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Mitigating the Risk for Glufosinate Resistance

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Mitigating the Risk for Glufosinate Resistance

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by

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ABSTRACT

While glufosinate-resistant weeds have not yet been identified in U.S. row crops, rapid evolution of glyphosate-resistance, and resulting cost to U.S. farmers, demonstrates the need to responsibly steward the limited number of herbicides available in agricultural systems. Field and laboratory experiments were conducted to: 1) Identify herbicide interactions that can occur in Enlist™ and Roundup Ready® Xtend® systems; 2) Identify herbicide interactions that occur when glufosinate is mixed with fomesafen, clethodim, and glyphosate; 3) Determine the optimum rate structure and application timings when multiple POST applications of glufosinate are applied to LibertyLink soybean; 4) Use ^{14}C techniques to determine why herbicide interactions (e.g., antagonism) occur in barnyardgrass and Palmer amaranth; and 5) Use data obtained from field experiments to refine herbicide-resistance simulation models for Palmer amaranth and barnyardgrass. Glufosinate + glyphosate, glufosinate + clethodim, glyphosate + 2,4-D, and glyphosate + dicamba were all antagonistic when applied to barnyardgrass. Few antagonistic interactions were observed for Palmer amaranth control. Results from various experiments show that nozzle selection (i.e., droplet size and spray volume) is important for maximizing efficacy of glufosinate plus 2,4-D, clethodim, dicamba, fomesafen, or glyphosate. When large weeds (≥10-cm) were present in the field, two applications of glufosinate at 882 g ai ha^{-1} made 7-10 days apart maximized weed control and soybean yield. When glufosinate was mixed with ^{14}C-glyphosate, reduced uptake and transport were observed in barnyardgrass and Palmer amaranth. Dicamba also reduced uptake of ^{14}C-glyphosate in barnyardgrass, and potentially explains antagonism observed in field experiments**.** Glyphosate-resistance simulation models for barnyardgrass demonstrated antagonism of glyphosate by synthetic auxin herbicides increased the risk of evolving resistance 17-fold over a 30 yr period. Although glufosinate +

glyphosate was also antagonistic in the field, the use of the mixture resulted in minimal risk of resistance in barnyardgrass. The Palmer amaranth resistance model suggests that intense management focused on depleting the soil seedbank is needed to mitigate the risk of evolving glufosinate-resistance, as all herbicide management programs evaluated in the model resulted in some level glufosinate-resistance after 30 yr.

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Chapter 1

Review of Literature

Glufosinate is one of the few broad-spectrum postemergence (POST) herbicides that has no confirmed cases of resistance in agricultural systems (Heap 2018). The LibertyLink® technology allows glufosinate to be applied POST in canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* [L.] Merr.). Acreage planted to glufosinate-resistant crops increased in response to widespread occurrence of glyphosate-resistance (Culpepper et al. 2010; Reddy and Norsworthy 2010). Glufosinate is effective at controlling glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.); however, as the use of glufosinate increases, so does the potential for improper management and likelihood of evolving resistance. Thus, identifying management strategies that maximize the effectiveness and utility of glufosinate in LibertyLink and other crop technologies is of great importance.

Glufosinate is a nonselective, broad-spectrum contact herbicide originally used in agriculture for burndown applications. Glufosinate is a competitive inhibitor of the enzyme glutamine synthetase and, when bound, prevents glutamine synthetase from producing the essential amino acid glutamine. As glutamine synthetase activity diminishes, the amount of available glutamine sharply declines, resulting in the accumulation of ammonia, a substrate of glutamine synthetase. The final result of glufosinate application is rapid death of affected plant tissue (Lea et al. 1984).

Glufosinate-Resistant Soybean

Introduction of the phosphinothricin-N-acetyl-transferase (*pat*) gene into the soybean genome resulted in the first glufosinate-resistant soybean cultivar. The *pat* gene encodes for a protein that rapidly acetylates glufosinate molecules, thereby detoxifying the herbicide. In plants, *pat* prevents glufosinate from inhibiting glutamine synthetase by rapidly inactivating the herbicide before it can bind to glutamine synthase (Dröge et al. 1992).

Glufosinate-resistant soybean varieties were commercially released in 1999. However, approval by the European Union for the importation of soybean containing the LibertyLink trait from the US was not granted until 2008. Direct comparisons of glufosinate and glyphosate determined glyphosate is generally more efficacious on common agricultural weeds in soybean (Culpepper et al. 2000). The broad adoption of Roundup Ready soybean prior to 2008 (USDA-ARS 2016), and the findings by Culpepper et al. (2000), may be two reasons glufosinate-resistant soybean is not widely adopted across the US. Glufosinate tends to be a better broadleaf herbicide than grass herbicide, sometimes requiring multiple applications to achieve acceptable control of grass weeds (Wiesbrook et al. 2001).

The acreage planted to glufosinate-resistant varieties has increased in recent years. The Enlist™ (2,4-D, glufosinate, and glyphosate-resistance) and Bollgard® II Xtendflex® (dicamba, glufosinate, and glyphosate-resistance) traits consist of varieties with stacks of herbicide-resistant traits that include glufosinate-resistance. As technologies containing glufosinate-resistance traits become more common, it will be necessary to develop and implement effective strategies to mitigate the likelihood of evolving glufosinate-resistance.

Troublesome Weeds

Palmer Amaranth. Glyphosate-resistant (GR) Palmer amaranth populations have been identified in 30 states in the U.S. (Heap 2018; Norsworthy, personal communication). Palmer amaranth populations with multiple resistance to acetolactate synthase (ALS)-inhibiting herbicides and glyphosate are widespread in the Midsouth (Bagavathiannan and Norsworthy 2013). Despite the emergence and implementation of management strategies for GR Palmer amaranth in the U.S, evolution of herbicide resistance across the U.S. shows no sign of slowing (Heap 2018).

PPO-inhibitor resistant Palmer amaranth was identified across Arkansas in 2017 (Salas et al. 2017; Varanasi et al. 2018) and PPO-inhibitor resistant *Amaranthus* spp. are increasingly common across the U.S. (Heap 2018). The evolution of PPO-inhibitor resistance in Palmer amaranth follows PPO-inhibiting herbicides becoming a common recommendation in glyphosate-resistant soybean (Owen and Zelaya 2005) in response to glyphosate-resistant weeds. Research into the confirmation and control of many glyphosate-resistant Palmer amaranth populations determined that many PPO-inhibiting herbicides, such as fomesafen, still provided excellent control (Chahal et al. 2017; Nandula et al. 2012). The evolution of PPO-inhibitor resistance once again illustrates the risk of mismanaging herbicides and the need for further research on how to maximize the utility of the decreasing pool of weed management tools.

Palmer amaranth has been documented as the most competitive of the *Amaranthus* spp. in regards to amount of plant volume, dry weight, and leaf area produced per plant (Horak and Loughin 2000; Sellers et al. 2003). It is also considered one of the most troublesome weeds across the Midsouth (Webster 2012, 2013), and GR Palmer amaranth has spread to most states in the Midwest. Within four weeks of emergence with the crop, Palmer amaranth can outgrow soybean by 20 cm and, at densities of 10 plants m^{-2}, can cause yield losses exceeding 60% (Bensch et al. 2003; Klingaman and Oliver 1994). Palmer amaranth emergence can exceed 1,000 plants m^{-2} $year^{-1}$ from a natural seedbank, demonstrating the importance of effective control to prevent rapid population growth (Jha and Norsworthy 2009).

Palmer amaranth possesses numerous characteristics favoring its survival in current cropping systems including: high seed production, rapid growth rate, erect growth habit, extended emergence pattern, rapid seed production (able to reproduce a few weeks after emergence), acclimation to shading, and drought tolerance (Bagavathiannan et al. 2015; Horak and Loughin 2000; Jha and Norsworthy 2009; Jha et al. 2009; Keeley et al. 1987; Norsworthy et al. 2008; Sellers et al. 2003). The extensive reproductive advantages Palmer amaranth utilizes increases the likelihood of its persistence and evolution of resistance to herbicides in modern production systems.

Neve et al. (2011) examined the likelihood of evolving GR Palmer amaranth using a population-based simulation model. Under a worst-case management scenario (five annual applications of glyphosate) among 10,000 populations (i.e., individual model runs), evolution of resistance was predicted in 39% of the populations after only five years. In other terms, setting the likelihood of glyphosate-resistance evolution at 5×10^{-10} (five per one-billion individuals) (Neve et al. 2011), only 4,000 plants producing 250,000 seeds plant^{-1} are required to result in five of those seeds possessing resistance to glyphosate (or even another herbicide from another SOA with a similar mutation rate). Considering over 33 million hectares were planted to soybean in the U.S. in 2015 (USDA-NASS 2016), prolific seed producers such as *Amaranthus* spp. are a serious threat for evolving resistance to any herbicide that is frequently used in production fields over a large geographical area. Therefore, rigorous weed management programs consisting of mechanical, cultural, and chemical control practices are still needed to manage herbicide-resistant *Amaranthus* spp. and prevent evolution of herbicide resistance to other SOA. Managing herbicide resistance culminates in higher costs. For instance, Legleiter et al. (2009) estimated the increased cost of controlling GR *Amaranthus* spp. in soybean to be \$48 ha^{-1}.

Barnyardgrass. *Echinochloa crus-galli* is an adaptive species that has thrived in Arkansas agricultural systems. It is a common weed in soybean and cotton and a persistent threat to rice production in Arkansas (Webster 2012; 2013). Glyphosate effectively controls barnyardgrass in glyphosate-resistant soybean; however, suspected glyphosate-resistant barnyardgrass has been identified in Tennessee (Steckel et al. 2017). Additionally, barnyardgrass has evolved resistance to seven other SOA used in the Southern United States, many of them utilized in rice production (Heap 2018). As an extremely prevalent, troublesome, and resistant-prone species, barnyardgrass must be managed appropriately in glufosinate-resistant technologies or evolution of glufosinate-resistance will happen all too soon.

Barnyardgrass shares many weedy characteristics with Palmer amaranth including extensive seed production, rapid C_4 growth habit, and extended emergence periods, but is not as competitive with crops as are *Amaranthus* species (Cowan et al. 1998). Emergence can take place from mid-April to late September and is highly dependent upon location (Bagavathiannan et al. 2011b). Bagavathiannan et al. (2011a) estimated that barnyardgrass produces up to 31,500 seed plant^{-1} when emerging with the soybean crop in the row-middle. Yield reductions in soybean were estimated by Vail and Oliver (1993) to be 0.25% per plant per m of row.

Johnsongrass. Johnsongrass was a major threat to crop production in the U.S. before the commercialization of glyphosate-resistant crops. By the end of the 19th century, johnsongrass had spread across most of the U.S. both as an agricultural weed and a means to prevent erosion, rapidly attracting the attention of lawmakers by 1890 (McWhorter 1971). Johnsongrass is capable of reproducing through seeds and rhizomes, giving it a selective advantage to survive under many weed management schemes. Rhizomes frequently exceed 1 m in length from the main plant and a single plant can produce 40 to 90 m of rhizomes per year (McWhorter and Jordan 1976).

Historically, preventing johnsongrass rhizomes from reproduction required intense tillage (i.e., multiple spring diskings) to cut and desiccate the underground stems. Tillage operations were then followed by applications of soil-applied herbicides to obtain acceptable control (Burt and Willard 1959; Johnson et al. 1997; McWhorter and Hartwig 1965). Introduction of glyphosate in the mid-1970s provided more effective control of johnsongrass than tillage, and glyphosate provided even better control when it was applied POST in GR crops (Johnson et al. 2003). Unfortunately, glyphosate-resistant johnsongrass was identified in 2007 in Arkansas, 2008 in Mississippi, and 2010 in Louisiana (Heap 2018; Riar et al. 2011), and johnsongrass could once again become a challenging weed to control in agriculture.

Contact herbicides (e.g., glufosinate) are not as effective on weeds with rhizomes compared to systemic herbicides (e.g., glyphosate) because the herbicide is not translocated to the rootstocks, allowing for regrowth. Johnson et al. (2003) concluded a single application of glufosinate alone is not sufficient to control johnsongrass. In this study, glufosinate application reduced johnsongrass biomass only by 56% 3 weeks after treatment (WAT) and allowed significant regrowth by 6 WAT. Sequential applications of glufosinate and mixtures with clethodim improved control over a single application of glufosinate alone (Johnson et al. 2014a; Johnson and Norsworthy 2014). To manage severe infestations or escapes, a two-pass POST program consisting of multiple effective sites of action (e.g., glufosinate plus clethodim followed by glufosinate plus clethodim) was effective at controlling small (15 cm) johnsongrass (Meyer et al. 2015b).

Fortunately, the spread of herbicide-resistant johnsongrass has not been as rapid as other glyphosate-resistant weeds such as Palmer amaranth. Even so, multiple resistant johnsongrass biotypes have been identified in Virginia with resistance to glyphosate and nicosulfuron (Smith et al. 2014) and in Arkansas with resistance to fusilade, glyphosate, and nicosulfuron (Bagavathiannan and Norsworthy 2014). Thus, effective control strategies and management tactics need to be implemented to effectively manage herbicide-resistant johnsongrass.

Large Crabgrass. Prior to the introduction of glyphosate, large crabgrass was a considerable pest in row crops, such as soybean. Glyphosate is highly effective on large crabgrass and is easily controlled with glyphosate in glyphosate-resistant soybean (Culpepper et al. 2001). Large crabgrass has remained a troublesome weed in specialty crops such as snap bean (Aguyoh and Masiunas 2003) and watermelon (Monks and Schultheis 1998). Large crabgrass becomes considerably more difficult to control after it begins to form adventitious roots at the stem internodes (Monks and Schultheis 1998). Large crabgrass populations are reported to have resistance to various ALS-, ACCase, and PSII- inhibiting herbicides (Heap 2018).

Broadleaf Signalgrass. Despite having no documented cases of herbicide resistance, broadleaf signalgrass remains one of the most common and troublesome weeds in Midsouth production systems (Webster 2012). Culpepper et al. (2000) demonstrated that control of broadleaf signalgrass with POST applications of glufosinate was lower than glyphosate; however, when a PRE was used in combination with glufosinate POST, control was equal to that of a PRE fb glyphosate. Glyphosate alone provides ≥ 90% control of broadleaf signalgrass (Culpepper et al. 2000; Scott et al. 2015). However, broadleaf signalgrass has persisted as a common agricultural weed even under intense glyphosate use. The presence of broadleaf signalgrass across a majority of the farm acres in the Midsouth implies that populations are exposed to a wide range of herbicides and could be at risk of evolving herbicide-resistance.

Glufosinate in Mixture

The processes by which a single POST herbicide enters a plant and causes plant death are intricate and dependent on various physical, chemical, and plant-related factors. These processes can quickly become convoluted when herbicides are applied in mixture. The upcoming release of crops with stacked herbicide resistance traits will allow even more products to be applied in mixture during the growing season. More information regarding the behavior of glufosinate in these mixtures is needed.

Some interactions of glufosinate with other postemergence herbicides have been reported, such as glufosinate plus 2,4-D (Craigmyle et al. 2013; Merchant et al. 2013), dicamba (Merchant et al. 2013), glyphosate (Bethke et al. 2013), clethodim (Gardner et al. 2006), and fomesafen (Culpepper et al. 2000). The aforementioned studies evaluated these combinations on various monocot and dicot species. The outcome of the herbicide interactions appears to be dependent upon the evaluated species and are variable, specifically when it involves grass species.

One of the current recommendations for LibertyLink soybean in the Midsouth includes an early POST application of glufosinate plus a fomesafen-containing product such as Flexstar or Prefix herbicide (Scott et al. 2015). Glufosinate + fomesafen is extremely effective against Palmer amaranth and other broadleaf weeds, but may not achieve the same levels of control on grass species (Culpepper et al. 2000; Scott et al. 2015). Culpepper et al. (2000) showed the addition of fomesafen to glufosinate either increased, or did not change, control of many grass and broadleaf weeds (e.g., broadleaf signalgrass and common lambsquarters). In situations where the first application of glufosinate or glufosinate + fomesafen did not adequately control grass weeds, a follow-up application of glufosinate and a graminicide, such as clethodim, may be needed to achieve acceptable control (Scott et al. 2015). Even so, the herbicide interactions that may occur between glufosinate + fomesafen, and glufosinate + clethodim, has not been thoroughly investigated on grass weeds.

