glufosinate, and dicamba plus glufosinate (not labeled) did not result in greater than 80% Palmer amaranth control, regardless of weed size. The mixture of dicamba plus glufosinate was antagonistic for Palmer amaranth control and percent mortality. A sequential application, when averaged over time intervals and herbicides, improved Palmer amaranth control 11- to 17-percentage points over a single application, regardless of weed size at application 28 days after final application. Palmer amaranth control with glufosinate followed by (fb) glufosinate and dicamba fb dicamba were optimized at a 7-, and 14- to 21-day interval, respectively. However, a single SOA postemergence system increases the likelihood for selection of resistant biotypes. Sequential applications that included both dicamba and glufosinate were optimized when dicamba was applied before glufosinate. Dicamba fb glufosinate at a 14-day interval was the only herbicide treatment that resulted in 100% control and mortality of Palmer amaranth when weed size was <10cm and was associated with optimal economic returns to the producer. When weeds are allowed to grow to larger size, economic analysis revealed dicamba fb dicamba to perform better than dicamba fb glufosinate without assigning a value for increased likelihood with herbicide resistance for sequential application using a single SOA. Further, margins in economic performance between treatment options widens with increasing weed size leading to greater risk for producers to incur yield loss and have more remaining Palmer amaranth to build soil weed seed bank. These findings highlight the importance of timely weed control and support sequential applications that incorporate two SOA to control Palmer amaranth.

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

Key words: Antagonism, cost-benefit analysis, dicamba, dicamba plus glufosinate, glufosinate, herbicide, Palmer amaranth, sequential, weed size

Introduction

Palmer amaranth has evolved resistance to seven sites of action (SOA) in the United States (Heap 2020). The perpetuating evolution of herbicide resistance in Palmer amaranth places stress on the few remaining PRE and POST herbicide options that are commonly used by U.S. soybean and cotton producers. The innovation of genetically modified (GM) soybean and cotton has enabled producers to apply over-the-top postemergence herbicides to combat evolving Palmer amaranth populations. However, a new herbicide SOA has not been developed in almost 30 years. Therefore, proper management of the few remaining effective SOA is imperative, especially in light of metabolic resistance (Duke 2005; Norsworthy et al. 2012).

Monsanto, now Bayer CropScience, commercially launched glyphosate/dicamba/glufosinate-tolerant cotton (XtendFlex®) in 2015 and the same trait system in soybean in 2021. The incorporation of multiple GM traits allows for use of postemergence applications of dicamba, glufosinate, and glyphosate. Applying two effective SOA in mixture mitigates the likelihood of target-site herbicide resistance more than applying the herbicides sequentially (Bagavathiannan et al. 2013; Bagavathiannan et al. 2014; Diggle et al. 2003); however, dicamba-containing products like XtendiMax® plus VaporGrip® (Monsanto Corporation, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) cannot be mixed with glufosinate (Anonymous 2018a; Anonymous 2018b). Therefore, dicamba and glufosinate in the XtendFlex® technology have to be applied sequentially.

Factors that can influence efficacy of sequential herbicide applications include but are not limited to: interval between sequential applications (Meyer et al. 2019), sequence of herbicides applied (Burke et al. 2005), weed size (Lee and Oliver 1982; Steckel et al. 1997; Wilson 2005), environmental conditions (Ahrens 1994; Anderson et al. 1993; Coetzer et al. 2001), application technique or nozzle selection (Etheridge et al. 2001; McKinlay et al. 1974; Meyer et al. 2015), and cost. To optimize herbicides used in the XtendFlex® technology, a clear understanding of how the aforementioned factors influence efficacy of sequential applications of dicamba and glufosinate is needed. Also, it would be beneficial to identify the treatment leading to the greatest relative net benefit to the producer from both a cost and effective control perspective.

The order that sequential postemergence herbicides are applied can influence weed control (Burke et al. 2005). When a contact herbicide like glufosinate is applied, a decrease in sequential herbicide absorption and translocation has been observed. Reductions in sequential herbicide absorption and translocation were attributed to the rapid necrosis of plant tissue following the glufosinate application (Burke et al. 2005). The reduction in absorption and translocation following a glufosinate application may suggest that applying glufosinate before dicamba will not optimize postemergence weed control in the XtendFlex® technology.

Applications of auxin herbicides like dicamba can have adverse effects on sequentially applied herbicides (Priess et al. 2019). Following an auxin herbicide application, sensitive plants have been observed to display abnormalities like epinasty, leaf abscission, and abnormal elongation of aerial structures (Grossman 2000). The plant symptomology that occurs after an auxin herbicide application may reduce the leaf surface area of subsequently treated sensitive broadleaf weeds. However, impacts from the reduction of leaf surface area on efficacy of sequentially applied herbicides have not been quantified. In addition, an application of an auxin herbicide causes an upregulation of detoxifying enzymes (glutathione transferase, cytochrome P450s), which can impact metabolism of the applied herbicide as well as subsequently applied pesticides (Cummins et al. 1999; Raghavan et al. 2005). Even though auxin herbicides have the potential to impact efficacy of sequentially applied contact herbicides, an increase in Palmer amaranth efficacy was observed when 2,4-DB was applied 7 days prior to lactofen or acifluorfen, when compared to sequential applications of 2,4-DB (Chahal et al. 2011).

To mitigate the probability of Palmer amaranth evolving resistance to either dicamba or glufosinate in the XtendFlex® technology, timing and order of sequential herbicide applications of the two SOA needs to be optimized. The objective of this research was to determine what timing interval and herbicide sequence between sequential applications of glufosinate only, dicamba only, dicamba followed by (fb) glufosinate, and glufosinate fb dicamba optimizes effectiveness on Palmer amaranth, and at what relative net benefit in dollar terms to the producer.

Material and Methods

Field trials. Field experiments were conducted in 2019 and 2020. In 2019, experiments were conducted in Keiser, AR (N 35.675128, W-90.07844), near Crawfordsville, AR (N 35.228428, W -90.336762), and near Marianna, AR (N 34.725784, W -90.735788). In 2020, the experiment was conducted in Fayetteville, AR (N 36.092002, W -94.187002), Keiser, AR (N 35.675128, W-90.07844), and near Marianna, AR (N 34.725784, W -90.735788). The experiment was designed as a single-factor randomized complete block with four replications (Table 1). Field location, Palmer amaranth size at the initial application, and soil information at each site are displayed in Table 2.

Treatments were initiated without a crop present to native Palmer amaranth populations at all locations besides Fayetteville, AR in 2020, where Palmer amaranth from Crittenden County, AR was over-seeded. Plot size at all locations were 1.93 m wide and 6 m long. Prior to the first herbicide application, two 0.25- to 0.5-m^{2} quadrants where the size of the quadrants depended on Palmer amaranth density, were established in each plot and plants were counted for a density assessment. After initial density assessments were recorded, either *S*-metolachlor or dimethenamid-P was applied over the entire test area at a rate of 1606 g ai ha^{1} or 736 g ai ha^{1}, respectively, to limit further Palmer amaranth emergence. Average Palmer amaranth height was also recorded prior to the initial herbicide application.

Herbicide applications were made with hand-held CO_2-pressurized sprayers calibrated to deliver 140 L ha^{-1} of spray solution at 6.4 kph. Dicamba applications were made with TTI 110015-VP (TeeJet, Springfield, IL 62703) nozzles to attempt to abide by the label requirement of an Ultra Course spray (Anonymous 2020a; Anonymous 2020b). Glufosinate applications were made with an AIXR 110015-VP (TeeJet, Springfield, IL 62703). The mixture of dicamba + glufosinate was made with TTI 110015-VP nozzles.

Following herbicide applications, Palmer amaranth control was visually rated and plants with live tissue were counted in the established 0.25- to 0.5-m^{2} quadrants, 28 days after the final application (DAFA) in each treatment. Estimates of Palmer amaranth control were rated on a scale of 0 to 100%, with 0 being no control and 100 being complete Palmer amaranth death 14 and 28 DAFA. Initial and final counts were used to calculate a quantitative mortality percentage for each treatment.

Economic analysis. Pricing for dicamba, the required volatility reducing agents (VRAs), drift reducing agent (DRA), and glufosinate products labeled for use over-the-top of XtendFlex®

crops were obtained from Helena Agri-Enterprises, Nutrien Ag Solutions, and Simplot locations in the midsouthern United States. Cost per liter, as averaged across the different retailers in the spring of 2021, were converted to cost per hectare utilizing labeled use rates of each product. Several VRAs were priced including Sentris (BASF, Lundwigshafen, Germany), VaporGrip® Xtra Agent (Bayer CropScience, St. Louis, MO), and Delta Lock (Loveland Products, Loveland, CO), use rates were used based on company and label recommendations and prices were converted to cost per hectare. Rebate programs were not calculated into the cost of the herbicide due to intricacies in the various programs and difficulty standardizing rebates across products. Given changing bio-tech trait availability no attempt was made to calculate longer term average cost differences across herbicide and VRAs.

Application cost also contributes to the overall expense of herbicide applications. To standardize treatments a custom application fee of \$21.98 ha^{-1} was added to each herbicide application, based on the average statewide cost of custom ground herbicide applications in Texas (Klose et al. 2019). A total cost of herbicide expense was calculated for each treatment in Table 1. Other factors that could impact the cost of these postemergence herbicide applications is the ability to mix residual herbicides to embed these applications in timely full-season herbicide programs to limit Palmer amaranth emergence; however, the use of residual herbicides is outside of the scope of this research.

To calculate the relative net monetary benefit a producer would experience across treatment options, @Risk v7.5 (Palisade Corporation, 2017) was used to fit triangular truncated probability density functions to Palmer amaranth mortality rates from experimental data for each of the treatments and to the initial Palmer amaranth density in the field. Since experimental trials were conducted under high initial Palmer amaranth densities, later distributions were scaled and truncated at 10,764 plants ha^{-1} based on the very high density found in the Palmer amaranth management software (Lindsay et al. 2017). Using Monte Carlo simulation with 10,000 iterations, Appendix Table A.1 lists the parameters describing probability density functions sampled from the fitted probability density functions for treatments where herbicide applications were made to < 10-cm tall plants as well as 13- to 20-cm sizes. Triangular probability density functions, truncated between software-selected minima greater than 0% or 0%, and maxima of software-selected maxima less than 100% or 100%, exhibited superior fit characteristics in comparison to beta, normal, exponential, gamma, Weibull, Pareto, Pearson, Inverse Gauss, Laplace, Levy, logistic, log logisitic, and lognormal distributions using the Kolmogorov-Smirnov Statistic reported by @RISK for a majority of the fit distribution comparisons for each treatment alternative.

Each simulation run randomly drew an initial Palmer amaranth density (PD) from its fitted distribution and then a mortality rate (MR_i) for each treatment alternative, *i*, from respective fitted distributions to calculate the estimated number of Palmer amaranth plants remaining (PR_i) after spraying individual herbicide treatments on the two weed sizes tested. Using PR_i, % yield losses (YL_i) were estimated for soybean as follows (Bensch et al. 2003):

[Eq. 1] $$YL_i = (104.6 \cdot \frac{PR_i}{10000})/(1 + (104.6 \cdot \frac{\frac{PR_i}{10000}}{86.9})/100$$

The yield loss percentages for each treatment alternative were then multiplied by a yield-based revenue expectation per hectare using a soybean price of \$0.37 kg^{-1} and an irrigated soybean yield of 4,370 kg ha^{-1} to reflect long-term average dollar loss expectations for a soybean producer in the study region, as an example.

Estimated dollar losses ($DL_i = YL_i$ multiplied by the revenue potential of soybean -- \$1606 ha^{-1}) due to lack of Palmer amaranth weed control across the *k* treatment alternatives were compared to get an estimate of the relative benefit (RB_i) a producer would obtain by choosing a particular herbicide treatment alternative *i* over the herbicide treatment with the largest dollar loss across the *k* alternatives:

[Eq. 2] $RB_i = \max_k DL_i - DL_i$

To obtain RB_i, or the least relative dollar loss, the producer spends different dollar amounts on weed control (WC_i) across alternatives. As such, relative cost (RC_i), is the difference between the least-expensive weed control option across the *k* alternatives and the chosen alternative *i* and reflects added cost for more expensive treatment alternatives in terms of herbicide cost itself as well as charges for application:

[Eq. 3] $RC_i = WC_i - \min_k WC_i$

Finally, the relative net benefit of a particular weed control method is a function of both RB and RC, summarizes the dollar impact from a revenue and cost side, and is calculated as follows:

[Eq. 4] $RNB_i = RB_i - RC_i$

Importantly, no cost is assessed to Palmer amaranth evolving herbicide resistance to alternatives that use the same SOA as would be the case for treatments using the same herbicide twice in the same growing season, nor is a value assigned for weed seed addition to the soil seedbank across treatment alternatives as a function of RP. Hence, RNB values are likely conservative in the sense that treatments with poor weed control have further costs.

In sum, a particular iteration run in the Monte Carlo simulation would depend on that particular iteration run's initial PD, which is the same across all treatment alternatives, and the randomly chosen mortality rate independently chosen form each treatment's fitted mortality rate probability distribution. Both a positive or negative RNB is possible as some treatments may be low cost but also lead to high DL given poor control or they could be effective weed control options (high RB) with a range of possible relative cost. Hence, the treatment alternative with the highest RNB is superior to the other treatment alternatives. Such RNB_i were iteratively calculated 10,000 times to report both an average RNB and also estimated cumulative probability density functions across 10,000 iterations to reflect differences in riskiness as well as relative profitability. Treatment alternatives with steeper curves and those with greater mean values would be preferred by the producer. Each treatments' cumulative distribution function (CDF) was developed for two Palmer amaranth sizes (<10 cm and 13- to 20-cm).

Since treatment effectiveness in terms of mortality rates is likely related across treatments, partial correlation coefficients were used as shown in Appendix Table A.2 and A.3, to develop CDFs with those correlations imposed to assess whether rankings of different treatments will change when treatment alternatives exhibit correlation.

Data analysis. Data were analyzed by Palmer amaranth size (<10 cm and 13- to 20-cm). A single factor ANOVA was used to assess herbicide treatments in SAS 9.4 utilizing the PROC GLIMMIX function (SAS Institute Inc., Cary, NC). A beta distribution was assumed for Palmer amaranth control 14- and 28-DAFA (McDonald and Xu 1995). Site-years were analyzed by weed size at the initial application. Experiments conducted at Crawfordsville, AR, in 2019 and Keiser, AR, in 2020 were considered labeled applications based on the average Palmer amaranth size at the initial application (Table 2). The other four experimental runs were pooled as Palmer amaranth averaged over 10 cm in height at the time of the initial application (Table 2). Means were separated using Tukey's HSD (α=0.05). Least significant mean contrasts were conducted for comparison of single applications versus (vs) sequential applications, dicamba followed by (fb) dicamba vs glufosinate fb glufosinate, dicamba fb glufosinate vs glufosinate fb dicamba, dicamba fb dicamba vs dicamba fb glufosinate, and glufosinate fb glufosinate vs dicamba fb glufosinate (α=0.05).

To evaluate the interaction of the unlabeled mixture of dicamba and glufosinate, Colby's method was utilized (Anonymous 2020a; Anonymous 2020b). Colby's method (Colby 1967) is a technique used to assess the type of interaction occurring when two herbicides are applied in mixture. Colby's method requires the calculation of an expected value (E), displayed in Equation 5:

[Eq. 5] $E = (X + Y) - (XY)/100$

where E is the expected value of the herbicide mixture and X and Y are values of herbicides when applied alone. A two-sided t-test was preformed comparing the expected value calculated from Colby's equation and the observed values of the mixture (α=0.05). If the expected value of the herbicide mixture was statistically greater than the observed value the mixture was considered antagonistic. If no difference was found between the observed and expected value the mixture was considered additive, and if the observed value was greater than the expected value the mixture was considered synergistic. The expected value calculated in this experiment may be considered inflated as glufosinate alone treatments were applied with an Air Induction Extended Range (AIXR) nozzle and the mixture of the two herbicides were applied with a Turbo Teejet

Induction (TTI) nozzle to attempt to abide by nozzle regulations of dicamba labels; even though, the mixture of dicamba plus glufosinate is not labeled (Anonymous 2020a and 2020b).

Results and Discussion

Site-years included in the analysis for labeled Palmer amaranth size at the time of the initial application were Crawfordsville, AR, 2019 and Keiser, AR, 2020. For Palmer amaranth control 14 and 28 DAFA and percent mortality the main effect of herbicide treatment was significant ($P=0.0005$) (Table 3). Site-years included in the larger-than-labeled Palmer amaranth size at the time of the initial application were Keiser 2019, Marianna 2019, Fayetteville 2020, and Marianna 2020. For Palmer amaranth control 14 DAFA, 28 DAFA, and percent mortality the main effect of herbicide treatment was significant ($P< 0.0001$). As mentioned previously, all experiments were over-sprayed with either *S*-metolachlor or dimethenamid-P prior to first application of treatments; therefore, control ratings and mortality percentages reflect emerged plants at the time of initial application.

Palmer amaranth control at 14 DAFA for sequential dicamba applications at 7-, 14-, and 21-day intervals were 4- to 19- and 4- to 11-percentage points lower than the 28 DAFA evaluation on labeled and larger-than-labeled weed sizes, respectively (Tables 3 and 4). At 14 DAFA, the systemic nature of sequential applications of dicamba had not reached maximum Palmer amaranth control; therefore, comparisons of sequential applications of dicamba to other sequential applications at 14 DAFA should not be made. The lack of rapid removal of Palmer amaranth from crops like cotton or soybean, unlike glufosinate, especially at high densities following application may have a negative effect on the crop if competition for resources are still occurring. Furthermore, the presence of weedy vegetation like injured Palmer amaranth and its reflected far-red light perceived by nearby plants are known to alter crop growth (Afifi and

Swantton 2012; Markham and Stolenberg 2009). However, evaluations at 28 DAFA allowed time for the maximized herbicide efficacy to be reached, and captured any regrowth that occurred from either dicamba or glufosinate (personal observation). In the presence of a crop, some of these sequential treatments may perform slightly different than observed here such as extent of Palmer amaranth regrowth from a dicamba or glufosinate application if the crop is approaching canopy formation as noted in previous research (Meyers and Norsworthy 2020).

Reductions in soybean and cotton yield increase as a function of Palmer amaranth density (Klingaman and Oliver 1994; Rowland et al. 1999). Critical weed-free periods and critical weed removal timings in cotton and soybean have been developed (Buchanan and McLaughlin 1975; Korres and Norsworthy 2015; Tursun et al. 2015; Tursun et al. 2016; Webster et al. 2009; Van Acker et al. 1993). Many factors can impact the critical weed-free period in cotton and soybean like row spacing, crop population, planting date, growing degree days, addition of a cover crop, weed species and density, fertility, and tillage (Buchanan and McLaughlin 1975; Korres and Norsworthy 2015; Tursun et al. 2015; Tursun et al. 2016; Webster et al. 2009). The competitive impact of weeds that survive contact and systemic herbicides like glufosinate and dicamba should be investigated in the future because Palmer amaranth plants appeared to rapidly regrow 14 days after a glufosinate application and slower death and limited regrowth was observed from plants treated with dicamba, in the absence of a crop (personal observation). The inability to quickly remove Palmer amaranth from crops following a dicamba application may result in competition for limited resources for an extended period following application of the herbicide. Conversely, the regrowth of glufosinate-treated Palmer amaranth 14 DAA may also influence the crop and weed interaction. Changes in the competitiveness of Palmer amaranth following a herbicide application in the presence of a crop would likely affect weed seed production.

Crop and weed interactions can also influence the ability of herbicide injured weeds to recover and produce seed (Evans et al. 2003; Jha and Norsworthy 2009). As crop density increases, weed biomass and the ability of weed interference to affect crop yield decreases (Tollenaar et al. 1994). Because these experiments were conducted without a crop present, Palmer amaranth had an improved opportunity to regrow. The presence of a crop would likely impact weed control of the herbicide treatments evaluated as observed elsewhere (Tollenaar et al. 1994). However, Palmer amaranth has been observed to partially acclimate to crop shading by increasing leaf area and total leaf chlorophyll concentrations (Jha et al. 2008). To evaluate the effectiveness of the herbicide treatments without crop competition the experiments were conducted without a crop present.

Single applications. Single applications of dicamba and glufosinate applied to less than 10-cm tall Palmer amaranth provided 76 and 65% control and caused 92 and 85% mortality, respectively, at 28 DAFA (Table 3). Larger-than-labeled Palmer amaranth plants were not controlled >90% by a single herbicide application. A single application of glufosinate or dicamba applied to larger-than-labeled weeds controlled Palmer amaranth 59 and 65% and led to 47 and 59% mortality, respectively (Table 4). Similarly, Merchant et al. (2013) and Coetzer et al. (2002) observed 71 to 74% control of 2- to 10-cm tall Palmer amaranth, and 75 to 76% control of 15- to 25-cm tall Palmer amaranth with a single application of glufosinate or dicamba, respectively.

Norsworthy et al. (2012) noted that the use of multiple SOA in mixture will lessen the risk of herbicide resistance due to an increase in efficacy and a reduction of selection pressure on a single herbicide. The mixture of dicamba plus glufosinate applied to Palmer amaranth less than 10 cm in height provided 76% control and did not differ from a single application of dicamba but did provide an increase of 11-percentage points in control when compared to a single application of glufosinate 28 DAFA. The mixture of dicamba plus glufosinate to larger-than-labeled Palmer amaranth did not result in increased control or mortality when compared to dicamba alone (Table 4). The mixture of dicamba plus glufosinate was antagonistic when compared to the expected value reducing Palmer amaranth control 15- and 28-percentage points and mortality 5- and 12-percentage points at the labeled and above-labeled weed sizes 28 DAFA, respectively (Table 5). These results are similar to those observed in other research (Meyer et al. 2019).

Antagonism from the mixture of dicamba plus glufosinate with reductions in Palmer amaranth control of 18-percentage points when compared to the expected value calculated by Colby's equation when 30-cm tall weeds were treated were observed by Meyer et al. (2019). When compared to the expected value, the poor efficacy of the mixture of two SOAs is likely attributed to the use of a TTI (Ultra-Coarse spray) nozzle and the inverse nature of the systemic and contact activity of the two herbicides. Glufosinate efficacy and reduction in drift potential was optimized at a droplet size of 605 µm (Extremely Coarse) (Butts et al. 2018). The TTI nozzle used in this experiment for postemergence applications of dicamba produces an ultra-coarse droplet, thus droplet size is not optimized for glufosinate efficacy (Anonymous 2018a; Anonymous 2018b; Butts et al. 2018). Contrarily, Merchant et al. (2013) observed an increase in efficacy when dicamba plus glufosinate was applied to Palmer amaranth 13- to 25-cm in height with a Fine to Coarse droplet nozzle. Meyer et al. (2020) also observed a 46% reduction in dicamba translocation when dicamba was mixed with glufosinate compared to dicamba alone. As mentioned previously, dicamba applications can cause an upregulation of cytochrome P450 and glutathione S-transferase enzymes, which can enhance herbicide metabolism (Cummins et al. 1999; Raghavan et al. 2005). All aforementioned factors including nozzle selection, reductions in systemic translocation of dicamba caused by rapid necrosis from glufosinate, and/or upregulation of detoxifying enzymes caused by dicamba could be possible reasons improved control was not observed when the two herbicides were mixed (Burke et al. 2005; Cummins et al. 1999; Meyer et al. 2020; Raghavan et al. 2005).

Palmer amaranth control or mortality percentages did not reach 100% when a single application of dicamba, glufosinate, or a mixture of the two herbicides was applied, regardless of weed size (Table 3 and 4). To mitigate the selection for resistant biotypes and addition of weed seed to the soil seedbank, a zero-tolerance policy should be implemented (Norsworthy et al. 2012; Norsworthy et al. 2016). Therefore, additional measures will be needed to control Palmer amaranth plants that survive a single application of either herbicide or mixture, regardless of weed size at the initial application.

Sequential applications. An increase of 5- to 11- and 16- to 17-percentage points in control occurred when sequential herbicide applications were made compared to single herbicide applications at 14 and 28 DAFA, regardless of weed size, respectively (Table 6). Sequential applications of glufosinate were optimized at a 7-day interval between applications when initially applied at a labeled weed size. When applied to labeled-sized Palmer amaranth (<10cm), glufosinate fb glufosinate at the 7-, 14-, and 21-day intervals provided 94, 78, and 72% control and 98, 92, and 88% mortality 28 DAFA, respectively (Table 3). Similarly, Meyer and Norsworthy (2020) observed that sequential applications of glufosinate at a 7- to 10-day interval optimized annual weed control. On larger-than-labeled Palmer amaranth sizes (13- to 20-cm), weed control and mortality among timing intervals of sequential glufosinate applications did not differ. Control and mortality of larger-than-labeled Palmer amaranth plants following sequential glufosinate applications ranged from 75 to 76% and 66 to 77% at 28 DAFA, respectively (Table 4). Likewise, Meyer and Norsworthy (2020) observed 84 and 80% Palmer amaranth control when glufosinate at 451 g ai ha^{-1} was applied sequentially at 7- and 14-day intervals, 3 weeks after application.

In terms of visual control ratings of less than 10-cm tall Palmer amaranth, sequential applications of dicamba were highest at the 14- and 21-day interval, 28 DAFA (Table 3). A distinctly superior interval between sequential applications of dicamba applied to 13- to 20-cm tall Palmer amaranth was not observed. Control and mortality of larger-than-labeled Palmer amaranth ranged from 82 to 85% and 88 to 90%, respectively. No differences in Palmer amaranth mortality were observed among sequential applications of dicamba at 7-, 14-, and 21-day intervals, regardless of weed size (Tables 3 and 4). No sequential application of dicamba or glufosinate resulted in 100% control or 100% mortality of Palmer amaranth (Tables 3 and 4). The risk for selection of resistant biotypes in the aforementioned single SOA postemergence systems is high and multiple SOA should be used to mitigate target-site based herbicide resistance (Norsworthy et al. 2012). The use of a single SOA for postemergence control reflects a glufosinate (LibertyLink™) or Roundup Ready™ Xtend™ system used in an area where Palmer amaranth has resistance to acetolactate synthase, 5-enolpyruvyl-shikimate-3-phosphate synthase, and protoporphyrinogen oxidase inhibitors. Additional control measures will have to be taken to mitigate Palmer amaranth seed replenishing the soil seedbank and furthering the selection for resistant biotypes.

The sequence of sequential herbicide applications influenced the control level observed in the postemergence two SOA XtendFlex® system. Averaged over intervals, dicamba fb glufosinate provided a 4-percentage point increase in control when compared to glufosinate fb dicamba sequentially applied to labeled and larger-than-labeled Palmer amaranth sizes based on a contrast (Table 6). Similarly, Ogden and Dotray (2021) found that Palmer amaranth control was increased when a dicamba application was made prior to a glufosinate application, compared to the inverse sequence. The increase in control observed when dicamba is applied prior to glufosinate is likely attributed to adequate absorption and translocation of both herbicides. When a contact herbicide like glufosinate is applied before a systemic herbicide like dicamba a reduction in absorption and translocation of the systemic herbicide is observed (Sung-Eun et al. 2005). Future work should assess to what extent dicamba absorption and translocation is affected by a prior glufosinate application at differing time intervals.

When weed sizes were less than 10 cm, >90% Palmer amaranth control was observed in all sequential herbicide treatments 28 DAFA that included two SOA, except dicamba fb glufosinate at the 0.2-day interval, and glufosinate fb dicamba at the 7- and 21-day intervals (Table 3). Dicamba fb glufosinate at the 0.2-day (6 hour) interval was consistently the lowest level of control observed when dicamba was applied prior to glufosinate 28 DAFA, regardless of weed size. This interaction can likely be attributed to the rapid reduction in Palmer amaranth groundcover following an auxin herbicide application (Priess et al. 2019). Following an application of dicamba with TTI nozzles, a 31- to 36-percentage point reduction in Palmer amaranth groundcover was observed (Priess et al. 2019). A dicamba application subsequently reduces Palmer amaranth groundcover and the surface area of the weed available for intercepting glufosinate. Even though the prior sequence and interval of the sequential herbicide treatment follows label requirements, an increase in herbicide cost, application cost, and reductions in Palmer amaranth efficacy does not make dicamba fb glufosinate at a 0.2-day (6 hour) interval a sequence likely for adoption by growers and applicators.

To optimize the use of the two SOAs on labeled weed sizes dicamba fb glufosinate at the 14-day interval was the only treatment that provided 100% control and 100% mortality of Palmer amaranth 28 DAFA (Table 3). On larger-than-labeled Palmer amaranth, dicamba fb glufosinate at the 14-day interval provided higher control than any other herbicide treatment besides dicamba fb glufosinate at the 21-day interval at 28 DAFA (Table 6). Findings from this research lead to the conclusion that dicamba fb glufosinate 14 days later optimizes Palmer amaranth control (Tables 3 and 4). Only when dicamba was applied to Palmer amaranth less than 10 cm in height and fb by glufosinate 14 days later was replenishing the Palmer amaranth soil seedbank and further selection of herbicide resistance mitigated by eliminating escapes (Neve et al. 2011; Shrestha 2004).

The optimized use of dicamba fb glufosinate at the 14-day interval may be explained by a reduction in the interaction between the two herbicides. Priess et al. (2019) observed that Palmer amaranth regrowth and an increase in Palmer amaranth groundcover occurred 14 days after a dicamba application. Therefore, when a sequential application of dicamba fb glufosinate at a 14-day interval is made, glufosinate would be applied to actively growing weeds with increased leaf surface area for herbicide contact when compared to closer time intervals between sequential applications. In addition, by delaying a subsequent herbicide application by 14 days and targeting actively growing weeds, interactions of herbicide absorption and translocation may be negligible. Reductions in herbicide absorption and translocation are often attributed to rapid necrosis of contact herbicides (Meyer et al. 2020). Scarponi et al. (2005) found that upregulation of herbicide detoxifying enzymes was maximized 3 days after a metabolic enzyme-inducing seed treatment was applied. An upregulation of herbicide detoxifying enzymes was not observed past 7 days. Since auxin herbicides are known to cause an upregulation of herbicide detoxifying enzymes delaying the subsequent herbicide application by 14-days may alleviate this interaction. Further research will be needed to confirm why dicamba fb glufosinate at the 14-day interval was superior to other sequential herbicide treatments and further studies should work to investigate the interactions.

Economic implications. Dicamba products labeled for use in Xtend® or XtendFlex® crops averaged \$34.05 ha^{-1} (including the addition of a necessary volatility reducing agent and drift reduction agent) and glufosinate products averaged \$29.33 ha^{-1}. Excluding technology fees, seed cost, residual herbicides, and herbicide rebate programs, the cost of dicamba and glufosinate are similar but increase with sequential applications given added application charges as shown in the HC or herbicide cost columns in Tables 7 and 8 for weed sizes < 10 cm and 13 to 20 cm, respectively.

Average mortality rates, as drawn from the fitted distributions, are shown in Table 7 and 8 along with estimated yield loss and associated relative revenue loss of a hypothetical soybean crop as calculated using Eq. 1. Note that the average mortality rates closely resemble those reported in Tables 3 and 4 but are slightly different since they are averages of 10,000 random draws from fitted mortality rate distributions as discussed above. Also, in Table 7 and 8 the relative benefit of a treatment is reported in relation to the most revenue robbing alternative (Eq. 2) using the average Palmer amaranth plant density (PD) before herbicide application of 5,194 plants ha^{-1} across all treatments (Appendix Table A.1). Added cost relative to the most inexpensive treatment reflects RC (Eq. 3) and showcases the single pass with glufosinate to be the cheapest alternative whereas sequential applications of dicamba are most costly.

The average net benefit calculated at average PD and average MR represents a point estimate on the distribution functions of RNB calculated. Treatment differences across RNB showcase dicamba fb glufosinate with a 14-d interval between applications to have the highest RNB and a second-best alternative of a single pass of dicamba at RNB difference of \$6.11 ha^{-1}

for Palmer amaranth plants < 10 cm in height (Table 7). Since MR differed not only in average but also in range, the average RNB numbers in Table 7 report average RNB's using the 10,000 randomly drawn observations with zero correlation among treatment alternative MR distributions. Averages reported are larger as randomly selected observations from different distributions lead to greater RNB values. Importantly, however, the ranking of treatment alternatives continues to highlight dicamba fb glufosinate at 14 d interval to showcase the highest RNB but now at a lesser average difference (\$3.16 ha^{-1}) in comparison to using dicamba alone. Imposing correlation across treatments does not alter the rankings nor the difference among the top RNB treatments as shown in the last column on Table 7 as RNB differences point to the same optimal treatment choice of using dicamba fb glufosinate with a 14-d interval and remain on average about \$3 ha^{-1} apart.