Many products are available in soybean that provide adequate control of barnyardgrass (Scott et al. 2015); however, these products must be managed to minimize the risk of evolving resistance. Herbicide recommendations resulting in antagonism between two herbicides are not an effective resistance management strategy (Norsworthy et al. 2012). As the interactions among glufosinate, fomesafen, and clethodim are not well documented on barnyardgrass and other resistant prone grasses such as goosegrass, a more-thorough investigation is needed to determine if antagonism is occurring with these applications.

Herbicide Mixtures in Enlist and Xtend Technologies

With the commercialization of dicamba and 2,4-D resistant crops, two and three-way combinations of glufosinate, glyphosate, and an auxinic herbicide will likely become a standard herbicide application. Glufosinate plus 2,4-D is very effective at controlling glyphosate-resistant Palmer amaranth (Merchant et al. 2014a) even when weeds are large (Merchant et al. 2014b). Glufosinate plus dicamba has also been determined to be a very effective POST application to control Palmer amaranth (Merchant et al. 2013).

Numerous techniques are available to assess the performance of herbicide mixtures, some of which require specific experimental designs or more complicated analyses (Hatzio and Penner 1985). Colby’s Method (Colby 1967) is one of the simpler analyses used to assess for herbicide interactions and is easily adaptable to many types of experiments. Thus, it persists as a widely used technique throughout the literature (Besançon et al. 2018; Flint et al. 1988; Kohrt and Sprague 2017). If a herbicide combination does not have POST activity on a given species, (e.g., dicamba on barnyardgrass), Colby's method cannot be used. Instead, a significant decrease in herbicidal activity from the mixture (e.g., glyphosate plus dicamba), compared to the herbicide with activity alone (e.g., glyphosate) is considered antagonism (Flint and Barrett 1989; O'Sullivan and O'Donovan 1980).

The behavior of glufosinate, glyphosate, dicamba, and 2,4-D in mixture on other hard-to-control species is not yet fully understood. Additionally, the extent that nozzle selection and spray volume contribute to interactions, and ultimately selection for resistance, is not well understood. Therefore, research involving mixtures of these products is needed to make appropriate herbicide program recommendations. Sound herbicide programs are needed to minimize evolution of resistance, especially when it involves controlling resistant populations of weeds, such as Palmer amaranth and barnyardgrass.

Effect of Droplet Size on Herbicide Interactions

Although the effect of droplet size on herbicide efficacy has been documented, little research has been conducted to evaluate if droplet size could influence herbicide interactions. However, a wide array of information is available on the effect of droplet size on single herbicides and herbicide mixtures. Small droplet size is more important for retention on upright, grass weeds than broadleaf weeds with horizontal structure (McKinlay et al. 1974; Etheridge et al. 2001). The ability of the droplet to spread on the leaf is dependent on the weed species, contributing to differential tolerances to the same herbicide between species (Norsworthy et al. 2001). As droplet spread and contact with the leaf affects herbicide uptake, it is not surprising that the effect of droplet size on herbicide efficacy appears to be dependent upon weed species. Also, the importance of adequate coverage, typically achieved with smaller droplets, has a more consistent effect on the efficacy of contact herbicides such as glufosinate (Etheridge et al. 2001).

The effect of nozzle selection and droplet size on efficacy of herbicide mixtures is well documented, but remains difficult to predict. The behavior of herbicides in mixture can be dependent upon all parameters of the application. The efficacy of contact herbicides, such as glufosinate, is more dependent upon the droplet size and coverage of the application than systemic herbicides (Etheridge et al. 2001; Meyer et al. 2015a). Thus, a component of an antagonistic interaction could be a change in droplet size that does not favor one, or more, herbicides in a mixture.

Herbicide Interactions and Evolution of Resistance

The ultimate goal of identifying and understanding potentially antagonistic herbicide interactions is to determine if any interactions will increase the likelihood of herbicide resistance. Herbicide-resistance simulation models suggest that applying multiple effective SOA can be a highly effective method for preventing evolution of herbicide resistance (Bagavathiannan et al. 2013; Bagavathiannan et al. 2014; Diggle et al. 2003). However, a situation in which mixing two herbicides results in antagonism should be avoided in a herbicide-resistance management program, if possible (Norsworthy et al. 2012). Thus, knowing what antagonistic interactions may occur in the field and whether those interactions may increase the likelihood of resistance is critical in order to make proper herbicide recommendations. Simulation models using STELLA visual programming language (iSee systems, Lebanon, NH) have been developed for Palmer amaranth (Neve et al. 2011) and barnyardgrass (Bagavathiannan et al. 2014) that are able to determine the effect of factors that decrease herbicide efficacy have on the evolution of herbicide resistance.

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Chapter 2

Timing and application rate for sequential applications of glufosinate are critical for maximizing control of annual weeds in LibertyLink® soybean

Preserving the utility of glufosinate in both LibertyLink soybean and other glufosinate-resistant crops is critical for managing herbicide-resistant weeds. An experiment with a two-factor factorial arrangement was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR, in 2015, 2016, and 2017 to evaluate the efficacy of glufosinate in single and sequential applications at various rates on 8 to 32 cm tall Palmer amaranth, barnyardgrass, and broadleaf signalgrass. Herbicide treatments consisted of glufosinate applied at 454, 595, 738, 882 g ai ha^{-1} (Factor 1) with either no sequential application, or a sequential application occurring 7, 10, 14, or 21 days after the initial application (DAI) (Factor 2). For treatments that contained a sequential application, the same rate used in the initial application (e.g., 451 g ai ha^{-1}) was also used in the sequential. The initial herbicide application occurred when weeds reached 20 to 30-cm in height. Regardless of species and rate, sequential applications were always superior to single applications. Palmer amaranth control 3 weeks after the final treatment (WAF) was 8% greater when the sequential application occurred 10 DAI compared to 21 DAI, averaged over glufosinate rates. When at least 595 g ai ha^{-1} glufosinate was used in a treatment, no differences between the 7-, 10-, 14- and 21-day sequential application intervals were observed for barnyardgrass or broadleaf signalgrass control, 3 WAF. Soybean yields were greater when the glufosinate applications occurred 7 or 10 d apart compared to 21 d, averaged over glufosinate rates. When large weeds are present in the field, these data suggest glufosinate should be applied sequentially with a 7- to 14-day interval between applications. If sequential applications of glufosinate are used in combination with a comprehensive weed control management program (using residual herbicides PRE and POST, tillage, etc.) the likelihood of yield reduction from weed competition and the evolution of glufosinate-resistant weeds should be greatly reduced, and the LibertyLink technology should remain a valuable weed management tool.

Nomenclature: glufosinate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; broadleaf signalgrass, *Urochloa platyphylla* (Nash); Palmer amaranth, *Amaranthus palmeri* S. Wats.

Key words: Glufosinate, application timing, sequential applications

Glufosinate-resistant cultivars began to increase in popularity as a tactic to help manage glyphosate-resistant weeds such as Palmer amaranth (Culpepper et al. 2010). Glufosinate is effective at controlling glyphosate-resistant Palmer amaranth; however, as the use of glufosinate increases, so does the potential for improper management and likelihood of evolving resistance. Glufosinate has no confirmed cases of resistance in row crops (Heap 2018). However, confirmation of glufosinate-resistant grass species in orchards highlights the importance of proper management (Heap 2018). Thus, identifying management strategies that maximize the effectiveness and utility of glufosinate in LibertyLink systems is of utmost importance.

One of the best management practices (BMPs) to mitigate against herbicide resistance is to use the full herbicide labeled rate (Norsworthy et al. 2012). In soybean, the single application use rate for glufosinate ranges from 450 to 882 g ai ha^{-1} with a yearly maximum of 1,734 g ai ha^{-1} (Anonymous 2016). The glufosinate label allows for multiple applications POST and sequential applications are needed for acceptable control of many broadleaf and grass species (Coetzer et al. 2002; Culpepper et al. 2000; Wiesbrook et al. 2001). Sequential applications are needed for acceptable control of Palmer amaranth (Coetzer et al. 2002) and glufosinate applications appear to be sensitive to weed heights, with control decreasing when heights increase from 10 to 15 cm (Steckel et al. 1997).

Although herbicide resistance management principles emphasize the importance of using a preemergence (PRE) herbicide at planting (Norsworthy et al. 2012), a variety of circumstances may result in a grower either not being able to apply a PRE herbicide or the PRE herbicide being ineffective. In a LibertyLink system, not applying a PRE herbicide would place a great pressure on glufosinate to control a broad spectrum and high density of weeds POST, a scenario highly undesirable for herbicide-resistance management.

Dry conditions can reduce the efficacy of PRE herbicides, resulting in poor control and weed escapes, which compete with the emerging crop and must be controlled POST. The availability of herbicides in the soil solution is critical for root uptake, particularly when the compound is lipophilic (Cobb and Reade et al. 2010). A moderate (1.2 to 2.5 cm) amount of rainfall within 2 weeks of application can be critical for maximizing efficacy of certain soil-applied herbicides, such as atrazine, too little, or too much, rainfall can reduce effectiveness (Splittstoesser and Derscheid 1962). Even so, the impact of soil moisture on herbicide efficacy also depends on the individual herbicide, with some (e.g., acetochlor) providing acceptable control under dry conditions and others (e.g., *S*-metolachlor) being more sensitive to rainfall for herbicidal activity (Jursík et al. 2015).

Poor weather and field conditions can create challenges to successfully complete timely field operations such as planting and spraying herbicides. For example, from May 1 through May 14, 2017, only 3.6 d were suitable for fieldwork in Arkansas during a period in which 23% of the soybean acreage would have been planted in a normal year (USDA-NASS 2018). Other factors, such as soil properties, can compound poor weather conditions. A large percentage of row crop acreage in Arkansas consists of somewhat poorly to poorly drained soils (DeLong et al. 2017; NRCS 2018; USDA-NASS 2018). For growers who plant soybean on poorly drained soils in the Mississippi Delta region, it is possible that weather and field conditions could make it impossible for PRE applications before crop emergence. With a limited number of POST herbicides available to control widespread glyphosate-resistant Palmer amaranth, proper management of glufosinate is critical in undesirable situations where no PRE herbicide is applied.

Palmer amaranth is capable of emerging at extremely high densities, exceeding 1,000 plants m^{-2} $year^{-1}$ in some situations (Jha and Norsworthy 2009). Within four weeks of emergence with the crop, Palmer amaranth can outgrow soybean by 20 cm and cause yield losses exceeding 60% at densities of only 10 plants m^{-2} (Bensch et al. 2003; Klingaman and Oliver 1994). Nine days after emergence, Palmer amaranth can reach and exceed a height of 10-cm (Horak and Laughlin 2000), the maximum height allowed on the glufosinate label for this particular weed (Anonymous 2016). The aforementioned findings demonstrate how rapidly Palmer amaranth can become unmanageable in agricultural systems.

The concept of the “critical period of weed control” (CPWC) is fundamental for understanding the risk of yield loss to early weed interference. The CPWC for a given cropping system is defined as the interval a crop must remain weed free to prevent unacceptable yield loss (Zimdahl 1980). Even within a given crop, the CPWC will vary with production practices (e.g., row spacing), environment, weeds present, and other variables (Cowan et al. 1998; Halford et al. 2001). More recently, crop competition studies have determined that early-season weed interference can cause irreversible physiological changes that can be associated with yield loss. For example, Green-Tracewicz et al. (2012) determined that from V1 to V3 (first trifoliate to third trifoliate), soybean is highly sensitive to changes in red:far-red (R:FR) light ratios caused by shading. Exposure to low R:FR ratios from V1 to V3 will cause an increase in plant height, internode length, and shoot:root ratio with decreases in biomass and leaf number.

When no PRE herbicide is applied and conditions are not suitable for fieldwork, weeds can emerge and compete with the crop during its critical early stages of development, causing yield loss. However, even in field situations with high densities of weeds rapidly overtaking a crop, it may still be desirable to keep the crop and initiate an aggressive weed management program instead of crop destruction and replanting. Even with alternatives to herbicides, such as inter-row cultivation, larger weeds in the soybean row will need to be controlled with herbicide applications. Alternatively, it is also possible that in situations where most of the weeds are a labeled weed size, a small portion of total population may exceed the label recommendations. The objective of this experiment was to evaluate single and sequential POST applications of glufosinate on large weeds and determine the optimum application window for sequential applications.

Materials and Methods

An experiment was conducted at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, AR to evaluate single and sequential glufosinate applications to determine optimum rate structure and interval between applications. Plots 3.7 by 9.1 m were established on a leaf silt loam in 2015 and 2017 (Fine, mixed, active, thermic Typic Albaquults) with 1.5% organic matter, pH of 5.6, 26% sand, 66% silt, and 8% clay, and a Captina silt loam in 2016 (fine-silty, siliceous, active, mesic Typic Fragiudults) with 2.0% organic matter, pH of 5.2, 18% sand, 63% silt, and 19% clay. A commercially available LibertyLink variety was planted at the time of trial establishment: Credenz 4748 LL in 2015 (322,800 seeds ha^{-1}) Pioneer P53T62LL in 2016 (321,100 seeds ha^{-1}), and Pioneer P48T67LL in 2017 (322,800 seeds ha^{-1}). Fertilizer and lime were applied based on a soil test and according to University of Arkansas recommendations. Plots were irrigated with an overhead lateral irrigation system (2015 and 2017) or furrow irrigated (2016) as needed.

The experimental design was a randomized complete block with a factorial treatment structure; Factor 1 was glufosinate rate (451, 595, 738, 882 g ai ha^{-1}) and Factor 2 was sequential application structure. Each experiment contained four replications. The five levels for the sequential application structure were: no sequential application, initial application followed by (fb) a sequential application 7 d after the initial application (DAI), initial fb sequential 10 DAI, initial fb sequential 14 DAI, and initial fb sequential 21 DAI. For treatments that contained a sequential application, the same rate used in the initial application (e.g., 451 g ai ha^{-1}) was also used in the sequential.

The first application for all treatments occurred when weeds achieved 20 to 30-cm in height and a list of weeds and their size at application is listed in Table 1. At the time of the initial application, soybean stages were V4-V5 in 2015 and 2017 and V5-V6 in 2016. *S*-metolachlor at 1,390 g ai ha^{-1} was included with the initial treatment to prevent new weed emergence. A list of planting dates, spray dates, and weather conditions at the time of all herbicide applications is compiled in Table 2. A CO_2-pressurized backpack sprayer was used to make all herbicide applications calibrated to deliver 141 L ha^{-1} spray volume at 276 kPa at 4.8 km hr^{-1} through nozzles spaced 51 cm apart. The boom was equipped with TeeJet (TeeJet Technologies, Springfield, Illinois) Turbo TeeJet (TT) 110015 nozzles.

Weed control ratings were collected 3 weeks after the final application and at harvest for Palmer amaranth, barnyardgrass, and broadleaf signalgrass. Weed control was visually evaluated on a scale of 0 (no control) to 100% (complete death of all plants) relative to the nontreated check. At the end of the season, plots were harvested for yield. Data from all three years were pooled (Blouin et al. 2011) and data were subjected to an analysis of variance (ANOVA) using JMP 13 (SAS Institute Inc., Cary, NC). Replication and year were included in the model as random effects. Means were separated using Fisher's protected least significant difference (LSD) ($\alpha = 0.05$). Variance components estimates for each ANOVA are shown in Table 3.

Results and Discussion

Palmer Amaranth. For Palmer amaranth control 3 weeks after the final application (WAF), the interaction between glufosinate rate and sequential application timing was not significant in the

ANOVA (p=0.08181) and was not interpreted. However, both the main effects of glufosinate rate ($p<0.0001$) and sequential application timing ($p<0.0001$) were significant for percent control 3 WAF and are presented in Table 4. As mentioned previously, all of the initial applications included *S*-metolachlor at 1390 g ha^{-1}; therefore, control ratings reflect emerged plants at the time of application. A glufosinate rate response was detected as the rate increased from 451 to 738 g ai ha^{-1}, control increased from 68% to 79%, averaged across sequential application timing. No difference was observed between the 738 and 882 g ai ha^{-1} rates. The main effect of sequential application timing showed a clear benefit of applying a sequential application. Control was lowest (51%) when no sequential application was applied, compared to 84% when a sequential application occurred 7 d after the initial application (DAI), averaged across glufosinate rates. As the length of time between sequential applications increases beyond 10 d, control tended to decrease. For example, control with the sequential application occurring 10 DAI was 86%, compared to 78% when the sequential application occurred 21 DAI.

At harvest, an interaction between glufosinate rate and sequential application timing was observed for percent control (p=0.00671) (Table 5). However, most of the trends observed for control 3 WAF held true for the assessment at harvest. Considering the 451 g ai ha^{-1} rate of glufosinate at harvest, control when the sequential application occurred 10 DAI was 81%, and control declined to 73% when time between applications increased to 21 d. A single application was always inferior to treatments that contained a sequential, even when comparing different rates. For example, a single application of 882 g ai ha^{-1} provided only 57% control of Palmer amaranth at harvest, whereas two applications of 451 g ai ha^{-1} 21 d apart provided 73% control.

These data clearly demonstrate that if large Palmer amaranth is present at the time of a glufosinate application, two applications of 451 g ai ha^{-1} 7 to 14 d apart would be highly preferable to using a comparable amount of active ingredient in a single application (902 vs. 882 g ai ha^{-1} total glufosinate for the sequential and single applications, respectively). It should be noted that 451 and 882 g ai ha^{-1} are the lowest and highest labeled rates, respectively (Anonymous 2016). These data do not suggest that using partial rates in sequential applications would provide effective control compared to a labeled rate. Norsworthy et al. (2012) recommended using full labeled rates is an integral part of herbicide resistance management. In fact, exposure to low doses is often used to rapidly generate herbicide resistance in greenhouse experiments. Busi and Powles (2009) produced a rigid ryegrass (*Lolium rigidum* Gaud.) population with resistance to glyphosate in three rounds of low-dose recurrent selection, and similar experiments produced a Palmer amaranth population resistant to dicamba (Tehranchian et al. 2017). Thus, applications should be made at rates appropriate to achieve complete control and be only one component of a weed management plan.

Barnyardgrass and Broadleaf Signalgrass. For both barnyardgrass and broadleaf signalgrass, an interaction between glufosinate rate and sequential application timing was observed for percent control 3 WAF (p=0.01401, p=.01074 for barnyardgrass and broadleaf signalgrass, respectively) (Table 6). Barnyardgrass control at 3 WAF ranged from 55 to 92% and 55 to 91% for broadleaf signalgrass. A sharp rate response was observed for the single applications (no sequential) as rates increased from 595 to 738 g ai ha^{-1}, for both species. For barnyardgrass, control 3 WAF with 595 g ai ha^{-1} was 58% and increased to 71% when 738 g ai ha^{-1} was applied with no sequential application. However, few differences were observed between sequential application timings within a given rate, except at the 451 g ai ha^{-1} rate. For example, broadleaf signalgrass control was greater when 451 g ai ha^{-1} was applied 14 d apart (86%) compared to 21 d apart (73%).

The analysis of percent control at harvest was similar for barnyardgrass and broadleaf signalgrass; the two-way interaction was not significant and both the main effects (glufosinate rate and sequential application timing) were significant in the each of models (refer to Table 7 for p-values). For both barnyardgrass and broadleaf signalgrass, control was not different among sequential application timings as long as a second application was made (Table 8). Making a sequential application 7 DAI improved barnyardgrass control by 21% and broadleaf signalgrass control by 19% compared to no sequential application, averaged across glufosinate rates. These results differ from Palmer amaranth control, for which control generally decreased as time between sequential applications increased. Thus, species may play a role in response to the length of time between two applications. The differences between species could also be due to morphological differences between monocots and dicots: the growing point on barnyardgrass and broadleaf signalgrass lies at the soil surface making it more difficult to intercept spray from a contact herbicide (i.e., glufosinate). Thus, grass species may be less responsive to time between sequential applications.

A gradual rate response was observed for both grass species for percent control assessed at harvest, averaged across sequential application timings. For both broadleaf signalgrass and barnyardgrass, control was greatest when at least 738 g ai ha^{-1} was applied. Control of both species was 79% for the 738 g ai ha^{-1} rate, averaged across application timings.

Grain Yield. The response in grain yield to these various treatments generally followed the responses in weed control. The interaction between glufosinate rate and sequential application timings was not significant in the model (Table 7), but both main effects were and data are presented in Table 9. Averaged across sequential application timings, soybean yield was greatest (2,760 kg ha^{-1}) when 882 g ai ha^{-1} was used. This result follows the weed control data, in which control tended to be greatest when at least glufosinate at 738 g ha^{-1} was applied, although control with 882 g ha^{-1} tended to be numerically greater than 738 g ha^{-1} (e.g., Tables 4 and 7). For the main effect of sequential application timing, grain yield was lowest (2,320 kg ha^{-1}) when no sequential application was applied, averaged across glufosinate rates. For comparison, yield from the nontreated check was 1,150 kg ha^{-1} (SE=101 kg ha^{-1}). The response in grain yield for the main effect of sequential application timing followed the response of Palmer amaranth control as opposed to the grass species; yield tended to decline as time between sequential applications increased. For example, soybean yield was 2,700 kg ha^{-1} when the sequential application occurred 7 DAI compared to 2,530 kg ha^{-1} when the second application occurred 21 DAI.

Practical Implications

Sequential applications of any rate provided superior weed control to a single application, implying the need to make two POST applications when large weeds are present at the time of the initial application. These results agree with Aulakh and Jhala (2015), who determined applications of glufosinate 3 to 4 weeks apart generally had greater control of a broad spectrum of weeds than single applications. Control of Palmer amaranth and grain yield declined as time between applications increased, suggesting the follow-up application should occur 7 to 14 DAI. Furthermore, at least 738 g ha^{-1} glufosinate should be used for both applications to maximize weed control. The use of a PRE herbicide and avoiding applications to weeds beyond recommended sizes is needed for herbicide resistance management (Norsworthy et al. 2012). In the same study conducted by Aulakh and Jhala (2015), control with herbicide programs that consisted of a PRE application followed by a POST application were usually superior to two applications of glufosinate POST. These experiments were designed to evaluate a worst-case scenario where all weeds present are large and at high densities to provide recommendations when field situations are similar. As noted previously, both Palmer amaranth and barnyardgrass plants that emerge with the crop can cause severe yield losses and irreversible physiological changes before yield loss is recognized (Cowan et al. 1998; Green-Tracewicz et al. 2012).

Other hard-to-control weed species may also present greater challenges than those evaluated in this experiment. Johnsongrass is a perennial species that has historically been a highly problematic weed in row-crop production (McWhorter and Hartwig 1965; McWhorter 1971). In the absence of glyphosate, Johnson et al. (2003) concluded a single application of glufosinate alone is not sufficient to control johnsongrass and sequential applications of glufosinate plus clethodim were generally needed for acceptable control (Johnson et al. 2014; Meyer et al. 2015a). Weed spectrum and weather conditions may play a role in the recommendations of specific herbicide programs, and everything should be done to maximize the utility of the herbicides (i.e., glufosinate) utilized in those programs to achieve maximum weed control and mitigate the likelihood of resistance. For example, glufosinate is a contact herbicide and the label recommends applications with medium to coarse droplet sizes and higher spray volumes ($\geq$141 L ha^{-1}), both of which improve the performance of glufosinate (Creech et al. 2015, 2016; Etheridge et al. 2001; Knoche 1994; Meyer et al. 2015b, 2016)

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Appendix

Table 1. Weed heights and densities for three species at time of the initial herbicide application in 2015, 2016, and 2017.

	2015		2016		2017	
Species	Height	Density	Height	Density	Height	Density
	cm	plants m^{-2}	cm	plants m^{-2}	cm	plants m^{-2}
Palmer amaranth	10 to 25	19	13 to 32	4	10 to 20	15
Barnyardgrass	20	1.5	20	1 to 7	10 to 18	2
Broadleaf signalgrass	23	10	8 to 20	1	9 to 15	12

Table 2. Dates of herbicide applications in 2015, 2016, and 2017 and weather conditions at the time of application at Fayetteville, AR.

	Year											
	2015				2016				2017			
Application timing	Date	Time	Temp	RH	Date	Time	Temp	RH	Date	Time	Temp	RH
Initial	7/24/15	8:30 AM	28	70	6/13/16	2:00 PM	33	81	6/15/17	4:00 PM	31	77
7 DAI	7/31/15	8:00 AM	28	69	6/20/16	11:00 AM	27	50	6/22/17	10:00 PM	27	70
10 DAI	8/3/15	3:30 PM	32	40	6/23/16	12:00 PM	28	62	6/26/17	10:30 AM	23	67
14 DAI	8/7/15	9:15 AM	27	60	6/27/16	9:00 AM	23	90	6/29/17	10:00 AM	29	55
21 DAI	8/14/15	9:30 AM	24	65	7/4/16	11:00 AM	32	79	7/6/17	8:00 AM	26	75

Abbreviations. DAI, days after initial application; RH, relative humidity; Temp, temperature;

Table 3. Variance components estimates obtained from the ANOVA for Palmer amaranth control, barnyardgrass control, broadleaf signalgrass control, and soybean yield.

Model effect	Variance Components Estimates						
	Palmer amaranth		Barnyardgrass		Broadleaf signalgrass		Soybean yield
	3 WAF	At harvest	3 WAF	At harvest	3 WAF	At harvest	At harvest
	--------------------% of Total--------------------						
Year	49.0	60.9	46.6	71.0	60.9	67.6	65.8
Rep(Year)	1.6	0.4	<0.1	1.2	<0.1	0.8	2.7
Residual	49.4	38.6	53.8	27.8	39.8	31.6	31.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Abbreviation. WAF, 3 weeks after the final application

Table 4. Palmer amaranth control 3 weeks after the final application for the main effects of glufosinate rate and sequential application timing, averaged over the other factor in Fayetteville, AR, in 2015, 2016, and 2017[a].

Main effect	Level	Control[b]
		-----%-----
Glufosinate rate[c]	451	68 c
	595	75 b
	738	79 a
	882	81 a
Sequential application timing	None	51 d
	7 DAI	84 ab
	10 DAI	86 a
	14 DAI	80 bc
	21 DAI	78 c

[a] Abbreviation: DAI, days after initial application.

[b] For a given main effect, means within a column followed by the same letter are not different according to Fisher's protected LSD (α =0.05).

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

Table 5. Effect of glufosinate rate and sequential application timing on Palmer amaranth control at harvest in Fayetteville, AR, in 2015, 2016, and 2017[a].

Glufosinate rate	Sequential application timing	Control[b]	
g ai ha^{-1}		------%------	
451	None	35	l
	7 DAI	80	fgh
	10 DAI	81	efg
	14 DAI	76	ghi
	21 DAI	73	i
595	None	43	k
	7 DAI	83	def
	10 DAI	83	def
	14 DAI	84	cdef
	21 DAI	75	hi
738	None	57	j
	7 DAI	86	bcde
	10 DAI	89	abcd
	14 DAI	84	cdef
	21 DAI	79	fgh
882	None	57	j
	7 DAI	94	a
	10 DAI	91	ab
	14 DAI	89	abc
	21 DAI	87	bcd

[a] Abbreviation: DAI, days after initial application.

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

Table 6. Effect of glufosinate rate and sequential application timing on barnyardgrass and broadleaf signalgrass control 3 weeks after the final application for the interaction between Fayetteville, AR, in 2015, 2016, and 2017[a].

Glufosinate rate	Sequential application timing	Control[b]	
		Barnyardgrass	Broadleaf signalgrass
g ai ha^{-1}		-------------------- % --------------------	
451	None	55 g	55 e
	7 DAI	82 cd	73 d
	10 DAI	86 bc	78 cd
	14 DAI	76 def	86 ab
	21 DAI	77 def	73 d
595	None	58 g	56 e
	7 DAI	87 abc	79 bcd
	10 DAI	87 abc	85 abc
	14 DAI	87 bc	88 a
	21 DAI	82 cde	87 a
738	None	71 f	73 d
	7 DAI	90 ab	88 a
	10 DAI	86 bc	88 a
	14 DAI	89 ab	86 ab
	21 DAI	86 bc	88 a
882	None	75 ef	72 d
	7 DAI	94 a	91 a
	10 DAI	92 ab	86 abc
	14 DAI	91 ab	90 a
	21 DAI	88 abc	85 abc

[a] Abbreviation: DAI, days after initial application.

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

Table 7. Model effects and p-values obtained from the ANOVA for barnyardgrass control, broadleaf signalgrass control, and soybean yield collected at harvest.

	Control		
Model effect	Barnyardgrass	Broadleaf signalgrass	Soybean yield
	------------------------p-value	------------------------	
Glufosinate rate	<0.0001	<0.0001	0.0006
Sequential application timing	<0.0001	<0.0001	<0.0001
Glufosinate rate* Sequential application timing	0.1937	0.2289	0.0726

Table 8. Percent control of barnyardgrass and broadleaf signalgrass at harvest for the main effects of glufosinate rate and sequential application timing, averaged over the other factor in Fayetteville, AR, in 2015, 2016, and 2017[a].

		Control[b]	
Main effect	Level	Barnyardgrass	Broadleaf signalgrass
		---------------- % ------------------	
Glufosinate rate[c]	451	69 c	68 c
	595	75 b	77 b
	738	79 a	79 ab
	882	80 a	81 a
Sequential application timing	None	59 b	59 b
	7 DAI	80 a	78 a
	10 DAI	80 a	81 a
	14 DAI	80 a	81 a
	21 DAI	80 a	82 a

[a] Abbreviation: DAI, days after initial application.

[b] For a given main effect, means within a column followed by the same letter are not different according to Fisher's protected LSD (α =0.05).

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

Table 9. Soybean grain yield for the main effects of glufosinate rate and sequential application timing, averaged over the other factor in Fayetteville, AR, in 2015, 2016, and 2017.

Main effect	Level	Yield[b]	
		kg ha^{-1}	
Glufosinate rate[c]	451	2526	b
	595	2620	b
	738	2526	b
	882	2762	a
Sequential application timing	None	2324	d
	7 DAI	2701	ab
	10 DAI	2849	a
	14 DAI	2661	bc
	21 DAI	2526	c

[a] Abbreviation: DAI, days after initial application.

[b] For a given main effect means within a column followed by the same letter are not different according to Fisher's protected LSD (α =0.05).

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

Chapter 3

What antagonistic interactions mean for Enlist™ and Roundup Ready Xtend® technologies

The commercial release of Roundup Ready® Xtend® and Enlist™ cropping systems increased the number of herbicide products that can be applied postemergence (POST) in soybean and cotton. As POST herbicide combinations of glyphosate, glufosinate, dicamba, and 2,4-D become more common, a greater understanding of how these herbicides are interacting in mixture is needed. Two field experiments were conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR, to evaluate potential herbicide interactions that could occur in Enlist (2,4-D Experiment) and RoundupReady Xtend (Dicamba Experiment) cropping systems. Various rates and combinations of glufosinate, glyphosate, dicamba, and 2,4-D were applied and evaluated for percent weed control. Control of barnyardgrass, Palmer amaranth, and prickly sida, by these herbicide treatments was evaluated 2 and 5 weeks after treatment (WAT) and analyzed for herbicide interactions based on Colby's method. In the 2,4-D experiment, glyphosate (dimethylamine salt) at 840 g ae ha^{-1} provided 88% barnyardgrass control, whereas a pre-mixture of glyphosate at 840 g ae ha^{-1} plus 2,4-D at 785 g ae ha^{-1} provided 80% control 5 WAT. Similarly in the Roundup Xtend experiment, glyphosate (potassium salt) at 865 g ae ha^{-1} provided 86% barnyardgrass control, compared to 79% with glyphosate at 865 g ae ha^{-1} plus dicamba at 560 g ae ha^{-1}. Antagonism was also identified for mixtures of glufosinate plus glyphosate for barnyardgrass in both experiments. For Palmer amaranth and prickly sida control, mixtures were generally equal to or greater than the individual herbicides alone, even though some mixtures were deemed antagonistic. If Roundup Xtend or Enlist cropping systems become

widely adopted, herbicide applicators need to be aware of antagonistic interactions and the implications of antagonism on herbicide resistance management.

Nomenclature: 2,4-D; dicamba; glufosinate; glyphosate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; prickly sida, *Sida spinosa* L.