Table 8 focuses on economic implications when spraying is delayed to a larger-than-labeled Palmer amaranth size. The best control program changes, yield losses are larger, sequential passes of herbicide are necessary and relative net benefit differences increase in comparison to Table 7. The best option to differentiate SOAs remains with dicamba fb glufosinate; however, treatment interval should be shorter (7 d) than for smaller sized weeds (14 d). The treatment option likely to lead to resistance but of highest RNB is the dicamba fb dicamba treatment with an interval of 21 d between herbicide application. The simulated mean difference between dicamba fb dicamba after 21 d and dicamba fb glufosinate after 7 d is \$27.41 ha^{-1}. A similar option of glufosinate fb glufosinate, in terms of increasing the likelihood of weed resistance is much less successful in avoiding soil seedbank accumulation. Assuming the producer chooses the dicamba fb dicamba with a 21-day interval, it is noteworthy that expected yield loss is nearly 5 times greater than if spraying were to occur in a timelier manner, promotes the likelihood of herbicide resistance, and leaves on average 9% more Palmer amaranth plants in the field.

To portray differences at the mean (50% percentile) and across the range of observations in relative net benefit by size of Palmer amaranth plant at time of application, Figure 1 plots differences across single and sequential pass treatments. To lessen the number of CDFs to compare, only the best treatments that incorporated sequential pass control options as highlighted in bold in Tables 7 and 8 are shown and represent RNB iterations with the correlation among treatments imposed. It is obvious from comparison of control options by Palmer amaranth size that timely application is less risky (CDFs are steeper and show a smaller range) not only in terms of profitability but also given better control with a lesser range in efficacy. A second observation is that the cheapest control option involving a single pass of glufosinate alone has the least downside risk but lags behind (CDF is furthest to the left) in terms of upside potential associated with superior Palmer amaranth control. There is a 43% and 37% chance of being least-cost when Palmer amaranth plants are large and <10 cm, respectively, for the glufosinate alone option. The best weed control options also indicate superior relative net benefit approximately 98% of the time in comparison to the cheapest option using dicamba alone (<10 cm) or dicamba fb dicamba after 21 d when Palmer amaranth plants are 13 to 20cm in size. Using a mixture of dicamba and glufosinate in a single pass also bests the cheapest option over 90% of the time when Palmer amaranth plants are large. Finally, while the CDF's are clearly differentiable in terms of producer preference when weed size is large, the distinction between dicamba alone vs. dicamba fb glufosinate after 14 d, for example is less obvious. At the mean (50%), the costlier option is preferred as indicated in the plot and Table 7; however, there is more downside risk with dicamba fb glufosinate after 14 d in comparison to dicamba alone as that treatment option is costlier. At the same time, the upside potential is larger with the costlier option. A risk averse producer may thus opt for dicamba alone as the range in relative profitability is smaller. At the same time, however, reduction in profit risk increases the soil seedbank given the 7% mortality rate difference between dicamba alone and dicamba fb glufosinate after 14 d.

Conclusions and practical implications. A single application of dicamba, glufosinate, or dicamba plus glufosinate alone did not control Palmer amaranth greater than 80%, regardless of weed size. Sequential applications of dicamba and glufosinate alone did not result in 100% control of Palmer amaranth at any time interval or regardless of weed size. In order to mitigate the selection of biotypes with reduced sensitivity to the few remaining effective postemergence herbicides in XtendFlex® soybean or cotton, producers will have to adopt sequential herbicide application regimes and other integrated weed management strategies to completely control Palmer amaranth. Both dicamba and glufosinate have already experienced a tremendous amount of selection. The risk for further selection of biotypes with reduced sensitivity to either herbicide in single SOA sequential herbicide systems is high. To increase the sustainability of herbicides, an optimized sequence and time interval between applications of dicamba and glufosinate should be utilized in the XtendFlex® technology. Dicamba fb glufosinate 14-days later was the only sequential postemergence system that provided 100% control and 100% mortality of Palmer amaranth less than 10 cm in height. On larger-than-labeled Palmer amaranth sizes, complete control and mortality was not achieved; therefore, weed size at the time of the initial application is still of the utmost importance and is reflected well in dollar terms in Figure 1. When weed size increases to 13-20 cm in height, incomplete control of Palmer amaranth leads to greater variability in producer returns not only for individual treatment options but also across treatment options. Economic returns were highest for dicamba fb dicamba after 21 d creating economic pressure to choose a weed control option that is more likely to lead to herbicide resistance than using dicamba fb glufosinate after 7 d, or the next best option in Table 8.

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Tables

Table 1. Experimental treatments, including herbicides, herbicide rate, the time interval between the sequential herbicide applications, and the associated cost of herbicide treatments are displayed below.

Herbicide	Rate	Time interval between sequential applications	Cost of herbicide treatment[a]
			USD ha^{-1}
Nontreated	-	-	0
Dicamba[b]	560 g ae ha^{-1}	-	56.03
Glufosinate	656 g ai ha^{-1}	-	51.31
Dicamba + glufosinate	560 g ae ha^{-1} + 656 g ai ha^{-1}	-	85.36
Dicamba fb dicamba	560 g ae ha^{-1} fb 560 g ae ha^{-1}	7, 14, and 21 days	112.06
Glufosinate fb glufosinate	656 g ai ha^{-1} fb 656 g ai ha^{-1}	7, 14, and 21 days	102.62
Dicamba fb glufosinate	560 g ae ha^{-1} fb 656 g ai ha^{-1}	0.2 (6 hours), 3, 7, 14, and 21 days	107.34
Glufosinate fb dicamba	656 g ai ha^{-1} fb 560 g ae ha^{-1}	0.2 (6 hours), 3, 7, 14, and 21 days	107.34

[a] Cost of herbicide treatment includes a custom application fee of $21.98 ha^{-1}.

[b] dicamba products priced included Engenia® and Xtendimax® plus VaporGrip®, additionally the average price of volatility reducing agents including Sentris®, VaporGrip Xtra®, and Delta Lock®, and the drift reduction agent Induce® was added to the dicamba price.

[c] Glufosinate products priced included Liberty® and Interline®

Table 2. Contains the year, location, Palmer amaranth size at the initial application, and soil information where the experiment was conducted.

			Palmer amaranth		
			Size at initial application	Density	
Year	Nearest town	Trial site	Average (range)	Average (range)	Soil information
			cm	plants ha^{-1}	
2019	Crawfordville, AR	Production field	7.6 (0.5-8.2)	2,400,000 (480,000-5,400,000)	Dundee silt loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs) with 11% sand, 77% silt, 12% clay, 1.95% organic matter, and a pH of 5.5
2019	Keiser, AR	Northeast Research and Extension Center	13 (0.5-15.4)	840,000 (120,000-1,400,000)	Sharkey silty clay (Very-fine, smectitic, thermic Chromic Epiaquerts)
2019	Marianna, AR	Lon Mann Cotton Research Station	13 (0.5-13.5)	800,000 (200,000-1,320,000)	Convent silt loam (Coarse-silty, mixed, superactive, thermic Fluvaquentic Endoaquepts) with 9% sand, 80% silt, 11% clay, 1.8% organic matter, and a pH of 6.3
2020	Fayetteville, AR	University of Arkansas-Agricultural Research and Extension Center	20 (1.5-25.4)	760,000 (16,000-2,240,00)	Leaf silt loam soil (Fine, mixed, active, thermic Typic, Albaqualts) with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and pH of 6.2
2020	Keiser, AR	Northeast Research and Extension Center	7.6 (0.5-8.0)	1,040,000 (240,000-1,920,000)	Sharkey silty clay (Very-fine, smectitic, thermic Chromic Epiaquerts)
2020	Marianna, AR	Lon Mann Cotton Research Station	20 (2-25.4)	1,280,000 (320,000-2,880,000)	Convent silt loam (Coarse-silty, mixed, superactive, thermic Fluvaquentic Endoaquepts) with 9% sand, 80% silt, 11% clay, 1.8% organic matter, and a pH of 6.3

Table 3. Percent control and mortality when <10-cm-tall Palmer amaranth was treated with single and sequential applications of dicamba and glufosinate averaged over two site-years.

		Palmer amaranth control[a]		Palmer amaranth mortality[a]
Herbicide	Interval between applications	14 DAFA[b]	28 DAFA[b]	28 DAFA[b]
	days	%		
Dicamba	Na[b]	80 EF[c]	74 IJ	92 BCD
Glufosinate	Na	76 FGH	65 K	85 E
Dicamba + glufosinate	Na	78 FG	76 HIJ	85 E
Dicamba fb[b] dicamba	7	82 DEF	86 DEFG	98 ABC
Dicamba fb dicamba	14	78 FG	97 AB	94 ABC
Dicamba fb dicamba	21	78 FG	97 AB	98 AB
Glufosinate fb glufosinate	7	92 AB	94 ABC	98 AB
Glufosinate fb glufosinate	14	83 CDEF	78 GHIJ	92 CD
Glufosinate fb glufosinate	21	61 I	72 JK	88 DE
Dicamba fb glufosinate	0.2	88 BCD	81 FGHI	94 ABCD
Dicamba fb glufosinate	3	95 AB	94 ABC	97 ABC
Dicamba fb glufosinate	7	98 A	94 ABC	95 ABC
Dicamba fb glufosinate	14	96 AB	100 A	100 A
Dicamba fb glufosinate	21	72 GH	95 ABC	98 ABC
Glufosinate fb dicamba	0.2	89 BCD	90 BCDE	93 BCD
Glufosinate fb dicamba	3	91 ABC	93 ABCD	97 ABC
Glufosinate fb dicamba	7	88 BCDE	83 EFGH	95 ABC
Glufosinate fb dicamba	14	77 FGH	91 ABCD	95 ABC
Glufosinate fb dicamba	21	69 H	87 CDEF	93 BCD

[a] Palmer amaranth control and mortality is expressed as percent of the nontreated

[b] Abbreviations: DAFA, days after final application; fb, followed by; Na, not applicable

[c] Means followed by the same letter within a column are not statistically different according to Tukey's HSD (α=0.05).

Table 4. Percent control and mortality when 13- to 25-cm-tall Palmer amaranth was treated with single and sequential applications of dicamba and glufosinate averaged over four site-years.

Herbicide	Interval between applications	Palmer amaranth control[a] 14 DAFA[b]	Palmer amaranth control[a] 28 DAFA[b]	Palmer amaranth mortality[a] 28 DAFA[b]
	Days	--------------------------------------	--%--------------------------------	---
Dicamba	Na[b]	62 EF[c]	65 GH	57 FG
Glufosinate	Na	54 F	59 H	49 G
Dicamba + glufosinate	Na	61 EF	59 H	66 EF
Dicamba fb[b] dicamba	7	81 BC	85 ABC	88 ABC
Dicamba fb dicamba	14	79 BC	85 ABC	90 A
Dicamba fb dicamba	21	73 CD	82 BCD	89 AB
Glufosinate fb glufosinate	7	81 BC	77 CDE	77 BCDE
Glufosinate fb glufosinate	14	78 BC	76 DEF	75 CDE
Glufosinate fb glufosinate	21	63 E	76 DEF	66 EF
Dicamba fb glufosinate	0.2	67 DE	68 FG	71 DEF
Dicamba fb glufosinate	3	77 BC	76 DEF	72 DE
Dicamba fb glufosinate	7	79 BC	69 FG	84 ABCD
Dicamba fb glufosinate	14	92 A	92 A	89 AB
Dicamba fb glufosinate	21	84 AB	87 AB	89 AB
Glufosinate fb dicamba	0.2	67 DE	65 GH	65 EF
Glufosinate fb dicamba	3	80 BC	79 BCDE	74 DE
Glufosinate fb dicamba	7	78 BC	75 DEF	80 ABCD
Glufosinate fb dicamba	14	75 CD	81 BCD	83 ABCD
Glufosinate fb dicamba	21	54 F	71 EFG	58 FG

[a] Palmer amaranth control and mortality is expressed as percent of the nontreated

[b] Abbreviations: DAFA, days after final application; fb, followed by; Na, not applicable

[c] Means followed by the same letter within a column are not statistically different according to Tukey's HSD (α=0.05).

Table 5. The effect of mixtures of dicamba and glufosinate on Palmer amaranth control at 14 and 28 days after treatment and Palmer amaranth mortality 28 days after treatment, separated by labeled and larger-than-labeled weed sizes.

		Palmer amaranth control						Palmer amaranth mortality[b]		
		14 days after final application			28 days after final application			28 days after final application		
Palmer amaranth size	Herbicide	Observed	Expected	P-value	Observed	Expected	P-value	Observed	Expected	P-value
cm		----------%----------			----------%----------			----------%----------		
<10[d]										
	dicamba	80			74			92		
	glufosinate	76			65			85		
	dicamba + glufosinate	78	95	<0.0001*[d]	76	91	<0.0001*	85	99	0.0025*
13 to 25[d]										
	dicamba	62			65			57		
	glufosinate	54			59			49		
	dicamba + glufosinate	61	83	<0.0001*	59	86	<0.0001*	66	78	0.0042*

[a] Abbreviation: Observed, observed value, Expected, expected value

[b] Palmer amaranth mortality is expressed as percent of the nontreated

[c] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E=(X + Y) - (XY)/100$].

[d] Labeled Palmer amaranth is <10 cm in height and larger-than-labeled Palmer amaranth is 13- to 20-cm in height.

[e] Significant P-values (≤0.05) are indicated by "*"

Table 6. Least significant means contrast conducted on single applications vs sequential applications and differing sequential applications vs differing sequential applications analyzed by Palmer amaranth size, evaluation timing, and averaged over site-year. Sequential applications were averaged over time intervals between sequential applications.

	Palmer amaranth less than 10 cm in height[a]			
	Control 14 DAFA[b]		Control 28 DAFA	
Contrast	Means	P-value	Means	P-value
	%		%	
Single application vs sequential application	78 vs 83	0.0014*[c]	72 vs 83	<0.0001*
Dicamba fb[b] dicamba vs glufosinate fb glufosinate	79 vs 79	0.7821	93 vs 81	<0.0001*
Dicamba fb glufosinate vs glufosinate fb dicamba	90 vs 83	<0.0001*	93 vs 89	0.0441*
Dicamba fb dicamba vs dicamba fb glufosinate	79 vs 90	<0.0001*	93 vs 93	0.7638
Glufosinate fb glufosinate vs dicamba fb glufosinate	79 vs 90	<0.0001*	81 vs 93	<0.0001*

	Palmer amaranth 13 to 25 cm in height[a]			
	Control 14 DAFA		Control 28 DAFA	
Contrast	Means	P-value	Means	P-value
	%		%	
Single application vs sequential application	59 vs 75	<0.0001*	61 vs 78	<0.0001*
Dicamba fb dicamba vs glufosinate fb glufosinate	78 vs 74	0.1935	84 vs 76	0.0014*
Dicamba fb glufosinate vs glufosinate fb dicamba	80 vs 71	<0.0001*	78 vs 74	0.0491*
Dicamba fb dicamba vs dicamba fb glufosinate	78 vs 80	0.1090	84 vs 78	0.0042*
Glufosinate fb glufosinate vs dicamba fb glufosinate	74 vs 80	0.4902	76 vs 78	0.4710

[a] Average Palmer amaranth height at the time of the initial application

[b] Abbreviations: DAFA, days after final application; fb, followed by

[c] Significant P-values (≤0.05) are indicated by "*"

Table 7. Relative comparisons across treatment alternatives using Monte Carlo simulation and hypothetical soybean revenue loss estimates associated with different weed control programs evaluated at average initial Palmer amaranth plant density (PD) and average mortality rates under varying assumptions of correlation among mortality rate probably distributions for simulations of weed control of Palmer amaranth of 10 cm size or less.

Herbicide	Interval	Herb. cost & app. chg. (HC[d])	Exp. Palmer amaranth mortality (MR[d])	Exp. # of remaining plants post spray (RP[d])	Est. yield loss (YL[d])	Est. revenue loss	Est. relative benefit (RB[d])	Added cost (RC[d])	Avg. RB – RC at avg. PD	Simulated avg. RNB[d] (no corr.)	Simulated avg. RNB[d] (corr.[b])
	d	$ ha^{-1}	%	plants ha^{-1}	%	$ ha^{-1}					
Dicamba	Na[c]	$56	92.0	415	4.1	$66	$53	$5	**$48.75**[a]	**$103.27**	**$83.99**
Glufosinate	Na	$51	85.0	781	7.5	$120	$0	$0	$0.00	$60.47	$41.13
Dicamba + glufosinate	Na	$85	93.6	333	3.4	$54	$66	$34	$32.04	$85.86	$66.41
Dicamba fb[b] dicamba	7	$112	96.5	181	1.8	$30	$90	$61	$29.46	$81.61	$62.69
Dicamba fb dicamba	14	$112	89.4	548	5.4	$86	$34	$61	-$27.22	$29.00	$9.75
Dicamba fb dicamba	21	$112	97.2	147	1.5	$24	$96	$61	**$34.97**	**$86.95**	**$68.10**
Glufosinate fb glufosinate	7	$103	97.7	120	1.2	$20	$100	$51	**$48.70**	**$100.58**	**$81.67**
Glufosinate fb glufosinate	14	$103	88.9	575	5.6	$90	$30	$51	-$21.75	$34.37	$15.70
Glufosinate fb glufosinate	21	$103	88.0	622	6.1	$97	$23	$51	-$28.59	$29.12	$9.47
Dicamba fb glufosinate	0.2	$107	93.9	316	3.2	$51	$69	$56	$12.81	$65.87	$47.33
Dicamba fb glufosinate	3	$107	95.7	222	2.3	$36	$84	$56	$27.60	$79.90	$61.14
Dicamba fb glufosinate	7	$107	91.7	430	4.3	$69	$51	$56	-$4.85	$49.46	$30.69
Dicamba fb glufosinate	14	$107	99.0	54	0.6	$9	$111	$56	**$54.86**	**$106.43**	**$87.63**
Dicamba fb glufosinate	21	$107	95.8	219	2.2	$36	$84	$56	$28.05	$80.64	$61.57
Glufosinate fb dicamba	0.2	$107	92.0	417	4.2	$67	$53	$56	-$2.89	$51.54	$32.35
Glufosinate fb dicamba	3	$107	96.6	175	1.8	$29	$91	$56	**$35.03**	**$87.08**	**$68.14**
Glufosinate fb dicamba	7	$107	95.1	255	2.6	$42	$78	$56	$22.27	$75.02	$56.24
Glufosinate fb dicamba	14	$107	94.9	263	2.7	$43	$77	$56	$21.04	$73.87	$54.74
Glufosinate fb dicamba	21	$107	88.8	583	5.7	$91	$28	$56	-$27.57	$28.90	$10.03

[a] Bold lettering indicates the top choice (highest RNB = RB – RC) among either single herbicide weed control treatments using different herbicides or their tank mix and again the most profitable time interval among weed control systems involving two sequential passes with different combinations of herbicides.

[b] See Appendix Table A.2 for partial correlation coefficients among weed control options.

[c] Na, not applicable

[d] Abbreviations: herbicide cost, HC; mortality rate, MR; remaining plants, RP; yield loss, YL; relative benefit, RB; relative cost, RC; plant density, PD; RNB, relative net benefit

Table 8. Relative comparisons across treatment alternatives using Monte Carlo simulation and hypothetical soybean revenue loss estimates associated with different weed control programs evaluated at average initial Palmer amaranth plant density (PD) and average mortality rates under varying assumptions of correlation among mortality rate probably distributions for simulations of weed control of Palmer amaranth of 13 to 25 cm in size.

Herbicide	Interval	Herb. cost & app. chg.	Exp. Palmer amaranth mortality	Exp. # of remaining plants post-spray	Est. yield loss	Est. revenue loss	Est. relative benefit (RB[c])	Added cost (RC[c])	Avg. RB – RC at avg. PD[c]	Simulated avg. RNB[c] (no corr.)	Simulated avg. RNB[c] (corr.[b])
	d	$ ha^{-1}	%	plants ha^{-1}	%	---------------------- $ ha^{-1} ----------------------					
Dicamba	Na	$56	55.7	2,301	18.8	$303	$79	$5	$74.47	$145.88	$134.78
Glufosinate	Na	$51	39.8	3,129	23.8	$382	$0	$0	$0.00	$83.11	$72.61
Dicamba + glufosinate	Na	$85	64.7	1,832	15.7	$252	$130	$34	**$95.62**[a]	**$161.53**	**$150.10**
Dicamba fb[b] dicamba	7	$112	85.9	732	7.0	$113	$269	$61	$208.18	$258.13	$248.15
Dicamba fb dicamba	14	$112	89.0	573	5.6	$90	$292	$61	$231.02	$278.85	$268.29
Dicamba fb dicamba	21	$112	89.7	536	5.3	$85	$297	$61	**$236.54**	**$283.44**	**$273.47**
Glufosinate fb glufosinate	7	$103	79.0	1,091	10.1	$162	$220	$51	**$168.50**	**$225.90**	**$214.70**
Glufosinate fb glufosinate	14	$103	64.2	1,858	15.9	$255	$127	$51	$75.47	$148.98	$139.44
Glufosinate fb glufosinate	21	$103	63.8	1,880	16.0	$258	$124	$51	$73.05	$145.82	$136.79
Dicamba fb glufosinate	0.2	$107	72.2	1,441	12.8	$206	$176	$56	$119.50	$183.66	$173.78
Dicamba fb glufosinate	3	$107	71.8	1,467	13.0	$209	$172	$56	$116.40	$181.15	$171.36
Dicamba fb glufosinate	7	$107	84.8	789	7.5	$121	$261	$56	**$204.81**	**$255.76**	**$246.06**
Dicamba fb glufosinate	14	$107	84.7	796	7.6	$122	$260	$56	$203.87	$255.00	$244.50
Dicamba fb glufosinate	21	$107	80.8	997	9.3	$150	$232	$56	$176.29	$231.85	$221.36
Glufosinate fb dicamba	0.2	$107	67.6	1,682	14.6	$235	$147	$56	$90.86	$160.88	$150.15
Glufosinate fb dicamba	3	$107	72.7	1,415	12.7	$203	$179	$56	$122.67	$186.55	$176.36
Glufosinate fb dicamba	7	$107	81.6	954	9.0	$144	$238	$56	**$182.03**	**$236.03**	**$225.97**
Glufosinate fb dicamba	14	$107	77.1	1,191	10.9	$175	$207	$56	$150.89	$205.43	$195.11
Glufosinate fb dicamba	21	$107	56.3	2,270	18.6	$300	$82	$56	$26.34	$97.57	$88.07

[a] Bold lettering indicates the top choice (highest RNB = RB – RC) among either single herbicide weed control treatments using different herbicides or their tank mix and again the most profitable time interval among weed control systems involving two sequential passes with different combinations of herbicides.

[b] See Appendix Table A.3 for partial correlation coefficients among weed control options.

[c] Abbreviations: relative benefit, RB; relative cost, RC; plant density, PD; RNB, relative net benefit

Figures

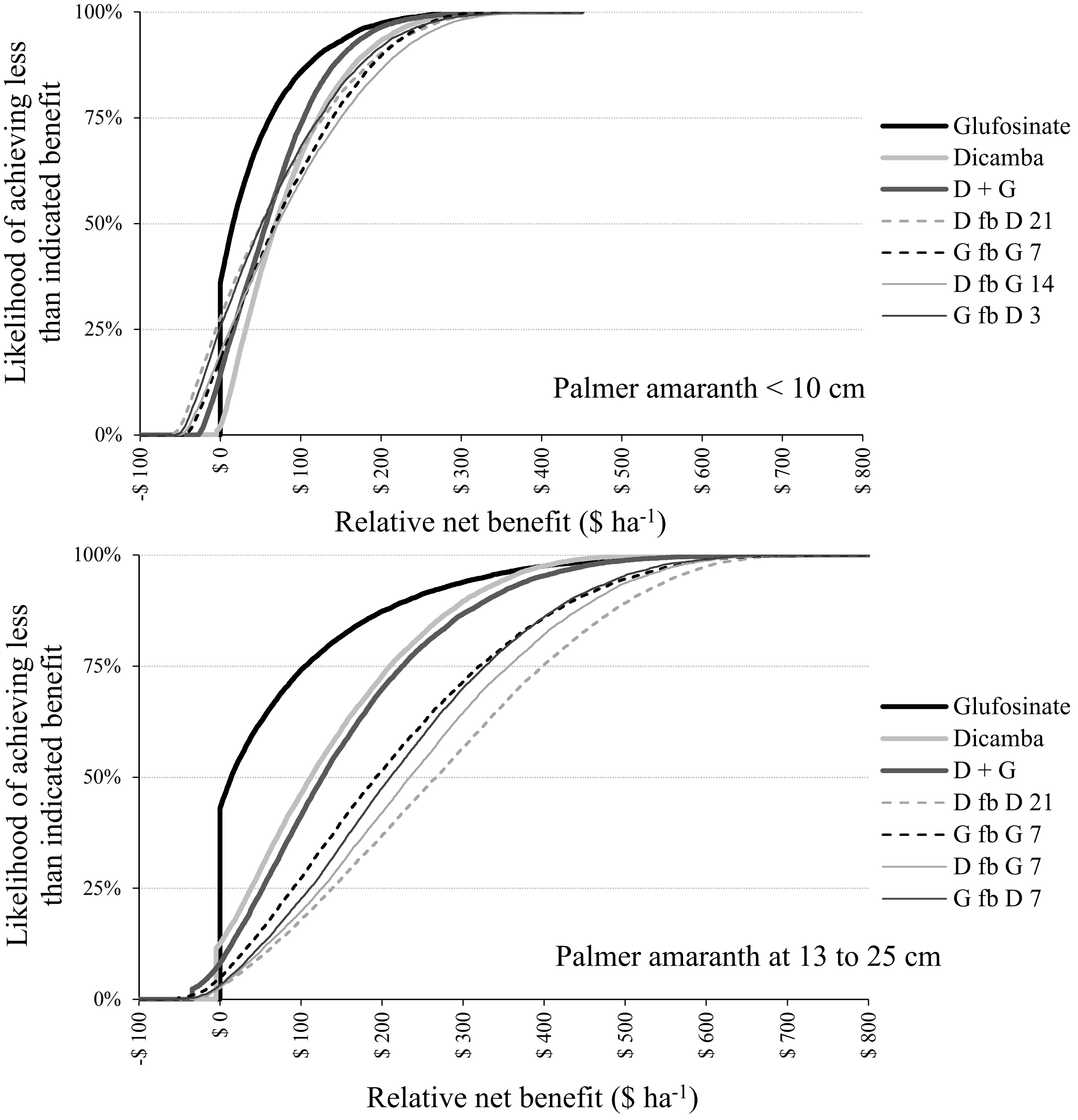

Figure 1. Comparison of simulated cumulative distribution functions of relative net benefit accounting for relative sales losses and relative weed control cost for single pass vs. sequential passes of herbicides when applied to large and small weeds using average soybean price and yield expectations as an example. In the legend, abbreviations, D, dicamba; G, glufosinate are used and the number following abbreviations is the interval in days between sequential applications.

Appendix

Appendix Table A.1. Parameter estimates and descriptive statistics for probability density functions for initial plant density of Palmer amaranth (PD in # ha^{-1}) and mortality rates (% of PD removed) across different herbicide treatments when applied to weeds at different sizes as sampled from triangular distributions fitted from experimental data using Monte Carlo simulation with 10,000 iterations with @Risk software.

	Palmer amaranth < 10 cm					Palmer amaranth at 13 to 25 cm				
				Percentiles					Percentiles	
Name[a]	Min.	Mean	Max.	5%	95%	Min.	Mean	Max.	5%	95%
Starting Palmer amaranth density	431	5,194	10,764	929	10,085	430	5,194	10,763	929	10,085
D	76.2	92.0	100	81.4	99.4	0.0	55.7	85.7	16.0	83.4
G	55.2	85.0	100	65.0	98.9	0.1	39.8	>99.9[b]	3.2	88.9
D + G	80.9	93.6	100	85.1	99.5	16.3	64.7	>99.9	31.7	93.1
D fb D 7	89.6	96.5	100	91.9	99.7	58.0	85.9	100	67.2	98.9
D fb D 14	68.5	89.5	100	75.4	99.2	67.0	89.0	100	74.3	99.2
D fb D 21	91.6	97.2	100	93.4	99.8	69.2	89.7	100	76.0	99.2
G fb G 7	93.1	97.7	100	94.6	99.8	37.4	79.0	100	51.1	98.4
G fb G 14	67.1	88.9	100	74.2	99.2	<0.1	64.2	100	17.1	97.3
G fb G 21	64.2	88.0	100	72.1	99.1	<0.1	63.8	100	16.3	97.2
D fb G 0.2	81.9	93.9	100	85.9	99.5	17.5	72.3	100	35.4	97.9
D fb G 3	87.3	95.7	100	90.1	99.7	15.5	71.8	100	34.2	97.9
D fb G 7	75.3	91.7	100	80.7	99.4	54.8	84.8	100	64.6	98.9
D fb G 14	96.9	99.0	100	97.6	99.9	54.2	84.7	100	64.3	98.8
D fb G 21	87.5	95.8	100	90.2	99.7	43.0	80.8	100	55.3	98.5
G fb D 0.2	76.0	92.0	100	81.3	99.4	3.6	67.6	100	24.6	97.5
G fb D 3	90.0	96.6	100	92.1	99.7	18.8	72.8	100	36.5	97.9
G fb D 7	85.4	95.1	100	88.6	99.6	44.9	81.6	100	57.2	98.6
G fb D 14	84.9	94.9	100	88.2	99.6	42.5	77.1	94.4	54.0	93.1
G fb D 21	66.5	88.8	100	73.9	99.1	<0.1	56.3	88.4	14.2	86.0

[a] First letter provides information about herbicide used (D = Dicamba, G = Glufosinate), fb stands for followed by if applicable and + stands for a tank mix, second letter (if applied in sequence) again informs about herbicide choice, and the number indicates the number of d between herbicide applications.

[b] >99.9 implies less than complete control and <0.01 implies nearly no mortality.

Appendix Table A.2. Partial correlation coefficients of among weed control treatment alternatives when herbicide was applied to Palmer amaranth at <10 cm size.

Herbicide[a]	D	G	D + G	DD 7	DD 14	DD 21	GG 7	GG 14	GG 21	DG .2	DG 3	DG 7	DG 14	DG 21	GD 0.2	GD 3	GD 7	GD 14	GD 21
Dicamba (D)	1.00																		
Glufosinate (G)	0.45	1.00																	
D + G	0.54	0.46	1.00																
D fb D 7	0.19	-0.61	0.23	1.00															
D fb D 14	0.27	-0.17	**0.73**	**0.67**	1.00														
D fb D 21	0.47	0.06	-0.32	0.17	-0.34	1.00													
G fb G 7	0.55	**0.87**	0.53	-0.47	-0.17	0.04	1.00												
G fb G 14	**0.82**[b]	0.47	0.46	-0.03	0.33	0.30	0.36	1.00											
G fb G 21	0.55	0.62	**0.87**	0.13	0.53	-0.01	0.53	0.47	1.00										
D fb G 0.2	-0.40	0.48	0.30	-0.55	-0.08	**-0.66**	0.46	-0.30	0.22	1.00									
D fb G 3	**0.85**	0.26	0.24	0.02	0.04	0.34	0.41	**0.72**	0.10	-0.47	1.00								
D fb G 7	0.33	0.39	0.51	0.00	0.56	-0.08	0.03	**0.69**	**0.63**	0.00	0.09	1.00							
D fb G 14	-0.05	0.43	0.32	-0.23	0.30	-0.22	-0.01	0.32	0.56	0.28	-0.31	**0.87**	1.00						
D fb G 21	**0.61**	0.28	-0.18	-0.23	-0.31	**0.71**	0.25	**0.72**	-0.03	-0.50	**0.66**	0.19	-0.07	1.00					
G fb D 0.2	0.49	0.27	**0.96**	0.39	**0.87**	-0.35	0.29	0.47	**0.82**	0.14	0.21	0.61	0.39	-0.22	1.00				
G fb D 3	-0.05	0.07	0.42	-0.13	0.35	-0.45	0.11	0.17	0.34	0.34	-0.18	0.34	0.34	-0.05	0.42	1.00			
G fb D 7	0.41	0.52	0.07	-0.14	-0.43	0.56	0.58	0.00	0.37	-0.05	0.20	-0.21	-0.11	0.21	-0.10	-0.39	1.00		
G fb D 14	**0.73**	0.45	**0.86**	0.22	**0.73**	-0.05	0.39	**0.82**	**0.79**	-0.03	0.47	**0.77**	0.45	0.24	**0.88**	0.37	-0.05	1.00	
G fb D 21	0.33	0.22	**0.82**	0.30	0.52	-0.37	0.53	0.01	0.58	0.39	0.11	-0.07	-0.17	-0.42	**0.72**	0.28	0.16	0.46	1.00

[a] First letter provides information about herbicide used (D = Dicamba, G = Glufosinate), fb stands for followed by if applicable and + stands for a tank mix, second letter (if applied in sequence) again informs about herbicide choice, and the number indicates the number of d between herbicide applications.