Key words: Antagonism, barnyardgrass, glufosinate + glyphosate, glyphosate + 2,4-D, glyphosate + dicamba, Palmer amaranth, herbicide interactions

With the commercialization of dicamba- and 2,4-D-resistant crops (i.e., RoundupReady® Xtend® and Enlist™), two- and three-way combinations of glufosinate, glyphosate, and an auxinic herbicide will likely become standard herbicide applications. Glufosinate plus 2,4-D is very effective at controlling glyphosate-resistant Palmer amaranth (Merchant et al. 2014a) even in salvage situations (Merchant et al. 2014b). Glufosinate plus dicamba has also been determined as a very effective POST application to control Palmer amaranth (Merchant et al. 2013).

However, the behavior of glufosinate, glyphosate, dicamba, and 2,4-D in mixture on other hard-to-control species is not yet fully understood. Therefore, more research involving mixtures of these herbicides is needed to make appropriate herbicide program recommendations. Effective POST mixtures are needed in Enlist and RoundupReady Xtend crops to control a broad weed spectrum and minimize evolution of resistance, especially when it involves controlling resistant populations of weeds such as Palmer amaranth and barnyardgrass.

Glyphosate is a highly effective tool for managing barnyardgrass in cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] (Payne and Oliver 2000; Scott et al. 2017). Even so, barnyardgrass persists as a highly common and troublesome weed in Midsouth USA soybean and cotton fields (Webster 2013). Glyphosate-resistant barnyardgrass was confirmed in Tennessee in 2017 (Steckel et al. 2017) and barnyardgrass has evolved various cases of multiple resistance to many different sites of action (Heap 2018), highlighting the need to preserve the efficacy of glyphosate in Enlist and RoundupReady Xtend crop systems.

Some interactions of glufosinate with other POST herbicides have been reported such as glufosinate plus 2,4-D (Craigmyle et al. 2013; Merchant et al. 2013), dicamba (Merchant et al. 2013), and glyphosate (Bethke et al. 2013). The aforementioned papers evaluated these combinations on various monocot and dicot species. The outcomes of the herbicide interactions appear to depend on the evaluated species and are inconsistent, specifically when they involve grass species.

In addition, apparent antagonism has been reported when glyphosate has been used in mixtures with a synthetic auxin such as dicamba or 2,4-D and applied to various grass species. A reduction in control has been observed when dicamba was added to glyphosate on barnyardgrass (Meyer et al. 2015) and johnsongrass [*Sorghum halepense* (L.) Pers.] (Flint and Barrett 1989). O'Sullivan and O'Donovan (1980) reported antagonism for glyphosate plus dicamba and glyphosate plus 2,4-D on three monocots. However, both Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) show that the identification of antagonism can be dependent upon rates of the herbicides and species evaluated. Thus, fully understanding these interactions on troublesome weeds common in the Midsouth may be vital for proper management.

When two herbicides are applied in a mixture, interactions can be described by the use of Colby's method (Colby 1967). In circumstances when one herbicide has no POST activity on a given species, (e.g., dicamba on barnyardgrass), Colby's method cannot be used because the model requires control greater than 0% from both herbicides. In such cases, considering a significant decrease in herbicidal activity from the mixture (e.g., glyphosate plus dicamba), compared to the herbicide with activity alone (e.g., glyphosate) can be considered antagonism. Such methodology was used by both Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980).

The formulation of an active ingredient may also play a role in the identification of herbicide interactions. For example, Kudsk and Mathiassen (2004) reported higher levels of synergism for mixtures of commercial products compared to the technical grade laboratory products, indicating the adjuvants in the commercially available products may be improving uptake of both products in mixture. Identification of herbicide interactions can also differ between commercial formulations of the same active ingredient (Nalewaja and Matysiak 1992).

Two products formulated as salts can interact in mixture leading to the formation of byproduct salts upon drying. For example, if an isopropylamine salt of glyphosate and ammonium salt of glufosinate are mixed, two glyphosate salts (isopropylamine and ammonium) will likely exist on the leaf after evaporation of the spray carrier, which could impact the herbicide interaction. Minor differences in the activity of the isopropylamine and ammonium glyphosate salts has been reported (Kudsk and Mathiassen 2002, 2004).

A change in formulations may not only impact the herbicide interaction in a physiochemical fashion, but could also alter the droplet spectra. Mueller and Womac (1997) reported differences in the droplet size produced between two formulations of glyphosate. When glyphosate is mixed with glufosinate, formulations of glyphosate that produce larger droplet sizes in mixture could negatively impact the performance of glufosinate compared to a different formulation of glyphosate. Glufosinate has been shown to have improved efficacy when smaller droplet sizes are used (Etheridge et al. 2001; Meyer et al. 2015). Thus, an increase in droplet size could also influence any potential antagonistic interactions.

The aforementioned research demonstrates the need to evaluate mixtures of various commercial products even if they contain the same active ingredient. The purpose of these experiments was to evaluate potential interactions of glufosinate with glyphosate, 2,4-D, and dicamba applied in various combinations and rates in 2,4-D- and dicamba-resistant cropping systems using products labeled or recommend in those systems.

Materials and Methods

Two field experiments were conducted at the Northeast Research and Extension Center in Keiser, AR, to evaluate the weed control of glufosinate, glyphosate, 2,4-D, and dicamba applied alone, and in mixtures. The 2,4-D Experiment was designed to evaluate herbicide interactions that could occur in the Enlist crop systems using products marketed in that system (Liberty, Durango, and Enlist Duo herbicides). The Dicamba Experiment was designed to evaluate herbicide interactions that could occur in Roundup Xtend cropping systems using products marketed in that system (e.g., Liberty, Roundup PowerMax II, and Clarity herbicides). For a complete list of herbicide products used in both experiments, refer to Table 1.

At the time of trial initiation in 2015, the formulations of dicamba that are now registered in RoundupReady Xtend crops, Engenia, FeXapan, and Xtendimax herbicides, were not commercially available. Instead, Clarity herbicide, a diglycoamine formulation of dicamba, was used. Additionally, a stand-alone product of 2,4-D choline (Enlist One herbicide) was also not commercially available and Weedar herbicide, a 2,4-D amine formulation, was used when needed.

Both the 2,4-D and Dicamba Experiments were randomized, complete block designs with herbicide as the single factor. Each treatment was replicated four times in a given experiment, each year. In the 2,4-D Experiment, various rates and combinations of glufosinate, the dimethylamine (DMA) form of glyphosate, 2,4-D amine, a pre-mixture of glyphosate (DMA) plus 2,4-D choline (Enlist Duo herbicide) and *S*-metolachlor were evaluated. In the Dicamba Experiment, various rates and combinations of glufosinate, the potassium salt of glyphosate (K), diglycoamine salt of dicamba, and *S*-metolachlor were tested. A complete list of treatments is shown in Table 2 for the 2,4-D Experiment and Table 3 for the Dicamba Experiment.

Plot sizes were 3.9 by 9.1 m, and experiments were established on a Sharkey clay (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept), pH 6.7, and 1.7% organic matter. Herbicides were applied at the same rates when applied alone as when applied as a mixture, and a nontreated check was included for comparison. Additionally, a nonionic surfactant (NIS) at 0.25% (v v^{-1}) (Induce, Helena Chemical Company, Collierville, TN) was added to all dicamba-containing treatments unless glyphosate was included as a part of the mixture because adjuvants were present in the formulated glyphosate. Any reference to dicamba alone refers to a solution of dicamba plus NIS. Air Induction Extended Range (AIXR 110015) nozzles (TeeJet Technologies, Springfield, IL) were used to apply herbicide solutions. AIXR 110015 nozzles are designated by the manufacturer as producing coarse droplets at 276 kPa. Applications were made with a CO_2-pressurized backpack sprayer calibrated to deliver 141 L ha^{-1} spray volume at 276 kPa at 4.8 km hr^{-1} through nozzles spaced 51 cm apart.

Before planting, barnyardgrass and prickly sida (*Sida spinosa* L.) seed were broadcast across the trial area. Glyphosate-resistant corn (*Zea mays* L.) was planted in 97-cm-wide rows to simulate the effect of a typical crop canopy on herbicide application. A Smart-Stax (Monsanto Company, St. Louis, MO) hybrid was planted because it is commercially available and can tolerate POST applications of all herbicides used in this experiment (glufosinate, glyphosate, 2,4-D, and dicamba). A DeKalb DD 1246 Smart-Stax hybrid was planted at 86,500 seeds ha^{-1} in 2015 and A DeKalb DKC46-36RIB Smart-Stax hybrid was planted at 101,000 seeds ha^{-1} in 2016. Corn was at or near V8 at the time of application. Plots were furrow irrigated to soil saturation as needed. Fertilizer and lime were applied based on a soil test and according to University of Arkansas recommendations, however, no nitrogen was applied to the corn in an effort to keep the crop from out-competing the weeds.

Applications were made to large, actively growing weeds, and a list of weed sizes is available in Table 4. In 2015, applications were made on July 28, 2015 at 9:00 AM for the 2,4-D Experiment and 2:00 PM for the Dicamba Experiment. In-field observations recorded a temperature of 32 C with 70% relative humidity (RH) at 9:00 AM and 36 C with 77% RH at 2:00 PM. In 2016, applications were made on July 18 at 3:00 PM for 2,4-D Experiment and 3:45 PM for the Dicamba Experiment. At the time of application in 2016, temperature was 33 C with 50% RH.

Weed control ratings were collected 2 and 5 weeks after treatment (WAT) for barnyardgrass, Palmer amaranth, and prickly sida. Weed control was visually evaluated and rated on a scale of 0 (no control) to 100% (complete death of all plants) relative to the nontreated check. For a given species, heights were also collected 5 WAT for 3 random individuals that survived the herbicide application in each plot.

Herbicide interactions were identified using Colby's method (Colby 1967), where an Expected value (E) is calculated using Equation 1.

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

Where E is the expected level of control of a given species when two herbicides are applied in a mixture, and variables X and Y represent the level of control of a given weed species provided by each herbicide applied individually. The observed and expected values were compared using a two-sided t-test ($\alpha = 0.05$). If E was significantly greater than the observed value for a given mixture, it was deemed antagonistic. When an herbicide mixture contained more than two herbicides (e.g., glufosinate plus glyphosate plus dicamba) an expected value was not calculated unless one of the components had no POST activity on a given species. For example, glufosinate plus glyphosate plus 2,4-D plus *S*-metolachlor has an expected value for barnyardgrass control

(Table 3) calculated from the two components (glufosinate and glyphosate) that have POST grass activity.

In addition to the field experiments, the herbicide treatments in the 2,4-D and Dicamba experiments were analyzed in a low-speed wind tunnel at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE. The wind tunnel uses a Sympatec Helos Vario KR particle-size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) to measure droplet spectra via laser diffraction as described in detail by Creech et al. (2015) and Henry et al. (2014). The laser is equipped with an R7 lens capable of detecting particle sizes in a range from 18 to 3,500 μm and is positioned 30-cm from the tip of the nozzle. A linear actuator moves the nozzle during spraying, moving the width of the nozzle plume across the laser. Windspeed was set to 24 km h^{-1} during the analysis to minimize spatial sampling bias. Each herbicide treatment in the 2,4-D and Dicamba Experiments was replicated three times, and the same products used in the field experiments were used for particle-size analysis. Spray parameters that were of interest were the Dv_{10}, Dv_{50} Dv_{90}, relative span (RS), and the percentage of fines. Dv_{10} is the diameter below which 10% of the liquid volume is atomized into smaller droplets. Dv_{50} and Dv_{90} are similar values for 50% and 90% of the volume, respectively. The percentage of driftable fines was classified as the percentage of the volume containing droplets with a diameter <150 μm ($\%_{vol}$ fines). The relative span (RS) is a parameter of the spray plume that has no units and describes the range of droplet sizes of the plume using Equation 2.

$$\mathrm{RS} = (D_{\mathrm{v}90} - D_{\mathrm{v}10})\ D_{\mathrm{v}50}^{-1} \qquad [2]$$

The statistical software JMP Pro 13 (SAS Institute Inc., Cary, NC) was used to subject all data to an analysis of variance (ANOVA) means were separated using Fisher’s protected least significant difference (LSD) test (α = 0.05). Natural-log transformation of weed height data was used if it improved the model fit. ANOVA was conducted on the transformed values and values were back-transformed for discussion and reporting. Replication and year were included in the ANOVA as random effects. Variance components estimates for each ANOVA are shown in Table 5. As previously stated, herbicide interactions were evaluated using Colby's method. Results from the ANOVA were also interpreted and comparisons were made to determine if the mixtures provided significantly greater, equal, or less control than the components. Comparisons from the ANOVA may, or may not, correlate with the results from Colby's method (e.g., Table 2). Data from the particle-size analysis did not have a blocking factor and no such factor was included in the analyses of these data. Additionally, a more-conservative Tukey adjustment ($\alpha = 0.05$) was used to identify differences among the means of the droplet size parameters.

Results and Discussion

Barnyardgrass

2,4-D Experiment. Antagonism was positively identified for glufosinate plus glyphosate (DMA) for both rates across evaluations (Table 2). Interestingly, glufosinate plus glyphosate DMA had greater control 2 WAT based on visual evaluations compared to either of its components alone, indicating the mixture appeared to be superior despite being antagonistic. However, by 5 WAT, glufosinate at 450 g ai ha^{-1} plus glyphosate DMA at 840 g ae ha^{-1} had less control (83%) than glyphosate alone at 840 g ae ha^{-1} (88%). Similar findings were also reported by Bethke et al. (2013) where glufosinate plus glyphosate had relatively higher ratings at earlier rating timings. These results demonstrate some of the important details and nuances of using an interaction analysis such as Colby's method, to evaluate herbicide mixtures. A mixture may provide greater control than either of its components alone while still being designated as antagonistic, as was the case for glufosinate plus glyphosate DMA control 2 WAT.

The pre-mixture of glyphosate DMA plus 2,4-D at the low rate (840 plus 785 g ae ha^{-1}) had less control (80%) compared to glyphosate DMA alone (88%) 5 WAT, indicating 2,4-D may be antagonizing glyphosate despite not having any POST activity on barnyardgrass. No differences between glyphosate DMA plus 2,4-D and glyphosate alone were observed at the higher rate (1,120 plus 1,065 g ae ha^{-1}) indicating that using higher rates of glyphosate may help overcome antagonistic effects in some cases.

As mixtures of glyphosate DMA plus glufosinate and glyphosate DMA plus 2,4-D appear to be antagonistic on barnyardgrass, it is not surprising a three-way combination of glufosinate plus glyphosate DMA plus 2,4-D is also antagonistic at the rate combinations included in this experiment. An expected value was calculated for these three-way mixtures on barnyardgrass from the performance of glufosinate and glyphosate, as 2,4-D has no POST activity. One of the combinations (595 g ai plus 1,120 g ae plus 1,065 g ae ha^{-1}, respectively) had less control 5 WAT (86%) than 1,120 g ae ha^{-1} glyphosate alone (91% control), with a similar conclusion from the height reduction evaluation.

For the three-way mixtures of glufosinate plus glyphosate plus 2,4-D, large increases in D_{v50} relative to glufosinate alone were observed and may be reducing the performance of glufosinate (Table 6). The D_{v50} and percentage of the volume with droplets <150 μm ($\%_{vol}$ fines) for glufosinate at 450 g ai ha^{-1} were 400 μm and 7.1%, respectively. When glufosinate at 450 g ai ha^{-1} was added to a pre-mixture of glyphosate at 840 g ae ha^{-1} plus 2,4-D at 785 g ae ha^{-1}, D_{v50} increased to 453 μm and the droplet classification was changed from Very Coarse to Extremely Coarse (ASABE 2009). Similar increases in droplet size were observed for the other rates of the three-herbicide mixture. Although the glufosinate label does not recommend Very Coarse (VC) droplets for application as a reduction in weed control could result (Anonymous 2016), further increases in droplet size will only reduce the performance of glufosinate more.

These data suggest that three-way mixtures may be a detriment for resistance management, as individuals are surviving application to two effective modes of action with exposure to a third herbicide (2,4-D) that at the very least is negatively impacting the performance of the glyphosate in the mixture. Additionally, the addition of glyphosate alone or 2,4-D alone to glufosinate typically did not cause an increase in D_{v50}; only mixtures of all three herbicides caused such large increases in droplet size relative to glufosinate alone. A more suitable and efficient management strategy to control a broad weed spectrum that includes barnyardgrass may be to apply glyphosate DMA plus 2,4-D in an early-POST application and follow up with an application of glufosinate late-POST, or vice-versa. More research may be needed to evaluate mixtures in herbicide programs in order to identify an optimum resistant-management strategy.