[b] Bold numbers indicate partial correlations statistically significantly different from zero at $p < 0.10$ and are based on 8 observations per treatment.

Appendix Table A.3. Partial correlation coefficients of among weed control treatment alternatives when herbicide was applied to Palmer amaranth at 13 to 25 cm size.

Herbicide[a]	D	G	D + G	DD 7	DD 14	DD 21	GG 7	GG 14	GG 21	DG .2	DG 3	DG 7	DG 14	DG 21	GD 0.2	GD 3	GD 7	GD 14	GD 21
Dicamba (D)	1.00																		
Glufosinate (G)	0.24	1.00																	
D + G	0.01	**0.47**	1.00																
D fb D 7	-0.39	0.04	0.42	1.00															
D fb D 14	0.11	0.08	0.16	0.13	1.00														
D fb D 21	-0.21	**-0.47**	-0.30	0.24	0.00	1.00													
G fb G 7	-0.29	0.10	0.40	0.20	-0.09	-0.27	1.00												
G fb G 14	-0.15	**0.44**	-0.08	-0.26	0.12	-0.31	-0.04	1.00											
G fb G 21	-0.01	**0.63**	0.14	0.00	0.06	-0.41	0.08	0.17	1.00										
D fb G 0.2	0.31	0.01	0.08	-0.10	-0.19	-0.41	**0.50**	-0.10	-0.25	1.00									
D fb G 3	0.31	-0.41	-0.08	-0.23	-0.26	0.02	-0.19	**-0.59**	-0.28	0.32	1.00								
D fb G 7	0.18	0.22	0.11	0.04	**-0.43**	0.01	0.20	-0.09	0.01	0.29	-0.06	1.00							
D fb G 14	-0.16	-0.07	-0.12	0.01	0.31	**0.46**	0.18	-0.12	0.04	-0.15	-0.03	-0.24	1.00						
D fb G 21	0.06	0.12	0.04	0.30	0.15	-0.16	0.19	0.10	0.13	0.14	-0.32	-0.06	-0.29	1.00					
G fb D 0.2	-0.02	0.23	0.24	-0.04	-0.40	**-0.49**	**0.45**	-0.29	**0.47**	**0.48**	0.28	0.27	-0.19	0.12	1.00				
G fb D 3	0.40	0.10	0.12	-0.19	**-0.51**	-0.40	0.12	**-0.43**	0.13	**0.62**	**0.69**	0.35	-0.36	-0.04	**0.77**	1.00			
G fb D 7	-0.29	0.35	0.37	0.36	**-0.47**	-0.01	0.19	-0.01	0.25	0.17	0.11	**0.47**	-0.14	-0.21	**0.50**	**0.44**	1.00		
G fb D 14	0.25	-0.02	-0.05	-0.37	0.25	-0.23	0.55	-0.01	-0.04	**0.51**	0.17	-0.21	**0.51**	0.05	0.14	0.10	-0.38	1.00	
G fb D 21	0.11	-0.37	-0.07	-0.34	0.26	0.10	-0.22	-0.12	-0.08	-0.01	0.56	-0.38	0.19	-0.39	0.01	0.13	-0.04	0.20	1.00

[a] First letter provides information about herbicide used (D = Dicamba, G = Glufosinate), fb stands for followed by if applicable and + stands for a tank mix, second letter (if applied in sequence) again informs about herbicide choice, and the number indicates the number of d between herbicide applications.

[b] Bold numbers indicate partial correlations statistically significantly different from zero at $p < 0.10$ and are based on 8 observations per treatment

Chapter 3

Interaction of dicamba, dicamba + glyphosate, and glufosinate on labeled and larger-than-labeled weed sizes

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Abstract. The XtendFlex® technology allows for applications of dicamba, glyphosate, and glufosinate over-the-top of soybean and cotton; however, little is known about the herbicide interactions that may occur when mixtures and sequential applications are utilized and how to optimize weed control. An interaction experiment was conducted over five site-years to assess mixtures and intervals between sequential applications of dicamba, dicamba plus glyphosate, and glufosinate on Palmer amaranth control. A weed size experiment was also conducted over six site-years to assess the efficacy of sequential applications of dicamba, dicamba plus glyphosate, and glufosinate on labeled and larger-than-labeled Palmer amaranth sizes. In the interaction experiment, a single application of dicamba, dicamba plus glyphosate, and glufosinate controlled labeled and above-labeled size Palmer amaranth 67 to 83% and 37 to 72%, respectively. For above-labeled weed sizes, the mixtures of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate were antagonistic. Palmer amaranth control was not improved by sequential applications when there was a 4-hour interval between sprays, regardless of the herbicides applied. In both experiments, dicamba plus glyphosate fb glufosinate or dicamba fb glufosinate at the 14-day interval optimized Palmer amaranth control. Incorporating a systemic and contact herbicide like dicamba and glufosinate, may optimize weed control by applying systemic dicamba and glyphosate 14 days prior to the contact herbicide glufosinate. In addition, applying herbicide treatments to labeled (<10 cm in height) Palmer amaranth improved efficacy of treatments; however, herbicide interactions were more commonly observed on larger-than-labeled weed sizes.

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

Key words: herbicide interactions, optimization, weed control, XtendFlex® technology, weed size

Introduction

Palmer amaranth is one of the most troublesome weeds in soybean and cotton in the southern United States (Riar et al. 2013a; Riar et al. 2013b; Schwartz-Lazzaro et al. 2018; Van Wychen 2016), primarily due to the weed's ability to evolve resistance to herbicides. Currently, Palmer amaranth has evolved resistance to acetolactate synthase inhibitors, microtubule inhibitors, 5-enolpyruvate shikimate 3-phosphate inhibitors, auxin mimics, hydroxyphenylpyruvate dioxygenase inhibitors, protoporphyrinogen oxidase inhibitors, very-long chain fatty-acid elongase inhibitors, and photosystem II-inhibiting herbicides in the United States (Brabham et al. 2019; Chahal et al. 2015; Heap 2020; Kumar et al. 2019; Wu et al. 2020). Adding genetically modified traits to soybean and cotton has allowed producers in some instances to apply multiple effective over-the-top herbicides to combat evolution of herbicide resistance in Palmer amaranth. However, a new herbicide site of action (SOA) has not been commercialized in 35 years (Duke 2012). Therefore, incorporation of optimized herbicide applications and integrated weed management techniques are needed to reduce the perpetuation of herbicide resistance in Palmer amaranth (Norsworthy et al. 2012).

The commercial launch of XtendFlex® cotton and soybean in 2015 and 2021, respectively, allows producers to apply dicamba, glufosinate, and glyphosate postemergence. Wide-spread glyphosate resistance in Palmer amaranth renders glyphosate ineffective on this weed (Culpepper et al. 2006; Culpepper et al. 2008). One strategy for mitigating target-site resistance is mixing two effective SOA (Bagavathiannan et al. 2013; Bagavathiannan et al. 2014; Diggle et al. 2003); however currently, dicamba-containing products like XtendiMax® plus VaporGrip® (Bayer CropScience, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) cannot be mixed with glufosinate (Anonymous 2021a, 2021b). Therefore, to incorporate two effective SOA in the XtendFlex® technology, dicamba and glufosinate have to be applied sequentially.

Many factors can influence the efficacy of sequential applications such as environmental conditions (Ahrens 1994; Anderson et al. 1993; Coetzer et al. 2001), sequence of herbicides applied (Burke et al. 2005; Vann et al. 2017), timing between sequential applications (Meyer et al. 2019), weed size (Lee and Oliver 1982; Steckel et al. 1997; Wilson 2005), and application techniques or nozzle selection (Etheridge et al. 2001; McKinlay et al. 1974; Meyer et al. 2015). To optimize POST applications in the XtendFlex® technology, a clear understanding of how dicamba, dicamba plus glyphosate, and glufosinate interactions in mixture and sequentially are needed.

Dicamba does not control monocot weed species; therefore, mixture with graminicides is needed to improve the weed spectrum (Lee and Oliver 1982). Conversely, glufosinate does provide control of monocot weed species (Meyer et al. 2020). When dicamba or glufosinate are mixed with glyphosate, an antagonistic response has been observed on monocot weed species (Besancon et al. 2018; Flint and Barret 1989; Meyer et al. 2017; Meyer et al. 2019; O'Sullivan and O'Donovan 1980). However, to increase the spectrum controlled over that with dicamba alone, glyphosate is commonly added.

Incorporation of multiple SOA in a full-season herbicide program is essential to mitigate the evolution and spread of herbicide-resistance (Norsworthy et al. 2012). To evaluate the interactions of postemergence herbicides, applications are made to labeled and above-labeled weed sizes (Meyer et al. 2019). Statistical differences between herbicide treatments are more often observed when above-label sized weeds are treated compared to applications when on-label sized weeds are treated (Kells et al. 1984; Lee and Oliver 1982; Meyer et al. 2019; Sellers et al.

2009; Steckel et al. 1997; Wilson 2005. In an attempt to optimize Palmer amaranth control while incorporating multiple SOA in the XtendFlex® technology, experiments were conducted to assess the effectiveness of single and sequential applications of dicamba, dicamba plus glyphosate, and glufosinate on labeled and above-labeled Palmer amaranth sizes at varying intervals between applications.

Material and Methods

Interactions of mixtures and sequential applications of dicamba, dicamba plus glyphosate, and glufosinate. A field experiment was conducted in 2019 and 2020 with a total of five site-years of data collected. Field locations were conducted near Crawfordsville, AR (N 35.228428, W -90.336762) in 2019, near Marianna, AR (N 34.725784, W -90.735788) in 2019 and 2020, and in Fayetteville, AR (N 36.092002, W -94.187002), and Keiser, AR (N 35.675128, W-90.07844) in 2020. The experiment was designed as randomized complete block with four replications in all locations.

Single applications of dicamba, glufosinate, and the combinations of dicamba plus glyphosate, dicamba plus glufosinate, dicamba plus glyphosate plus glufosinate were included to assess the interactions of herbicide mixtures. To evaluate the interaction of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate, efficacy of single applications of dicamba, dicamba plus glyphosate, and glufosinate were utilized in the Colby's equation to obtain expected values of the mixtures (Anonymous 2021a; Anonymous 2021b; Colby 1967).

Colby's method is a technique commonly used to assess the type of interaction that occurs when differing herbicides are applied in mixture. Colby's method requires the calculation of an expected value (E), which is displayed in equation 1,

$$E = (X + Y) - (XY)/100 \quad [1]$$

where E is the expected value of the herbicide mixture and X and Y represent the weed efficacy or mortality percentage of the individual herbicides applied alone. Observed efficacy of the herbicide mixtures are compared to the expected value. Herbicide mixtures that are observed to control less than, equal to, or greater than the expected values are determined to be antagonistic, additive, or synergistic, respectively.

To further assess the interaction between the aforementioned herbicides, sequential applications were made. Dicamba fb glufosinate, dicamba plus glyphosate fb glufosinate, and the inverse sequences were applied at 4-hour and 14-day intervals. Herbicide treatments were applied without a crop present to native Palmer amaranth populations at each location, besides Fayetteville, where Palmer amaranth from Crittenden County, AR was over-seeded. Field preparation included disking, hipping, and knocking down rows that were 91- to 97-cm wide. Plots dimensions at all locations were 1.8 to 1.9 m wide and 6 m long. Before the initial herbicide treatment was applied, two 0.25 to 0.5 m^2 quadrants were established in each plot and live Palmer amaranth plants were counted. Quadrant size varied by site-year due to densities of Palmer amaranth. Where lower densities occurred, a 0.5 m^2 quadrant was used to capture at least 15 plants $quadrant^{-1}$. Either dimethenamid-P or *S*-metolachlor [Weed Science Society America (WSSA) Group 15 herbicides] at a rate of 736 g ai ha^{-1} or 1606 g ai ha^{-1}, respectively, were applied to the entire experiment 1 to 3 days prior to the initial herbicide treatment to minimize further Palmer amaranth emergence. The WSSA Group 15 herbicides were reapplied on biweekly intervals to further mitigate any Palmer amaranth emergence through evaluations.

Herbicide treatments were applied with CO_2-pressurized backpack sprayers calibrated to deliver 140 L ha^{-1} of spray solution at 6.4 kph. All dicamba and dicamba plus glyphosate treatments were applied with Turbo TeeJet Induction (TTI) 110015 nozzles (TeeJet, Springfield, IL 62703). All glufosinate applications were made with Air Induction Extended Range (AIXR) 110015 nozzles (TeeJet, Springfield, IL 62703). All herbicide treatments were made between 9 am and 5 pm on days with less than 50% cloud cover to abide by guidelines on the glufosinate label (Anonymous 2020).

After treatments were applied, plots were visually rated and Palmer amaranth plants with live tissue were counted in the established quadrants 28 days after the final application (DAFA) in each treatment. Prior Palmer amaranth counts and final counts were utilized to provide a percent mortality. Palmer amaranth was visually rated on a scale of 0 to 100%, with 0% being no symptomology, no reductions in growth/vigor, and no reductions in biomass, and 100% being complete Palmer amaranth death (Frans and Talbert 1977).

Weed size and sequential interval. Field experiments were conducted in 2018, 2019, and 2020 in Arkansas. A total of six site-years of data were collected. Field locations included Crawfordsville, AR (N 35.228428, W -90.336762) in 2018 and 2019, Keiser, AR (N 35.675128, W-90.07844) in 2019 and 2020, Fayetteville, AR (N 36.092002, W -94.187002) in 2020, and Marianna, AR (N 34.725784, W -90.735788) in 2020. The experiment was designed as a randomized complete block with four replications and a three factor-factorial treatment structure. The three factors were herbicide sequence [dicamba followed by (fb) glufosinate, dicamba plus glyphosate fb glufosinate, glufosinate fb dicamba, and glufosinate fb dicamba plus glyphosate], application interval (3 and 14 days), and weed size (2.5 to 9.5cm and 35 to 40.6cm). Palmer amaranth sizes at initial application and soil information at each location are represented in Table 1. Experiments located at Keiser, AR and Marianna, AR were irrigated when 10 consecutive days of no precipitation occurred. Experiments located at Fayetteville, AR and Crawfordsville, AR were not irrigated. Plot size, experiment maintenance, application techniques, nozzle selection, and data collection were identical to the previously discussed interaction.

Data analysis. Site-years within the Weed Size experiment were pooled in analysis by inputting replication, site-year, and location within the model as random effects. A three-factor factorial model statement was built for the Weed Size experiment, utilizing the main effects: weed size, sequential herbicide interval, herbicide, and the respective interactions in PROC GLIMMIX model in SAS 9.4 (SAS Institute Inc., Cary, NC). A beta distribution for Palmer amaranth control and percent mortality was assumed as Palmer control and mortality data failed to fit normality assumptions in both experiments.

In the Interaction experiment, a single factor ANOVA was used to assess herbicide treatments in SAS 9.4 utilizing the PROC GLIMMIX function. A beta distribution was assumed for Palmer amaranth control and percent mortality. Site-years were analyzed by weed size at the initial application. Experiments conducted in Crawfordsville in 2019 and Fayetteville in 2020 were pooled as weed size at the time of the initial application abided by label requirements (Table 1). Site-years in Marianna in 2019, 2020, and Keiser in 2020 were pooled as the range of weed sizes at initial application were above-label requirements for both dicamba and glufosinate products (Table 1). Observed values of Palmer amaranth control and percent morality of dicamba plus glufosinate, and dicamba plus glyphosate plus glufosinate mixtures were compared via a paired t-test in the Match Pair platform in JMP 15.1 (SAS Institute Inc., Cary, NC) to the expected value calculated through the aforementioned Colby's equation. If the observed value was less than, greater than, or not different from the expected value then the interaction was deemed antagonistic, synergistic, or additive, respectively.

Results and Discussion

Interaction experiment. Single applications of dicamba, dicamba plus glyphosate, and glufosinate did not exceed 67, 83, or 68% control of Palmer amaranth when applications were made to labeled sized Palmer amaranth at either 14 or 28 DAFA, respectively (Table 2). On above-labeled weed sizes, a single application of dicamba, dicamba plus glyphosate, and glufosinate did not exceed 72, 69, or 37% control at either 14 DAFA or 28 DAFA, respectively (Table 2). The fecundity of Palmer amaranth in this trial was not quantified, but it appeared that survivors of the herbicide applications did produce seed. For a production system to remain sustainable, the weed seedbank must remain static or declining (Norsworthy et al. 2012).

Mean Palmer amaranth mortality following single applications of dicamba, dicamba plus glyphosate, and glufosinate did not exceed 73, 86, and 80% for labeled weed sizes and 65, 62, and 27% for above-labeled weed sizes 28 DAFA, respectively (Table 2). The initial soil seedbank density is an influential factor in determining acceptable control/mortality of weed species (Neve et al. 2011). Weed seedbank densities in the Corn Belt often range from 600 to 162,000 seed m^{-2} (Forcella et al. 1992). Paired with the fact, Palmer amaranth has been observed to produce an excess of 500,000 seeds per plant; the likelihood for evolution of herbicide resistance after repeated use of these single applications cannot be ignored (Norsworthy et al. 2012).

Current literature suggests a shift from annual economic thresholds to thresholds that aim beyond a single growing season and incorporate the long-term cost of herbicide resistance (Bauer et al. 1992; Cardina and Norquay 1997; Norris et al. 1999; Norsworthy et al. 2012; Sattin et al. 1992; Swanton et al. 1999). The idea proposed was to adopt near-zero- or zero-tolerance threshold to mitigate replenishment of the weed seedbank to help reduce the development of herbicide resistance (Norsworthy et al. 2012). To incorporate integrated weed management strategies with the XtendFlex® technology, the use of multiple SOAs in mixture or sequentially, as well as other management practices, should be evaluated; as single applications of dicamba, dicamba plus glyphosate, and glufosinate will not successfully control Palmer amaranth and mitigate replenishment of the weed seedbank.

Mixtures of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate that were applied to labeled weed sizes resulted in less than 84 and 86% control and 90 and 85% Palmer amaranth mortality (Table 2). The combination of dicamba plus glyphosate plus glufosinate was found to be antagonistic when the observed value of 83% Palmer amaranth control was compared to the expected value of 94% (P-value=0.0134) (Table 3). Furthermore, the mixtures of multiple SOA did not differ from dicamba plus glyphosate alone in terms of Palmer amaranth control or mortality (Table 2). Contact and systemic herbicides should not be mixed in most instances because the contact herbicide often antagonizes the systemic herbicide.[11] In addition, the aforementioned mixtures are prohibited by current labels (Anonymous 2021a; Anonymous 2021b).

Interactions of herbicide mixtures were more likely to be observed on large weed sizes where herbicide efficacy decreased (Meyer et al. 2019). On larger-than-labeled weed sizes, efficacy of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate did not exceed 69% control or 56% mortality (Table 2). Both herbicide mixtures were antagonistic for control of Palmer amaranth at both evaluations. Similarly, antagonism from the mixture of dicamba plus glufosinate on Palmer amaranth was observed in previous research, with a decrease in efficacy of 18-percentage points between the observed and expected value (Meyer et al. 2019).

When both herbicides are needed as part of a resistance-management strategy, sequential applications may be superior to mixtures for reducing the risks of resistance (Duke 2012). In the XtendFlex® technology where Palmer amaranth plant harbor multiple resistance mechanisms to other herbicides, dicamba and glufosinate may be the only effective postemergence options in soybean and cotton; therefore, there is a need to evaluate the utility of sequential applications (Heap 2020).

Sequential herbicide applications were made at 4-hour and 14-day intervals. The 4-hour interval was designed to simulate a producer spraying a field once and then reloading the sprayer and applying the alternative herbicide sequentially. The 14-day interval was included to assess the efficacy of two SOA as a first and second postemergence sequential program, excluding preemergence and residual herbicides.

When Palmer amaranth at a labeled size was treated with sequential applications of dicamba fb glufosinate, dicamba plus glyphosate fb glufosinate or the inverse sequence, >85% control was achieved 28 DAFA (Table 2). Dicamba plus glyphosate fb glufosinate at the 14-day interval was the only herbicide treatment to reach 100% control and 100% mortality of Palmer amaranth; therefore, making it the only herbicide treatment to abide by the proposed zero-tolerance policy (Norsworthy et al. 2012). The use of dicamba plus glyphosate fb glufosinate at the 14-day interval provided a 17- and 15-percentage point increase in control compared to the mixtures of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate, respectively, 28 DAFA (Table 2). However; dicamba plus glyphosate fb glufosinate at the 14-day interval was not different from other sequential applications when labeled weed sizes were treated.

The larger-than-labeled Palmer amaranth was controlled 97% by dicamba plus glyphosate fb glufosinate at the 14-day interval, and this treatment provided a 28- and 41-

percentage point increase in control compared to dicamba plus glufosinate or dicamba plus glyphosate plus glufosinate 28 DAFA, respectively (Table 2). While, numerically Palmer amaranth control with dicamba plus glyphosate fb glufosinate at the 14-day interval was consistently the best across weed sizes, other trends are of importance.

Interval between sequential applications influenced Palmer amaranth control. On larger-than-labeled weed sizes averaged across herbicide treatments, sequential applications made at the 14-day interval provided a 13- and 15-percentage point increase in Palmer amaranth control when compared to the 4-hour interval at 14- and 28-DAFA, respectively. Therefore, regardless of herbicide sequence, producers should wait to apply sequential herbicides 14 days after the initial application. In addition, this finding also eludes to the mechanism of antagonism of dicamba and dicamba plus glyphosate in mixture with glufosinate. The combination of dicamba plus glufosinate did not differ from any sequential herbicide treatment at the 4-hour interval. Therefore, a reaction within spray solution is not a likely cause for the observed antagonism as control was not increased when dicamba, dicamba plus glyphosate, and glufosinate were sequentially applied at the 4-hour interval.

Antagonism of herbicide mixtures and reductions in sequential herbicide efficacy at the 4-hour interval can likely be attributed to the opposing physiological response of Palmer amaranth to glufosinate and dicamba. Meyer et al. (2019) observed a higher absorption of ^{14}C-dicamba when mixed with glufosinate relative to ^{14}C-dicamba alone. This was attributed to a reduction in spray-solution pH when dicamba and glufosinate were applied in mixture and a likewise conversion of dicamba salt to dicamba acid. The conversion of dicamba salt to dicamba acid was the assumed reason for the increase in ^{14}C dicamba absorption. Therefore, spray solution interactions of dicamba and glufosinate would likely increase Palmer amaranth efficacy.

However, Meyer et al. (2019) also observed a 77% reduction in dicamba translocation from the treated leaf when glufosinate was added in mixture. The reduction in dicamba translocation can be attributed to the relatively fast necrosis caused by glufosinate; hence, Palmer amaranth control did not increase when sequential applications were applied at the 4-hour interval.

Glufosinate and dicamba metabolism has been observed in Palmer amaranth populations (Meyer et al. 2020; Jansen et al. 2000). An application of an auxin herbicide causes an upregulation of detoxifying enzymes (e.g. glutathione *s*-transferase, cytochrome P-450) which can increase herbicide metabolism (Cummins et al. 1999; Raghavan et al. 2005; Yu and Powles 2014). Therefore, by applying dicamba and glufosinate in mixture or in short (i.e. 4-hour) time intervals may lead to an increase in glufosinate metabolism. The study of herbicide physiological interactions is comprised of many intricacies and unknowns. The use of labeled herbicide rates in mixture can mask the interactions observed in field trials (Ou et al. 2018). Further research of the mechanism of antagonism of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate is needed to aid efforts to mitigate reductions in control.

Weed size experiment. A significant three-way interaction of herbicide treatment x weed size x interval between sequential applications was observed for Palmer amaranth control 28 DAFA (P-value=0.0081). Palmer amaranth control at 14 DAFA and Palmer amaranth mortality 28 DAFA were not affected by a three-way interaction of the factors tested (P-value=0.2015 and 0.6627, respectively); however, there was a significant two-way interaction of interval x weed size (P-value= <0.0001 and 0.0008, respectively) and significant main effect of herbicide (P-value=<0.0001 and <0.0001, respectively).

In general, as weed size increased, interval between sequential applications decreased, and glufosinate was used prior to dicamba vs dicamba before glufosinate, Palmer amaranth control at 28 DAFA decreased. The interval between sequential applications and weed size influenced the level of Palmer amaranth control and mortality (Table 2). Excluding glufosinate fb dicamba at the 3-day interval, control at 14- and 28-DAFA, and mortality at 28 DAFA only varied by 11-percentage points when labeled Palmer amaranth was treated. Glufosinate fb dicamba at the 3-day interval provided 74% control of labeled Palmer amaranth 28 DAFA; which, was less than any other treatment at this size. Thus, glufosinate fb dicamba at the 3-day interval does not optimize the postemergence options in the XtendFlex® technology and should be avoided. In addition, the only treatments that provided above 90% Palmer amaranth control (14- and 28-DAFA) and mortality (28 DAFA) was dicamba + glyphosate fb glufosinate at the 3- and 14-day interval. However, other treatments achieved similar control and mortality when Palmer amaranth <10 cm was treated.

Palmer amaranth control at 14- and 28-DAFA and mortality 28 DAFA was reduced 13- to 24-percentage points as weed size increased from labeled (2.5 to 9.5 cm) to larger-than-labeled (35 to 41 cm). Similarly, Meyer and Norsworthy (2019) observed higher levels of weed control with herbicide mixtures on 10 cm weeds versus 30 cm weeds. To abide by label restrictions, applications of dicamba and glufosinate should be applied to Palmer amaranth <10cm in height (Anonymous 2021a; Anonymous 2021b); however, due to inclement weather and various other factors, the ability to make timely applications to <10 cm Palmer amaranth are not always practical.

A greater difference in herbicide efficacy on larger-than-labeled compared to labeled weed sizes when the same treatments were applied was observed by Meyer et al. (2019). By applying herbicide treatments to larger-than-labeled Palmer amaranth a better understanding of the interactions may be understood. Dicamba + glyphosate fb glufosinate at the 14-day interval provided 82 to 83% Palmer amaranth control and mortality, which, numerically was the highest level achieved at the 35- to 41-cm tall weed size. Dicamba + glyphosate fb glufosinate at the 14-day interval achieved a higher level of weed control than any other treatment besides dicamba fb glufosinate at the 14-day interval, on larger-than-labeled weeds, 28 DAFA. On larger-than-labeled Palmer amaranth sizes (35 to 41 cm), applying dicamba or dicamba plus glyphosate 14 days prior to glufosinate optimized Palmer amaranth control.

Overall, multiple sequences of dicamba, dicamba plus glyphosate, and glufosinate as well as several intervals between sequential applications provided similar control and mortality when Palmer amaranth <10 cm in height was treated. Palmer amaranth control and mortality was numerically optimized when dicamba plus glyphosate fb glufosinate or dicamba fb glufosinate at the 14-interval was used on larger-than-labeled weed sizes. Future research should compare dicamba or dicamba plus glyphosate fb glufosinate at the 14-day interval to other sequences and intervals to understand the changes in likelihood of either dicamba or glufosinate resistance evolving.

Conclusions and practical implications. Single applications of dicamba, dicamba plus glyphosate, and glufosinate controlled labeled and larger-than-label sized Palmer amaranth 67 to 83% and 37 to 72%, respectively. The high genetic diversity and prolific seed producing ability of Palmer amaranth may imply single applications of dicamba, dicamba plus glyphosate, and glufosinate may not be sustainable in terms of resistance mitigation. The use of multiple effective SOAs in mixture has been proposed as a solution to mitigate the evolution of resistance in weed populations, if the two or more herbicides are not antagonistic in mixture. The mixture of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate reduced Palmer amaranth control 19- to 31-percantage points and 16- to 30-percentage points when compared to expected values calculated by Colby’s analysis across evaluation timings, respectively.

Sequential applications of dicamba, dicamba plus glyphosate, and glufosinate at the 4-hour interval did not increase Palmer amaranth control. Assumptions that can be implied are, but not limited to, reductions in weed control of mixtures that include dicamba or dicamba plus glyphosate and glufosinate are not due to interactions in the spray solution but likely occur from differing physiological responses of Palmer amaranth. An application of dicamba plus glyphosate fb glufosinate at the 14-day interval consistently achieved above 90% and 82% control of labeled and larger-than-label sized Palmer amaranth, respectively across experiments. By lengthening the time interval between sequential applications to 14-days and applying the systemic herbicides dicamba and glyphosate prior to the contact herbicide glufosinate, negative physiological interactions may have been avoided. Future research should assess what impacts applying dicamba or dicamba plus glyphosate in close time intervals to glufosinate and the implications of applying glufosinate prior to dicamba and glyphosate have on of resistance mitigation.

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Tables

Table 1. Trial year, location, Palmer amaranth size at the initial application, and soil series where the experiments were conducted.

				Palmer amaranth		
				Size at initial application	Density	
Experiment	Year	Nearest City	Location	Average (range)	Average (range)	Soil Series[b]
				cm	plants ha^{-1}	
Weed Size	2018	Crawfordville, AR	Production field	Na[a]	1,240,000 (320,000-5,840,000)	Dundee silt loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs)
Weed Size	2019	Crawfordville, AR		Na	1,320,000 (240,00-5,160,000)	
Interaction	2019	Crawfordville, AR		6.3 (1.3-10.1)	1,240,000 (520,000-3,800,00)	
Weed Size	2019	Keiser, AR	Northeast Research and Extension Center	Na	880,000 (360,000-3,440,000)	Sharkey silty clay (Very-fine, smectitic, thermic Chromic Epiaquerts)
Weed Size	2020	Keiser, AR		Na	1,680,000 (1,200,000-5,440,000)	
Interaction	2020	Keiser, AR		9.5 (2.5-15.2)	1,040,000 (680,000-4,000,000)	
Interaction	2019	Marianna, AR	Lon Mann Research and Extension Center	15.2 (2.5-30)	280,000 (120,000-1,200,000)	Convent silt loam (Coarse-silty, mixed, superactive, thermic Fluvaquentic Endoaquepts)
Weed Size	2020	Marianna, AR		Na	1,520,000 (360,000-6,160,000)	
Interaction	2020	Marianna, AR		10.1 (6.3-15.2)	1,520,000 (320,000-4,000,000)	
Weed Size	2020	Fayetteville, AR	University of Arkansas-Agricultural Research and Extension Center	Na	3,240,000 (1,280,000-4,880,00)	Leaf silt loam soil (Fine, mixed, active, thermic Typic, Albaqualts)
Interaction	2020	Fayetteville, AR		7.2 (1.3-9.5)	2,560,000 (520,000-5,840,000	

[a] Na, not applicable

[b] Soil series were obtained from Web Soil Survey database (53)

Table 2. Percent control and morality of label sized (<10cm tall) and above-label sized (>10cm tall) Palmer amaranth following herbicide treatments in Crawfordsville and Marianna AR, in 2019 and Fayetteville, Keiser, Marianna, AR in 2020. Experimental runs conducted at Crawfordsville, AR in 2019 and Fayetteville, AR in 2020 were pooled as weed size at the time of the initial application abided by height requirements required by the herbicide label. Experimental runs conducted in Marianna, AR in 2019 and 2020, and Keiser, AR in 2020 were pooled as Palmer amaranth height at the time of the initial application exceeded all product label requirements.

		Palmer amaranth size					
		Labeled			Above-labeled		
		Control[a]		Mortality[a]	Control[a]		Mortality[a]
Herbicide treatment	Interval	14 DAFA	28 DAFA	28 DAFA	14 DAFA	28 DAFA	28 DAFA
		%					
Dicamba	0	67 D	67 D	73 D	72 ABC	70 D	65 BCD
Dicamba + glyphosate	0	83 BC	76 CD	86 ABC	65 BC	69 D	62 CD
Glufosinate	0	68 D	66 D	80 BCD	27 E	37 F	27 F
Dicamba + glufosinate	0	83 BC	84 BC	90 ABC	44 D	69 D	56 DE
Dicamba + glufosinate + glyphosate	0	83 BC	86 BC	85 ABCD	45 D	56 E	42 EF
Dicamba fb glufosinate	4 hours	85 BC	83 BC	88 ABC	73 ABC	73 D	67 BCD
Dicamba fb glufosinate	14 days	93 AB	94 AB	97 A	78 AB	89 AB	77 ABC
Dicamba + glyphosate fb glufosinate	4 hours	94 AB	91 AB	92 AB	66 BC	74 CD	65 BCD
Dicamba + glyphosate fb glufosinate	14 days	100 A	99 A	100 A	89 A	97 A	86 A
Glufosinate fb dicamba	4 hours	91 ABC	91 AB	97 A	60 C	71 D	61 D
Glufosinate fb dicamba	14 days	87 BC	91 AB	96 A	73 ABC	79 BCD	70 BCD
Glufosinate fb dicamba + glyphosate	4 hours	83 BC	82 BC	79 CD	59 C	69 D	61 D
Glufosinate fb dicamba + glyphosate	14 days	80 C	85 BC	91 ABC	78 AB	87 ABC	78 AB

[a] Palmer amaranth control and mortality is expressed as percent of the nontreated

[b] Abbreviations: DAFA, days after final application; fb, followed by

[c] Means followed by the same letter within a column are not statistically different according to Tukey's HSD (α=0.05).

Table 3. The effect of mixtures of dicamba plus glufosinate and dicamba plus glyphosate plus glufosinate on Palmer amaranth control at 14 and 28 days after treatment and Palmer amaranth mortality 28 days after treatment, separated by labeled and above-label sized weeds.

		Palmer amaranth								
		Control 14 DAFA[a]			Control 28 DAFA[a]			Mortality 28 DAFA[a]		
Weed size	Herbicide mixture	Obs[a]	Exp[a]	P-value	Obs	Exp	P-value	Obs	Exp	P-value
		-----%-----			------%-----			-----%----		
Labeled[d]										
	dicamba + glufosinate	83[b]	89	0.3050	84[b]	87	0.6191	90[b]	94	0.6152
	dicamba + glufosinate + glyphosate	83	94	0.0134*[c]	86	91	0.1387	85	98	0.0548
Above Label										
	dicamba + glufosinate	48	79	0.0023*	56	75	0.0358*	54	66	0.3661
	dicamba + glufosinate + glyphosate	45	61	0.0339*	42	72	<0.0001 *	40	60	0.0042*

[a] Abbreviation: DAFA, days after final application, Obs, observed value, Exp, expected value

[b] Palmer amaranth control and mortality is expressed as percent of the nontreated

[c] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E=(X + Y) - (XY)/100$].

[d] Labeled Palmer amaranth is <10 cm in height and above-label sized Palmer amaranth is >10 cm in height.

Table 4. Palmer amaranth control and mortality of sequential applications of dicamba, dicamba plus glyphosate, and glufosinate made at 3- day and 14-day intervals to two weed sizes pooled over six site-years of data.

				Palmer amaranth					
				Control[c]				Mortality[c]	
Factors	Sequential herbicide treatment	Weed size[a]	Interval[b]	14 DAFA		28 DAFA		28 DAFA	
		cm		---------------------%----------------------					
Herbicide x interval x weed size									
	dicamba fb[d] glufosinate	3 to 10	3 day	86		88	AB[e]	85	
	dicamba fb glufosinate	3 to 10	14 day	88		89	AB	88	
	glufosinate fb dicamba	3 to 10	3 day	73		74	D	73	
	glufosinate fb dicamba	3 to 10	14 day	84		85	B	84	
	dicamba + glyphosate fb glufosinate	3 to 10	3 day	92		94	A	92	
	dicamba + glyphosate fb glufosinate	3 to 10	14 day	90		91	A	90	
	glufosinate fb dicamba + glyphosate	3 to 10	3 day	82		93	A	82	
	glufosinate fb dicamba + glyphosate	3 to 10	14 day	93		83	BC	93	
	dicamba fb glufosinate	35 to 41	3 day	52		54	FG	52	
	dicamba fb glufosinate	35 to 41	14 day	76		77	CD	76	
	glufosinate fb dicamba	35 to 41	3 day	48		51	G	48	
	glufosinate fb dicamba	35 to 41	14 day	64		63	E	64	
	dicamba + glyphosate fb glufosinate	35 to 41	3 day	54		56	F	55	
	dicamba + glyphosate fb glufosinate	35 to 41	14 day	83		83	BC	82	
	glufosinate fb dicamba + glyphosate	35 to 41	3 day	57		59	EF	57	
	glufosinate fb dicamba + glyphosate	35 to 41	14 day	73		72	D	73	
Interval x weed size									
		3 to 10	3 day	92	A			88	A
		3 to 10	14 day	87	B			88	A
		35 to 41	3 day	62	D			66	B
		35 to 41	14 day	69	C			82	A
Herbicide									
	dicamba fb glufosinate			74	B			81	BC
	dicamba + glyphosate fb glufosinate			87	A			88	A
	glufosinate fb dicamba			72	B			75	B
	glufosinate fb dicamba + glyphosate			83	A			84	AB

[a] Weed size at the time of the initial application

[b] Interval between the sequential applications

[c] Percent control and mortality of Palmer amaranth 28 days after the final application in each treatment

[d] Abbreviation: fb, followed by, herbicide one fb (followed by) herbicide two

[e] Means followed by the same letter within a column and within a factor level are not statistically different according to Tukey's HSD (α=0.05).

Chapter 4

Effects of applying metabolic inhibitors to sequential applications of dicamba and glufosinate

Jason K Norsworthy, Andy Mauromoustakos, Thomas R Butts, Trenton L Roberts

Abstract. In some geographies, the only effective postemergence (POST) options remaining to control Palmer amaranth in soybean and cotton are auxin herbicides and glufosinate due to the evolution of herbicide resistance. An experiment was conducted in 2018, 2019, and 2020 including a total of six-site years and four locations. The objective of this experiment was to assess if metabolic inhibitors [amitrole, malathion, piperonyl butoxide (PBO), NBD-Cl] or combinations of multiple metabolic inhibitors when applied between an application of dicamba followed by (fb) glufosinate at a 3-day interval improved Palmer amaranth control. The application of metabolic inhibitors between a dicamba fb glufosinate sequential application at a 3-day interval did not increase the efficacy or mortality of Palmer amaranth. The metabolic inhibitors and combinations of metabolic inhibitors did increase visual injury to cotton at 7- and 21-days after final application (DAFA) compared to the herbicides alone. However, mean cotton injury did not exceed 21% where metabolic inhibitors or combinations of metabolic inhibitors were used, 7 or 21 DAFA. Relative cotton height was not affected by the metabolic inhibitors applied between dicamba fb glufosinate at a 3-day interval, 21 DAFA. Amitrole, malathion, NBD-Cl, and PBO may be applicable to use over-the-top of cotton because of the relatively low cotton injury observed; however, the use of metabolic inhibitors alone or in combination did not provide an increase in Palmer amaranth control or mortality when added between the sequential applications of dicamba fb glufosinate at a 3-day interval.

Introduction

The perpetuating evolution of herbicide resistance in Palmer amaranth has left producers with limited postemergence (POST) herbicide options for weed control. Palmer amaranth has evolved resistance to the auxin herbicides 2,4-D and dicamba, acetolactate synthase (ALS) inhibitors, dinitroanilines, glyphosate, 4-hydroxyphenylpyruvate dioxygenase inhibitors, protoporphyrinogen oxidase inhibitors, *S*-metolachlor, and triazine herbicides in the United States (Brabham et al., 2019; Chahal et al., 2015; Kumar et al., 2019; Wu et al., 2020). Herbicide resistance in Palmer amaranth can be divided into two classifications (i) target-site resistance and (ii) nontarget-site resistance (Nakka et al., 2017).

Target-site-based resistance can be defined as an amino acid substitution in the target-site enzyme that reduces herbicide binding affinity (Tranel et al., 2016). Currently, many cases of target-site resistance in weed populations have been reported and have not been overcome. In weeds such as giant ragweed (*Ambrosia trifida* L.), kochia [*Bassia scoparia* (L.) A.J. Scott], and four *Amaranthus* species a total of 26 amino acid substitutions have been identified across 8 amino acid positions on the ALS gene (Tranel et al., 2016). Specifically, 11 substitutions at the Pro-197 amino acid position of the ALS gene have been observed in Palmer amaranth populations have been observed (Burgos et al., 2001; Foes et al., 1999; Guttieri et al., 1995; Patzoldt & Tranel, 2002; Varanasi et al., 2015).

Nontarget-site resistance includes but is not limited to detoxification/metabolism of the herbicide through cytochrome P450 monooxygenase enzymes (Christopher et al., 1994), and glutathione *S*-transferase (GST) enzymes (Brabham et al., 2019; Ma et al., 2016). Development of nontarget-site resistance is troublesome due to cross-resistance to multiple sites of action (SOA) that can occur (Varanasi et al., 2019). Nontarget-site resistance that utilizes metabolism through cytochrome P450- or GST-enzymes has been alleviated through the addition of metabolic-inhibiting compounds such as amitrole (Oliveira et al., 2017), malathion (Ma et al., 2013; Oliveira et al., 2017), piperonyl butoxide (PBO), and 4-chloro-7-nitrobenzofurazan (NBD-Cl) (Ma et al., 2016) prior to or in mixture with the herbicide applied (Varanasi et al., 2018). Amitrole, malathion, and PBO are known cytochrome P450 inhibitors and NBD-Cl is a known GST inhibitor, all of which have been used to decipher the mechanism of herbicide metabolism and reverse metabolic-herbicide resistance in some weed species (Brabham et al., 2019; Ma et al., 2016; Oliveira et al., 2017; Varanasi et al., 2018).

There is a lack of research associated with enhanced herbicide metabolism due at least partially to the complexity of the experiments (Yu & Powles, 2014). The lack of research associated with this topic may be due to the complexity of the study. In plants, cytochrome P450 enzymes make up approximately 1% of the genome. Cytochrome P450 enzymes are divided into 47 families and grouped into 11 clans (Nelson & Werk-Reichhart, 2011). Similarly, 54 GST genes have been identified in Arabidopsis [*Arabidopsis thaliana* (L.) Heynh], which have been constituted in seven distinct classes in plants (Dixon et al., 2002, and 2009). Minimal research has been conducted to associate specific P450- and GST-enzymes with herbicide metabolism or sequestration; therefore, inhibiting specific enzymes involved in herbicide degradation is challenging. In addition, the specificity of metabolic inhibitors like amitrole, malathion, PBO, and NBD-Cl is unknown.

The discovery of metabolic resistance has changed the perspective of how to mitigate the probability of resistance evolving (Norsworthy et al., 2012; Yu & Powles, 2014). Utilizing two SOAs may be as detrimental to metabolic-resistance mitigation as repeated use of a single SOA, if the two SOAs are metabolized by the same enzymes or if the prior herbicide causes an upregulation of herbicide degrading enzymes (Burnet et al., 1993a, 1993b; Burnet et al., 1994; Yu & Powles, 2014). With auxin herbicides and glufosinate being the only effective POST options for Palmer amaranth control in some geographies, novel approaches to mitigate metabolic-resistance should be evaluated.

XtendFlex® cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] were commercially launched in 2015 and 2021, respectively, and confer herbicide tolerance that allow for over-the-top applications of dicamba, glufosinate, and glyphosate. It has been observed that mixing two effective SOAs mitigates the likelihood of target-site resistance development (Bagavathiannan et al., 2013; Bagavathiannan et al., 2014; Diggle et al., 2003); however, dicamba-containing products like XtendiMax® plus VaporGrip® (Bayer CropScience, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) cannot be mixed with glufosinate, due to regulatory limitations (Anonymous, 2020a; Anonymous, 2020b). Therefore, dicamba and glufosinate have to be applied sequentially in the XtendFlex® technology. An application of dicamba fb glufosinate at a 14-day interval optimized Palmer amaranth control in the XtendFlex® technology, which was most apparent on larger-than-labeled weed sizes (Priess et al., 2019).

When dicamba fb glufosinate was applied at a 6-hour and 3-day interval, reductions in Palmer amaranth control were observed on larger than labeled weed sizes (Priess et al., 2019). Applications of auxin herbicides similar to dicamba can have adverse effects on sequentially applied herbicides (Cummins et al., 1999; Raghavan et al., 2005; Yu & Powles, 2014). Auxin herbicides have been observed to cause an upregulation of detoxifying enzymes (glutathione *S*-transferase, cytochrome P450s), which can impact metabolism of subsequently applied pesticides (Cummins et al., 1999; Raghavan et al., 2005). Yu & Powles, (2014) observed that a pretreatment of 2,4-D resulted in a 10-fold increase in diclofop rate needed to control 50% of a rigid ryegrass (*Lolium rigidum* Gaudin) susceptible population, due to an induction of cytochrome P450 enzymes. The increase in diclofop rate needed to control pretreated rigid ryegrass was reversed when a metabolic inhibitor (malathion) was sprayed prior to the diclofop treatment. Based on these findings it is believed that auxin herbicides may cause an upregulation of detoxifying enzymes; therefore, the objective of this experiment was to determine whether an application of metabolic inhibitors (amitrole, malathion, PBO, NBD-Cl) to the sequential application of dicamba fb glufosinate at a 3-day interval improved Palmer amaranth control.

Materials and Methods

A field experiment was conducted in 2018, 2019, and 2020. The locations, year, cotton growth stage, soil information, and Palmer amaranth size at the initial application for each run of the experiment are displayed in Table 1. The field experiment was conducted as a single-factor randomized complete block design with four replications, with the single factor being herbicide treatment with or without the addition of metabolic inhibitors prior to a glufosinate application (Table 2). Metabolic inhibitors were applied after the dicamba application but prior to the glufosinate application to assess the influence that potentially upregulated herbicide-degrading enzymes may have on subsequent glufosinate efficacy. Cytochrome P450 inhibitors (amitrole at 14 g ai ha^{-1}, PBO at 1500 g ai ha^{-1}, malathion at 2000 g ai ha^{-1}), and GST inhibitor (NBD-Cl at 269 g ai ha^{-1}) were applied 4 hours, or 2 days prior to the subsequent glufosinate application, respectively, according to recommendations from previously published research on these metabolic inhibitors (Brabham et al. 2019; Ma et al. 2016; Oliveira et al. 2017; Varanasi et al. 2018).

Combinations of metabolic inhibitors were applied to assess if multiple enzymes may be responsible for subsequent herbicide metabolism. Multiple cytochrome P-450 inhibitors were used to compensate for the extensive nature of the enzyme family and the fact that amitrole, malathion, and PBO have been observed to reduce herbicide metabolism in weedy species (Brabham et al., 2019; Ma et al., 2016; Nelson & Werk-Reichhart, 2011; Oliveira et al., 2017; Varanasi et al., 2018).

Prior to experiment initiation, fields were cultivated and 91 cm raised beds were formed in Fayetteville, AR, and 96 cm raised beds at other locations (Crawfordsville, AR; Marianna, AR; and Keiser, AR). XtendFlex® DP 1518B2XF (Bayer CropScience, St. Louis, MO, 63141) cotton was planted at 98,800 to 118,000 seeds ha^{-1} in locations where native Palmer amaranth populations were present and were allowed to go to seed the previous year. Plot size at all locations were 0.91 to 0.96 m wide and 6 m long. At cotton planting, *S*-metolachlor (Dual II Magnum, Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 401 g ai ha^{-1} to delay Palmer amaranth emergence and allow cotton to achieve the second node growth stage prior to the initial application of the treatment.

Treatments were applied with a hand-held CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} of spray solution at 6.4 kph. Rates for herbicides and metabolic inhibitors are displayed in Table 1. Dicamba (XtendiMax® plus VaporGrip®, Bayer Crop Science, St. Louis, MO) was applied with Turbo TeeJet Induction (TTI) 110015-VP (Teejet, Springfield, IL, 62703) nozzles. Metabolic inhibitors and glufosinate (Liberty, BASF, Ludwigshafen, Germany) were applied with Air Induction Extended Range (AIXR) 110015-VP (Teejet, Springfield, IL, 62703) nozzles.

An application of *S*-metolachlor or dimethenamid-P (Outlook, Syngenta Crop Protection, Greensboro, NC) at a rate of 1606 g ai ha^{-1} or 736 g ai ha^{-1}, respectively, was made to the entire experiment 3 to 7 days prior to the application of the initial herbicide treatment. Applications of a WSSA group 15 herbicide was made once every two weeks through the duration of the assessments to minimize further Palmer amaranth emergence and limit the impact of newly emerged plants on observations.

Prior to initial application of treatments, two 0.25- to 0.5-m^2 quadrants were established in each plot, and Palmer amaranth plants were counted. Palmer amaranth plants with live tissue were counted in the established quadrants 28 days after the final application (DAFA). Initial and final Palmer amaranth densities in each plot were used to calculate percent mortality of Palmer amaranth. Following treatment applications, Palmer amaranth control was visually evaluated 28 DAFA on a 0 to 100 scale, with 0 being no control and 100 being Palmer amaranth death (Frans & Talbert, 1977). Cotton injury was visually rated at 7 and 21 DAFA on 0 to 100 scale, with 0 being no injury and 100 being cotton death. The height of 3 to 5 cotton plants in each plot were recorded 21 DAFA in five of the six site years. Cotton height was expressed as relative to the nontreated control in each replication of the experimental run.

Data analysis. Percent control and mortality of Palmer amaranth 28 DAFA were assumed to have a beta distribution, and cotton injury at 7 and 21 DAFA was assumed to follow a gamma distribution by assessing AICc and BIC values in the distribution platform of JMP Pro 15.2 (SAS Institute Inc., Cary, NC). Relative cotton height was assumed to follow a normal distribution. The effect of the single-factor herbicide treatments was assessed in PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). Site-year, location, and replication were considered random effects. Means were separated using Tukey’s HSD at an alpha value of 0.05.

Results and Discussion

Palmer amaranth control and mortality. The use of metabolic inhibitors did not impact Palmer amaranth control and Palmer amaranth mortality with P-values of 0.0726 and 0.3686, respectively (Figures 1 and 2). The differences in Palmer amaranth control were not deemed relevant even though the effect of metabolic inhibitors was close to statistically significant. An application of dicamba fb glufosinate at a 3-day interval achieved greater than 89% mean Palmer amaranth control and mortality (Figures 1 and 2). Because dicamba fb glufosinate at a 3-day interval resulted in Palmer amaranth control greater than 89%, the ability to detect differences among treatments may be negligible from a practical standpoint. In future work, where metabolic additives are applied in sequence or added to efficacious herbicides, applications should be made to larger weeds or a reduced rate of herbicide should be used.

The copious amounts and complexity surrounding cytochrome P450- and GST-enzymes in plant species also may have hindered the ability to observe responses in Palmer amaranth control. NBD-Cl has been observed to be an acceptable substrate for *PvGm*GSTs but lacks affinity for other GST enzymes (Chronopoulou et al., 2018). Even though amitrole, malathion, NBD-Cl, and PBO have been shown to mitigate herbicide metabolism in some plants; more research is needed to understand what enzymes if any are responsible for metabolizing glufosinate when dicamba is applied previously. With a better understanding of the nature of glufosinate metabolism, more specific metabolic inhibitors could be selected.

Even more troublesome is the fact that combinations of multiple metabolic inhibitors did not increase Palmer amaranth control or mortality (Figures 1 and 2). The addition of four metabolic inhibitors (amitrole, NBD-Cl, malathion, PBO) did not increase Palmer amaranth control or mortality. Herbicide metabolism has been observed to occur in a process of steps.

P450 enzymes can catalyze herbicide arylhydroxlation or alkylhydroxylation, which can be followed by GST catalyzed conjugation (De Prado & Franco, 2004; Yu & Powles, 2014). The complexity of these interactions of multiple enzymes did not appear to be affected by the metabolic inhibitors chosen. In addition, NBD-Cl the only GST inhibitor used in the experiment, is photodegraded in the presence of ultra violet light, and not a formulated product for commercial use; therefore, the addition of adjuvants may have improved NBD-Cl uptake (Norsworthy personal communication).

The use of metabolic inhibitors to overcome herbicide metabolism is a complex study that is not well understood, because of the lack of information surrounding the mechanism and selectivity of cytochrome P450 and GST enzymes (Dixon et al., 2010; Dixon & Edwards, 2009; Edwards et al., 2000; Gullener et al., 2018; Marrs, 1996). In some geographies, producers are relying solely on auxin herbicides and glufosinate for Palmer amaranth control, because of wide-spread herbicide resistance that has evolved in the weed species (Heap, 2021). Further research is needed to assess the utility of metabolic inhibitors to improve efficacy of POST options in the XtendFlex® technology and mitigate the development or consequences of metabolic resistance.

Kumar et al. (2019) and Priess et al. (unpublished data) recently confirmed Palmer amaranth biotypes that are resistant to dicamba and glufosinate, respectfully. The reduction of dicamba and glufosinate efficacy may influence the results from this experiment. The utility of metabolic inhibitors may influence levels of control if the herbicide-resistance mechanism is metabolic in nature. Future work should assess to what extent glufosinate metabolism was impacted by an application of the metabolic inhibitors used in the experiment. If glufosinate metabolism was reduced by metabolic inhibitors, these inhibitors may mitigate the likelihood of metabolic resistance evolving.

Cotton response. Overcoming herbicide metabolism in weedy species with an application of metabolic inhibitors is limited if increased crop injury occurs. At 7 and 21 DAFA, an application of metabolic inhibitors prior to glufosinate increased cotton injury (P-values of <0.0001 and 0.0004, respectively) (Figures 3 and 4). At 7 DAFA, mean cotton injury averaged over six site-years did not exceed 15%. Certain combinations of metabolic inhibitors affected the variability of cotton injury. For example, the 3-way combination of amitrole, PBO, and NBD-Cl resulted in injury ranging from 0 to 70% while the nontreated control only ranged from 0 to 20% (Figure 3). In general, the addition of metabolic inhibitors to dicamba fb glufosinate herbicide treatments did increase the likelihood of seeing a response to the cotton, but mean cotton injury over six-site years of data were comparable to labeled herbicides (Chachalis & Galanis, 2007).

An application of amitrole generally increased the mean level of cotton injury observed 7 and 21 DAFA (Figure 3 and 4). Amitrole does have herbicidal activity on a number of weed species and cotton (Clor et al., 1964; Smith & Wiese, 1972). Therefore, cotton injury following an amitrole application was expected. Cotton recovered from the application of amitrole as evidenced by a reduction in cotton injury from 7 to 21 DAFA.

An application of malathion, NBD-Cl, or PBO did not cause a significant increase in cotton injury when compared to dicamba fb glufosinate at a 3-day interval, 7 DAFA (Figure 3). Malathion is labeled for use in cotton, and prior research indicated that malathion did not cause injury to cotton; therefore, cotton injury was not expected to occur (Anonymous, 2015; Snipes & Seifert, 2003). Piperonyl butoxide also has been reported to not cause injury to cotton (Selim & Testman, 1999). When NBD-Cl was added to glufosinate less than 20% cotton injury was observed (Priess & Norsworthy, 2020) and results from the experiment conducted led to the conclusion that NBD-Cl injury to cotton is negligible.

An application of multiple metabolic inhibitors following a dicamba application but prior to the glufosinate application generally increased the level of cotton injury. The addition of amitrole + malathion + PBO + NBD-Cl to the sequential application of dicamba fb glufosinate at a 3-day interval resulted in a mean of 15% injury to cotton 7 DAFA. The addition of multiple metabolic inhibitors has been shown to increase susceptibility of weeds to herbicides (Letouzé & Gasquez, 2013), and an assumption could be a likewise response to cotton could be expected. Metabolic degradation of herbicides such as glufosinate may incorporate multiple enzymes, and use of multiple metabolic inhibitors may be needed to overcome herbicide metabolism (Cagnac et al., 2004; Cummins et al., 1999, 2009; Cummins & Edwards, 2004; Iwakami et al., 2014; Sika et al., 2014). Reducing crop injury should be a primary focus when screening for combinations of metabolic inhibitors to overcome herbicide metabolism.

An application of metabolic inhibitors did not influence cotton height relative to the nontreated control 21 DAFA (P-value=0.1385) (Figure 5). Amitrole, PBO, malathion, and NBD-Cl or combinations of multiple metabolic inhibitors did not influence cotton height or result in mean cotton injury equal to or less than 21%. Thus, the use of the metabolic inhibitors tested alone or in combination may be a viable option for future use in cotton if a reduction in herbicide metabolism in weed species is discovered.

Conclusions and practical applications. An application of amitrole, malathion, NBD-Cl, and PBO or combinations of metabolic inhibitors did not improve control or mortality of dicamba- and glufosinate-susceptible Palmer amaranth populations when compared to dicamba fb glufosinate at a 3-day interval. Differences in treatments may have been masked due to dicamba fb glufosinate at a 3-day interval providing above 89% control and mortality of Palmer amaranth less than 10 cm in height. While no response was observed, an upregulation of herbicide degrading enzymes when dicamba is applied 3-days prior to glufosinate cannot be ruled out from the data collected in this experiment. The high level of control from dicamba fb glufosinate at the 3-day interval may have masked any effect that the metabolic inhibitors had. Additionally, enzymes not inhibited by amitrole, malathion, NBD-Cl, and PBO or the combination of multiple metabolic inhibitors could be responsible for subsequent glufosinate metabolism, if present.

Future research should assess the impact prior applications of auxin herbicides have on subsequent glufosinate metabolism and if the addition of metabolic inhibitors improve herbicide efficacy on recently documented dicamba- and glufosinate-resistant populations. Additionally, future work should assess if cytochrome P450 or GST inhibitors limit glufosinate metabolism, as this may be an avenue to mitigate resistance evolving or spreading among weed populations. Averaged over six-site years of data, mean cotton injury of the metabolic inhibitors alone or in combination did not exceed 21%, 7 or 21 DAFA. Relative cotton height was not impacted by the application of metabolic inhibitors to dicamba fb glufosinate at the 3-day interval; therefore, an application of amitrole, malathion, NBD-Cl, and PBO may be viable options to mitigate herbicide metabolism via cytochrome P450- or GST-enzymes.

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Tables

Table 1. Location, year(s), coordinates, Palmer amaranth size, cotton stage and soil information are displayed for each site-year where the experiment was conducted.

Location	Year(s)	Coordinates	Palmer amaranth size	Cotton stage	Soil information
			Range (Avg[a]) cm	Range (Avg) nodes	
Crawfordville, AR	2018	N 35.228428, W -90.336762	0.5 to 7.6 (6.2)	2 to 4 (3)	Dundee silt loam
	2019	N 35.228428, W -90.336762	0.5 to 12.5 (7.6)	3 to 6 (4)	Dundee silt loam
Fayetteville, AR	2020	N 36.092002, W -94.187002	2.5 to 20.4 (8.5)	2 to 5 (3)	Leaf silt loam soil
Keiser, AR	2019	N 35.675128, W-90.07844	0.5 to 8.2 (6.6)	3 to 6 (4)	Sharkey silty clay
	2020	N 35.675128, W-90.07844	2.5 to 8.4 (6.8)	4 to 5 (5)	Sharkey silty clay
Marianna, AR	2020	N 34.725784, W -90.735788	0.5 to 10.6 (7.6)	5 to 6 (5)	Convent silt loam

[a] Abbreviation, Avg (average)

Table 2. Treatment structure of the experiment that was conducted in six-site years with cotton present.

Treatment	Herbicide	Metabolic additives[a]
1	Nontreated	None
2	Dicamba fb[b] glufosinate[c]	None
3	Dicamba fb glufosinate	Amitrole
4	Dicamba fb glufosinate	PBO[b]
5	Dicamba fb glufosinate	Malathion
6	Dicamba fb glufosinate	NBD-Cl[b]
7	Dicamba fb glufosinate	Amitrole, NBD-Cl
8	Dicamba fb glufosinate	Amitrole, Malathion
9	Dicamba fb glufosinate	Amitrole, PBO
10	Dicamba fb glufosinate	NBD-Cl, PBO
11	Dicamba fb glufosinate	Amitrole, NBD-Cl, PBO
12	Dicamba fb glufosinate	Amitrole, NBD-Cl, PBO,
13	Dicamba fb glufosinate	NBD-Cl, PBO, Malathion
14	Dicamba fb glufosinate	Amitrole, NBD-Cl, PBO
15	Dicamba fb glufosinate	Amitrole, Malathion, PBO
16	Dicamba fb glufosinate[d]	Amitrole, Malathion, NBD-Cl, PBO[d]

[a] Metabolic additives including amitrole, malathion, and PBO were applied 4 hours prior to the glufosinate application. NBD-Cl was applied 2 days prior to the glufosinate application.

[b] Abbreviation: fb, followed by; NBD-Cl, 4-chloro-7-nitrobenzofurazan; PBO, piperonyl butoxide

[c] Dicamba fb glufosinate: dicamba was sprayed 3 days prior to glufosinate

[d] Rates: Amitrole 14 g ai ha^{-1}; dicamba, 560 g ae ha^{-1}; glufosinate, 565 g ai ha^{-1}, Malathion, 2000 g ai ha^{-1}; NBD-Cl, 269 g ai ha^{-1}; PBO, 1500 g ai ha^{-1}

Figures

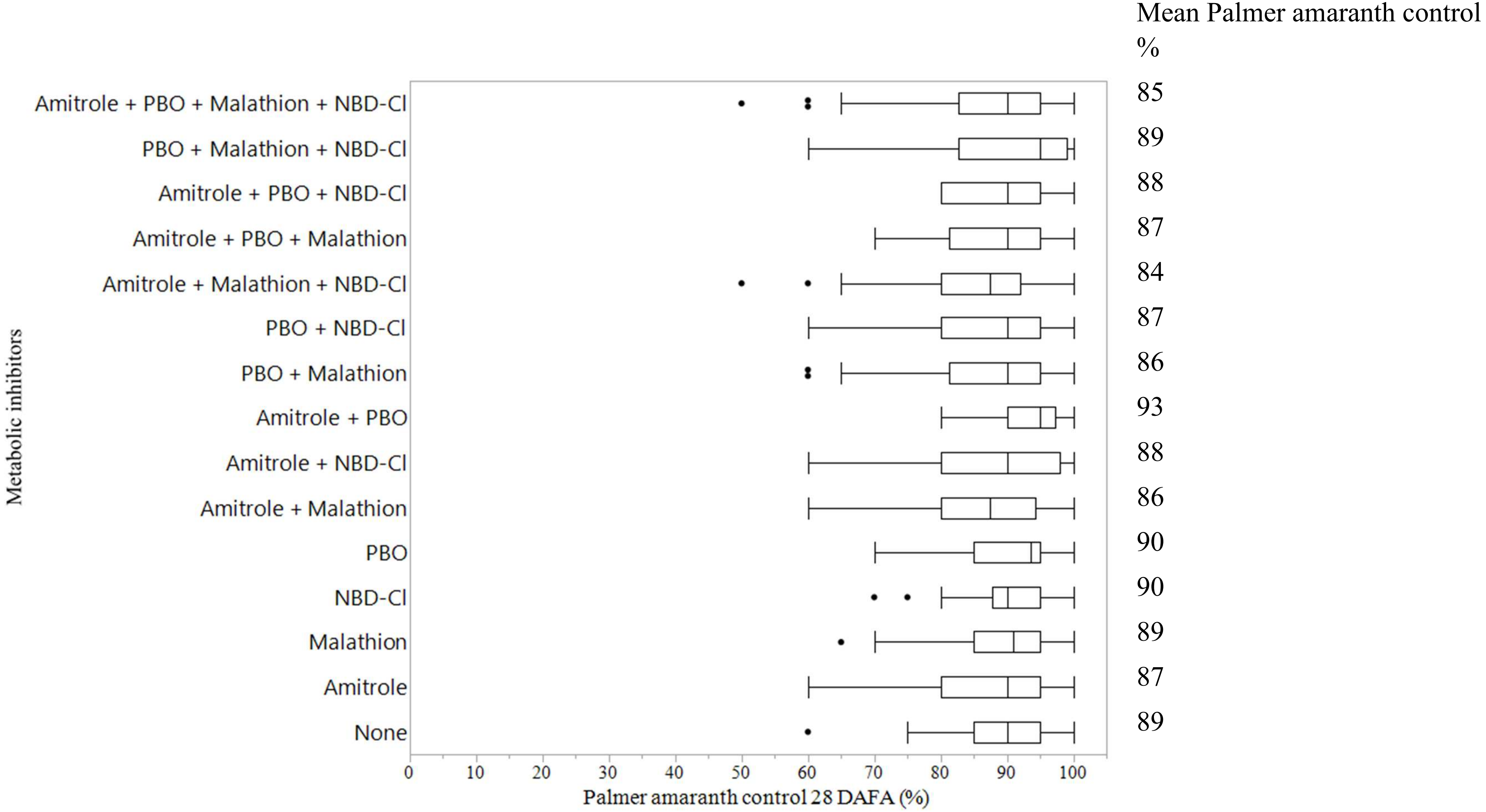


Figure 1. Percent Palmer amaranth control 28 days after the final application averaged over six site-years of data located near Crawfordsville, AR, Marianna, AR, and Keiser, AR and in Fayetteville, AR. Representation of the data using box and whisker plots provides an estimate of the variability in cotton response to the various treatments in differing environments (sites and years) evaluated. All treatments included an application of dicamba at 560 g ae ha^{-1} followed by glufosinate at 565 g ai ha^{-1} 3 days later. Rates of metabolic inhibitors were the following: amitrole 14 g ai ha^{-1}; malathion 2000 g ai ha^{-1}; NBD-Cl 269 g ai ha^{-1}; PBO 1500 g ai ha^{-1}. Abbreviations: DAFA, days after the final application, fb, followed by