Dicamba Experiment. Antagonism was identified for the mixtures across all assessments (Table 3) in the Dicamba Experiment. Glufosinate appears to be antagonizing the activity of glyphosate K at all rate combinations and even had less height reduction (58%) than glyphosate K alone (73%). As was observed in the 2,4-D experiment, when glyphosate K was used in a mixture with an auxinic herbicide (in this case, dicamba), an antagonistic effect was observed. Glyphosate K plus dicamba at the low rate (865 plus 560 g ae ha^{-1}) provided less control than the equivalent rate of glyphosate K alone 5 WAT (79 and 86%, respectively). Glyphosate K plus dicamba at the higher rate of glyphosate (1,260 plus 560 g ae ha^{-1}) also resulted in less control than the same rate of glyphosate K alone. For the three-way combination of glufosinate plus glyphosate K plus dicamba, percent control 5 WAT was less than glyphosate K alone, likely a result of both glufosinate and dicamba antagonizing the systemic activity of glyphosate.

Much like the 2,4-D Experiment, the droplet spectra of the herbicide combinations were most impacted by the three-way mixtures of glufosinate plus glyphosate plus dicamba relative to glufosinate alone (Table 7). Additionally, if this mixture were to be an approved tank-mixture, it would have to be applied using a nozzle that is classified as producing Ultra Coarse droplets, as is required by the Xtendimax herbicide label (Anonymous 2018). Even though this experiment utilized the Clarity product, a different formulation of the DGA salt of dicamba, it is reasonable to assume a three-way mixture that included Xtendimax instead of Clarity would behave similarly in regards to the changes in droplet spectra. Although a variety of issues exist surrounding obtaining approval for a three-way mixture of glufosinate plus glyphosate plus dicamba in RoundupReady Xtend crops, the effect of relatively large droplets on glufosinate efficacy would be one concern as it relates to the weed control of the mixture.

Palmer amaranth

2,4-D Experiment. No mixtures were considered to be antagonistic for Palmer amaranth control in the 2,4-D Experiment (Table 8). Glyphosate alone (DMA and K in the 2,4-D and Dicamba Experiments, respectively) provided 24-28% control of the glyphosate-resistant Palmer amaranth population (Tables 8 and 9). When comparing the pre-mixture of glyphosate DMA plus 2,4-D to 2,4-D alone, the pre-mixture did not have improved control over 2,4-D alone for either rate for control 5 WAT or for height reduction. However, for the mixture of glufosinate plus glyphosate DMA (595 g ai plus 1120 g ai ha^{-1}), control of Palmer amaranth 5 WAT was greater (91%) than either glufosinate alone (85%) or glyphosate DMA alone (24%).

Dicamba Experiment. Glyphosate K at 865 g ae ha^{-1} plus dicamba at 560 g ae ha^{-1} provided greater control (92%) than dicamba alone (80%) 5 WAT (Table 9), once again reinforcing the concept of the inclusion of glyphosate in a mixture applied to a glyphosate-resistant population still provides some value. Generally, mixtures of herbicides had improved control over their individual components, suggesting the value of mixtures for control of Palmer amaranth in RoundupReady Xtend crops.

In this experiment, glufosinate alone at 595 g ai ha^{-1} had improved control over dicamba alone 5 WAT (88 and 80% for glufosinate and dicamba, respectively). Even so, the mixtures of glufosinate plus dicamba still had improved control over glufosinate alone. For glufosinate at 450 g ai ha^{-1} plus dicamba at 560 g ae ha^{-1}, control was greater with the mixture than with either herbicide applied alone even though it was deemed antagonistic. When a higher rate of glufosinate (595 g ai ha^{-1}) was mixed with dicamba at 560 g ae ha^{-1}, percent control 5 WAT and height reduction were not different between the mixtures (94 and 90% for high and low rates, respectively). However, the mixture with the higher rate of glufosinate (595 g ai ha^{-1}) did not deviate as far from its expected value and was not considered antagonistic. Typically, increasing the rate of the fast-acting contact herbicide in a mixture of contact and systemic herbicides is associated with an increased level of antagonism (Wehtje et al. 2008). Either due to the high expected values calculated for these treatments or another mechanism, it does not appear glufosinate is inhibiting the activity of dicamba on Palmer amaranth when used in a mixture.

Any changes in droplet spectra do not appear to be playing a role in the interaction between glufosinate and dicamba. When dicamba was added to glufosinate at 595 g ai ha^{-1}, D_{v50} increased from 385 to 413 µm and $\%_{vol}$ fines decreased (Table 7), an effect that should reduce the performance of glufosinate. The droplet spectra for the lower rate of glufosinate (450 g ai ha^{-}

^{1}) did not differ for D_{v50} or $\%_{vol}$ fines from glufosinate alone. It may be important that the mixture of glufosinate plus dicamba dramatically reduced D_{v50} and increased $\%_{vol}$ fines relative to dicamba alone, which is one factor restricting this from being a possible mixture under current tank-mix partner guidelines.

Prickly Sida

2,4-D Experiment. At 5 WAT, two-way mixtures had improved control of prickly sida compared to the individual herbicides alone, with the exception of glufosinate plus *S*-metolachlor and glufosinate at 450 g ai ha^{-1} plus glyphosate DMA at 1120 g ae ha^{-1} plus 2,4-D at 1065 g ae ha^{-1} (Table 10). Glufosinate at 595 g ai ha^{-1} plus 2,4-D at 1065 g ae ha^{-1} and the pre-mixture of glyphosate DMA plus 2,4-D (1120 plus 1065 g ae ha^{-1}) also had greater height reduction relative to the individual herbicides applied alone. Glufosinate plus glyphosate DMA (both rates) was also considered antagonistic for control 5 WAT. Even so, it appears mixtures of two or more of these herbicides generally improve control of prickly sida over the use of individual products.

Dicamba Experiment. Most two-way mixtures performed better than their component herbicides (Table 11). One of the exceptions was glufosinate plus glyphosate (595 g ai plus 1260 g ae ha^{-1}) which was antagonistic, and did not perform better than glufosinate or glyphosate alone. In both the 2,4-D and Dicamba Experiments, at least one mixture of glyphosate plus glufosinate was antagonistic 5 WAT. Much like with barnyardgrass, it is likely glufosinate is reducing the uptake or transport of glyphosate, resulting in antagonism.

Practical Implications

The results from these experiments identify mixtures or individual herbicides that may be selected for optimum control of a given weed, but also present a challenge for selecting treatments that will perform best on a broad range of species. Barnyardgrass assessments showed clear antagonism for mixtures of glyphosate plus glufosinate and glyphosate plus 2,4-D. For some mixtures, (e.g., glyphosate DMA plus 2,4-D) a decline in barnyardgrass control relative to glyphosate DMA alone was observed (Table 2). Additionally, mixtures of glufosinate plus glyphosate were antagonistic for prickly sida despite showing improvements in percent control compared to the individual herbicides. Adding to the conflict is the presence of glyphosate-resistant Palmer amaranth populations rampant throughout the Midsouth that require alternatives to glyphosate for effective control. Although the glyphosate in a mixture did provide some benefit for control of a glyphosate-resistant Palmer amaranth population, another product, such as glufosinate or a synthetic auxin, is clearly needed. An antagonistic mixture that is better than the individual herbicides may provide some benefit in managing that given weed. However, the benefit provided by the mixture may not be as much as is expected and may result in more survivors. Thus, care should be taken when mixtures are utilized, and fields should be properly managed to mitigate the likelihood of resistance evolution.

Field use rates of various herbicides (e.g. glyphosate, glufosinate, etc.) were evaluated in the current experiments and certain herbicides provided high levels of control on a given species (e.g., glyphosate at 1120 g ae ha^{-1} provided 91% barnyardgrass control 5 WAT). Analyzing for herbicide interactions is better when the herbicides doses used provide approximately 50% control (Colby 1967). Herbicide interactions can vary depending on the rates used in mixture and when single herbicides provide >90% control alone identifying synergy is essentially impossible using Colby's method (Riley and Shaw 1988; Scott et al. 1998). Other methods to assess herbicide interactions can be used to more robustly analyze for interactions, but those methods also require more specific experimental designs (Streibig and Jensen 2000; Wehtje and Gilliam 2015). The intent of the current experiments was to evaluate for interactions at field use rates. Additional research may be needed to fully understand how the herbicides evaluated behave in mixture.

It is not clear why dicamba and 2,4-D antagonize the activity of glyphosate on grass species. Applications of dicamba are known to disrupt phloem loading, and thus may be impacting glyphosate translocation throughout the plant. Additionally, the synthetic auxin response is a complicated and dynamic pathway that may be causing other physiological changes which could affect the ability of glyphosate to reach its target site in the whole plant (e.g., sequestration). Applications of dicamba disrupt natural hormone signaling, with stimulation of ethylene biosynthesis occurring within hours of application and growth inhibition setting in within the first 24 h (Grossman 2010). Evidence suggests abscisic acid, auxins and gibberellins are involved with phloem loading and unloading (Lalonde et al. 2003) and disrupting native hormone signaling may impact herbicide transport. Additionally, glyphosate inhibits synthesis of the amino acid tryptophan, a precursor involved in the plant biosynthesis of indole acetic acid (Taiz and Zeiger 2006). Hormone signaling is described as a complex signal transduction cascade and often involves more than one phytohormone. Therefore, it is possible that the inhibition of auxin biosynthesis with concurrent exposure to high concentrations of a synthetic auxin could result in the antagonism observed in this experiment.

These results present a unique challenge for controlling a broad spectrum of weeds in Enlist and RoundupReady Xtend technologies. The addition of more products to control one species (i.e., glyphosate-resistant Palmer amaranth) may negatively impact the control of another (i.e., barnyardgrass) and thus growers and applicators need to successfully manage all species present in a given field. To mitigate the antagonism and reductions in control identified for some herbicide mixtures on a given species, mixtures of two herbicides should be utilized in a POST application and high labeled rates should be selected, specifically if glyphosate is part of the mixture. Herbicides should always be applied to labeled weed sizes (<10-cm) and full labeled rates, thus, following these principles when mixtures are used should also help mitigate antagonism.

The weed species present in a field, specifically the problematic ones, should dictate the herbicide program used in that field. If a field was infested with Palmer amaranth, a mixture of glufosinate + 2,4-D or dicamba would be an effective treatment, but may be limited by label restrictions (Anonymous 2018). In contrast, a field infested solely with barnyardgrass would be best controlled by a single herbicide, preferably glyphosate, as a function of antagonism. As all fields will have a range of species present, this research would suggest a two-pass program may be needed (e.g., glufosinate + 2,4-D fb glyphosate alone 7-14 days later, if grass species are a concern).

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Appendix

Table 1. Herbicide information for all products used in the experiments.[a]

Herbicide common name	Herbicide trade name	Rate	Manufacturer	Address	Website	Adjuvant[b]
		g ai or g ae ha^{-1}				
Glyphosate	Durango					
Glufosinate	Liberty	594	Bayer CropScience LP	Research Triangle Park, NC	www.bayercropscienceus.com	
2,4-D	Weedar	1065	Nufarm Inc.	Burr Ridge, IL	www.nufarm.com/US/Home	
Glyphosate + 2,4-D	Enlist Duo	834 + 785	Dow AgroSciences LLC	Indianapolis, IN	http://www.dowagro.com/en-US	
S-metolachlor	Dual Magnum	1068	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	
Glyphosate	Roundup PowerMax	867	Monsanto Company	St. Louis, MO	www.monsanto.com	
Dicamba	Clarity	560	BASF Corporation	Research Triangle Park, NC	www.basf.com	NIS

[a] Abbreviation: NIS, nonionic surfactant (Helena Chemical Company, Collierville, TN)

[b] Adjuvant rate: NIS, 0.25% v v^{-1}

Table 2. Barnyardgrass control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the 2,4-D Experiment.

		Percent control[ab]								Height reduction[abc]			
		2 WAT				5 WAT							
Treatment	Rate	Obs		Exp	p[d]	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
Nontreated		0				0				0			
Glufosinate	450	81				78				37			
Glufosinate	595	89				85				60			
Glyphosate	840[e]	80				88				67			
Glyphosate	1120[e]	86				91				76			
2,4-D	785	0				0				10			
2,4-D	1065	0				0				14			
Glyphosate + 2,4-D	840[e] + 785[e]	78	NS			80	∨			61	NS		
Glyphosate + 2,4-D	1120[e] + 1065[e]	84	NS			89	NS			72	NS		
Glufosinate + glyphosate	450 + 840[e]	90	∧	96	*	83	∨	97	*	55	NS	79	*
Glufosinate + glyphosate	595 + 1120[e]	95	∧	98	*	93	NS	99	*	59	∨	92	*
Glufosinate + 2,4-D	450 + 785[e]	84	NS			82	NS			40	NS		
Glufosinate + 2,4-D	595 + 1065[e]	85	NS			81	NS			60	NS		
Glufosinate + glyphosate + 2,4-D	450 + 840[e] + 785[e]	88	NS	96	*	84	NS	97	*	53	NS	79	*
Glufosinate + glyphosate + 2,4-D	450 + 1120[e] + 1065[e]	89	NS	97	*	87	NS	98	*	64	NS	87	*
Glufosinate + glyphosate + 2,4-D	595 + 1120[e] + 1065[e]	90	NS	98	*	86	∨	99	*	61	∨	92	*
Glufosinate + *S*-metolachlor	450 + 1390	84	NS	-	NS	79	NS	-	NS	47	NS	-	NS
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	450 + 840[e] + 785[e] + 1390	91	∧	96	*	86	NS	97	*	63	NS	79	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 3. Barnyardgrass control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the Dicamba Experiment.

		Percent control[ab]											
		2 WAT				5 WAT				Height reduction[abc]			
Treatment	Rate	Obs		Exp	p[d]	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
Nontreated		0				0				0			
Glufosinate	450	84				78				52			
Glufosinate	595	88				79				62			
Glyphosate	865[e]	79				86				73			
Glyphosate	1260[e]	86				92				81			
Dicamba	560[e]	0				0				11			
Glyphosate + dicamba	865[e] + 560[e]	80	NS			79	∨			73	NS		
Glyphosate + dicamba	1260[e] + 560[e]	79	∨			81	∨			76	NS		
Glufosinate + dicamba	450 + 560[e]	80	NS			77	NS			50	NS		
Glufosinate + dicamba	595 + 560[e]	84	NS			79	NS			60	NS		
Glufosinate + glyphosate	450 + 867[e]	84	NS	97	*	82	NS	97	*	58	∨	87	*
Glufosinate + glyphosate	595 + 1260[e]	91	NS	98	*	91	NS	98	*	70	NS	94	*
Glufosinate + glyphosate + dicamba	450 + 865[e] + 560[e]	83	NS	97	*	78	∨	97	*	62	NS	87	*
Glufosinate + glyphosate + dicamba	595 + 1260[e] + 560[e]	90	NS	98	*	88	NS	98	*	66	∨	94	*
Glufosinate + glyphosate + dicamba	450 + 1260[e] + 560[e]	89	NS	98	*	87	NS	98	*	69	NS	91	*
Glufosinate + *S*-metolachlor	450 + 1390	83	NS			79	NS			62	NS		
Glufosinate + glyphosate + dicamba + *S*-metolachlor	450 + 865[e] + 560[e]+ 1390	80	NS	97	*	80	∨	97	*	65	NS	87	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 4. Weed sizes and densities of four grass weeds at herbicide application evaluated in the 2,4-D and Dicamba Experiments in 2015 and 2016.

	2,4-D Experiment				Dicamba Experiment			
	2015		2016		2015		2016	
Species	Height	Density	Height	Density	Height	Density	Height	Density
	cm	plants m^{-2}	cm	plants m^{-2}	cm	plants m^{-2}	cm	plants m^{-2}
Barnyardgrass	14	22	26	20	32	20	24	21
Palmer amaranth	19	8	22	6	18	7	21	4
Prickly sida	15	12	12	2	15	7	13	2

Table 5. Variance components estimates obtained from the ANOVA for barnyardgrass, Palmer amaranth, and prickly sida control, height reduction, and density reduction for the 2,4-D and Dicamba Experiments[a].