Mean Palmer amaranth control
%
91
91
95
87
94
92
91
94
94
92
94
89
94
91
92

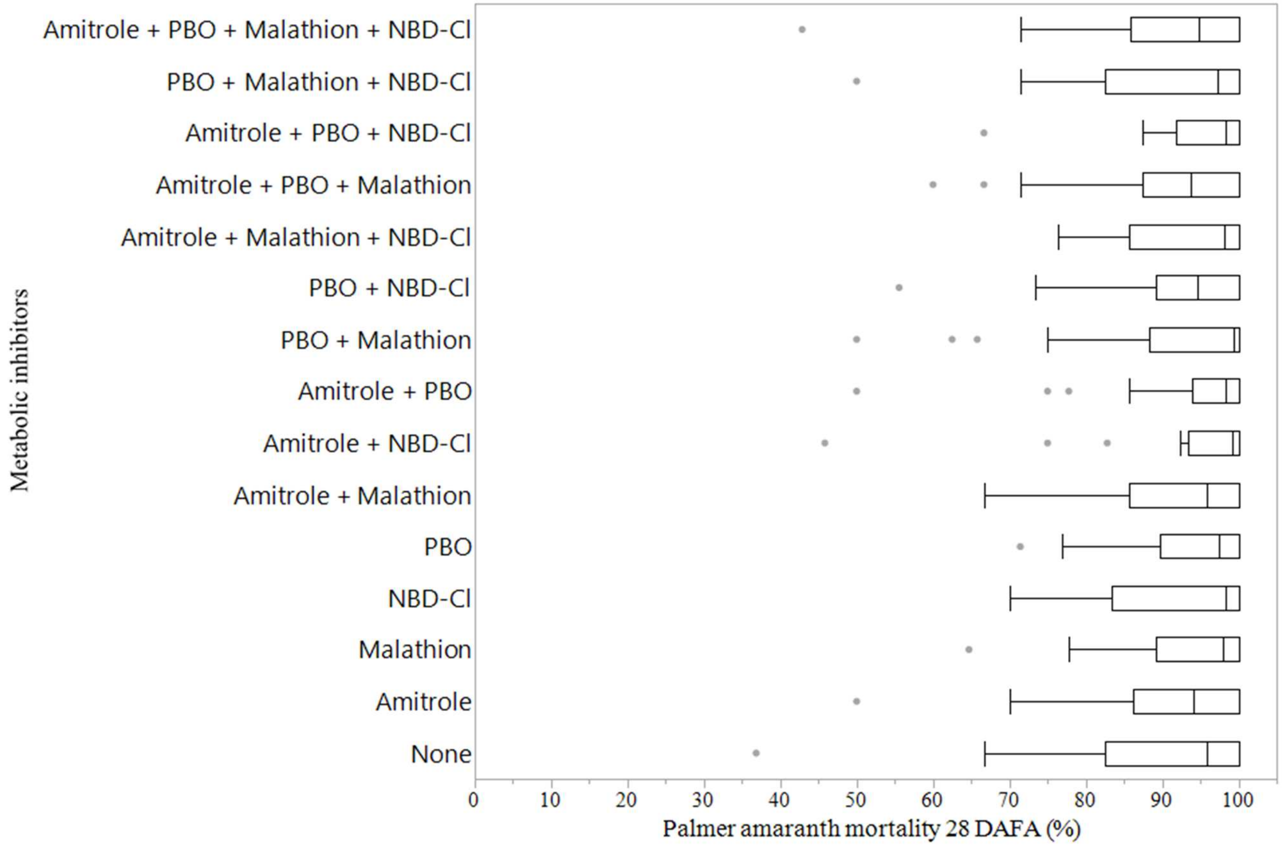
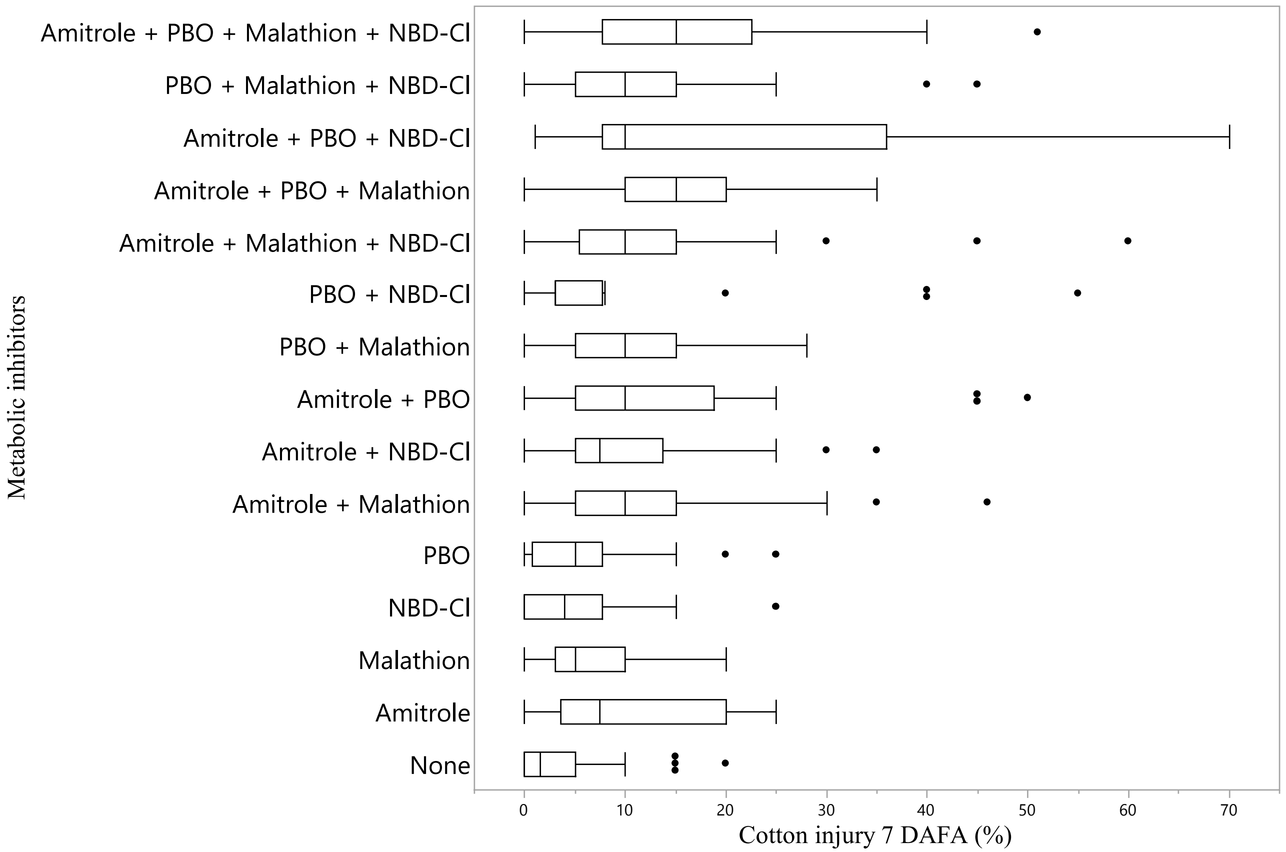


Figure 2. Percent Palmer amaranth mortality 28 days after the final application averaged over six site-years of data located near Crawfordsville, AR, Marianna, AR, and Keiser, AR and in Fayetteville, AR. Representation of the data using box and whisker plots provides an estimate of the variability in cotton response to the various treatments in differing environments (sites and years) evaluated. All treatments included an application of dicamba at 560 g ae ha^{-1} followed by glufosinate at 565 g ai ha^{-1} 3 days later. Rates of metabolic inhibitors were the following: amitrole 14 g ai ha^{-1}; malathion 2000 g ai ha^{-1}; NBD-Cl 269 g ai ha^{-1}; PBO 1500 g ai ha^{-1}. Abbreviations: DAFA, days after the final application, fb, followed by



Mean cotton injury 7 DAFA %	
17	AB
12	ABCDE
21	A
16	ABC
15	ABCD
9	BCDE
11	BCDE
14	ABCDE
10	BCDE
13	ABCDE
6	DE
5	DE
7	CDE
11	BCDE
5	E

Figure 3. Percent cotton injury 7 days after the final application averaged over six site-years of data located near Crawfordsville, AR, Marianna, AR, and Keiser, AR and in Fayetteville, AR. Representation of the data using box and whisker plots provides an estimate of the variability in cotton response to the various treatments in differing environments (sites and years) evaluated. All treatments included an application of dicamba at 560 g ae ha^{-1} followed by glufosinate at 565 g ai ha^{-1} 3 days later.

Rates of metabolic inhibitors were the following: amitrole 14 g ai ha^{-1}; malathion 2000 g ai ha^{-1}; NBD-Cl 269 g ai ha^{-1}; PBO 1500 g ai ha^{-1}. Abbreviations: DAFA, days after the final application, fb, followed by

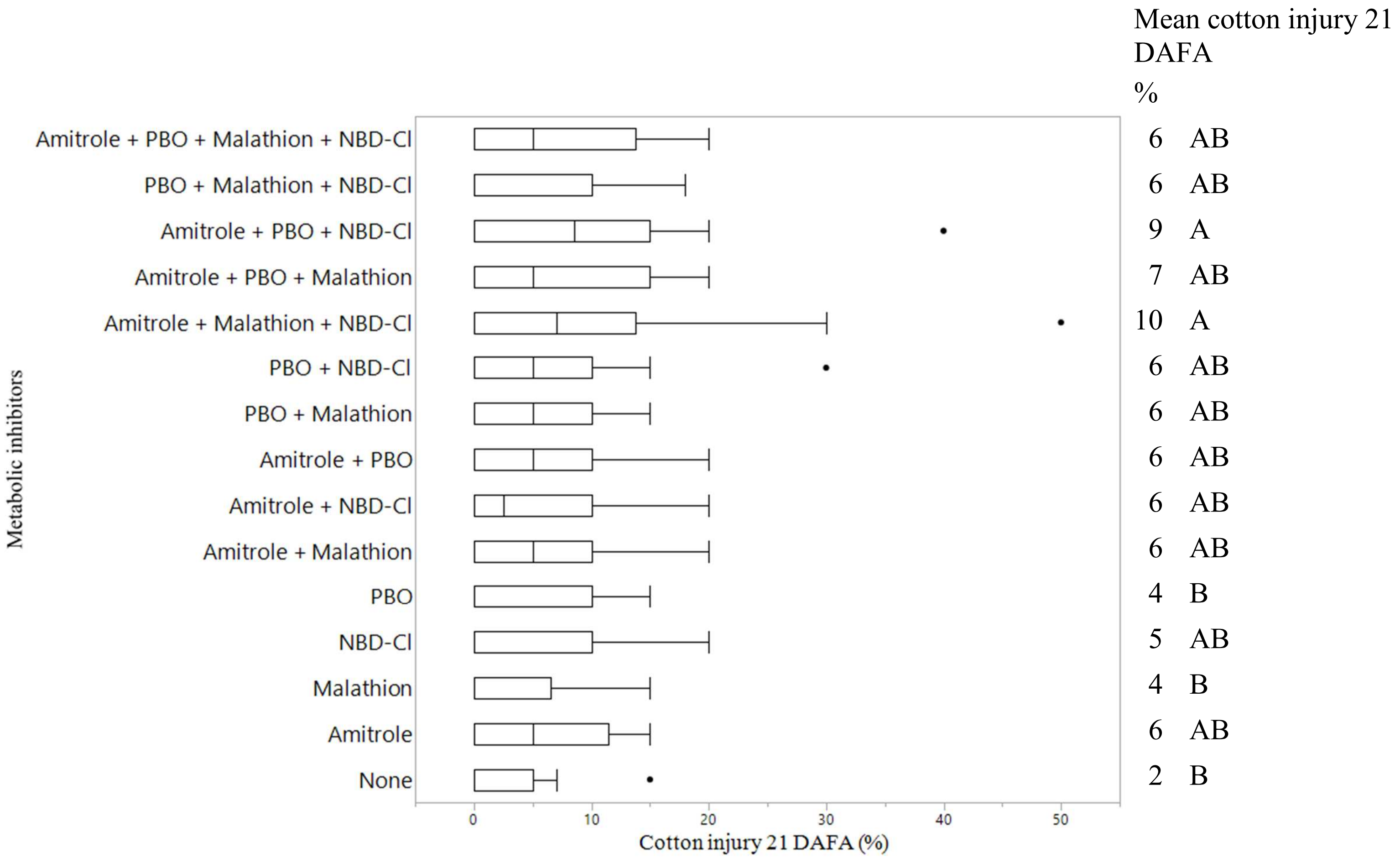


Figure 4. Percent cotton injury 21 days after the final application averaged over six site-years of data located near Crawfordsville, AR, Marianna, AR, and Keiser, AR and in Fayetteville, AR. Representation of the data using box and whisker plots provides an estimate of the variability in cotton response to the various treatments in differing environments (sites and years) evaluated. All treatments included an application of dicamba at 560 g ae ha^{-1} followed by glufosinate at 565 g ai ha^{-1} 3 days later.
Rates of metabolic inhibitors were the following: amitrole 14 g ai ha^{-1}; malathion 2000 g ai ha^{-1}; NBD-Cl 269 g ai ha^{-1}; PBO 1500 g ai ha^{-1}. Abbreviations: DAFA, days after the final application, fb, followed by

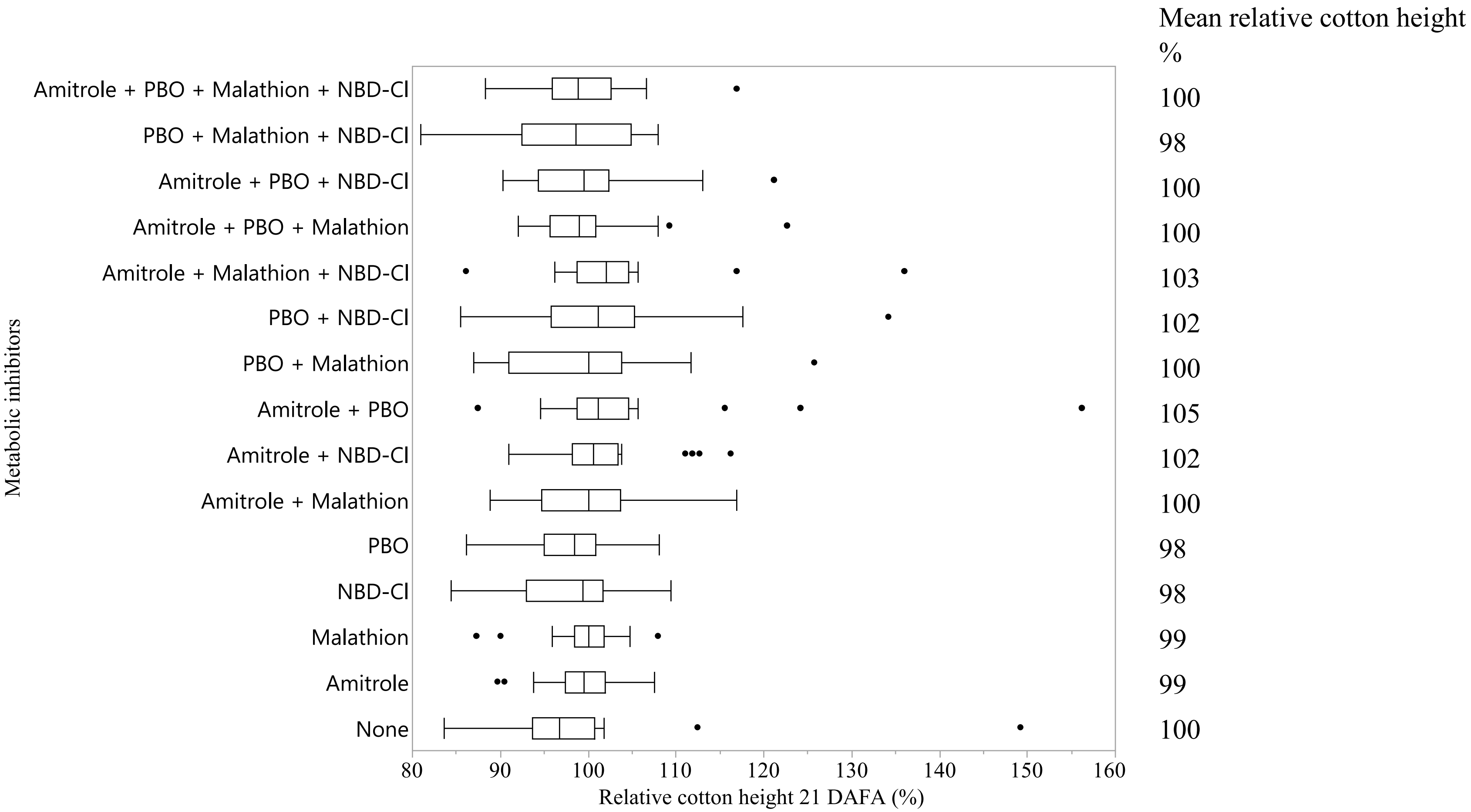


Figure 5. Cotton height relative to the nontreated 21 days after the final application averaged over six site-years of data located near Crawfordsville, AR, Marianna, AR, and Keiser, AR and in Fayetteville, AR. Representation of the data using box and whisker plots provides an estimate of the variability in cotton response to the various treatments in differing environments (sites and years) evaluated. All treatments included an application of dicamba at 560 g ae ha^{-1} followed by glufosinate at 565 g ai ha^{-1} 3 days later. Rates of metabolic inhibitors were the following: amitrole 14 g ai ha^{-1}; malathion 2000 g ai ha^{-1}; NBD-Cl 269 g ai ha^{-1}; PBO 1500 g ai ha^{-1}. Abbreviations: DAFA, days after the final application, fb, followed by

Chapter 5

Impact of Auxin Herbicides on Palmer amaranth Groundcover

Jason K Norsworthy, Rodger B Farr, Andy Mauromoustakos, Thomas R Butts, Trenton L Roberts

Abstract. In current and next-generation weed control technologies, sequential applications of contact and systemic herbicides for postemergence control of troublesome weeds are needed to mitigate the evolution of herbicide resistance. A clear understanding of the impact auxin herbicide symptomology has on Palmer amaranth groundcover will aid optimization of sequential herbicide applications. Field and greenhouse experiments were conducted in Fayetteville, AR and a laboratory experiment was conducted in Lonoke, AR, in 2020 to evaluate changes in Palmer amaranth groundcover following an application of 2,4-D and dicamba with various nozzles, droplet sizes, and velocities. Field experiments utilized three nozzles: Extended Range (XR), Air Induction Extended Range (AIXR), and Turbo TeeJet Induction (TTI), to assess the effect of spray droplet size on changes in Palmer amaranth groundcover. Nozzle did not affect Palmer amaranth groundcover when dicamba was applied. However, nozzle selection did impact groundcover when 2,4-D was applied; the following nozzle order XR>AIXR>TTI reduced Palmer amaranth groundcover the greatest in both site-years of the field experiment. This result (XR>AIXR> TTI) matches percent spray coverage data for 2,4-D and is inversely related to spray droplet size data. Rapid reductions of Palmer amaranth groundcover from 100% at time zero to 39.4 to 64.1% and 60.0 to 85.8% were observed 180 minutes after application in greenhouse and field experiments, respectively, regardless of herbicide or nozzle. In one site-year of the greenhouse and field experiments, regrowth of Palmer amaranth occurred 10080 minutes (14 days) after an application of either 2,4-D or dicamba to larger than labeled weeds. In all experiments, complete reduction of live Palmer amaranth tissue was not observed 21 days after application with any herbicide or nozzle combination. Control of Palmer amaranth escapes with reduced groundcover may potentially lead to increased selection pressure on sequentially applied herbicides due to a reduction in spray solution contact with the targeted pest.

Nomenclature: 2,4-D; dicamba; Palmer amaranth, *Amaranthus palmeri* (S.) Wats.

Keywords: Digital imagery analysis; symptomology; herbicide interaction; leaf area; field crops; application equipment.

Introduction

Dow AgroSciences commercially launched Enlist™ cotton in 2018, which allowed 2,4-D, glufosinate, and glyphosate to be used as postemergence options for control of troublesome weeds. Current label regulations allow for 2,4-D choline to be added in mixture or sequence with glufosinate over-the-top of Enlist™ crops, providing two effective SOA's for control of HR *Amaranthus* spp. (Anonymous 2019a; Merchant et al. 2014). Adding two effective SOA's in mixture reduces selection for target-site herbicide resistance in weeds; however, this practice is not always utilized (Norsworthy et al. 2012). Enlist One® (2,4-D choline) and Enlist Duo® (2,4-D choline plus glyphosate) labels also allow for application of both products with spray nozzles that provide better coverage than the Turbo TeeJet nozzles (Ultra Coarse spray classification) that are required by the Xtend® system (Anonymous 2018a; Anonymous 2018b; Anonymous 2019a; Anonymous 2019b; Meyer et al. 2016; Ramsdale and Messersmith 2001).

XtendFlex**®** cotton was commercially launched by Monsanto, which allowed POST applications of dicamba, glufosinate, and glyphosate. Xtendimax® plus VaporGrip® (Monsanto Corporation, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) labels currently do not allow for mixture with glufosinate (Anonymous 2018a; Anonymous 2018b). These label restrictions force producers to apply dicamba and glufosinate sequentially. However, limited work has been conducted to optimize sequential applications of dicamba and glufosinate. Understanding what sequence and duration between sequential applications of the two herbicides best optimizes efficacy of troublesome weeds will likely mitigate the perpetuating evolution of herbicide resistance (Norsworthy et al. 2012).

From past literature, applying a contact herbicide like glufosinate will decrease absorption and translocation of sequential systemic herbicide applications (Burke et al. 2005). Reductions in herbicide absorption and translocation were attributed to the rapid necrosis caused by the prior glufosinate application. Furthermore, Meyer et al. (2020) observed a 46% reduction in dicamba translocation in Palmer amaranth when dicamba plus glufosinate was applied in mixture compared to dicamba alone. Following a glufosinate application, the reduction of absorption and translocation of the sequentially applied herbicide may suggest that applying glufosinate before dicamba will not optimize the postemergence options in the XtendFlex® system.

In contrast, little work has evaluated the effects of applying auxin herbicides prior to contact herbicides. Dicamba and 2,4-D are synthetic auxin herbicides that cause leaf and stem epinasty to sensitive vegetation shortly after application (Al-Khatib and Peterson 1999; Anderson et al. 2004; Auch and Arnold 1978; Kelley et al. 2005; Wax et al. 1969). The resulting symptomology from an auxin herbicide application may be a concern if weeds are not effectively controlled, and a sequential application of a contact herbicide is needed.

Synthetic auxins affect dicot weeds in three phases; the stimulation phase, inhibition phase, and decay phase (Cobb 1992; Fedtke and Duke 2005; Grossman 2007; Sterling and Hall 1997). The stimulation phase is associated with the activation of ethylene biosynthesis through the induction of 1-amioncyclopropane-1-carboxylic acid in shoot tissues (1 to 2 hours after application) resulting in subsequent leaf epinasty, tissue swelling, and stem curling that occurs 3 to 4 hours after an application. The resulting epinasty, tissue swelling, and stem curling likely affects the spray retention of sequential herbicide applications (Butler Ellis et al. 2004; Konoche 1994). Spray droplet adhesion decreases with an increase in leaf angle, droplet impact velocity, diameter, and leaf roughness factor (Forster et al. 2005; Nairn et al. 2013). The resulting symptomology that follows an auxin herbicide application changes the leaf/stem angles and exposes shoot tissue of sensitive species that would not typically be contacted by a pesticide application.

When using the XtendFlex® technology, glufosinate can only be applied in sequence of dicamba. In terms of glufosinate; several factors play contributing roles in optimizing efficacy. While not limited to, these include: light-intensity (Ahrens 1994), growing vigor of targeted species (Anderson et al. 1996); humidity (Coetzer et al. 2000); and coverage of spray solution (Etheridge et al. 2001; Meyer et al. 2015). The coverage of spray solution of glufosinate and other contact herbicides will likely be impacted by a prior auxin herbicide application due to the subsequent auxin herbicide symptomology observed. The adoption of Enlist™ and XtendFlex® crops, increases the likelihood of sequential applications that include auxin and contact herbicides, i.e. glufosinate. Currently, the effects of auxin symptomology on subsequent coverage of contact herbicides is unknown. Therefore, quantification of groundcover of weed species following an auxin herbicide application is needed to understand if reduced-rate selection of subsequently applied herbicides is occurring in the XtendFlex® and Enlist™ technologies. The objective of this research was to quantify the extent of changes in groundcover of Palmer amaranth following dicamba and 2,4-D applications in several environments across an assortment of nozzle types.

Materials and Methods

Greenhouse experiment. A greenhouse experiment was conducted in April of 2020 and repeated in May of 2020 at the University of Arkansas Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR. Each experimental run was conducted as a two-treatment, completely randomized design with six replications. Fifteen, 50-cell trays (25 cm by 50 cm) (Greenhouse Megastore, Danville, IL, USA) were planted with Palmer amaranth seed collected from a population collected from a production field in Crittenden County, AR, with confirmed resistance to acetolactate synthase (ALS) inhibitors, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, an 5-enolpyruvyl shikimate-3-phosphate synthase inhibitor , microtubule assembly inhibitors (dinitroanilines), protoporphyrinogen oxidase (PPO) inhibitors, and very-long-chain fatty acid elongase-inhibiting herbicides (data not shown) at a population of 50 plants per tray. The Palmer amaranth accession chosen for the experiment was not screened for dicamba or 2,4-D resistance. Each tray represented an experimental unit.

Palmer amaranth plants were grown in mediated potting soil (Sungro® Horticulture, Agawam, MA, USA) until the one leaf stage and then were transplanted into mediated potting soil one plant cell^{-1} in 50 cell trays. Moist potting mix was maintained throughout the experiment through daily irrigation. Greenhouse conditions throughout the experiment are displayed in Table 1. When Palmer amaranth reached a height of 7.6 and 10.6 cm, in experimental run 1 and 2, respectively, dicamba (Xtendimax® plus VaporGrip®, Monsanto Corporation, St. Louis, MO 63167) and 2,4-D (Enlist One®, Dow AgroSciences, Indianapolis, IN, 46268) were applied at 560- and 1065 g ae ha^{-1}, respectively. Applications were made using a two-nozzle track sprayer equipped with TeeJet 1100067 nozzles (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA). The stationary spray chamber equipped with a track sprayer was calibrated to deliver 190 L ha^{-1} at 1.61 km h^{-1}. Environmental conditions during application and after application are displayed in Table 1. Photos of each flat were taken 64 cm above the center of the flat using a Canon PowerShot SX10IS (One Cannon Park, Melville, NY, 11747) mounted to a stationary tripod. The camera was positioned horizontally directly above the flat to avoid angled photos. Black felt was placed under the flats to avoid background interference in the picture analysis. Images of each flat were repeatedly taken at time intervals of 0, 30, 60, 90, 120, 180, 210, 240, 270, 300, 360, 420, 480, 540, 600, 660, 720, 1440, 2880, 4320, 5760, 7200, 8640, 10080, 14400, 20160 (14 days), and 30240 (21 days) minutes after application to assess reductions in Palmer amaranth groundcover.

Images were analyzed using the Turf Analyzer 1.0.4 (TurfAnalyzer, Fayetteville, AR) software to determine the proportion of green pixels in each photograph, which represents the groundcover achieved by Palmer amaranth. The proportion of green pixels in each image was considered the groundcover of Palmer amaranth and was reported relative to the tray/plot image taken immediately prior to application (t = 0 min). Butts et al. (2016), Purcell (2000), Priess et al. (2020a), and Priess et al. (2020b) have used similar image analysis techniques to estimate the groundcover of crop canopies. These image analysis techniques have proven more accurate than visual estimates or manual height and width measurements (i.e. soybean volume calculations). Therefore, visual estimates were not taken to verify the image analysis.

Field experiment. Field experiments were initiated at AAREC in Fayetteville, AR, on May 18, 2020, and the experiment was repeated on August 21, 2020. The experimental design was a randomized complete block with a two-factor factorial treatment structure. The two factors were herbicide: dicamba (Xtendimax® plus VaporGrip®) and 2,4-D (Enlist One®) at 560- and 1065 g ae ha^{-1}, respectively, and nozzle selection: Extended Range (XR) 110015, Air Induction Extended Range (AIXR) 110015, and Turbo TeeJet Induction (TTI) 110015 (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA).

The soil in Fayetteville was composed of a Leaf silt loam (Fine, mixed, active, thermic Typic, Albaquults) with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.8.

The field where the experiment was conducted was over-seeded with the same Palmer amaranth biotype that was used in the greenhouse experiment. The plot size was 1 m^2, with a distance of 2.1 m between plots. The area outside of each 1 m^2 plot was roto-tilled to remove any green vegetation. The entire experiment was over-sprayed after roto-tilling with *S*-metolachlor at 1605 g ai ha^{-1}. Herbicide treatments were applied to Palmer amaranth with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 4.8 km hr^{-1}.

In site one and two, Palmer amaranth at application was an average of 12.7 cm (0.5- to 14.5-cm range) and 7.6 cm (0.5- to 10-cm range) tall and had an average density of 420,000 and 482,000 plants ha^{-1}, respectively (Table 1). The variability in size was likely influenced by rainfall events that promoted differing germination. Photos were taken at 0, 30, 60, 90, 120, 180, 210, 240, 270, 300, 420, 480, 540, 600, 660, 720, 1440, 2880, 4320, 5760, 7200, 8640, 10080, 14400, 20160 (14 days), and 30240 (21 days) minutes after application to assess changes in groundcover. Image analyses were performed similarly to the previous greenhouse experiment.

Droplet size and velocity experiment. An experiment was conducted at the Lonoke Extension Center in Lonoke, AR on October 14, 2020. Droplet size and velocity for each treatment was measured using the VisiSize Portable P15 Oxford image particle analyzer (Oxford Lasers, Imaging Division, Oxford U.K.). Images were analyzed in real time with the VisiSize Particle Sizing Software that linked to the VisiSize Portable P15 Oxford image particle analyzer. The system analyzed the droplet spectrum by utilizing a technique called Particle/Droplet Image Analysis (Carvalho et al. 2017). The system measures droplets with a diameter greater than 5 µm. In addition to the droplet diameter measurement, the system calculates velocity of droplets in real time through sequential images taken at a set time interval apart similar to other particle/droplet image analysis equipment and research (Butts et al. 2018a). The system was programmed to measure diameter and velocity of 2500 droplets per repetition. Treatments were repeated three times to allow diameter and velocity measurement of a total of 7500 droplets per treatment.

Treatments included applications of 2,4-D and dicamba with XR- (1100067, 110015, 11004), AIXR- (110015, 11004), and TTI- (110015, 11004) nozzles. A Generation 4 Research Track Sprayer (Devries Manufacturing, Hollandale, MN) was calibrated to deliver 147 L ha^{-1} of spray solution at 1.46 m s^{-1} and 276 kPa. Applications were made with the spray pattern oriented perpendicular in between the two image housings of the VisiSize Portable P15 Oxford image particle analyzer to allow for droplet measurements to be taken from the entire spray plume. The distance from nozzle to image frame was 50 cm to allow droplet measurements to be taken as the droplet would be contacting the target. The treatments in this study were compared using the $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$ size measurements and velocity. Droplet diameters of $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$ represent that 10, 50, and 90% of the spray volume was comprised of droplets of a smaller diameter, respectively.

Spray coverage. A spray coverage experiment was conducted at the AAREC in Fayetteville, AR on November 6, 2020. The spray coverage experiment conducted utilized water sensitive sprays cards to assess the coverage of the aforementioned treatments in the droplet size and velocity experiment. Three different application methods were utilized due to the change in nozzle orifice size and a desired constant 147 L ha^{-1} spray volume. XR 1100067 nozzles were applied in a two-nozzle track sprayer at 1.61 km h^{-1}. Nozzles with orifice sizes of 110015 were applied with CO_2-pressurized backpack sprayers at 4.8 km h^{-1}. Nozzles with orifice sizes of 11004 were applied with a Bowman Mudmaster Multi-Purpose Sprayer (Bowman Manufacturing Co., Inc., Newport, AR) at 11.2 km h^{-1}. All application methods were calibrated to deliver 147 L ha^{-1} at 276 kPa.

Prior to application SpotOn water sensitive spray cards (51 x 76mm) (Innoquest.inc, Woodstock, IL) were placed horizontal to the spray pattern, 50cm below the nozzle orifice. This process was repeated for four applications per nozzle and size, providing four replications per treatment. The yellow water sensitive spray cards turned blue where spray solution contacted the card. After application, the sprayed water sensitive cards were allowed to dry before handling. Spray cards were scanned and imported into the Deposit Scan Software (USDA-ARS). A coverage analysis was conducted in the Deposit Scan Software to provide a percentage of card that was covered by the spray solution. This methodology and software have proven useful for calculating percentage spray coverage by Hoffmann and Hewitt (2005).

Data analysis. Percent groundcover of Palmer amaranth after application is reported relative to initial percent groundcover prior to application in the greenhouse and field experiments. Relative groundcover estimates were analyzed in the Fit Curve Platform of JMP Pro 15.2 (SAS Institute Inc., Cary, NC). A biexponential 4P curve (y= a * Exp (-b * minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) was found to be the best fit when AICc, BIC, SSE, MSE, and R^2 values were used to model the percent groundcover of Palmer amaranth. Similarly, Dornai et al. (1991) used biexponential models to assess changes in cotton growth following trifluralin applications. Individual non-linear biexponential 4P curves were fit by site-year (due to differences in weed size), herbicide, and nozzle in the greenhouse and field experiment, respectively. Parameter estimates and R^2 values for the non-linear lines fitted are displayed in Table 2. Predictions of Palmer amaranth groundcover and associated standard errors (α=0.05) were made at 0, 180, 360, 4320, 10080, 20160, and 30240 minutes after an auxin herbicide application. Differences between the predicted Palmer amaranth groundcover between herbicide or among nozzles within site-year were determined by comparison of the predicted values + or – the associated standard error. If the predicted values + or – the associated standard error did not overlap with the compared predicted value + or – the associated standard error the two predictions were considered different.

The droplet size distribution and coverage experiments were designed as a completely randomized experiment with a 2 x 3 x 2 three-factor factorial treatment structure, with the three factors being herbicide (dicamba and 2,4-D), nozzle (XR, AIXR, and TTI), and nozzle size (110015 and 11004). The XR 1100067 treatments were not included in the analysis and means of the treatments will be presented. Droplet size, velocity, and percent coverage data were subjected to an analysis of variance (ANOVA) in the Generalized Linear Mixed Model Platform of JMP 15.2 (SAS institute Inc., Cary, NC). Droplet size and velocity data were assumed to have a gamma distribution while coverage data was assumed to have a normal distribution. Means were separated using Fisher's LSD at an alpha value of 0.05.

Results and Discussion

Greenhouse experiment. From these data collected, the effect of site-year was evident through comparison of trendlines. Therefore, biexponential 4P lines were fit by experimental run and herbicide. Several factors can influence the efficacy of a herbicide including weed size and environmental conditions (Ehleringer 1981; Wright et al. 1999). The method of transplanting Palmer amaranth at the one leaf stage increased the variability of size of plants in each tray. Flats were treated when 50% of the plants in the tray were 7.6 to 10.1 cm in height or at the 5-leaf stage (Table 1). In experimental run two, a delay in treatments occurred allowing for the range in plant height to increase. The authors suggest the difference in experimental runs were caused by plants that exceeded 15 cm at the time of application in site-year two. A higher survival rate of the Palmer amaranth plants that exceeded 15 cm at the time of application in site-year two likely contributed to differences in groundcover between the two site-years.

Generally, across experimental runs, rapid reductions in groundcover were observed in the first 180 minutes (Table 3). Dicamba and 2,4-D reduced groundcover of Palmer amaranth in the first 180 minutes from 100% at time zero to 69.8 to 84.6% and 60.0 to 85.8%, regardless of experimental run, respectively. From 180 to 360 minutes after application reductions in groundcover were 11.8- to 14.3-percentage points in site-year 1 and only 1.1- to 3.2-percentage points in site-year 2. General differences in trends in groundcover response between experimental run 1 and 2 were observed 360 minutes after application. In experimental run one, where Palmer amaranth weed size was shorter at the time of application, a general decrease in Palmer amaranth groundcover from 180 to 30240 minutes after application, regardless of herbicide, was observed. In experimental run2, reductions in Palmer amaranth groundcover ceased after 4320 minutes regardless of herbicides. From 10080 to 30240 minutes after an application of 2,4-D or dicamba, an increase of 10.6- and 19.3-percentage points in Palmer amaranth groundcover was observed, respectively (Table 3; Figure 1).

Based on the images captured and data collected, it was observed that neither treatment provided 100% control of Palmer amaranth, meaning that there were escapes for both treatments. For both herbicides, the most rapid reduction occurred within the first 180 minutes following application while also reaching a maximum or near-maximum reduction of groundcover one week following application. Coupled with the lack of complete control of Palmer amaranth by either herbicide, the reduction of groundcover may be detrimental to future efforts to control the weed within fields. At 20160 minutes or 14 days after application, the amount of plant material for sequential herbicide applications to contact on Palmer amaranth increased in one of the two experimental runs, regardless of herbicide. This increase in plant material would likewise increase the amount of herbicide intercepted by actively growing plant tissue. Further research should be conducted to investigate the efficacy of applications at different time intervals following 2,4-D and dicamba applications to determine the best timing between sequential herbicide applications for Palmer amaranth control.

Field experiment. In general, rapid reductions in groundcover of Palmer amaranth were observed after application regardless of nozzle selection or herbicide (Figures 2 and 3). In site 1 where larger plants were treated, changes in Palmer amaranth groundcover were significantly less than changes observed in site 2 (Tables 4 and 5; Figures 2 and 3). The variability in Palmer amaranth groundcover changes between site 1 and 2 are likely attributed to the differences in Palmer amaranth size, density, and to a lesser extent environmental factors at the initial application (Table 1). Observations from previous research concluded that weed size, weed density and environmental factors can influence the rate of growth and ability of Palmer amaranth to survive a herbicide application (Ehleringer 1981; Forseth et al. 1984; Guo and Al-Khatib 2003; Meyer and Norsworthy 2019; Shell and Lang 1976; Stewart et al. 2010; Wright et al. 1999). While differences in the factors mentioned above contributed to variability between sites, the primary focus of the experiment was to quantify the extent to which an auxin herbicide application influences Palmer amaranth groundcover.

In general, reductions in groundcover of Palmer amaranth were observed up to 4320 minutes after application in site-year 1, regardless of herbicide or nozzle. A 3.2 to 28.2 percentage point increase in Palmer amaranth groundcover was observed from 4320 minutes (3-

days) to 30240 minutes (21-days) regardless of herbicide or nozzle (Table 4). While an increase in groundcover of Palmer amaranth represents regrowth at 30240 minutes, the regrowth did not achieve groundcover to what was observed before herbicide application (Table 4). Additionally, Palmer amaranth in site 2 was at a labeled size at application for both herbicides (Anonymous 2018a and 2018b; Anonymous 2019b); however, complete control in both sites was not achieved with a single application of either herbicide based on observed regrowth at 14 days after application or failure to remove all living (green) biomass. Thus, surviving plants with reduced groundcover will need to be controlled with a sequential herbicide application.

When treating labeled sized plants (<10.2 cm height), a general decline in Palmer amaranth groundcover following application occurred through the final assessment at 30240 minutes, regardless of nozzle and herbicide. The continued decline in groundcover through all time intervals indicates the performance of the herbicides regardless of nozzle-selection. However, at 30240 minutes, Palmer amaranth still maintained between 8.6 to 24.2% groundcover. Even though applications were made to Palmer amaranth that was 7.6 cm tall, Palmer amaranth with green tissue was still present at 30240 minutes. Unlike site 1, regrowth of Palmer amaranth after 4320 minutes was not observed, therefore determining the best timing recommendation for sequential applications of a contact herbicide is unlikely from the data collected on auxin herbicide applications made to 7.6 cm Palmer amaranth.

Droplet size and velocity experiment. The three-factor interaction of herbicide X nozzle X nozzle size was significant when droplet diameters $D_{v0.1}$, $D_{v0.5}$, $D_{v0.9,}$ and velocity were analyzed (P-values=<0.0001). Overall trends showed that as orifice size increased, nozzle selection changed in order of XR to AIXR to TTI, and 2,4-D was used when compared to dicamba an increase in droplet diameter was observed (Table 6). For spray droplet velocity the general trend was such as when 2,4-D was used over dicamba, nozzle selection changed in order of TTI to XR=AIXR, and orifice size increased from the 110015 to 11004 the velocity of spray droplets increased (Table 6).

In the analysis of the spray solution coverage data a significant interaction of herbicide X nozzle (P-value=0.0173) and a main effect of nozzle size (P-value=<0.0001) was observed. In general, the percent coverage of 2,4-D treatments was reduced, when averaged over nozzle size, by 8.8- and 14.3-percentage points when the XR nozzle was compared to the AIXR nozzle and the AIXR nozzle was compared to the TTI nozzle, respectively (Table 7). Spray coverage (%) of dicamba was reduced, when averaged over nozzle size, by 14.8-percenatge points when a XR nozzle was compared to an AIXR nozzle. No change in spray coverage was observed between AIXR and TTI nozzles, when dicamba was applied (Table 7). This observation may be confusing as $D_{v0.5}$ nearly doubled from the AIXR to TTI nozzle; however, the number of spray depositions are likely a contributing factor. The number of spray depositions on the water sensitive cards calculated by the DepositScan software did not accurately represent the true number of depositions due to the spray solution volume used and the overlapping of spray depositions (Salyani et al. 2013). From Figure 4, a number of spray deposits can be observed to increase from XR to AIXR to TTI nozzles. However, spreading of large droplets on the water sensitive spray cards likely compensated for the reduction in spray deposits (Figure 4). Further spray coverage averaged over herbicide and nozzle was 44.7% for the 110015-orifice size and 34.7% for the 11004-orifice size. In the field experiment conducted in this manuscript, applications were applied through nozzles with 110015 orifice sizes. Commercial application equipment are often equipped with orifice sizes larger than 11004; therefore, the effect of nozzle selection may be more apparent as orifice size increases and a likewise decrease in spray solution coverage occurs.

Dicamba nozzle selection. Different nozzle types impact droplet size and efficacy of herbicide applications (Butts et al. 2018a, Butts et al. 2018b; Meyer et al. 2015; Meyer et al. 2016). Palmer amaranth control was indirectly captured through the quantitative assessment of the amount of green plant tissue at the time of the photographs. In site 1 and 2, nozzle selection did not affect the groundcover of Palmer amaranth differently in the first 360 minutes. Less than a 10-percentage point difference in Palmer amaranth groundcover was observed following dicamba applications in regards to nozzle selection from 4320 to 10080 minutes after application; however, these differences were not believed to be impactful to real-world scenarios. No relationship between nozzle selection and Palmer amaranth groundcover at 30240 minutes was observed when dicamba was applied. Nozzle selection for dicamba applications did not impact the groundcover of Palmer amaranth sufficiently to form different sequential herbicide application recommendations. As mentioned previously, no change in dicamba spray coverage was observed between the AIXR and TTI nozzle (Table 7); therefore, changes in Palmer amaranth groundcover in regards to nozzle selection would not be expected to be apparent. Additionally, only a TTI nozzle is labeled for POST applications of XtendiMax® plus VaporGrip® and Engenia® (Anonymous 2018a; Anonymous 2018b) therefore, it is unlikely that applications of dicamba POST will be made with AIXR or XR nozzles.

This observation coincides with previous literature where nozzle selection did not impact the efficacy of dicamba at 140 to 187 L ha^{-1} spray solution (Legleiter et al. 2018; Meyer et al. 2016; Nuyttens et al. 2009). If lower volumes of spray solutions are used, a nozzle effect should be anticipated (Meyer et al. 2016; Nuyttens et al. 2009). While this research did not evaluate the effect of spray solution volume on changes in Palmer amaranth groundcover; previous research observed a reduction in dicamba efficacy when a Coarse through Ultra Coarse spray is used in combination with low spray volumes (94 L ha^{-1}) (Butts et al. 2018b; Meyer et al. 2016). The reduction in dicamba efficacy with Coarse through Ultra Coarse spray producing nozzles at lower spray volumes would likely lead to a decrease in the reduction of Palmer amaranth groundcover and hasten regrowth of escapes.

2,4-D nozzle selection. In general, decreases in Palmer amaranth groundcover were similar across nozzle type up to 4320 minutes after application when 2,4-D was applied, in both site-years (Figures 2 and 3). After 4320 minutes, the effect of the nozzle used during application became apparent. At 10080, 20160, and 30240 minutes after a 2,4-D application, groundcover of Palmer amaranth was reduced greatest by order of the following nozzles XR>AIXR>TTI, in both site-years. These data, coincide with the spray coverage and droplet diameter data collected as spray coverage increases and droplet size decreases in the following order of nozzle XR to AIXR to TTI. The XR (Fine spray classification) nozzle reduced Palmer amaranth's groundcover at 30240 minutes after application 10.9- and 19.2-percentage points greater than the TTI (Ultra Coarse spray classification) nozzle, in site 1 and 2, respectively. Previous research has observed that as droplet size decreased likewise weed control of multiple species increased (Ennis and Williamson 1963; Lake 1977; Knoche 1994; Mckinlay et al. 1972 and 1974). These data contradict the general observations made by Butts et al. (2019), which observed that a Very Coarse to an Ultra Coarse spray optimized the efficacy of 2,4-D plus glyphosate on several weed species. However, in some site-years where high humidity and low wind speeds were present, a Fine to Coarse-sized spray optimized the efficacy of the 2,4-D plus glyphosate mixture (Butts et al. 2019). In the experiment conducted, humidity levels were between 67 to 84%, and wind speeds were negligible, below 0.89 m s^{-1}, thus allowing for smaller spray droplets produced by the XR and AIXR nozzle to reach the intended target without off-target movement or substantial in-air evaporation (De Cock et al. 2017). Under low humidity and higher wind speeds, the efficacy of a Coarse to Ultra Coarse spray may outperform a Fine spray and impact the reductions in groundcover observed.

Practical implications and conclusions. In current and next-generation technologies, the use of sequential applications of contact and systemic herbicides are needed to control escapes from the first application and reduce the risk for herbicide resistance. A rapid reduction in Palmer amaranth groundcover from 100% at time zero to 39.4 to 64.1% and 60.0 to 85.8% following an auxin herbicide application was observed 180 minutes after application, in greenhouse and field experiments, respectively. The reductions in groundcover of targeted weed species could be troublesome to sequential applications. Reductions in groundcover reduce the surface area for sequentially applied herbicides to contact; therefore, reducing the rate of the sequentially applied herbicide that individual plants are exposed to. In site 1 of the field experiment and site 2 of the greenhouse experiment, regrowth of Palmer amaranth was observed at 20160 (14 days) after the initial application. If Palmer amaranth regrowth occurs following an auxin herbicide application, sequential herbicide efficacy may be optimized if applied at 20160 minutes after the initial application. In addition, further work is needed to optimize coverage, rate, and timing of sequentially applied herbicide to overcome the reduction in groundcover of Palmer amaranth following an auxin herbicide application. If coverage, rate, or timing of sequentially applied herbicides cannot be adjusted to combat reductions in Palmer amaranth groundcover, an increase in selection pressure on sequentially applied herbicides should be expected due to selection of reduced rate exposure.

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Tables

Table 1. Environmental condition at the time of application and averages calculated for the 21 days following application by experiment and site-year.

		Environmental conditions						
		At application					21-days following application	
					Palmer amaranth			
Location	Site-year	Wind speed	Air temp.	Relative humidity	Height	Density	Air temp.	Relative humidity
					average (range)	average (range)	average (range)	average
		m s^{-1}	C	%	cm	plants/plot	C	%
Greenhouse								
	1	NA	35.2	84	7.6 (1-8.4)	50	30.8 (28.2-41.7)	86
	2	NA	37.3	76	10.6 (5.2-18.8)	50	34.8 (29.2-42.1)	82
Field								
	1	0	27.2	82	12.7 (5.2-20.2)	42 (22-85)	25.2 (18.3-36.1)	65
	2	0.89	28.9	67	7.6 (1-10.6)	28 (17-41)	27.5 (19.1-37.1)	62

Table 2. Biexponential 4P curve (y= a * Exp (-b * minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) fit to by site-year, herbicide in the greenhouse experiment and by site-year, herbicide, and nozzle in the field experiment; R^2 values represent the amount of variability explained by the fit of the line.

				Parameter estimates				
Experiment	Site year	Herbicide	Nozzle	Scale 1	Decay rate 1	Scale 2	Decay rate 2	R^2
Greenhouse								
	1							
		2,4-D		40.66	$0.22e^{-4}$	59.12	$0.51e^{-2}$	0.91
		Dicamba		40.47	$2.87e^{-5}$	66.23	$0.82e^{-2}$	0.93
	2							
		2,4-D		38.24	$-1.11e^{-6}$	139.05	0.03	0.92
		Dicamba		35.82	$-1.83e^{-5}$	69.35	0.16	0.86
Field								
	1							
		2,4-D	XR[a]	54.42	$-4.14e^{-6}$	2.39	$0.51e^{-2}$	0.90
			AIXR[b]	54.56	$-8.53e^{-6}$	46.51	$0.31e^{-2}$	0.86
			TTI[c]	63.89	$-7.81e^{-6}$	38.03	$0.31e^{-2}$	0.86
		Dicamba	XR	54.49	$-6.57e^{-6}$	46.89	$0.23e^{-2}$	0.79
			AIXR	49.45	$-1.22e^{-5}$	56.44	$0.33e^{-2}$	0.92
			TTI	46.73	$-1.18e^{-5}$	58.96	$0.33e^{-2}$	0.92
	2							
		2,4-D	XR	64.01	$6.44e^{-5}$	36.14	0.04	0.93
			AIXR	60.39	$0.45e^{-4}$	39.44	0.04	0.81
			TTI	60.64	$3.68e^{-5}$	38.02	0.02	0.86
		Dicamba	XR	29.37	$8.37e^{-6}$	41.73	$0.17e^{-3}$	0.79
			AIXR	1.22	$-0.12e^{-3}$	71.83	$0.10e^{-3}$	0.82
			TTI	2.76	$-3.31e^{-5}$	69.94	$0.14e^{-5}$	0.89

[a] XR (Extended Range nozzle)

[b] AIXR (Air Induction Extended Range nozzle)

[c] TTI (Turbo TeeJet Induction nozzle)

Table 3. Predicted groundcover of Palmer amaranth (PA) and the associated standard error for the biexponential (y= a * Exp (-b * minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) nonlinear curves that were fit to data in site-year 1 and 2 of the greenhouse experiment following an application of dicamba and 2,4-D.

		Herbicide			
		Dicamba		2,4-D	
Site-year	Time	Groundcover of PA	Standard error	Groundcover of PA	Standard error
	min[a]	%		%	
1	180	55.3[b]	0.99[c]	64.1[b]	1.05[c]
	360	43.5	0.76	49.8	0.97
	4320	35.7	0.78	36.9	0.86
	10080	30.3	0.98	32.4	0.97
	20160	22.7	1.51	25.9	1.56
	30240	17.0	1.76	20.7	1.96
2	180	39.4	0.79	39.5	0.48
	360	36.2	0.88	38.4	0.54
	4320	38.8	0.85	40.1	0.49
	10080	43.1	0.84	42.7	0.51
	20160	51.9	1.34	47.7	0.86
	30240	62.4	2.45	53.3	1.48

[a] Abbreviations: min = minutes after application of the auxin herbicide; PA = Palmer amaranth.

[b] The predicated values of Palmer amaranth groundcover relative to time prior to application.

[c] Associated standard error of the predicted value of Palmer amaranth groundcover.

Table 4. Predicted groundcover of Palmer amaranth and the associated standard error of utilizing the biexponential 4P (y= a * Exp (-b * minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) nonlinear curves that were fit to the data in site-year 1 of the field experiment following an application of dicamba and 2,4-D.

		Predicted groundcover of Palmer amaranth					
		Nozzle					
Herbicide	Time	XR[a]		AIXR[b]		TTI[c]	
	min[d]	%	Std. error	%	Std. error	%	Std. error
Dicamba	180	84.6[e]	2.06[f]	80.5[e]	1.21[f]	79.2[e]	1.28[f]
	360	74.3	2.58	66.6	1.44	64.7	1.53
	4320	60.5	2.56	52.6	1.44	49.2	1.52
	10080	61.4	2.14	57.8	1.25	52.7	1.31
	20160	63.1	2.87	63.2	1.54	59.4	1.67
	30240	64.8	4.71	71.4	2.74	66.9	2.95
2,4-D	180	74.3	1.30	81.1	1.32	85.8	1.04
	360	62.3	1.18	69.7	1.62	76.6	1.29
	4320	55.4	1.29	56.6	1.62	66.1	1.28
	10080	56.8	1.12	59.5	1.39	69.1	1.10
	20160	59.7	1.57	64.8	1.98	74.8	1.57
	30240	61.7	2.59	70.6	3.49	80.9	2.75

[a] XR (Extended Range nozzle)

[b] AIXR (Air Induction Extended Range nozzle)

[c] TTI (Turbo TeeJet Induction nozzle)

[d] min = minutes and after application of the auxin herbicide

[e] Predicated values of Palmer amaranth groundcover relative to time prior to application.

[f] Associated standard error of the predicted value of Palmer amaranth groundcover.

Table 5. Predicted groundcover of Palmer amaranth and the associated standard error for the biexponential 4P (y= a * Exp (-b * minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) nonlinear curves that were fit to the data in site-year 2 of the field experiment following an application of dicamba and 2,4-D.

		Predicted groundcover of Palmer amaranth					
		Nozzle					
Herbicide	Time	XR[a]		AIXR[b]		TTI[c]	
	min[d]	%	Std. error	%	Std. error	%	Std. error
Dicamba	180	69.8[e]	1.61[f]	70.7[e]	1.28[f]	71.0[e]	1.28[f]
	360	68.5	1.51	69.5	1.22	69.3	1.21
	4320	48.1	3.22	48.4	2.05	41.4	2.45
	10080	34.3	2.93	30.2	2.11	21.0	2.18
	20160	26.1	2.98	21.6	3.16	9.6	2.71
	30240	23.0	8.05	24.2	17.22	8.6	8.84
2,4-D	180	63.3	0.94	60.0	1.37	60.8	1.05
	360	62.5	0.93	59.4	1.37	59.8	1.18
	4320	48.5	0.82	49.7	1.12	51.7	0.92
	10080	33.4	1.15	38.3	1.54	41.8	1.16
	20160	17.5	1.24	24.2	2.01	28.8	1.63
	30240	9.1	0.99	15.4	1.95	19.9	1.75

[a] XR (Extended Range nozzle)
[b] AIXR (Air Induction Extended Range nozzle)
[c] TTI (Turbo TeeJet Induction nozzle)
[d] min = minutes after application of the auxin herbicide
[e] Predicated values of Palmer amaranth groundcover relative to time 0 prior to application.
[f] Associated standard error of the predicted value of Palmer amaranth groundcover.

Table 6. Droplet diameter and velocity of dicamba and 2,4-D when applied through XR, AIXR, and TTI nozzles at orifices sizes of 1100067, 110015, and 11004.

Nozzle	Herbicide	$Dv_{0.1}$[a]		$D_{v0.5}$[a]		$D_{V0.9}$[a]		Velocity	
		µm		µm		µm		m s^{-1}	
XR[b] 1100067	2,4-D	96		156		220		1.21	
	dicamba	87		145		211		1.17	
XR 110015	2,4-D	104	GH[e]	175	F	267	G	1.83	D
	dicamba	94	H	168	F	309	G	1.69	E
XR 11004	2,4-D	115	G	211	E	325	G	2.92	B
	dicamba	98	H	184	EF	311	G	2.51	C
AIXR[c] 110015	2,4-D	155	EF	305	D	543	F	1.83	D
	dicamba	147	F	308	D	551	EF	1.64	E
AIXR 11004	2,4-D	179	D	390	C	623	E	3.03	A
	dicamba	163	E	402	C	701	D	2.52	C
TTI[d] 110015	2,4-D	312	A	688	B	1095	C	1.65	E
	dicamba	297	B	707	B	1088	C	1.43	F
TTI 11004	2,4-D	259	C	684	B	1198	B	1.71	E
	dicamba	307	AB	878	A	1537	A	1.69	E

[a] Dv-0.1, 0.5, and 0.9 represents the diameter of which 10%, 50%, and 90% of spray solution is atomized into smaller droplets, respectively.

[b] XR (Extended Range nozzle); XR 1100067 droplet data was not used in the analysis therefore letter separation is not displayed in the table

[c] AIXR (Air Induction Extended Range nozzle)

[d] TTI (Turbo TeeJet Induction nozzle)

[e] Means not represented with like letters are statistically different within columns based on Fisher's protected LSD (α = 0.05).

Table 7. Spray solution coverage of dicamba and 2,4-D when applied through XR, AIXR, and TTI nozzles on water sensitive spray cards, averaged over orifice size.

Herbicide	Nozzle	Coverage	
		%	
2,4-D	XR[a]	56.4	A[d]
	AIXR[b]	47.5	B
	TTI[c]	33.2	C
Dicamba	XR	44.2	B
	AIXR	29.3	C
	TTI	27.7	C

[a] XR (Extended Range nozzle)

[b] AIXR (Air Induction Extended Range nozzle)

[c] TTI (Turbo TeeJet Induction nozzle)

[d] Means not represented with like letters are statistically different within columns based on Fisher's protected LSD (α = 0.05).

Figures

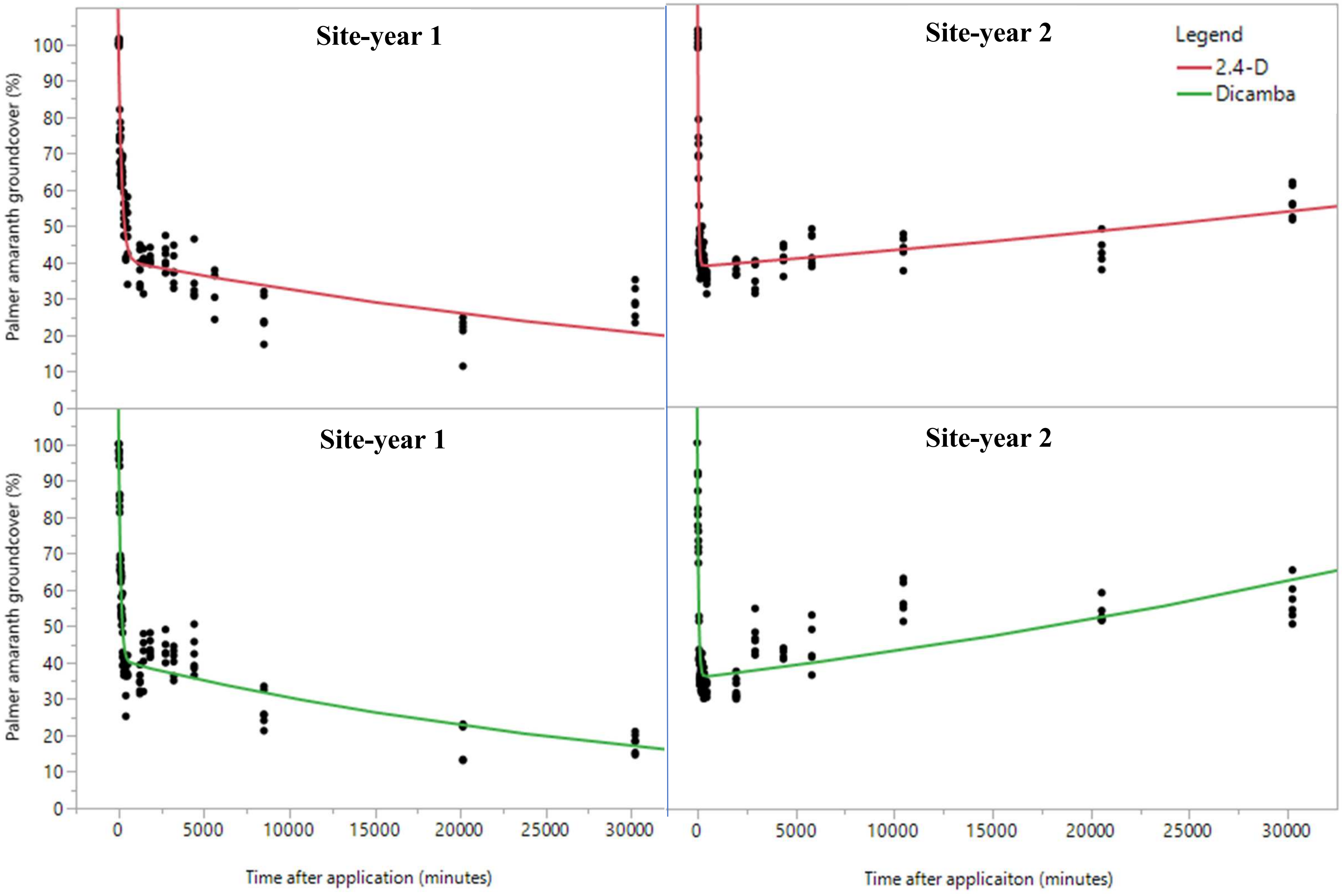

Figure 1. Biexponential 4P curves fit the greenhouse data by site-year and herbicide. Palmer amaranth groundcover was made relative to groundcover prior to the application.

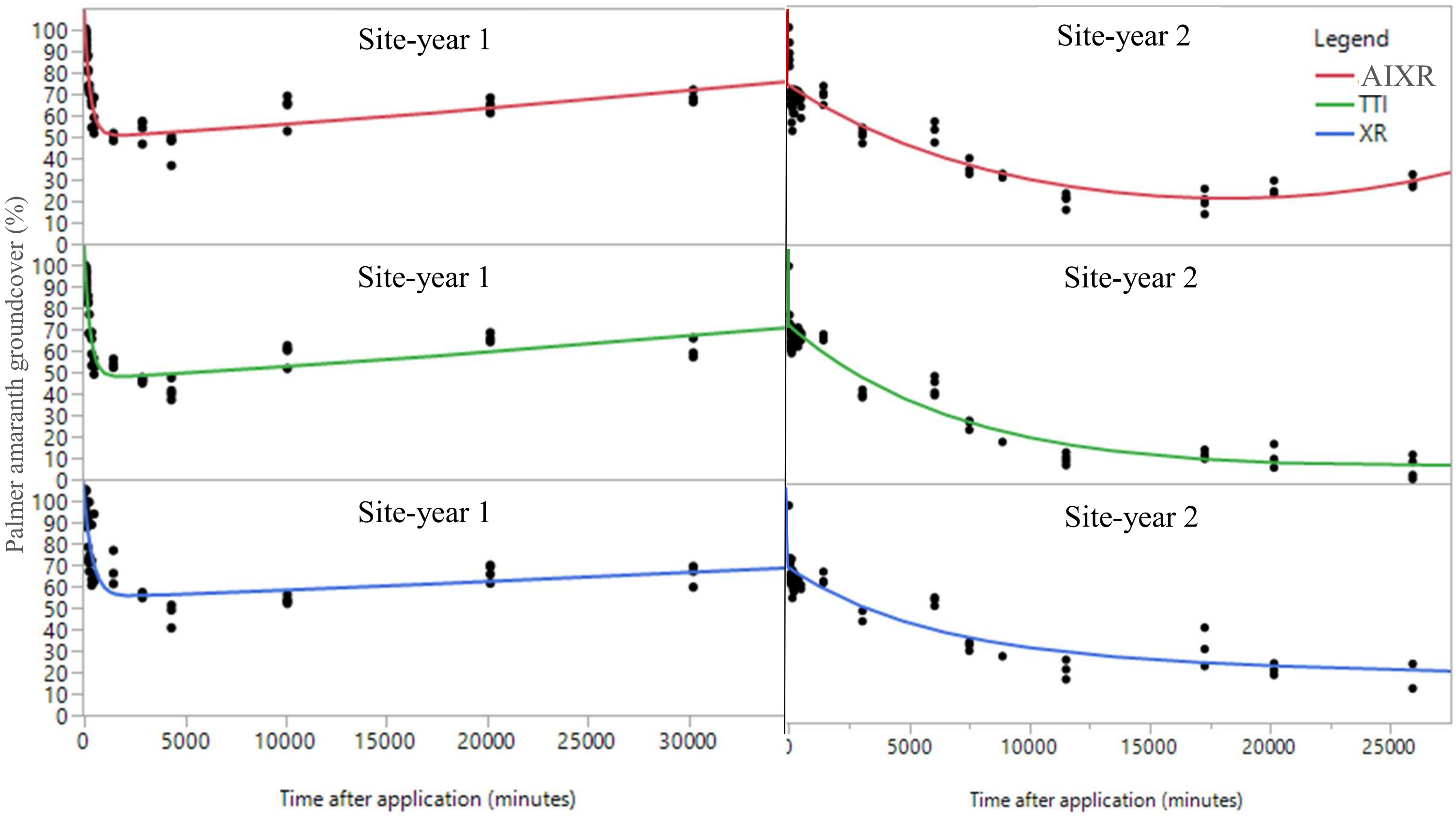

Figure 2. Biexponential 4P (y= a * Exp(-b*minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2)curve to estimate percent reduction in Palmer amaranth groundcover by nozzle following a dicamba application relative to Palmer amaranth groundcover prior to the application.