		Variance Components Estimates								
		Barnyardgrass			Palmer amaranth			Prickly sida		
Experiment	Model effect	2 WAT	5 WAT	Height reduction	2 WAT	5 WAT	Height reduction	2 WAT	5 WAT	Height reduction
		% of total								
2,4-D	Rep(Year)	<0.1	<0.1	0.2	<0.1	<0.1	15.8	0.8	<0.1	<0.1
	Year	11.4	0.2	2.6	35.0	7.8	5.9	54.0	51.7	43.1
	Residual	88.7	99.8	97.2	65.0	92.2	78.3	45.2	48.2	56.9
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Dicamba	Rep(Year)	<0.1	4.7	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
	Year	10.9	4.4	17.0	45.2	48.3	25.4	58.2	56.9	54.2
	Residual	89.1	90.9	83.0	55.2	51.5	74.6	41.8	43.1	45.8
	Total	100.0	100.0	100.0	100.4	100.0	100.0	100.0	100.0	100.0

[a] Abbreviation: WAT, weeks after treatment.

Table 6. Spray characteristics of various herbicide combinations in the 2,4-D Experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm.

Treatment	Rate	Droplet spectra parameter[a]				
		D_{v10}	D_{v50}	D_{v90}	Relative span[b]	<150 µm
	g ai ha^{-1}	---------------------µm---------------------			-	$\%_{vol}$
Water		249 a	453 abc	668 a-e	0.93 ef	1.8 h
Glufosinate	450	175 ef	400 hij	663 b-f	1.22 abc	7.1 bcd
Glufosinate	595	166 f	385 k	641 fg	1.23 ab	7.9 b
Glyphosate	840^{c}	217 c	446 bcd	682 abc	1.04 d	3.6 f
Glyphosate	1120^{c}	205 cd	440 cde	682 abc	1.08 d	4.6 e
2,4-D	785	237 b	459 ab	682 ab	0.97 e	2.8 fg
2,4-D	1065	239 ab	460 a	687 a	0.97 e	2.5 gh
Glyphosate + 2,4-D	840^{e} + 785^{c}	230 b	420 fg	606 h	0.90 f	2.5 gh
Glyphosate + 2,4-D	1120^{e} + 1065^{c}	233 b	429 ef	636 g	0.94 ef	2.3 gh
Glufosinate + glyphosate	450 + 840^{c}	151 g	400 hij	656 d-g	1.27 a	9.9 a
Glufosinate + glyphosate	595 + 1120^{c}	176 ef	402 hij	648 efg	1.17 bc	6.9 cd
Glufosinate + 2,4-D	450 + 785^{c}	171 ef	398 ijk	651 efg	1.21 abc	7.5 bc
Glufosinate + 2,4-D	595 + 1065^{c}	172 ef	395 jk	659 c-g	1.23 a	7.2 bcd
Glufosinate + glyphosate + 2,4-D	450 + 840^{c} + 785^{c}	237 ab	453 ab	675 a-d	0.97 e	2.3 gh
Glufosinate + glyphosate + 2,4-D	450 + 1120^{c} + 1065^{c}	201 d	435 de	679 a-d	1.10 d	5.0 e
Glufosinate + glyphosate + 2,4-D	595 + 1120^{c} + 1065^{c}	181 e	409 ghi	660 b-f	1.17 c	6.6 d
Glufosinate + *S*-metolachlor	450 + 1390	217 c	410 ghi	606 h	0.95 ef	2.9 fg
Glufosinate + glyphosate + 2,4-D + S-metolachlor	450 + 840^{c} + 785^{c} + 1390	217 c	411 gh	610 h	0.96 e	3.0 fg

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] Relative span is a unitless index of the range of droplet sizes in the spectrum.

[c] Rate is in g ae ha^{-1}.

Table 7. Spray characteristics of various herbicide combinations in the Dicamba Experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm.

Treatment	Rate	Droplet spectra parameters[a]				
		D_{v10}	D_{v50}	D_{v90}	Relative span[b]	<150 µm
	g ai ha^{-1}	---------------------µm---------------------			-	$\%_{vol}$
Water		249 a	453 a	668 bc	0.93 i	1.8 f
Glufosinate	450	175 ij	400 i	663 bcd	1.22 ab	7.1 ab
Glufosinate	595	166 j	385 j	641 de	1.23 a	7.9 a
Glyphosate	865[c]	199 ef	432 bcd	677 abc	1.10 fg	5.0 cd
Glyphosate	1260[c]	197 e-h	433 bc	681 ab	1.12 defg	5.2 cd
Dicamba	560[c]	237 ab	460 a	695 a	1.00 h	2.5 ef
Glyphosate + dicamba	865[c] + 560[c]	199 efg	422 cde	659 cd	1.09 g	4.8 d
Glyphosate + dicamba	1260[e] + 560[c]	185 hi	402 hi	634 e	1.11 efg	5.9 bcd
Glufosinate + dicamba	450 + 560[c]	185 ghi	412 e-i	658 cd	1.15 de	6.1 bc
Glufosinate + dicamba	595 + 560[c]	186 f-i	413 e-h	675 abc	1.19 bc	5.9 bcd
Glufosinate + glyphosate	450 + 867[c]	191 e-h	416 ef	668 bc	1.15 de	5.5 cd
Glufosinate + glyphosate	595 + 1262[c]	188 fgh	416 efg	668 bc	1.16 cd	5.8 cd
Glufosinate + glyphosate + dicamba	450 + 865[c] + 560[c]	225 bc	439 b	667 bc	1.00 h	2.7 ef
Glufosinate + glyphosate + dicamba	595 + 1260[c] + 560[c]	195 e-h	421 de	671 bc	1.13 def	5.1 cd
Glufosinate + glyphosate + dicamba	450 + 1260[c] + 560[c]	203 de	436 b	682 ab	1.10 fg	4.7 d
Glufosinate + *S*-metolachlor	450 + 1390	214 cd	404 ghi	592 f	0.93 i	3.0 ef
Glufosinate + glyphosate + dicamba + *S*-metolachlor	450 + 865[c] + 560[c] + 1390	214 cd	408 f-i	598 f	0.94 i	3.2 e

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] Relative span is a unitless index of the range of droplet sizes in the spectrum.

[c] Rate is in g ae ha^{-1}.

Table 8. Palmer amaranth control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the 2,4-D Experiment

		Percent control[ab]											
		2 WAT				5 WAT				Height reduction[c]			
Treatment	Rate	Obs		Exp	p[d]	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
Nontreated		0				0				0			
Glufosinate	450	87				83				58			
Glufosinate	595	89				85				66			
Glyphosate	840[e]	26				22				29			
Glyphosate	1120[e]	33				24				46			
2,4-D	785	88				90				64			
2,4-D	1065	89				93				79			
Glyphosate + 2,4-D	840[e] + 785[e]	92	NS	91	NS	88	NS	91	NS	68	NS	75	NS
Glyphosate + 2,4-D	1120[e] + 1065[e]	95	∧	94	NS	92	NS	94	NS	72	NS	79	NS
Glufosinate + glyphosate	450 + 840[e]	92	∧	90	NS	86	NS	85	NS	56	NS	69	NS
Glufosinate + glyphosate	595 + 1120[e]	94	∧	93	NS	91	∧	90	NS	74	NS	82	NS
Glufosinate + 2,4-D	450 + 785[e]	98	∧	98	NS	96	NS	98	NS	89	∧	86	NS
Glufosinate + 2,4-D	595 + 1065[e]	99	∧	99	NS	96	NS	99	NS	96	NS	92	NS
Glufosinate + glyphosate + 2,4-D	450 + 840[e] + 785[e]	98	∧			96	NS			87	∧		
Glufosinate + glyphosate + 2,4-D	450 + 1120[e] + 1065[e]	99	∧			95	NS			95	NS		
Glufosinate + glyphosate + 2,4-D	595 + 1120[e] + 1065[e]	99	∧			99	∧			97	NS		
Glufosinate + *S*-metolachlor	450 + 1390	92	∧			87	NS			63	NS		
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	450 + 840[e] + 785[e] + 1390	98	∧			95	NS			87	NS		

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] NS denotes no significance between observed and expected values. Expected values are based on Colby's equation [E = (X + Y) - (XY)/100]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 9. Palmer amaranth control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the Dicamba Experiment

		Percent control[ab]											
		2 WAT				5 WAT				Height reduction[abc]			
Treatment	Rate	Obs		Exp	p[d]	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
										0			
Glufosinate	450	86				84				60			
Glufosinate	595	93				88				74			
Glyphosate	865[e]	34				31				31			
Glyphosate	1260[e]	33				28				49			
Dicamba	560[e]	86				80				81			
Glyphosate + dicamba	865[e] + 560[e]	91	NS	90	NS	92	∧	86	NS	86	NS	92	NS
Glyphosate + dicamba	1260[e] + 560[e]	89	NS	90	NS	85	NS	86	NS	81	NS	90	NS
Glufosinate + dicamba	450 + 560[e]	90	NS	98	*	90	∧	97	*	86	NS	92	NS
Glufosinate + dicamba	595 + 560[e]	96	NS	99	NS	94	∧	98	NS	88	NS	95	NS
Glufosinate + glyphosate	450 + 867[e]	86	NS	90	NS	81	NS	89	*	64	NS	72	NS
Glufosinate + glyphosate	595 + 1260[e]	92	NS	95	NS	87	NS	91	NS	76	NS	86	NS
Glufosinate + glyphosate + dicamba	450 + 865[e] + 560[e]	91	NS			91	∧			86	NS		
Glufosinate + glyphosate + dicamba	595 + 1260[e] + 560[e]	95	NS			95	∧			83	NS		
Glufosinate + glyphosate + dicamba	450 + 1260[e] + 560[e]	92	NS			94	∧			90	NS		
Glufosinate + *S*-metolachlor	450 + 1390	92	∧			86	NS			74	NS		
Glufosinate + glyphosate + dicamba + *S*-metolachlor	450 + 865[e] + 560[e]+ 1390	93	NS			93	∧			83	NS		

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 10. Prickly sida control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the 2,4-D Experiment

		Percent control^{ab}											
		2 WAT				5 WAT				Height reduction^{abc}			
Treatment	Rate	Obs		Exp	p^{d}	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
Nontreated		0				0				0			
Glufosinate	450	93				85				82			
Glufosinate	595	95				89				75			
Glyphosate	840^{e}	93				84				76			
Glyphosate	1120^{e}	92				86				72			
2,4-D	785	72				70				67			
2,4-D	1065	73				74				72			
Glyphosate + 2,4-D	840^{e} + 785^{e}	90	NS	97	NS	92	∧	94	NS	73	NS	91	NS
Glyphosate + 2,4-D	1120^{e} + 1065^{e}	96	NS	98	NS	94	∧	96	NS	98	∧	91	NS
Glufosinate + glyphosate	450 + 840^{e}	95	NS	99	*	91	∧	97	*	90	NS	95	NS
Glufosinate + glyphosate	595 + 1120^{e}	98	NS	99	NS	95	∧	98	*	89	NS	90	NS
Glufosinate + 2,4-D	450 + 785^{e}	98	∧	97	NS	97	∧	95	NS	93	NS	94	NS
Glufosinate + 2,4-D	595 + 1065^{e}	98	NS	98	NS	95	∧	97	NS	92	∧	91	NS
Glufosinate + glyphosate + 2,4-D	450 + 840^{e} + 785^{e}	97	NS			93	∧			90			
Glufosinate + glyphosate + 2,4-D	450 + 1120^{e} + 1065^{e}	96	NS			89	NS			90			
Glufosinate + glyphosate + 2,4-D	595 + 1120^{e} + 1065^{e}	98	NS			98	∧			98			
Glufosinate + *S*-metolachlor	450 + 1390	92	NS			88	NS			84			
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	450 + 840^{e} + 785^{e} + 1390	97	NS			97	∧			99			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 11. Prickly sida control 2 and 5 weeks after treatment (WAT) and height reduction as affected by treatment for the Dicamba Experiment.

		Percent control[ab]								Height reduction[abc]			
		2 WAT				5 WAT							
Treatment	Rate	Obs		Exp	p[d]	Obs		Exp	p	Obs		Exp	p
	g ai ha^{-1}	%		%		%		%		%		%	
										0			
Glufosinate	450	95				87				72			
Glufosinate	595	95				88				74			
Glyphosate	865[e]	94				86				79			
Glyphosate	1260[e]	94				92				77			
Dicamba	560[e]	63				67				76			
Glyphosate + dicamba	865[e] + 560[e]	91	NS	97	NS	93	^	95	NS	78	NS	91	NS
Glyphosate + dicamba	1260[e] + 560[e]	95	NS	97	NS	91	NS	96	NS	80	NS	89	NS
Glufosinate + dicamba	450 + 560[e]	96	NS	98	NS	93	^	94	NS	90	NS	90	NS
Glufosinate + dicamba	595 + 560[e]	98	NS	99	NS	96	^	97	NS	99	^	90	NS
Glufosinate + glyphosate	450 + 865[e]	97	NS	99	NS	93	^	97	NS	78	NS	90	NS
Glufosinate + glyphosate	595 + 1260[e]	97	NS	99	*	91	NS	99	*	87	NS	90	NS
Glufosinate + glyphosate + dicamba	450 + 865[e] + 560[e]	98	NS			97	^			95	^		
Glufosinate + glyphosate + dicamba	595 + 1260[e] + 560[e]	99	^			98	^			94	^		
Glufosinate + glyphosate + dicamba	450 + 1260[e] + 560[e]	95	NS			96	NS			94	^		
Glufosinate + *S*-metolachlor	450 + 1390	97	NS			92	^			97	^		
Glufosinate + glyphosate + dicamba + *S*-metolachlor	450 + 865[e] + 560[e] + 1390	96	NS			97	^			99	^		

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "^" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction is expressed as a percent of the nontreated control.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Chapter 4

Influence of weed size on herbicide interactions for Enlist™ and Roundup Ready® Xtend® technologies

Weed size is an important component of herbicide performance and can influence herbicide interactions in mixtures. To effectively control a broad range of species in soybean or cotton, POST herbicides mixtures will likely be commonplace in Roundup Ready® Xtend® and Enlist™ technologies. The impact of weed size on herbicide interactions that could occur in Roundup Ready® Xtend® or Enlist™ crops was assessed in two field experiments conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR. Combinations of glufosinate, glyphosate, dicamba, and 2,4-D were applied to either 10-cm or 30-cm weeds and evaluated for percent weed control, height reduction, and density reduction, collected 5 weeks after treatment (WAT). Colby's method was used to analyze treatments for herbicide interactions for control of barnyardgrass, Palmer amaranth, and pitted morningglory. Antagonism was identified with at least one treatment on all species. Almost all treatments were antagonistic for percent weed control, height reduction, and density reduction on barnyardgrass. When glyphosate in mixture with 2,4-D or dicamba to 30-cm barnyardgrass, control declined 9% for both mixtures relative to glyphosate alone. Glufosinate + glyphosate was antagonistic when applied to both 30-cm pitted morningglory and barnyardgrass. Glufosinate + dicamba provided less control and density reduction of Palmer amaranth than what was expected from Colby's equation. Overall, antagonism was more likely to be identified when applications were made to 30-cm weeds compared to 10-cm weeds. The utility of a given herbicide mixture will depend on the species present in the field and the size of those species at the time of application.

Nomenclature: 2,4-D; dicamba; glufosinate; glyphosate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; pitted morningglory, *Ipomoea lacunosa* L.

Key words: Antagonism, Colby’s method, glufosinate + glyphosate, glyphosate + 2,4-D, glyphosate + dicamba, herbicide interactions,

Approximately 94% of soybean [*Glycine max* (L.) Merr.], and 91% of cotton (*Gossypium hirsutum* L.) hectares in the US were planted to a variety containing a herbicide resistance trait in 2018 (USDA-NASS 2018). The herbicide-resistance traits vary with the individual technology, but Enlist™, LibertyLink®, and Bollgard II® Xtendflex® technology all contain a glufosinate-resistant trait, increasing the likelihood glufosinate will be applied in mixture with synthetic auxins or glyphosate. Prior research demonstrated glufosinate plus 2,4-D and glufosinate plus dicamba provided excellent control of glyphosate-resistant Palmer amaranth (Merchant et al. 2013; 2014a; 2014b). However, evidence suggests glufosinate + glyphosate and glyphosate + a synthetic auxin, are antagonistic when applied to various grass species (Besançon et al. 2018; Flint and Barrett 1989; Meyer et al. 2017; O'Sullivan and O'Donovan 1980).