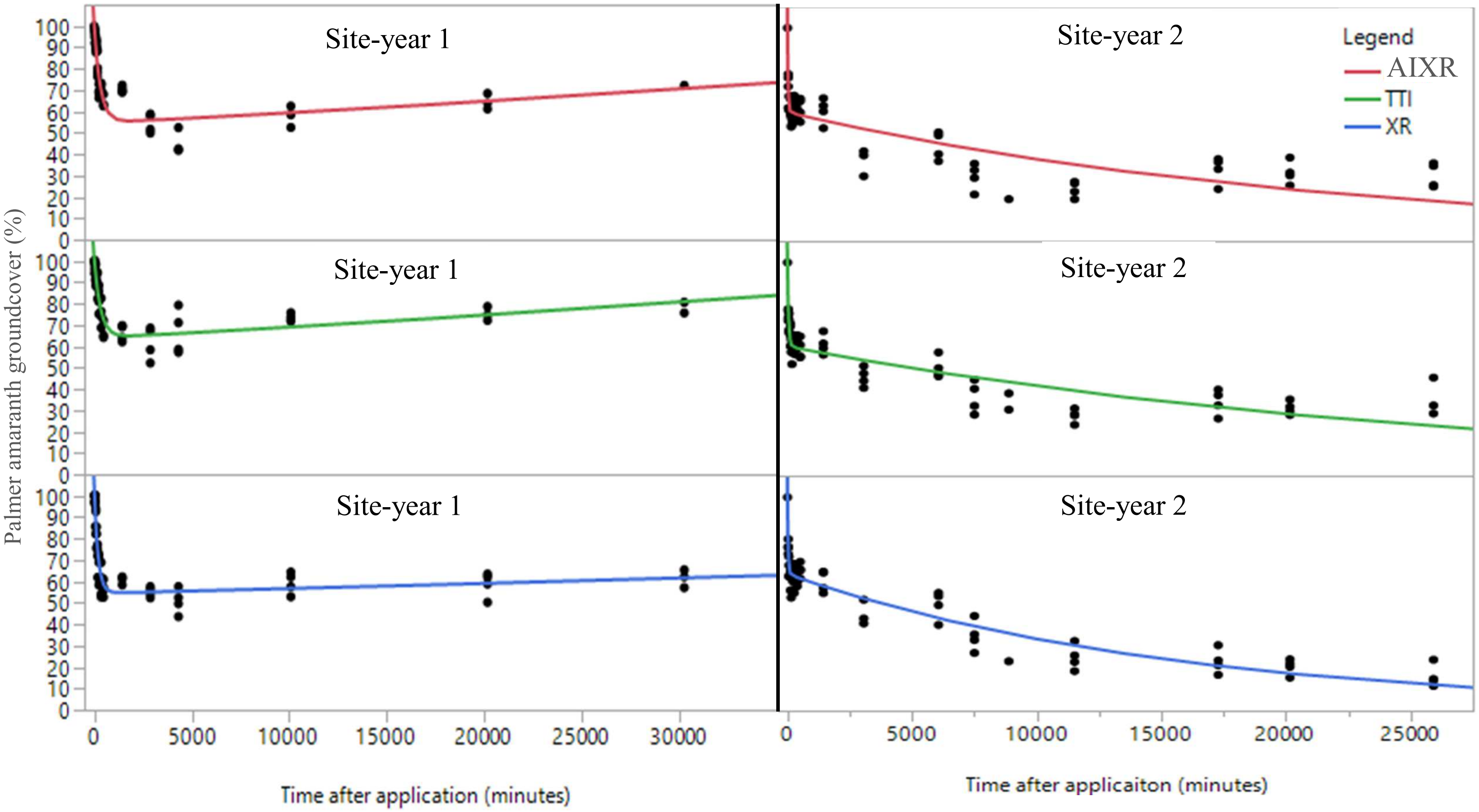

Figure 3. Biexponential 4P (y= a * Exp(-b*minutes after application) + c * Exp (-d * minutes after application), a = scale 1, b = decay rate 1, c = scale 2, d = decay rate 2) curve to estimate percent reduction in Palmer amaranth groundcover by nozzle following a 2,4-D application relative to Palmer amaranth groundcover prior to the application

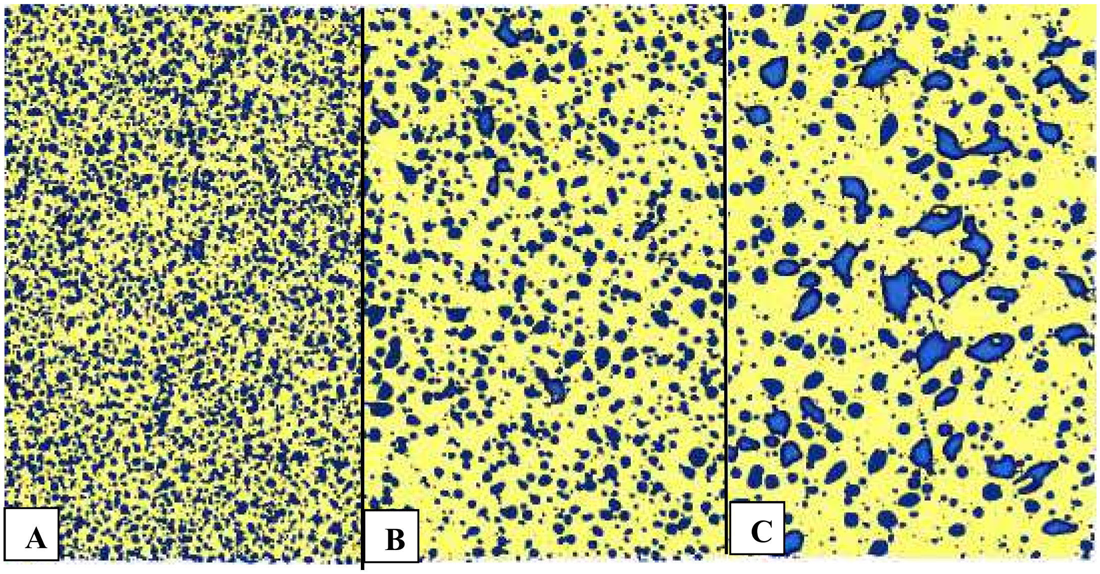

Figure 4. Water sensitive spray cards that received dicamba at 560 g ae ha^{-1} at 140 L ha^{-1} through A) Extended Range-, B) Air Induction Extended Range-, C) Turbo TeeJet Induction-110015 nozzles.

Chapter 6

Effects of sequential applications of dicamba and glufosinate on herbicide absorption, translocation, and metabolism.

Jason K Norsworthy, Jeong-In Hwang, Andy Mauromoustakos, Trenton L Roberts, Thomas R Butts

Abstract. Interactions of contact and systemic herbicides can deleteriously affect weed control. The objective of this research was to determine the effect of application interval or sequence of applications of dicamba and glufosinate on absorption, translocation, and metabolism of both herbicides. Dicamba and glufosinate were applied separately, in mixture, and at 3- and 14-day intervals, allowing assessment of dicamba followed by (fb) glufosinate and glufosinate fb dicamba. Compared to ^{14}C-dicamba and ^{14}C-glufosinate alone, dicamba absorption increased when dicamba and glufosinate were applied in mixture; however, dicamba translocation was decreased by 22-percentage points. Glufosinate absorption and translocation was unaffected when mixed with dicamba. Reductions in dicamba translocation occurred when glufosinate was applied prior to dicamba; therefore, the prior application of glufosinate may be detrimental to the activity of dicamba on Palmer amaranth. When dicamba was applied before glufosinate, no impact on glufosinate absorption or translocation was observed; however, only when dicamba fb glufosinate was applied at the 14-day interval was metabolism of glufosinate similar to glufosinate alone. Dicamba fb glufosinate at the 14-day interval avoided interactions involving absorption, translocation, and metabolism, while the mixture, glufosinate fb dicamba at 3- or 14-days, or dicamba fb glufosinate at 3-days were observed to impact absorption, translocation, or metabolism. Thus, to avoid potential negative interactions dicamba should be applied 14-days prior to glufosinate.

Nomenclature: dicamba; glufosinate; Palmer amaranth, *Amaranthus palmeri* (S.) Wats.

Key words: dicamba, glufosinate, herbicide interactions, sequential applications

Introduction

Dicamba and glufosinate are key herbicides to control troublesome weeds like Palmer amaranth in cotton (*Gossypium hiristum* L.), corn (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.]. XtendFlex® crops allow for over-the-top applications of dicamba, glufosinate, and glyphosate. Broad-spectrum weed control can be achieved in XtendFlex® crops when applying dicamba, glufosinate, and glyphosate as part of a complete program (Meyer and Norsworthy 2019; Meyer et al. 2015). The labeled dicamba-containing products (e.g., XtendiMax®, Engenia®) for use over-the-top of XtendFlex® crops currently do not allow for dicamba to be mixed with glufosinate (Anonymous 2020a, Anonymous 2020b); therefore, dicamba and glufosinate have to be applied sequentially.

Dicamba and glufosinate are systemic and contact herbicides, respectively. When systemic and contact herbicides are mixed antagonism is likely and may increase the likelihood of resistance evolving (Norsworthy et al. 2012). Meyer et al. (2020) observed that when dicamba and glufosinate were applied to Palmer amaranth in mixture a 12-percentage point increase in dicamba absorption resulted; however, only 4% and 52% of the absorbed dicamba was translocated out of the treated leaf when the mixture of glufosinate plus dicamba, and dicamba alone was applied, respectively. When dicamba and glufosinate were applied in mixture, dicamba absorption increased, dicamba translocation decreased, and glufosinate was unaffected. To mitigate antagonism and reductions in herbicide translocation, systemic and contact herbicides are often applied sequentially (Neve et al. 2003; Walsh and Powles 2007).

Sequential applications of contact and systemic herbicides can sometimes have deleterious effects. When glufosinate was applied at 7- or 14-days prior to clethodim, a 50-percentage point reduction in goosegrass (*Eleusine indica* (L.) Gaertn.) control was observed when compared to clethodim alone (Burke et al. 2005). The findings from Burke et al. (2005) displays that applying contact herbicides prior to systemic herbicides may be detrimental to weed control and resistance mitigation. Contrarily, pretreatment of 2,4-D (a systemic herbicide) on rigid ryegrass (*Lolium rigidum* Gaud.) increased the rate of diclofop that was needed to control a susceptible population by 10-fold (Yu and Powles 2014). Merchant et al. (2013) also reported that a pretreatment of 2,4-D reduced the efficacy of later applied glufosinate in Texas millet (*Urochloa texana* Buckl.) and broadleaf signalgrass (*Urochloa platyphylla* Nash). Thus, interaction of sequential herbicide applications may be more complex than previously believed and require further investigation.

Herbicide interactions are commonly only assessed when herbicides are applied in mixture. These studies can be complex and dependent on species (Meyer and Norsworthy 2019, O'Sullivan and O'Donovan 1980), herbicide rate (Flint and Barrett 1989), weed size (Meyer and Norsworthy 2019, Flint and Barrett 1989b), and individual herbicide products (Flint and Barrett 1989a, Kudsk and Mathiassen 2004). Fewer studies have assessed the interactions of herbicides when applied sequentially, likely due to the added complexity of explaining interactions if observed. In addition to the aforementioned factors that affect herbicide interactions in mixture, herbicide interactions when applied sequentially can be affected by time between sequential applications (Priess et al. 2019a), changes in weed groundcover following the prior application (Priess et al. 2019b), and changes in absorption, translocation, or metabolism (Burke et al. 2005; Yu and Powles 2014).

One aspect of sequential herbicide applications lacking investigation is how herbicide applications affect absorption, translocation, and metabolism of a later applied herbicide. The objectives of the present study were to utilize ^{14}C-labeled herbicides to determine what application order and time interval allowed for similar absorption and translocation of dicamba and glufosinate, without negatively affecting metabolism of either herbicide. These experiments will help refine the best management practices for in-season use of both dicamba and glufosinate over-the-top of XtendFlex® crops, while mitigating the likelihood for resistance evolving in Palmer amaranth populations.

Materials and Methods

Plant materials. Seeds of a Palmer amaranth population collected from Crittenden County, AR in 2018 was used in absorption, translocation, and metabolism experiments. Seeds were planted in mediated potting mix (Sungro® Horticulture, Agawam, MA) at the University of Arkansas Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR. When plants reached the cotyledon to one-leaf stage a single plant was transplanted into mediated potting mix contained in 10 cm diameter plastic pots (Growers Supply, Dyersville, IA). Moist potting mix was maintained throughout the experiment through daily irrigation. The first run of each experiment explained below was initiated on April 1, 2021, and the second run was initiated on April 28, 2021.

Uptake and translocation experiments. Uptake and translocation experiments were conducted for each ^{14}C-labeled herbicide used (i.e. dicamba and glufosinate). Non-radiolabeled formulated dicamba as XtendiMax® plus VaporGrip® (Bayer CropScience, St. Louis, MO), and glufosinate as Liberty® (BASF, Lundwigshafen, Germany) were applied at 560 g ae ha^{-1} and 595 g ai ha^{-1}, respectively. These non-radiolabeled spray solutions were spiked with radiolabeled glufosinate or dicamba to create a spotting solution. ^{14}C-glufosinate [RS-glufosinate ammonia, (3,4-^{14}C)] and [phenyl-U-^{14}C]dicamba were used to evaluate glufosinate and dicamba absorption, translocation, and metabolism of the parent compound. The radiolabeled spotting solutions used for both dicamba and glufosinate contained the same concentrations of the respective herbicides used in the non-radiolabeled applications. For example, the spotting solution for radiolabeled applications of dicamba contained 287 µl of water, 3 µl of formulated dicamba, and 20 µl of radiolabeled dicamba solution. The glufosinate spotting solution contained, 158 µl of water, 2 µl of formulated glufosinate, and 160 µl of radiolabeled glufosinate solution.

When Palmer amaranth plants reached the 5- to 7-leaf stage, plants were treated with non-radiolabeled dicamba and/or glufosinate in a stationary spray chamber with a mounted two-nozzle track sprayer equipped with TeeJet 1100067 nozzles (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA) calibrated to deliver 190 L ha^{-1}, at 1.61 km h^{-1}. Immediately after the non-radiolabeled herbicide application, plants were transported to the radioactive laboratory where radiolabeled spotting solutions were applied. Methodology for application of radiolabeled herbicide solutions were modified from Nadula and Vencill (2003) and Meyer et al. (2020). Radioactive working solutions were applied to the second-oldest fully expanded leaf. A micropipette was used to apply four 0.5 µl droplets of spotting solution to the adaxial surface of the leaf on either side of the midvein. A total of 240,000 and 320,000 disintegrations per minute (DPM) of radiolabeled glufosinate and dicamba were applied to each plant, respectively.

After application of radiolabeled herbicides, three plants were immediately sampled for reference and the rest of the plants were allowed to sit for 30 min to allow the spotting solution to dry before transporting the plants to a growth chamber; which was set at a constant temperature of 28 C, 65% humidity, 16-hour light/8-hour dark photoperiods with a light intensity of 600 μmol photons m^{-2} s^{-1}. Plants remained in the growth chamber for 48 hours until harvest was initiated. Irrigation of treated plants was accomplished without water contacting plant foliage. Plants were harvested only at 48 hours after application. Prior literature has reported that the majority (≥80%) of herbicide absorption occurs in the first 48 hours (Besançon et al. 2018; Everman et al. 2009; Lorentz et al. 2014; Ou et al. 2018; Everman et al. 2009; Young et al. 2003).

At harvest (48 hours after treatment), plants were dissected into four plant parts: above the treated leaf (ATL), treated leaf (TL), below the treated leaf (BTL), and roots. Roots were gathered by washing all soil from the rootzone with tap water and clipping the stem at the soil surface. The treated leaf after harvest was rinsed with 5 mL of methanol in a 20 mL plastic vial. Following the leaf rinse, 5 mL of scintillation cocktail (Ultima Gold™, PerkinElmer Inc., Waltham, MA) was added to each rinsate vial. Effective removal and recovery of the radiolabeled herbicides was confirmed by rinsing leaves with methanol within 2 min after application. The recovery of ^{14}C-dicamba and -glufosinate were 98% and 92% of the amount of radioactivity applied when the methanol rise methodology was utilized, respectively.

Plant sections were dried in a freeze dryer at -50 C for 36 hours (Model 18DX48SA, Botanique Preservation Equipment, Inc., Phoenix, AZ, USA). Samples were then combusted using a biological oxidizer (OX-700, R.J. Harvey Instrument, Tappan, NY, USA). The $^{14}CO_2$ gas was trapped in 15 mL of liquid scintillation cocktail (Ultima Gold™, PerkinElmer Inc., Waltham, MA). Quantification of the captured ^{14}C following oxidation and rinsing of treated leaves was determined using a Tri-Carb 2900TR Liquid Scintillation Analyzer (LSA; PerkinElmer Inc, Waltham, MA, USA).

The amount of ^{14}C recovered from plant parts and rinse solutions 48 hours after treatment was 86% and 84% of the total applied radioactivity for dicamba and glufosinate, respectively. Similarly, Meyer et al. (2020) achieved 90% and 83% recovery of ^{14}C-dicamba and -glufosinate using similar methods. The percentage of herbicide absorbed was calculated by dividing the amount in the plant by the total amount recovered in the plant and leaf wash (total detected). The percentage of herbicide in each plant part is reported as a percentage of total radioactivity recovered and sums to the percentage absorbed.

Metabolism experiment. Plant preparation and herbicide treatments, both non-radiolabeled and radiolabeled, were identical to the absorption and translocation experiment. The absorption, translocation, and metabolism experiments were initiated at the same time. Plant preparation for high-performance liquid chromatography (HPLC) utilized methodology adapted from Küpper et al. (2018), Meyer et al. (2020), and Zaccaro et al. (2020). The methodology for plant harvest differed from the absorption and translocation experiments. Plants were harvested by rinsing soil from roots, and the treated leaf was rinsed in a 100% methanol solution 48 hours after treatments were applied. The whole plants were placed into paper envelops and freeze dried for 36 hours at -50 C (Model 18DX48SA, Botanique Preservation Equipment, Inc., Phoenix, AZ, USA). The methodology used to extract ^{14}C-dicamba and ^{14}C-glufosinate differed and will therefore be discussed separately.

Dicamba extraction. Plants were removed from the freeze dryer and cut in 0.25 cm sections and placed in 10 mL of 90:10% methanol:water (HPLC grade) solution contained in a 50 mL Eppendorf tube (VWR, Randor, PA, 19087-8660). The plant material was then homogenized in the solution for one minute using a Polytron Homogenizer (Brinkmann instruments, Inc, Westbury, NY 11590). After homogenization was complete, samples were centrifuged at 6000 x *g* for 6 minutes. The supernatant was then extracted and filtered through Whatman Quantitative number 42 filter paper, ashless grade (VWR, Randor, PA, 19087-8660) into a separate 50 mL Eppendorf tube. Then 10 mL of 100% HPLC grade methanol was added to the residue not extracted with the supernatant. Samples containing the 100% methanol solution and plant residues were mixed with a VWR Mini Vortexer (VWR, Randor, PA, 19087-8660) for approximately one minute. The mixture of plant residue and methanol was then filtered with Whatman Quantitative number 42 filter paper (ashless grade) into the same 50 mL Eppendorf tube containing the extracted supernatant. The filtered solution was then evaporated with an Xcelvap (Horizon technologies, Inc, Lake Forest, CA) until less than 1 mL of each sample remained. Methanol was added to each sample to obtain a final volume of 1 mL. The solution was then pushed through a 0.2 µl syringe filter (VWR, Randor, PA, 19087-8660) into a HPLC compatible 2 mL glass vial.

Glufosinate extraction. The glufosinate extraction method used in the present study mimicked the methodology from Meyer et al. (2020). Plants were removed from the freeze dryer and immediately ground with mortar and pestle, until plant material formed a powder. Plant material was transferred to a 2.5 mL Eppendorf tube and 600 µl of 90:10% methanol:water solution was added. Samples were mixed by placing the Eppendorf tubes on a Mini Vortexer for approximately 30 seconds. Samples were then centrifuged at 6000 x *g* for 6 min. The supernatant was extracted into a separate vial. This process was repeated utilizing 90:10% acetonitrile:water in the second, and 10:90% methanol:water for the third and fourth time. All solvents used were HPLC grade. The supernatants were pooled and evaporated to less than 1 mL with a Xcelvap (Horizon technologies, Inc, Lake Forest, CA). Methanol was added to each sample to equate to 1 mL solution. Samples were then filtered into HPLC compatible 2 mL glass vials using a 0.2 µl syringe filter.

High-performance liquid chromatography analysis. For ^{14}C-dicamba analysis, the HPLC mobile phases consisted of 0.1% phosphoric acid (A) and methanol (B). Solvents were run for 28 min in seven stages: (i) a 6-min plateau at 80% solvent A; (ii) 4-min linear gradient from 80% to 30% of solvent A; (iii) 5-min linear gradient from 30% to 0% of solvent A; (iv) a 5-min plateau at 0% solvent A; (v) 3 min linear gradient from 0% to 100% of solvent A; (vi) 2 min linear gradient from 100% to 80% of solvent A; and (vii) 3-min plateau at 80% solvent A. A reverse phase HPLC column [Columbus^{TM} 5 µm C18 110 Å LC column, 250 (L) × 4.6 mm (ID), Phenomenex Co., Torrance, CA, USA] was used with along with a guard column (SecurityGuard^{TM} Guard Cartridge Kit with 3.0 mm C18 column, Phenomenex Co.). The column temperature was kept at 40°C and the flow rate was 1.0 mL min^{-1}. On average, recovery was 88% of the ^{14}C-dicamba applied.

For ^{14}C-glufosinate-ammonium analysis, the mobile phases consisted of 50 mmol ammonium acetate (C) in water and HPLC reagent grade water (D). Solvents were run for 1-min plateau at 15% solvent C, 5-min linear gradient from 15% to 30% of solvent C plateauing for 2 min, followed by a linear gradient returning to 15% solvent C in 5 min. The column was then flushed with 15% solvent C for 2 min. A SeQuant ZIC-pHILIC 5µm polymeric LC column [100 (L) × 4.6 mm (ID), Merck KGaA, Darmstadt, Germany] was used for glufosinate. The column temperature was kept at 40 C and the flow rate was 0.5 mL min^{-1}. On average, 93% of the applied ^{14}C-glufosinate-ammonium was recovered.

Statistical analysis. All experiments were established using a randomized complete block design that included three replications and two experimental runs. The distribution of all data was assessed with the Shapiro wilk test ($\alpha = 0.05$) and homogeneity of variances was assessed with the Levene's test ($\alpha = 0.05$). All data were analyzed in JMP 15.2 (SAS, Institute, Cary, NC). In the absorption and translocation experiment, data were presented as percent of total absorption and percent radioactivity per plant part of the ^{14}C-herbicide treated and replications and runs were set is random effects in the model statement. Total absorption values were determined by summation of the four plant parts. Total absorption data were analyzed where herbicide treatment was a single factor and separated by ^{14}C-herbicide; therefore, comparisons should not be made across ^{14}C-herbicides. Means were separated with Tukey's HSD with an alpha value of 0.05.

For the translocation experiment, a split-plot treatment structure with the whole-plot factor being treatment and subplot factor being plant section was utilized. An ANOVA was used to analyze the ^{14}C-herbicides separately. Replications and runs were pooled and set as random effects. Means were separated with Tukey's HSD with an alpha value of 0.05.

For the metabolism experiment, data were presented as the percent of ^{14}C-herbicides remaining in the parent compound. Data were analyzed with ANOVA, the single factor being herbicide treatment, and separate analyses were conducted for the two ^{14}C herbicides. Again, replications and runs were pooled and set as random effects within the model statement, and means were separated with Tukey's HSD with an alpha value of 0.05.

Results and Discussion

Herbicide absorption. No changes in glufosinate absorption were observed when glufosinate was applied in combination with dicamba or applied sequentially following dicamba (Table 1). Thus, a similar amount of glufosinate enters the plant regardless of mixture or application sequence with dicamba. Hence, impacts on efficacy should not be attributed to changes in glufosinate absorption. A potential shortcoming of this research is that the amount of glufosinate that contacted the dicamba pretreated Palmer amaranth plant was not quantified. Priess et al. (2019b) found that Palmer amaranth treated with auxin herbicides suffered a 40 to 50 percentage point reduction in groundcover at 3 and 14 days after a dicamba application, due to the auxin symptomology that occurred. The reduction in groundcover of targeted weed species likely reduces interception of subsequent herbicide applications. Because ^{14}C-glufosinate was applied directly to plant material, the aforementioned factor was not accounted for. Therefore, absorption data collected should not be compared directly to field scenarios but referenced only for plant uptake as reductions in herbicide contact may be a considerable variable that was not accounted for.

Dicamba absorption increased when mixed with glufosinate (Table 1). It is unclear why this occurred but these results were also observed by Meyer et al. (2020). A possible explanation from Meyer et al. (2020) is that the addition of glufosinate reduces the spray solution pH and likewise increases the passive diffusion of dicamba (a weak acid) through the electrochemical gradients present in plant cuticles (Roskamp et al. 2013). Supporting this hypothesis, when ammonium sulfate, a common tank additive that reduces spray solution pH, was added to dicamba an increase in broadleaf weed control was observed (Sterling 1994); however, further investigation is needed to determine the effects of spray solution pH on dicamba uptake in weedy species.

No changes in ^{14}C-dicamba absorption were observed when sequential applications of glufosinate fb dicamba were compared to dicamba alone. Contrarily, Burke et al. (2005) observed a 50 percentage-point reduction in goosegrass [*Eluesine indica* (L.) Gaertn] control when glufosinate was applied less than 7 days prior to clethodim. The reduction in goosegrass control was attributed to the rapid necrosis of plant tissue that resulted from an application of glufosinate. However, ^{14}C-dicamba absorption was not affected by a prior glufosinate application (Table 1).

Translocation. ^{14}C-dicamba translocation was reduced when mixed with glufosinate or when dicamba was applied 3 or 14 days after glufosinate compared to dicamba alone. When dicamba was applied alone 19, 9, and 2% of the ^{14}C-dicamba translocated to ATL, BTL, and roots, respectively. Thus 30% of the treated dicamba translocated from the treated leaf (Table 2). When the mixture of dicamba plus glufosinate or glufosinate fb dicamba at the 3-day interval was applied only 9 and 8% of treated dicamba translocated from the treated leaf. When glufosinate was applied 14 days prior to dicamba a total of 18% of the ^{14}C-dicamba translocated out of the treated leaf. Thus, the mixture or the use of sequential applications at 3 and 14 day-intervals were found to have different rates of dicamba translocation as dicamba alone. Compared to the mixture or use of glufosinate 3 days prior to dicamba, dicamba translocation increased when the glufosinate followed dicamba by 14 days. The authors believe this may be due to the fact Palmer amaranth plants were beginning regrowth 14 days after the glufosinate application. The regrowth may have increased the plants ability to circumvent dead tissue and allow dicamba to take alternative paths throughout the plant, thus increasing dicamba translocation; however, dicamba translocation for the glufosinate fb dicamba at the 14 day-interval treatment was still less than dicamba alone. The current label restrictions that prohibit a dicamba and glufosinate mixture may be detrimental to dicamba-resistance mitigation if the practice of applying glufosinate prior to dicamba or the prohibited mixture of dicamba and glufosinate are adopted.

A maximum of 3% of the applied glufosinate translocated out of the treated leaf, following any treatment evaluated. Prior research has hypothesized that glufosinate limits its own translocation because of rapid necrosis, especially in species highly sensitive to the herbicide (Beriault et al. 1999; Everman et al. 2009; Steckel et al. 1997). In this experiment, no differences in glufosinate translocation were observed; however, further investigation may be needed to determine if glufosinate translocation is affected when glufosinate-resistant Palmer amaranth plants are subjected to treatments used in this experiment.

Metabolism. Only 37 to 75% of the absorbed radioactivity recovered remained in the parent form of dicamba 48 h after treatment (Table 3). Previous research conducted by Meyer et al. (2020) found that 95 to 99% of dicamba remained in the parent form when Palmer amaranth populations from a similar geography were treated. A limitation of the research conducted was that only one sampling time was collected, 48 h after treatment. If more sampling times were collected, metabolism of dicamba could be referenced in time and may aid in explaining the higher levels of metabolism observed compared to the results of Meyer et al. (2020). Additionally, the geography that seed was collected from for this experiment had three more years of exposure to herbicides and outcrossing with adjacent fields. These factors may contribute to variation in metabolism between this study and the study conducted by Meyer et al. (2020).

Metabolism of dicamba occurred at the highest rate when dicamba was applied alone; only 37% of the absorbed radioactivity remained in the parent form of dicamba (Table 3). When dicamba was mixed with glufosinate or glufosinate was applied 3 or 14 days prior to dicamba, 57 to 75% of absorbed ^{14}C-dicamba remained in the parent form. However, a reduction in ^{14}C-dicamba translocation may explain the reduction in metabolism and not suggest a lower risk for metabolic resistance (Table 2). Westburg and Coble (1992) found that a pretreatment of acifluorfen (a contact herbicide) reduced the translocation of chlorimuron-ethyl (a systemic herbicide) and likewise reduced chlorimuron-ethyl metabolism. The lowest amount of ^{14}C-dicamba observed in the parent form other than dicamba alone, occurred when glufosinate was applied 14 days prior to dicamba and translocation of ^{14}C-dicamba was similar to dicamba alone (Tables 2 and 3).

The amount of glufosinate metabolism that occurred 48 h after treatment ranged from 29 to 59% (Table 3). Similarly, Meyer et al. (2020) observed 62 to 68% of glufosinate was metabolized 48 h after treatment. Significantly more glufosinate stayed in the parent form when glufosinate was mixed with dicamba or dicamba was sprayed 3 days prior to glufosinate. Past literature has shown that increased herbicide metabolism likewise decreases herbicide efficacy (Yu and Powles 2014). Similarly, crop safety to herbicides has been improved with the addition of seed treatments that increase herbicide metabolism (Hatzios and Burgos 2004). A plausible conclusion could be stated, that when glufosinate is mixed with dicamba or applied 3 days after dicamba, mitigating glufosinate metabolism may be beneficial in delaying the evolution of -metabolic-resistance and result in higher levels of weed control. However; many factors (i.e. nozzle type used for application of the mixture, weed size, etc.) not evaluated in the present experiment likely influence treatment efficacy and may offset the reduction in metabolism.

The mechanism responsible for the reduction in glufosinate metabolism when glufosinate was applied in mixture with dicamba or 3 days after dicamba is unknown. In depth, physiological/metabolic studies will be needed to understand why glufosinate metabolism was impacted by dicamba. A potential theory is the increase in sources needed to metabolize two herbicides may impede the ability of Palmer amaranth to detoxify glufosinate. It is well known that dicamba is a fast-acting herbicide that causes visual symptomology within a few hours after application (Priess et al. 2019b). It has also been noted that a key enzyme family involved in dicamba metabolism are cytochrome P-450 enzymes (Dellaferrera et al. 2018). Glufosinate metabolism has not been linked to specific enzymes in Palmer amaranth; however, Meyer et al. 2020 found that 3-methylphosphinico-propanoic acid (MPP) was the main glufosinate metabolite. MPP differs from glufosinate structurally by the lack of an amine group. Cytochrome-P-450 enzymes are known for hydroxylating or oxygenating xenobiotics (Bolwell et al. 1994); therefore, they are unlikely candidate enzymes for accomplishing the metabolism of glufosinate to MPP. If the aforementioned speculation regarding glufosinate metabolism is true, dicamba and glufosinate are metabolized by different enzyme families and complex interactions between enzyme expression may be responsible for the reduction in glufosinate metabolism when glufosinate is mixed with dicamba or applied shortly after dicamba. A significant amount of in-depth research will be needed to support the mentioned theory.

The metabolism of glufosinate when applied 14 days after dicamba was similar to when glufosinate was applied alone (Table 3). Thus, it is apparent that by waiting 14 days after a dicamba application, the subsequent glufosinate application would act similarly, in terms of metabolism as the glufosinate alone treatment. The interaction observed when dicamba and glufosinate were mixed or applied at the 3-day interval could be avoided if the subsequent glufosinate application was made later. Priess et al. (2019a) found that the use of sequential applications of dicamba and glufosinate were optimized for Palmer amaranth control when dicamba was applied 14 days prior to glufosinate. Since metabolism was similar when glufosinate was applied alone or 14 days after dicamba, likely impacts from the prior dicamba application would not influence the rate that metabolic glufosinate-resistance may evolve.

Conclusions and practical implications. An overall assessment of differences in absorption, translocation, and metabolism between treatments is needed to draw practical applications and implement best-management techniques When dicamba and glufosinate were applied in mixture an increase in ^{14}C-dicamba absorption was observed and ^{14}C-glufosinate absorption was unaffected; however, ^{14}C-dicamba did not translocate out of the treated leaf. Overall, the mixture of dicamba and glufosinate may not be the best option to maximize the activity of both herbicides in Palmer amaranth due to the reduction in translocation of dicamba when glufosinate is added in the mixture.

When glufosinate was applied 3 or 14 days prior to dicamba, no changes in ^{14}C absorption were observed when compared to dicamba alone. Translocation of dicamba was inhibited when glufosinate was applied 3 days prior to dicamba, but similar amounts of ^{14}C dicamba translocation were observed when the 14-day interval between applications was used. Further, dicamba was metabolized to a lesser extent when glufosinate was applied 3 days prior to dicamba when compared to dicamba alone, likely due to the limited translocation from the treated leaf. To allow for similar amounts of dicamba translocation as the dicamba alone treatment, glufosinate should be treated 14 days prior to dicamba.

When the use of sequential applications is needed to control Palmer amaranth populations dicamba fb glufosinate at the 14-day interval allowed for similar absorption, translocation, and metabolism as the herbicides alone, minimizing interactions between the herbicides. When utilizing both dicamba and glufosinate as postemergence options in the XtendFlex® technology, Priess et al. (2019) observed the greatest level of Palmer amaranth control when dicamba was fb glufosinate at a 14-day interval. Data collected from the present study supports that interactions involving herbicide absorption, translocation, and metabolism are avoided when glufosinate is applied 14 days after dicamba. Further, utilizing two SOA in a single growing season will likely mitigate the evolution of herbicide resistance if the interaction between the two herbicides are not antagonistic (Norsworthy et al. 2012).

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Tables

Table 1. Absorption of ^{14}C-dicamba and ^{14}C-glufosinate in Palmer amaranth as affected by herbicide treatment and harvested 48 h after application.

^{14}C herbicide	Treatment	Interval	Absorption[a]	
		days	%	
dicamba	dicamba alone	0	46	b[b]
	dicamba + glufosinate	0	88	a
	glufosinate fb dicamba	3	48	b
	glufosinate fb dicamba	14	52	b
Glufosinate[c]	glufosinate alone	0	70	
	dicamba + glufosinate	0	58	
	dicamba fb glufosinate	3	71	
	dicamba fb glufosinate	14	62	

[a] Absorption is represented as a percentage of the amount of radioactivity applied

[b] Means within a column and ^{14}C-herbicide can be compared with displayed letter separation, means with the same letter are not considered different utilizing Tukey's HSD at an alpha value of 0.05.

[c] Glufosinate absorption was not significantly affected by herbicide treatment; therefore, no letter separation is present

Table 2. Translocation of ^{14}C-dicamba and ^{14}C-glufosinate in Palmer amaranth as affected by herbicide treatment and harvested 48 h after application.

^{14}C herbicide	Treatment	Interval	ATL[a]	TL	BTL	R	Across column HSD[c]
		days					
dicamba	dicamba alone	0	19[b]	16	9	2	6
	dicamba + glufosinate	0	5	81	2	1	
	glufosinate fb dicamba	3	3	40	5	0	
	glufosinate fb dicamba	14	11	33	7	0	
	within column HSD and ^{14}C-herbicide			6			
glufosinate	glufosinate alone	0	1	67	1	1	4
	dicamba + glufosinate	0	0	56	1	1	
	dicamba fb glufosinate	3	0	70	1	0	
	dicamba fb glufosinate	14	1	60	2	0	
	within column HSD and ^{14}C-herbicide			5			

[a] ATL, above treated leaf; TL, treated leaf; BTL, below treated leaf; R, roots

[b] Translocation is represented as a percentage of the amount of radioactivity applied

[c] Means within a column and ^{14}C-herbicide can be compared utilizing the within column LSD and means across columns and within 14C-herbicides can be compared utilizing the across column HSD. HSDs were calculated using Tukey's HSD at an alpha value of 0.05 for a split plot experimental design.

Table 3. Dicamba and glufosinate metabolism, displayed as a percentage of applied radioactivity, as affected by herbicide treatment in Palmer amaranth as determined by high-performance liquid chromatography.

^{14}C-herbicide	Treatment	Interval	Parent compound[a]	
		days	---------%----------	
dicamba	dicamba alone	0	37[b]	c
	dicamba + glufosinate	0	64	ab
	glufosinate fb dicamba	3	75	a
	glufosinate fb dicamba	14	57	b
glufosinate	glufosinate alone	0	34	c
	dicamba + glufosinate	0	64	a
	dicamba fb glufosinate	3	48	b
	dicamba fb glufosinate	14	34	c

[a] Metabolism is represented as a percentage of the amount of radioactivity applied of each herbicide, respectively

[b] Means within a column and ^{14}C-herbicide can be compared utilizing letter separation. HSDs were calculated using Tukey's HSD at an alpha value of 0.05.

Chapter 7

Confirmation of glufosinate- and 2,4-D-resistant Palmer amaranth and response to other herbicides.

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Abstract. The ability of weed populations to evolve resistance to efficacious herbicides impact management strategies and profitability of crop production. The objective of this research was to screen three putative-resistant Palmer amaranth accessions from Arkansas for glufosinate and dicamba as well as 2,4-D in one accession. Additional efforts focused on the effectiveness of various herbicides, across an assortment of sites of action, on each putative-resistant accession. The putative glufosinate- and dicamba-resistant accessions were selected from 60 Palmer amaranth accessions collected in 2019 and 2020 and screened in response to 0.5x and 1x rates of glufosinate and dicamba. A dose-response experiment was conducted including the herbicides 2,4-D, dicamba, and glufosinate on accession A2019, and only dicamba and glufosinate on accessions A2020, and B2020, due to limited seed quantities. The effectiveness of various preemergence- and postemergence-applied herbicides were evaluated on each accession. Resistance ratios of A2019, A2020, and B2020 to glufosinate ranged from 5.1 to 27.4 when comparing LD_{50} values to two susceptible accessions, thus all three accessions were resistant to glufosinate. A resistance ratio of 8.8 to 9.5 was also observed for A2019 when the herbicide 2,4-D was applied. Dicamba results were inconclusive and require further research to identify if the accession were susceptible or resistance to dicamba. All three accessions (A2019, A2020, and B2020) were found to have a reduction of at least 20-percentage points in mortality relative to a susceptible standard to five herbicide sites of action. Herbicides from nine different sites of action controlled A2019 at least 20-percentage points less than the susceptible standard, which points to a need for additional research to characterize the response of this accession.

Key words. Multiple herbicide resistance, glufosinate resistance, Palmer amaranth, 2,4-D resistance.

Nomenclature. Palmer amaranth; *Amaranthus palmeri* (S.) Wats.

Introduction

Herbicides are valuable tools in agricultural production systems to remove troublesome weeds. In row-crop production systems, herbicides are often the best option to control weedy plants, due to the relatively low cost and ease of implementation. However, the widespread use of herbicides since the 1940's has led to selection for herbicide-resistant biotypes.

Herbicide-resistant biotypes have typically been controlled by the use of a herbicide with a different site of action (SOA); however, this approach may aid in selection for multiple herbicide-resistant biotypes. Weed species that harbor multiple resistance mechanisms include but are not limited to black grass (*Alopecurus myosuroides* Huds.), common waterhemp (*Amaranthus tuberculatus* (Moq.) JD Sauer), Palmer amaranth (*Amaranthus palmeri* S. Wats), barnyardgrass (*Echinochloa crus-galli*), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*), rigid ryegrass (*Lolium rigidum* Gaudin)*,* wild radish (*Raphanus raphanistrum*) (Bailey et al. 2012; Preston et al. 1996; Owen et al. 2015; Shergill et al. 2018; Spaunhorst et al. 2019, Schwartz-Lazaro et al. 2017; Tehranchian et al. 2019; Yu et al. 2009). Weed species like rigid ryegrass, Palmer amaranth, and barnyardgrass have been observed to harbor resistance to seven, six, and five different herbicide SOA in a single biotype, respectively (Heap 2021; Shyam et al. 2020). With an increase in weeds that harbor multiple resistance mechanisms, the number of effective herbicides available in crops like soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) has diminished.

Following the evolution of acetolactate synthase (ALS)-, photosystem II-, 5-enolpyruvate shikimate 3-phosphate- (EPSPS), and protoporphyrinogen oxidase- (PPO) inhibitor resistance in Palmer amaranth populations, glufosinate-resistant crops and the use of glufosinate became a commonly used option to control emerged weeds in soybean and cotton (Heap 2021). Since the commercial launch of glufosinate-resistant soybean and cotton in the United States, in-season annual use of glufosinate has increased from 34,375 kg in 2007 to 4,705,000 kg in 2019. Thus, in-season glufosinate use has increased by 137-fold over a 12-year period in the United States (USDA-NASS 2021). In the past, overreliance on a single SOA has led to evolution of herbicide resistance in weed populations (Peres-Jones et al. 2005; Powles et al. 1997; Simarmata et al. 2005). Currently, glufosinate resistance has not been reported in broadleaf weed species throughout the world (Heap 2021).

Dicamba and 2,4-D were registered for commercial use in the late 1960s and were primarily used as a preplant burndown herbicide or early POST herbicide in corn (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* L. Moench.) (Anonymous 2014). The deregulation of dicamba-resistant cotton and soybean in 2015 and 2016, respectively, eventually led to the registration of XtendiMax® (Monsanto Corporation, St. Louis, MO 63167) and Engenia® (BASF Corporation, Research Triangle Park, NC 27709) for in-crop applications beginning in 2016. The registration of Enlist™ crops and 2,4-D-containing products like Enlist One® and Enlist Duo® have likewise increased the use of 2,4-D in the United States (USDA-NASS 2021).

With an increase in 2,4-D and dicamba use nationwide, a likewise increase in selection pressure on weed populations is expected. Tehranchian et al. 2017 observed after three years of sublethal selection with dicamba Palmer amaranth evolved near 3-fold reduced sensitivity to the herbicide. Bernards et al. (2012) documented a common waterhemp (*Amaranthus tuberculatus* (Moq.) JD Sauer) population that was 10-fold resistant to 2,4-D, and had a 3-fold reduced sensitivity to dicamba. More recently, 2,4-D resistance has been observed in a Palmer amaranth population in Kansas (Kumar et al. 2019). In 2020, the first case of dicamba-resistant Palmer amaranth was confirmed in Kansas and Tennessee (Peterson et al. 2019; Steckel 2020).

Materials and Methods

Dose Response. A preliminary study was conducted by collecting 30 Palmer amaranth accessions from soybean and cotton fields in the state of Arkansas in 2019 and 2020 (60 total accessions). Accessions were collected from fields where either dicamba or glufosinate had been sprayed during the growing season and seed-producing Palmer amaranth plants persisted. Accessions were collected and brought back the Altheimer Laboratory at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR. The accessions were planted and grown to the 5- to 6-leaf stage and then subjected to dicamba at 280 (0.5x) and 560 g ae ha^{-1} (1x) and glufosinate at 297 (0.5x) and 595 g ai ha^{-1} (1x).

Three accessions that were not effectively controlled by a 0.5x or 1x rate of either dicamba or glufosinate were selected for use in the dose-response experiment. Only one accession was selected in 2019 that was suspected for harboring reduced sensitivity to both dicamba and glufosinate, and two accessions were selected in 2020 with suspected resistance to glufosinate. Two additional susceptible accessions collected from Arkansas in 2001 were also included in the experiment for comparison purposes. For the two susceptible and three putative-resistant accessions, two experimental runs were completed. Each experimental run was conducted as a completely randomized design with three spatial replications, with each spatial replication containing 15 to 20 Palmer amaranth plants. A minimum of 100 plants per herbicide dose was treated.

Palmer amaranth plants were grown in trays containing mediated potting soil (Sungro® Horticulture, Agawam, MA, USA) until the cotyledon to one-leaf stage. A single plant cell^{-1} was transplanted into mediated potting soil in 20 cell trays (Greenhouse Megastore, Danville, IL, USA). Moist potting mix was maintained throughout the experiment through daily irrigation. Plants were grown in a greenhouse at 25 ± 8 C and light was supplemented to provide 1000 ± 320 µmol m^{-2} s^{-1} at plant height in a 16-hour day.

The three putative-resistant accessions (A2019, A2020, B2020) and two susceptible accessions (S1 and S2) were grown to the 5- to 6-leaf stage. When plants reached the 5- to 6-leaf stage herbicide treatments were applied. Treatments applied to susceptible accessions included 2,4-D at 0, 133, 266, 533, 1065, and 2130 g ae ha^{-1}; dicamba at 0, 35, 70, 140, 280, 560, and 1120 g ae ha^{-1}; and glufosinate at 0, 37.2, 74.3, 148.8, 297.5, 595, 1190 g ai ha^{-1}. Putative-resistant accessions were subjected to a log scale of six herbicide rates based on their previous response to dicamba and glufosinate. For 2,4-D, dicamba, and glufosinate, a 1X field rate of each herbicide was considered to be 1065 g ae ha^{-1}, 560 g ae ha^{-1}, and 595 g ai ha^{-1}, respectively. A2019 was the only putative-resistant accession that 2,4-D was tested against due to limited seed quantities for A2020 and B2020. Differing rate structures were used to account for the variability in herbicide sensitivity among biotypes.

Applications were made using a two-nozzle track sprayer equipped with TeeJet 1100067 nozzles (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA). The track sprayer was calibrated to deliver 187 L ha^{-1} at 1.61 km hr^{-1}. Prior to application the number of live plants were counted, and again 28 days after application (DAA) the remaining live plants were counted. These values were used to calculate percent mortality of Palmer amaranth 28

DAA. Putative-resistant plants that survived greater than a 1x rate were kept to increase seed production for additional experiments; therefore, biomass was not assessed.

Response to labeled herbicide rates. In addition to the dose-response study, sensitivity of the three putative-resistant accessions and S1 were evaluated to herbicides from 11 distinct SOA. The study was set up similar to the dose-response experiment, with two experimental runs completed. A minimum of 100 plants per postemergence herbicide and a total of 300 seeds per preemergence-herbicide were subjected to treatments, a sample size that has been shown to be sufficient to assess for herbicide resistance (Burgos et al. 2013), albeit confirmation of resistance was not the intent of this experiment. Plants were grown in similar manner and under the same greenhouse conditions as the dose-response experiment.

Postemergence applications were made to 6- to 8-leaf Palmer amaranth plants and included the following herbicides: 2,4-D (Enlist One® 3.8 L), atrazine (Aatrex® 4L), dicamba (XtendiMax® plus VaporGrip® 2.9 L), diuron (Direx® 4L), fomesafen (Reflex® 2 SL), glyphosate (Roundup PowerMAX II® 4.5L), imazethapyr (Pursuit® 2 L), mesotrione (Callisto® 4 SC), paraquat (Gramoxone® 3 SL), tembotrione (Laudis® 3.5 L). Respective WSSA herbicide group numbers, common names, family names, adjuvants, and use rates are included in Table 1. Use rates of herbicides are representative of 1x rates applied in corn, cotton, and soybean.

Field soil characterized as a Leaf silt loam (Fine, mixed, active, thermic Typic, Albaqualts) with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and pH of 5.9 was sieved and used to test sensitivity of accessions to preemergence-applied herbicides, specifically pendimethalin (Prowl® 3.3 EC) and *S*-metolachlor (Dual II Magnum® 7.34 EC). Field soil was placed in 30cm by 17cm flats and wetted. After wetting, 50 Palmer amaranth seeds were spread and lightly covered with approximately 0.25 to 0.5 cm of field soil. A total of three replications on per herbicide were included in each run, thus a total 300 seeds were treated per herbicide. All herbicides were applied using the same methodology as the dose-response experiment, and herbicides were incorporated through overhead irrigation to simulate approximately 1.5 cm of rainfall.

The number of total plants sprayed at the time of application was recorded, and live plants that persisted 28 DAT were counted to capture mortality percentages. For the assessment of preemergence herbicide efficacy, the number of Palmer amaranth plants with one true leaf were counted at 14 DAT, and number of emerged plants were reported as a percentage relative to the nontreated to account for variability in germination and emergence among accessions.

Data analysis.

Dose response. In the dose-response experiment, the percent mortality of Palmer amaranth data were analyzed in the Fit Curve Platform of JMP Pro 15.2 (SAS Institute Inc., Cary, NC). A Weibull growth curve ($y= a * (1 - \mathrm{Exp}(- (\mathrm{rate}/b)^{c}))$, a = asymptote, b = inflection point, c = growth rate) was found to be the best fit compared to other models, including but not limited to, Exponential 3P, Mechanistic growth, Gompertz, Logistic 3P, etc., when AICc, BIC, SSE, MSE, and R^2 values were used to model the percent mortality of Palmer amaranth. The Weibull growth curve has been used to fit dose-response data in ecotoxicology, weed science, and other types of research (Christensen et al. 1984; Knezevic et al. 2007; Ritz 2010). Data were pooled over experimental runs and individual non-linear Weibull growth models were fit to each accession by herbicide. Parameter estimates and R^2 values for models fit are displayed in Table 2. Predictions of the herbicide rate needed to kill 50% of the population (e.g. LD_{50}) and 80% of the population (e.g. LD_{80}) were made along with the lower and upper estimates of the 95% confidence interval. Confidence intervals were used to determine if the LD_{50} and LD_{80} predictions were different from other accessions sprayed with the same herbicide. If confidence intervals of prediction estimates did not overlap, the predications were considered different, and resistant-fold values were calculated by dividing the LD_{50} or LD_{80} estimate of the resistant biotype by the respective LD_{50} or LD_{80} estimate of the susceptible biotypes.

Response to labeled herbicide rates. Analysis of variance confirmed that there were no differences between experimental runs (P=0.6857); therefore, data were pooled over runs. Moss et al. (1999) and Walsh et al. (2004) used 20% survival as a threshold for classifying a weed as resistant to a labeled rate of various herbicides when screening for multiple resistance, but as methodologies have improved to classify weed species as herbicide-resistant over the last 20 years, this experiment will only be used to assess effectiveness of alternative control options relative to a standard accession.

Results and Discussion

Dose Response

Glufosinate. The two susceptible accessions were proven to be sensitive to glufosinate. When the LD_{50} values of accessions A2019, A2020, and B2020 were compared to the susceptible accessions there was a 5- to 6-, 17- to 19-, and 24- to 27-fold increase in the rate of glufosinate needed to achieve comparable mortality of the putative-resistant accessions, respectively (Table 3). The glufosinate dose required to kill 80% of the three putative-resistant accessions was 5.7 to 21.0 times greater than the susceptible accessions (Table 3). As of 2021, glufosinate resistance has not been documented in any broadleaf weed (Heap 2021). The rate of glufosinate needed to kill 50% of the resistant Palmer amaranth accessions (A2019, A2020, B2020) was 0.46 to 2.5 kg ai ha^{-1}. Based on the LD_{50} and LD_{80} values, all three accessions that were suspected of having resistance to glufosinate can be deemed "resistant". All three fields where accession A2019, A2020, and B2020 originated had at least one glufosinate application fail to control Palmer amaranth plants in 2019 or 2020, and some plants in the 2019 field survived as many as five applications of glufosinate.

Dicamba. Results gathered from the dicamba dose-response were considered inconclusive and further research will focus on experiments with differing rate structures to generate sound dose-response curves.

2,4-D. The 2,4-D rate needed to kill 50% of the plants for accessions S1 and S2 was 302 and 211 g ae ha^{-1}, respectively (Table 3). The maximum labeled rate for 2,4-D choline use over-the-top of Enlist^{TM} crops is 1065 g ae ha^{-1}, thus, S1 and S2 were deemed sensitive to the herbicide (Anonymous 2019). The LD_{50} of A2019 when treated with 2,4-D was 1853 g ae ha^{-1}, a rate exceeding that listed on the label. Accession A2019 had a 8.8- to 9.5-fold resistance to 2,4-D when compared to the two susceptible accessions based on LD_{50} predictions.

Previous literature has reported a waterhemp population with 10-fold resistance to 2,4-D and 3-fold resistance to dicamba (Bernards et al. 2012). There have also been reports of a 3-fold level of 2,4-D resistance in waterhemp from Missouri (Shergill et al. 2018). A field application of 2,4-D was not made in the ten years prior to seed collection of accession A2019; however, low-dose exposure due to herbicide drift, pollen flow, or development of a mechanism(s) that confers multiple-herbicide resistance may be responsible for low efficacy of 2,4-D (Vieira et al.

2020). 2,4-D was not applied to the field and therefore, has not been observed to fail in field scenarios, although the lethal dose to kill 80% of A2019 was nearly 2x the labeled rate.

Effectiveness of Labeled Herbicides on Glufosinate-Resistant Palmer amaranth

The same S1 standard accession collected in 2001 and used in the previous dose-response experiments was used to confirm sensitivity of Palmer amaranth to the tested herbicides. Unfortunately, imazethapyr resulted in 0% mortality of the standard in both runs of the experiment (Table 4). This finding is not surprising as Palmer amaranth populations with resistance to acetolactate synthase-inhibiting herbicides, including imazethapyr, were first documented in 1994 in Arkansas (Heap 2021). The standard accession used in the experiment appeared to be effectively controlled by all other herbicides tested, with mortality ranging from 77 to 100%. In contrast, accessions A2019, A2020, and B2020 were not effectively controlled by several herbicides (Table 4).

Accession A2020 displayed at least a 20-percentage point reduction in mortality when compared to the susceptible standard following an application of 2,4-D, glyphosate, glufosinate, and mesotrione (Table 4). Greater than 46% mortality was not observed when A2020 was treated with labeled rates of 2,4-D, glyphosate, glufosinate, imazethapyr, or mesotrione, thus, rendering these herbicides ineffective control options. A2020 is suspected to harbor multiple resistance to 2,4-D, glyphosate, glufosinate, imazethapyr, and mesotrione, but further experiments would be needed to confirm resistance. Pendimethalin and *S*-metolachlor, both preemergence-applied herbicides, resulted in more than 85% mortality of A2020. Postemergence application of atrazine, diuron, and paraquat also resulted in above 85% mortality of A2020, while dicamba and fomesafen resulted in 74 and 82% mortality, respectively (Table 4).

When labeled rates (shown in Table 1) of glyphosate, glufosinate, imazethapyr, and mesotrione were applied to accession B2020, no more than 9% mortality was observed. Additionally, only 62% morality was observed when B2020 was treated with fomesafen, which was a 25-percentage point reduction when compared to the susceptible standard (Table 4). Labeled rates of *S*-metolachlor, pendimethalin, atrazine, dicamba, diuron, and paraquat resulted in greater than 85% mortality of B2020, thus potential options for chemical control of this accession exist.

As mentioned previously, dose response analysis revealed resistance to 2,4-D and glufosinate. Soil-applied pendimethalin and *S*-metolachlor resulted in only 77% and 46% mortality, respectively, of the A2019 accession, which was more than 20-percentage points less effective than the susceptible standard. Mortality of A2019 following a postemergence application of 2,4-D, diuron, fomesafen, glyphosate, glufosinate, mesotrione, and tembotrione was 20-percentage points less than the susceptible standard, and imazethapyr resulted in 0% mortality (Table 4). Additionally, mortality percentages declined by 18- and 14-percentage points when postemergence applications of dicamba and atrazine were made to A2019, respectively. Atrazine and paraquat were the only herbicide options tested that resulted in greater than 85% mortality of A2019 (Table 4). Again, A2019 is suspected to harbor resistance to at least nine sites of action, with these including WSSA groups 2, 3, 4, 7, 9, 10, 14, 15, and 27. To date, there has been no population of Palmer amaranth with resistance to more than 6 sites of action (Shyam et al. 2020). Likewise, there is no documented resistance to a Group 7 herbicide in this weed. The failure of diuron on this accession is not surprising because Group 7 herbicides have been used repeatedly for control of Palmer amaranth in this field in years when cotton was grown.

Practical implications and conclusions

All three accessions of Palmer amaranth for which glufosinate failed to provide control in the field in 2019 or 2020 may harbor multiple herbicide resistance. Resistance to glufosinate was confirmed in A2020 and B2020 with resistance ratios of 16.9 to 27.4. Resistance to 2,4-D (Group 4) and glufosinate (Group 10) were documented in A2019 based on dose response analysis. Further efforts should focus on determining what other herbicide sites of action to which this accession is resistant. The number of useful herbicide options to control Palmer amaranth in cotton and soybean in the southern United Stated is diminishing. With few herbicide options left in soybean and cotton, additional non-chemical control strategies will be needed to combat these Palmer amaranth populations. In the future, any novel herbicide that is brought to market is likely to undergo increased selection due to the lack of alternative in-crop herbicide options for Palmer amaranth control in cotton and soybean (Culpepper et al. 2006; Perez-Jones et al. 2005; Powles et al. 1997; Simarmata et al. 2005). Furthermore, the selection for resistance to an auxin herbicide without any recently known use of such herbicide is a concern for the long-term sustainability of effective herbicide-based weed control programs.

Multiple resistance to glufosinate and 2,4-D in Palmer amaranth further limits control options for corn, cotton, and soybean growers. Rotation to a crop like rice (*Oryza sativa* L.) where the field can be flooded as a non-chemical means of control was utilized in 2020 for control of this A2019 accession. Other strategies such as drill-seeded or narrow-row crops, cover crops, deep tillage, and harvest weed seed control techniques are additional options that may aid long-term management of this weed (Norsworthy et al. 2012).

In the future, accessions A2019, A2020, and B2020 will undergo additional testing to confirm resistance to other sites of action and elucidate the mechanisms responsible for herbicide failure. Additional research should also assess if any fitness penalty is associated with the resistant mechanisms, especially considering that A2019 did not appear to exhibit as vigorous growth as the others accession tested. Field research should also aim at identifying the most effective herbicide combinations and programs that effectively control these accessions. Mixtures of herbicides may also increase control and should be evaluated on these populations as potential chemical options.

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Tables

Table 1. Timing of applications, WSSA group number (s), herbicides, herbicide family, product names, and use rates of the treatments applied to accessions SUS, A2019, A2020, and B2020.

Timing of application	WSSA group number	Herbicide	Herbicide family	Product	Use rate
					g ai ha^{-1} or g ae ha^{-1}
PRE					
	3	pendimethalin	Dinitroaniline	Prowl H2O® 3.8 L	970
	15	*S*-metolachlor	Chloroacetamide	Dual II Magnum® 7.34 EC	1067
POST					
	2	imazethapyr[a]	Imidazolinone	Pursuit® 2 L	72
	4	2,4-D[a]	Phenoxy	Enlist One® 3.8 L	1064
	4	dicamba[a]	Benzoic acid	XtendiMax® plus VaporGrip® 2.9 L	560
	5	atrazine[c]	Triazine	Aatrex 4 L	1120
	7	diuron[a]	Ureas	Direx 4 L	894
	9	glyphosate	Glycine	Roundup Powermax II® 4.5 L	866
	10	glufosinate	Phosphinic acid	Liberty® 2.34 L	595
	14	fomesafen[a]	Diphenyl ethers	Reflex® 2 SL	395
	22	paraquat[a]	Bipyridylium	Gramoxone® 3 SL	709
	27	mesotrione[b]	Triketone	Callisto® 4 SC	105
	27	tembotrione[c]	Triketone	Laudis® 3.5 L	92

[a] nonionic surfactant (NIS) at 0.25% (v/v) will be included.
[b] crop coil concentrate (COC) at 1% (v/v) will be included.
[c] methylated seed oil at 1% (v/v) will be included.

Table 2. Weibull growth curve (y= a * (1 - Exp(- (rate/b)^c)), a = asymptote, b = inflection point, c = growth rate) fit to data by herbicide and Palmer amaranth accession; S1 and S2 are susceptible standards and A2019, A2020, and B2020 are putative-resistant accessions. R^2 values display the percentage of variability explained by the fit of the line.

Herbicide	Accession	Asymptote	Inflection point	Growth rate	R^2
Glufosinate	S1	100.00	0.08	2.50	0.99
	S2	98.53	0.08	1.56	0.98
	A2019	91.99	0.41	2.09	0.97
	A2020	99.22	1.50	1.53	0.98
	B2020	92.23	1.74	4.74	0.99
2,4-D[a]	S1	100.01	0.23	2.33	0.99
	S2	100.00	0.21	2.48	0.98
	A2019	89.44	1.79	7.55	0.98

[a] A2020 and B20202 were not evaluated in response to 2,4-D because of limited seed availability.

Table 3. LD$_{50}$ predictions from glufosinate and 2,4-D dose-response experiments conducted on accessions S1, S2, A2019, A2020, and B2020.

			Confidence interval (95%)				
Herbicide		Accession	Predicted rate	Lower	Upper	Level of resistance to S1	Level of resistance to S2
			g ai ha^{-1} or g ae ha^{-1}[a]			resistance ratio[b]	
2,4-D	LD$_{50}$	S1	230	221	237		
		S2	221	210	224		
		A2019	1853	1583	2123	8.8*[c]	9.5*
	LD$_{80}$	S1	302	282	322		
		S2	275	257	293		
		A2019	2188	1845	2391	7.0*	7.7*
Glufosinate	LD$_{50}$	S1	42	36	48		
		S2	36	30	42		
		A2019	214	184	244	5.1*	5.9*
		A2020	708	583	833	16.9*	19.7*
		B2020	988	898	1071	23.5*	27.4*
	LD$_{80}$	S1	60	54	65		
		S2	65	60	71		
		A2019	339	309	369	5.7*	5.4*
		A2020	1232	1107	1357	21.0*	19.6*
		B2020	1202	1119	1291	20.5*	19.1*

[a] Resistance ratio determined by dividing the predicted value of the putative resistant (R) accession by the predicted value of the susceptible (S) accession.

[b] Predicted 2,4-D rates are shown in g ae ha^{-1}, and glufosinate in g ai ha^{-1}

[c] Significant R/S ratios based on 95% confidence intervals are indicated by an "*".

Table 4. Percent mortality of Palmer amaranth accessions A2019, A2020, and B2020 following applications of various preemergence (PRE) and postemergence (POST) herbicides.

				Palmer amaranth mortality 28 DAA		
	WSSA group number	Herbicide	Herbicide family	A2019	A2020	B2020
				% (percentage point difference from susceptible)		
PRE	3	Pendimethalin	Dinitroaniline	77 (20)*	86 (11)	87(10)
	15	*S*-metolachlor	Chloroacetamide	48 (52)*	88 (12)	98 (2)
POST	2	imazethapyr[a]	Imidazolinone	0 (0)	4 (-4)	0 (0)
	4	2,4-D[a]	Phenoxy	47 (39)*	43 (43)*	77 (9)
	4	dicamba[a]	Benzoic acid	72 (18)	74 (16)	87 (3)
	5	atrazine[c]	Triazine	86 (14)	100 (0)	97 (3)
	7	diuron[a]	Ureas	58 (42)*	100 (0)	100 (0)
	9	glyphosate	Glycine	0 (84)*	4 (80)*	2 (82)*
	10	glufosinate	Phosphinic acid	80 (20)*	46 (54)*	6 (94)*
	14	fomesafen[a]	Diphenyl ethers	4 (83)*	82 (5)	62 (25)*
	22	paraquat[a]	Bipyridylium	100 (0)	100 (0)	100 (0)
	27	mesotrione[b]	Triketone	2 (76)*	9 (69)*	45 (33)*
	27	tembotrione[c]	Triketone	7 (70)*	73 (4)	73 (4)

[a] nonionic surfactant (NIS) at 0.25% (v/v) will be included.

[b] crop coil concentrate (COC) at 1% (v/v) will be included.

[c] methylated seed oil at 1% (v/v) will be included.

Chapter 8

General Conclusions

Mitigating the evolution or spread of dicamba- and glufosinate-resistant Palmer amaranth will require optimization of herbicide applications, recognition of interactions that are detrimental to weed control, and use of integrated weed management strategies that limit weed seed production or weed emergence. Overall, timely herbicide applications improved weed control and expanded the potential sequences and intervals of sequential applications of dicamba and glufosinate that resulted in greater than 90% Palmer amaranth control. However, effective control options for Palmer amaranth over 10 cm in size at the time of application were limited to dicamba fb dicamba at the 14- to 21-day interval, dicamba plus glyphosate fb dicamba plus glyphosate at the 14- to 21-day interval, or dicamba fb glufosinate at the 14-day interval. Economic analysis was conducted and determined that either dicamba fb dicamba or dicamba fb glufosinate provided the highest relative net return.

When glufosinate was applied prior to dicamba, less efficacious Palmer amaranth control was observed compared to alternative treatments. Radiolabeled herbicides revealed that when glufosinate (a contact herbicide) was applied prior to dicamba a reduction in dicamba translocation occurred. This reduction in dicamba translocation was likely the cause for the reduction in weed control. When dicamba was applied prior to glufosinate, a rapid reduction in Palmer amaranth groundcover resulted. The reduction in Palmer amaranth groundcover may limit the spray interception of latter glufosinate applications and thus resulting in less herbicide uptake. When glufosinate was applied 14-days after a dicamba application, the best control utilizing the two herbicides was observed. The 14-day interval between the dicamba fb glufosinate treatment allowed for enough time for Palmer amaranth to begin to regrow and regain groundcover.

Palmer amaranth accessions from Arkansas were discovered to have 3- to 27-fold resistance to glufosinate. Additionally, one accession harbored resistance to auxin herbicides and glufosinate. Further experiments will be needed to determine if sequential applications of dicamba and glufosinate will control these troublesome accessions. To mitigate the risk or spread of dicamba- and glufosinate-resistant Palmer amaranth, herbicide programs that incorporate residual herbicides, multiple effective sites of action within a growing season, and additional control strategies other than chemical options are needed.