Despite the prevalence of weeds across the U.S. that evolved resistance to glyphosate (Heap 2018), preserving the effectiveness of glyphosate on sensitive species is still of value. For example, in multiple resistant crop technologies (i.e., Enlist™ and Bollgard II® Xtendflex) alternative herbicides to glyphosate, such as glufosinate, often require sequential applications or additional herbicides for effective control of grass species including giant foxtail (*Setaria faberi* Herrm.) and johnsongrass (*Sorghum halepense* L.) (Wiesbrook et al. 2001, Meyer et al. 2015a). In the Midsouth, barnyardgrass is still a highly prevalent and problematic species in cotton and soybean fields (Van Wychen 2016). Although glufosinate is effective in controlling small barnyardgrass, glyphosate provides excellent control of small and large barnyardgrass (Meyer 2015a; Payne and Oliver 2000; Scott et al. 2017). Effective management of both glufosinate and glyphosate is needed to mitigate the likelihood of resistance evolution, specifically when antagonism in various mixtures may be present.

Mixtures of glyphosate plus a synthetic auxin herbicide have been reported as antagonistic when applied to monocots. Meyer et al. (2015b) observed a reduction in barnyardgrass control with glyphosate when dicamba was added to the solution. Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) also observed antagonism with mixtures of glyphosate + dicamba and glyphosate + 2,4-D on species including johnsongrass and wild oat (*Avena fatua* L.). However, the identification of antagonism depended on the specific rates and species in question.

A common and relatively straightforward technique to evaluate herbicide interactions, particularly when evaluating many different herbicides in a field setting, is Colby's method (Colby 1967). Colby's method calculates an expected value for a mixture based on the performance of the individual herbicides alone. However, when one herbicide has no POST activity on a given species, (e.g., 2,4-D on barnyardgrass), Colby's method is not suitable for making comparisons between the observed and expected values. When Colby's method is not applicable, typically a significant reduction in herbicidal activity of the mixture (e.g., glyphosate plus 2,4-D), compared to the herbicide with activity alone (e.g., glyphosate) is considered antagonism. Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) both considered significant deviations of the mixtures from the products alone as an antagonistic interaction.

A wide range of variables can influence interactions that occur between two herbicides, and the size of the weed can also play a role. Antagonism between glufosinate and glyphosate was more likely to be identified when applied to large weeds compared to small weeds (Miller et al. 2015). Similarly, the antagonistic interaction between clethodim and an ALS-inhibitor was more severe when applied to 6-8 leaf goosegrass [*Eleusine indica* (L.) Gaertn.] compared to 3-4 leaf goosegrass (Burke et al. 2002). Identifying herbicide interactions may be more likely on weed sizes beyond the range listed on herbicide labels, but understanding if antagonism is present and how mixtures perform on various weed sizes is important for selecting optimum herbicide mixtures.

Effective POST mixtures are needed in Enlist and RoundupReady Xtend crops to control a broad weed spectrum and minimize evolution of resistance, especially when it involves controlling herbicide-resistant populations of weeds such as Palmer amaranth and barnyardgrass. Therefore, the objective of these experiments was to identify the impact of weed size on the identification of antagonism potential for antagonism in 2,4-D- and dicamba-resistant cropping systems using products labeled or recommend in those systems.

Materials and Methods

The impact of weed size on the identification of antagonism with mixtures of glufosinate, glyphosate, 2,4-D, and dicamba was evaluated in two field experiments at Northeast Research and Extension Center in Keiser, AR, in 2015 and 2016. Both experiments (hereon referred to as the 2,4-D and Dicamba Experiments) were randomized complete block designs with two factors: herbicide treatment and weed size. Each experiment contained four replications and each experiment was repeated twice. In the 2,4-D Experiment, herbicide products and combinations that could occur in the Enlist crop systems (i.e., Liberty, Durango, and Enlist Duo herbicides) were evaluated on weed populations at two different application timings. The first herbicide application occurred when weeds reached approximately 10-cm in height and the second application at 30-cm (Table 1). Weed sizes were determined by measuring 3 plants for each species in the nontreated check plots. When the tallest species were approximately 10 and 30-cm in height, applications were initiated.

For the Dicamba Experiment, products associated with the Roundup Xtend cropping system were used (e.g., Liberty, Roundup PowerMax II, and Clarity herbicides) and also applied to two weed sizes (Table 2). All herbicide products used in both experiments are listed in Table 3. Some herbicide treatments (e.g., glufosinate + glufosinate) were used in both experiments, however different herbicide products for the same active ingredient were used (e.g., Roundup PowerMax and Durango herbicides). Prior research suggests changes in formulation and adjuvant load can impact mixture efficacy and herbicide interactions (Kudsk and Mathiassen 2004; Nalewaja and Matysiak 1992).

When trials were initiated in 2015, Xtendimax® and Engenia® herbicides (dicamba), now registered in RoundupReady Xtend crops, were not commercially available. Thus, a commercially available diglycoamine formulation of dicamba was used (Table 3). For the 2,4-D Experiment, a premix of glyphosate DMA + 2,4-D choline was used (Enlist Duo herbicide). However, no stand-alone product of 2,4-D choline (Enlist One herbicide) was available and a 2,4-D amine formulation was used when needed.

Experiments were established on a Sharkey clay (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept) with pH 6.7, and 1.7% organic matter. Plot size was 3.9 by 9.1 m in both years. In 2015, a DeKalb (Monsanto Company, St. Louis, MO) DD1246 SmartStax corn (*Zea mays* L.) hybrid was planted at 86,500 seeds ha^{-1} in 97-cm-wide rows in the trial area to mimic a crop canopy. In 2016, a DeKalb DKC46-36RIB Smart-Stax hybrid was planted at 101,000 seeds ha^{-1}. Fertilizer and lime were applied based on a soil test and according to University of Arkansas recommendations, however, no nitrogen was applied to the corn in an effort to keep the crop from out-competing the weeds. A Smart-Stax hybrid was selected for these experiments because it was commercially available and able to tolerate POST applications of 2,4-D, dicamba, glufosinate, glyphosate, and *S*-metolachlor. Plantings occurred on June 16, 2015 and June 10, 2016. The first herbicide application occurred approximately 4 wk after trial establishment when weeds reached 10-cm in height (Table 4). Corn was at or near V5 at the time of the first application and V8 at the time of the second application, in both years. Furrow irrigation to soil saturation was used as needed throughout the growing season.

When a herbicide was applied in mixture, it was applied at the same rate when applied alone. If the treatment contained dicamba, a nonionic surfactant (NIS) at 0.25% (v/v) (Induce, Helena Chemical Company, Collierville, TN) was added to the solution. Any reference to dicamba alone refers to a solution of dicamba plus NIS. If dicamba was mixed with a glyphosate product, no NIS was added because of adjuvants present in the glyphosate product. A CO_2-pressurized backpack sprayer was used to spray all herbicide treatments. At the time of application, the sprayer was calibrated to deliver 141 L ha $^{-1}$ spray volume at 276 kPa with application made 4.8 km hr^{-1}. Nozzles were spaced 51 cm apart and the boom was equipped with TeeJet (TeeJet Technologies, Springfield, IL) 110015 Air Induction Extended Range (AIXR) nozzle tips. One day after experimental treatments were applied, a blanket application of *S*-metolachlor was made to all plots unless a plot already received an application of *S*-metolachlor as part of the experimental treatment.

Weed control ratings, heights, and densities were collected 5 weeks after treatment (WAT) for barnyardgrass, Palmer amaranth, and pitted morningglory. Weed control was visually evaluated by comparing a treated plot to the nontreated check plots included in both experiments. Weeds were rated by species on a scale of 0 (no control) to 100% (complete death of all plants). Weed densities for each species were determined by counting individuals in two 1-m^2 quadrats. If 1 or fewer individuals were counted in at least one of the quadrats, all of the individuals in the plot were counted. Heights of 3 individuals of each species were collected in each plot. In order to use Colby's method on height and density assessments, data for each plot were converted to a percentage of the untreated check. For ease of discussion, heights and densities are presented as height and density reductions, so that a 100% reduction (0 plants m^2 or 0 cm) corroborates with 100% visual control (complete death of all plants).

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), shown in Equation 1.

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

Where E is the expected level of control of a given species when two herbicides are applied in a mixture, and variables X and Y represent the level of control of a given weed species provided by each herbicide applied individually. The Expected value for a mixture was compared to the observed value from the field using a two-sided t-test ($\alpha = 0.05$). When the Expected value was significantly greater than the observed value, the mixture was considered antagonistic. If a treatment contained more than two herbicides (e.g., glufosinate plus glyphosate plus dicamba) an expected value was not calculated for the mixture. However, if one component of a three-herbicide mixture had no POST activity on a given species, an expected value was calculated from the two herbicides that did provide control. Thus, the Expected value for barnyardgrass control for glufosinate plus glyphosate plus dicamba was equal to glufosinate plus glyphosate, as dicamba has no POST activity on barnyardgrass. In addition to the analysis used for herbicide interactions, the data were also subjected to an analysis of variance (ANOVA) using JMP Pro 13 (SAS Institute Inc., Cary, NC). Data from both years were combined, and replication and year were included in the analysis as random effects. Variance componesnts estimates for the random effects obtained from each ANOVA are listed in Table 5. As previously stated, herbicide interactions were evaluated using Colby's method. The results from the ANOVA were used to compare mixtures to their individual components in addition to the comparisons made using Colby's method. Comparisons from the ANOVA provide additional information to the herbicide interaction analysis by showing that an antagonistic herbicide mixture may actually provide control greater than either component alone (e.g., Table 6). Treatment means were separated using Fisher's protected least significant difference (LSD) test ($\alpha = 0.05$).

Results and Discussion

Palmer amaranth

2,4-D Experiment. All herbicide mixtures were considered additive for control 5 weeks after treatment (WAT), height reduction, and density reduction (Table 6). Control with glyphosate dimethylamine (DMA) alone was minimal (≤ 31%) but may be of some value for control of glyphosate-resistant populations. For example, control of 30-cm Palmer amaranth with a premix of 2,4-D + glyphosate DMA was significantly greater (92%) than control with 2,4-D alone (83%). When 2,4-D was applied with glufosinate, control of 30-cm Palmer amaranth was also greater than control provided by either 2,4-D or glufosinate alone, indicating the mixture may provide some benefit toward mitigating the likelihood of resistance evolution.

Dicamba Experiment. Two mixtures were identified as antagonistic for Palmer amaranth control 5 WAT in the dicamba experiment when applied to 30-cm weeds, glufosinate + glyphosate potassium (K) and glufosinate + dicamba (Table 7). Both percent control and density reduction were antagonistic for glufosinate + dicamba, with observed values being 9 and 10% less than expected values, respectively. No herbicide mixtures were antagonistic when applied to 10-cm

Palmer amaranth. One of the treatments identified as antagonistic applied to 30-cm weeds (glufosinate + dicamba), was additive and provided greater control than either glufosinate or dicamba alone when applied to 10-cm weeds. Thus, identification of a specific herbicide interaction appears to be dependent on weed size. Dicamba + glufosinate + glyphosate K + *S*-metolachlor provided 99% control, height reduction, and density reduction. The four-way mixture provided the greatest control of all other treatments, even more than dicamba + glufosinate + glyphosate K. *S*-metolachlor has no POST activity, and all plots received an application of *S*-metolachlor 24 h after experimental treatments were applied, except for the treatment that already contained *S*-metolachlor. Thus, the improvement in control is likely due to the adjuvants in the *S*-metolachlor product (Dual Magnum).

Pitted morningglory

2,4-D Experiment. The only mixture considered antagonistic for control of pitted morningglory in the 2,4-D experiment was glufosinate + glyphosate DMA applied to 30-cm weeds (Table 8). For percent control conferred by glufosinate + glyphosate DMA, the observed value was 91%, 8% lower than the expected value. The same treatment was also considered antagonistic for density reduction, where the observed value was 7% lower than what was expected from the combination of glufosinate + glyphosate DMA. Glufosinate and 2,4-D alone provided ≥ 91% control of pitted morningglory, depending on weed size, and control was not improved when another herbicide was added. Although a mixture may not improve control of one species, herbicides are commonly mixed to broaden spectrum of activity or improve control of other species.

Dicamba Experiment. Similar to the 2,4-D experiment, the mixture of glufosinate + glyphosate (in this experiment, glyphosate K) was considered antagonistic for pitted morningglory control when applied to 30-cm weeds (Table 9). Glufosinate + dicamba was antagonistic for both percent control and density reduction when applied to 30-cm weeds, but not 10-cm weeds. Glufosinate + dicamba was also antagonistic for Palmer amaranth control and density reduction, indicating this mixture may have reduced performance on broadleaf weeds relative to what would be expected based on the performance of the herbicides alone. It is not clear why dicamba may antagonize glufosinate, or vice versa, and more research is needed to identify a mechanism for such antagonism.

Barnyardgrass

2,4-D Experiment. All herbicide mixtures evaluated with Colby's method were antagonistic for barnyardgrass control and density reduction at both weed sizes (Table 10). All treatments were also antagonistic for height reduction, except for glufosinate + glyphosate and 2,4-D + glufosinate + glyphosate K + *S*-metolachlor applied to 10-cm weeds, which was additive. As explained previously, expected values for the treatments containing three and four herbicides were calculated for barnyardgrass from the two herbicides that have POST activity (glufosinate and glyphosate). When glyphosate DMA was applied as a premix with 2,4-D, a reduction in barnyardgrass control was observed relative to glyphosate DMA alone for both application timings (10- and 30-cm weeds) (O'Sullivan and O'Donovan 1980). The premix of glyphosate DMA + 2,4-D was also antagonistic for height and density reduction, but only for the 30-cm weeds application timing.

Dicamba Experiment. All herbicide mixtures evaluated with Colby's method were antagonistic for barnyardgrass control and height reduction (Table 11). All mixtures were also antagonistic for density reduction, except dicamba + glufosinate + glyphosate K + *S*-metolachlor, which was additive. Much like the antagonism observed with glyphosate DMA plus 2,4-D in the 2,4-D experiment, a reduction in barnyardgrass control was observed when glyphosate K + dicamba was applied to 30-cm weeds, compared to glyphosate K alone. Meyer et al. (2015b) observed a reduction in barnyardgrass control from mixtures glyphosate + dicamba. Both Flint and Barrett (1989) and O'Sullivan and O'Donovan (1980) identified antagonism of glyphosate + dicamba when applied to monocot species. Glyphosate K provided similar levels of control and density reduction as all other treatments when applied to 10-cm weeds, except for dicamba alone. However, when applied to 30-cm weeds, glyphosate K alone provided greater control (91%) and density reduction (86%) than all other treatments except for 2,4-D + glufosinate + glyphosate K + *S*-metolachlor.

Practical implications

Antagonism was identified on all three species investigated in this experiment (barnyardgrass, Palmer amaranth, and pitted morningglory), but was dependent upon the herbicide mixture, weed size, and parameter evaluated (e.g., weed density). More antagonistic mixtures were identified when applications were made to 30-cm weeds compared to 10-cm weeds (Burke et al. 2002; Miller et al. 2015). Antagonism was more common when the herbicide mixtures were evaluated on barnyardgrass, compared to the other species. Some mixtures resulted in a significant reduction in barnyardgrass control relative to one of its components (e.g., glyphosate + 2,4-D, glufosinate + glyphosate, and glufosinate + glyphosate + dicamba applied to 30-cm weeds).

The current experiments evaluated herbicides at field use rates, and obtained high levels of control for certain herbicides on a given species (e.g., glyphosate K 865 g ae ha^{-1} provided 91-96% barnyardgrass control). Colby (1967) explained that analyzing for herbicide interactions is better when the herbicides are applied alone at a dose that provides around 50% control. Riley and Shaw (1988) and Scott et al. (1998) showed that synergy is more likely when applied at reduced rates and herbicide interactions can vary for two herbicides when mixed at low rates compared to high rates. Other methods by such as that proposed by Streibig and Jensen (2000) and utilized by Wehtje and Gilliam (2015) likely provide a more robust analysis of how two herbicides behave in a plant when mixed. However, the purpose of the current experiments was to determine if mixtures at field use rates have reduced performance than expected (i.e., antagonism) and further research may be needed at reduced rates and various mixture ratios to fully understand how the herbicides evaluated behave in mixture.

Mixtures that compromise control of one species (e.g., barnyardgrass) in favor of improving control of another (e.g., Palmer amaranth) should be avoided to mitigate the likelihood of evolving resistance. Unfortunately, mixtures may often be needed to control both glyphosate-resistant Palmer amaranth and grass species in Enlist or Bollgard II Xtendflex in order to avoid complete reliance on glufosinate POST. For many mixtures, control was not greater than control with one of the component herbicides alone (e.g., glufosinate + 2,4-D vs. glufosinate alone on all species at the 10-cm size). However, it should be noted that when large weeds were present, glufosinate + 2,4-D provided better control of Palmer amaranth (99%) than either 2,4-D or glufosinate alone. Thus, to maximize the utility and efficiency of herbicide applications in both Enlist and RoundupReady Xtend technologies, herbicide treatments should be selected based on the weed spectrum and size of those weeds.

The optimum herbicide treatment for a given scenario will depend on the crop trait technology, weeds present, and weed size. If Palmer amaranth is the dominant weed, and barnyardgrass is also present but small in size, the preferred treatment is glufosinate (595 g ai ha^{-1}) + 2,4-D (1065 g ae ha^{-1}). Glufosinate + 2,4-D performed better than glufosinate alone on large Palmer amaranth, provided 2 sites of action (SOA) POST, and resulted in good control of small barnyardgrass. Weed management decisions are likely to be driven by Palmer amaranth because it is the most troublesome weed across the Midsouth (Van Wychen 2016) and has a rapid growth rate that can quickly overcome recommended weed sizes on herbicide labels (Horak and Loughin 2000; Sellers et al. 2003). Applying glufosinate + 2,4-D with a residual herbicide POST would further reduce the likelihood of resistance (Norsworthy et al. 2012) and is recommended for controlling any challenging weed species, such as Palmer amaranth.

If barnyardgrass, or another grass species, is the dominant weed in soybean or cotton field, the herbicide recommendation becomes more challenging. Glufosinate alone did not provide adequate control of large barnyardgrass, and 2,4-D has no POST activity on grass species. If no glyphosate-resistant weeds are present, an unlikely situation in the Midsouth, the recommended POST herbicide treatment would be glyphosate plus a residual herbicide because it would provide excellent control of large and small barnyardgrass. With the prevalence of glyphosate-resistance, a herbicide mixture, or sequential applications, will likely be needed to control a broad spectrum of weeds in the field. Although not evaluated in this experiment, sequential applications are a known strategy to overcome antagonism when two herbicides are mixed (Burke et al. 2003; Green 1989). Thus, if large grasses are present in the field and glyphosate-resistant weeds have not yet emerged, glyphosate plus a residual herbicide followed by glufosinate + 2,4-D 7-14 days later would likely provide excellent control of all species.

Glufosinate is an invaluable weed management tool in many current herbicide-resistant crop technologies for control of both grass and broadleaf weeds. In these experiments, glufosinate provided comparable barnyardgrass control to glyphosate K when applied to the recommended weed size (10-cm), although glyphosate alone was the preferred treatment when large (30-cm) barnyardgrass was present in the field. Due to the widespread occurrence of glyphosate-resistant weeds, the utility of glufosinate needs to be protected by utilizing Best Management Practices, as outlined by Norsworthy et al. (2012). However, the effectiveness of glyphosate on many grass species should not be ignored. In light of the antagonism identified in these experiments, both glufosinate and glyphosate need to be properly managed in the Enlist and RoundupReady Xtend technologies to enable effective weed control programs.

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Appendix

Table 1. Weed sizes and densities of barnyardgrass, Palmer amaranth, and pitted morningglory at both herbicide application timings in the 2,4-D experiment evaluated in 2015 and 2016.

	2015			2016		
	Height			Height		
Species	First[a]	Second	Density	First	Second	Density
	-------cm-------		plants m^{-2}	-------cm-------		plants m^{-2}
Barnyardgrass	11	29	5	8	25	24
Palmer amaranth	12	21	4	5	22	7
Pitted morningglory	15	25	4	6	19	1

[a] First and second application timing, to approximately 10 and 30-cm weeds, respectively

Table 2. Weed sizes and densities of barnyardgrass, Palmer amaranth, and pitted morningglory at both herbicide application timings in the dicamba experiment evaluated in 2015 and 2016.

	2015			2016		
	Height			Height		
Species	First[a]	Second	Density	First	Second	Density
	-------cm-------		plants m^{-2}	-------cm-------		plants m^{-2}
Barnyardgrass	11	32	8	8	25	22
Palmer amaranth	13	31	2	5	22	5
Pitted morningglory	13	33	2	6	19	2

[a] First and second application timing, to approximately 10 and 30-cm weeds, respectively

Table 3. Herbicide information for all products used in the 2,4-D and Dicamba Experiments

Herbicide common name	Herbicide Trade Name	Rate	Manufacturer	Address	Website	Adjuvant[b]
		g ai or g ae ha^{-1}				
Glyphosate	Durango					
Glufosinate	Liberty	594	Bayer CropScience LP	Research Triangle Park, NC	www.bayercropscienceus.com	
2,4-D	Weedar	1065	Nufarm Inc.	Burr Ridge, IL	www.nufarm.com/US/Home	
Glyphosate + 2,4-D	Enlist Duo	834[a] + 785[a]	Dow AgroSciences LLC	Indianapolis, IN	http://www.dowagro.com/en-US	
S-metolachlor	Dual Magnum	1068	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	
Glyphosate	Roundup PowerMax	867	Monsanto Company	St. Louis, MO	www.monsanto.com	
Dicamba	Clarity	560	BASF Corporation	Research Triangle Park, NC	www.basf.com	NIS

[a] Abbreviations: NIS, nonionic surfactant (Helena Chemical Company, Collierville, TN); MSO, methylated seed oil (Helena Chemical Company, Collierville, TN)

[b] Adjuvant rates: NIS, 0.25% v/v; MSO, 1% v/v

Table 4. Application dates, times, and weather conditions at the time of application for the Dicamba and 2,4-D Experiments.

		2,4-D Experiment				Dicamba Experiment			
Year	Timing	Application date	Time	Temp	RH	Application date	Time	Temp	RH
				C	%			C	%
2015	10-cm	July 16	8:30 AM	30	79	July 16	8:00 AM	36	77
	30-cm	July 28	10:00 AM	32	75	July 28	3:00 PM	35	76
2016	10-cm	June 29	8:45 AM	26	75	June 29	9:30 AM	26	75
	30-cm	July 18	1:30 PM	33	52	July 18	2:15 PM	33	52

Abbreviation: Temp, temperature; RH, relative humidity

Table 5. Variance components estimates obtained from the ANOVA for barnyardgrass, Palmer amaranth, and pitted morningglory control, height reduction, and density reduction for the 2,4-D and Dicamba Experiments[a].

		Barnyardgrass			Palmer amaranth			Pitted morningglory		
Experiment	Model effect	5 WAT	Height reduction	Density reduction	5 WAT	Height reduction	Density reduction	5 WAT	Height reduction	Density reduction
						% of total				
2,4-D	Rep(Year)	<0.1	<0.1	<0.1	4.0	10.2	8.6	5.4	2.4	1.1
	Year	18.5	16.3	2.6	1.0	23.3	2.5	2.5	14.5	9.2
	Residual	81.5	83.7	97.4	95.0	66.4	88.9	92.2	83.1	89.7
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Dicamba	Rep(Year)	<0.1	<0.1	<0.1	1.5	0.8	<0.1	<0.1	<0.1	<0.1
	Year	1.2	2.4	42.9	<0.1	0.7	1.5	16.6	<0.1	<0.1
	Residual	98.8	97.6	57.1	98.5	98.5	98.5	83.4	99.9	99.9
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Abbreviation: WAT, weeks after treatment.

Table 6. Palmer amaranth control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D Experiment.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Common name	Rate	Size	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[a]	10	31				13				27			
		30	20				10				6			
2,4-D	785[e]	10	89				72				85			
		30	83				77				85			
Glufosinate	595	10	96				79				95			
		30	87				72				88			
Glyphosate + 2,4-D	840[e] + 785[e]	10	94	NS	93	NS	81	NS	74	NS	94	NS	90	NS
		30	92	^	87	NS	81	NS	78	NS	89	NS	86	NS
Glufosinate + glyphosate	595 + 840[e]	10	97	NS	97	NS	66	NS	80	NS	93	NS	96	NS
		30	89	NS	89	NS	84	NS	76	NS	86	NS	89	NS
Glufosinate + 2,4-D	595 + 785[e]	10	97	NS	99	NS	70	NS	93	NS	96	NS	99	NS
		30	99	^	98	NS	89	NS	92	NS	99	^	98	NS
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	98	NS			89	NS			98	NS		
		30	95	^			94	NS			96	NS		
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[e] + 1390	10	98	NS			85	NS			99	NS		
		30	99	^			98	^			99	^		
LSD			6				18				10			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] A "^" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 7. Palmer amaranth control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the Dicamba Experiment.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Common name	Rate	Size	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	32				13				17			
		30	24				11				6			
Dicamba	560[e]	10	93				89				84			
		30	85				53				74			
Glufosinate	595	10	93				75				90			
		30	84				71				85			
Glyphosate + dicamba	865[e] + 560[e]	10	95	NS	97	NS	80	NS	90	NS	90	NS	84	NS
		30	87	NS	91	NS	71	NS	57	NS	83	NS	78	NS
Glufosinate + glyphosate	595 + 865[e]	10	94	NS	95	NS	73	NS	77	NS	84	NS	81	NS
		30	81	NS	89	*	74	NS	75	NS	79	NS	89	NS
Glufosinate + dicamba	595 + 560[e]	10	99	∧	99	NS	99	NS	98	NS	98	NS	93	NS
		30	89	NS	98	*	80	NS	93	NS	88	NS	98	*
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560	10	99	∧			99	NS			99	NS		
		30	92	∧			86	∧			90	NS		
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1390	10	100	∧			98	NS			99	NS		
		30	99	∧			99	∧			99	∧		
LSD			6				15				11			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; LSD, least significant difference

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 8. Pitted morningglory control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D Experiment.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Common name	Rate	Size	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[e]	10	84				68				81			
		30	65				30				47			
2,4-D	785[e]	10	94				98				98			
		30	91				97				98			
Glufosinate	595	10	97				97				94			
		30	95				96				94			
Glyphosate + 2,4-D	840[e] + 785[e]	10	98	NS	99	NS	98	NS	99	NS	97	NS	99	NS
		30	96	NS	96	NS	86	NS	96	NS	95	NS	98	NS
Glufosinate + glyphosate	595 + 840[a]	10	97	NS	99	NS	88	NS	97	NS	97	NS	98	NS
		30	91	NS	98	*	86	NS	90	NS	89	NS	96	*
Glufosinate + 2,4-D	595 + 785[e]	10	95	NS	99	NS	94	NS	99	NS	93	NS	99	NS
		30	95	NS	99	NS	94	NS	99	NS	96	NS	99	NS
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	97	NS			94	NS			94	NS		
		30	93	NS			92	NS			93	NS		
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[a] + 1390	10	95	NS			90	NS			94	NS		
		30	96	NS			89	NS			98	NS		
LSD			6				12				8			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; LSD, least significant difference

[b] NS indicates the mixture was similar to both of the herbicides alone based on the LSD.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation $[E = (X + Y) - (XY)/100]$. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 9. Pitted morningglory control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the Dicamba Experiment.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Common name	Rate	Size	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	87				66				85			
		30	72				54				57			
Dicamba	560[e]	10	92				91				91			
		30	90				88				89			
Glufosinate	595	10	95				99				99			
		30	89				97				99			
Glyphosate + dicamba	865[e] + 560[e]	10	96	NS	98	NS	90	NS	96	NS	91	NS	98	NS
		30	90	NS	92	NS	93	NS	95	NS	97	∧	95	NS
Glufosinate + glyphosate	595 + 865[e]	10	96	NS	99	NS	95	NS	99	NS	96	NS	99	NS
		30	87	NS	96	*	87	NS	98	NS	96	NS	99	NS
Glufosinate + dicamba	595 + 560[e]	10	98	NS	99	NS	97	NS	99	NS	99	NS	99	NS
		30	89	NS	98	*	94	NS	99	NS	94	NS	99	*
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560[e]	10	98	NS			97	NS			97	NS		
		30	94	NS			84	∨			94	NS		
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1390	10	97	NS			95	NS			97	NS		
		30	95	NS			96	NS			97	NS		
LSD			8				11				7			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; LSD, least significant difference

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 10. Barnyardgrass control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the 2,4-D Experiment.

Common name	Rate	Size	Control[ab]				Height reduction[abc]				Density reduction[abc]			
			Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	840[e]	10	97				74				97			
		30	93				72				95			
2,4-D	785[e]	10	0				0				9			
		30	0				3				5			
Glufosinate	595	10	96				66				92			
		30	84				57				82			
Glyphosate + 2,4-D	840[e] + 785[e]	10	91	∨			74	NS			94	NS		
		30	84	∨			53	∨			85	∨		
Glufosinate + glyphosate	595 + 840[e]	10	98	NS	99	*	74	NS	86	NS	91	NS	99	*
		30	87	∨	99	*	69	NS	88	*	95	NS	99	*
Glufosinate + 2,4-D	595 + 785[e]	10	94	NS			65	NS			92	NS		
		30	82	NS			57	NS			96	NS		
Glufosinate + glyphosate + 2,4-D	595 + 840[e] + 785[e]	10	94	NS	99	*	72	NS	86	*	88	∨	99	*
		30	84	∨	99	*	57	NS	88	*	88	∨	99	*
Glufosinate + glyphosate + 2,4-D + *S*-metolachlor	595 + 840[e] + 785[e] + 1390	10	93	NS	99	*	78	NS	86	NS	95	NS	99	*
		30	91	NS	99	*	65	NS	88	*	86	∨	99	*
LSD			5				16				7			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; LSD, least significant difference

[b]. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 11. Barnyardgrass control 5 weeks after treatment (WAT), height reduction, and density reduction as affected by herbicide treatment and weed size for the Dicamba Experiment.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Common name	Rate	Size	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	cm	%		%		%		%		%		%	
Glyphosate	865[e]	10	96				74				92			
		30	91				71				86			
Dicamba	560[e]	10	0				2				4			
		30	0				4				8			
Glufosinate	595	10	95				66				84			
		30	78				57				63			
Glyphosate + dicamba	865[e] + 560[e]	10	93	NS			52	∨			86	NS		
		30	82	∨			64	NS			68	∨		
Glufosinate + glyphosate	595 + 865[e]	10	95	NS	99	*	60	∨	92	*	86	NS	98	*
		30	85	∨	98	*	69	NS	87	*	66	∨	92	*
Glufosinate + dicamba	595 + 560[e]	10	95	NS			74	NS			93	NS		
		30	82	NS			67	NS			60	NS		
Glufosinate + glyphosate + dicamba	595 + 865[e] + 560	10	95	NS	99	*	64	NS	92	*	89	NS	98	*
		30	85	∨	98	*	61	NS	87	*	63	∨	92	*
Glufosinate + glyphosate + dicamba + S-metolachlor	595 + 865[e] + 560[e] + 1390	10	95	NS	99	*	63	NS	92	*	86	NS	98	*
		30	89	NS	97	*	72	NS	87	*	80	NS	92	NS
LSD			5				11				14			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; LSD, least significant difference

[b] A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Chapter 5

Herbicide interactions between glufosinate and three fomesafen-containing products as affected by weed and droplet size

Although protoporphyrinogen oxidase (PPO)-inhibitor resistant weeds are spread across the Midwest and Midsouth, fomesafen, and other PPO-inhibiting herbicides, are still commonly applied in soybean. In LibertyLink soybean production, a fomesafen-containing herbicide is often mixed with glufosinate and applied POST. However, research has not been conducted to determine if mixtures of glufosinate and fomesafen are antagonistic, specifically when applied to grass species. An experiment was conducted at the Northeast Research and Extension Center in Keiser, Arkansas, to evaluate mixtures of glufosinate and three fomesafen-containing products for weed control and herbicide interactions using Colby’s method. Glufosinate was applied at two rates (451 and 595 g ai ha^{-1}) alone or in mixture with fomesafen (Reflex® or Flexstar® herbicides) and a premix of fomesafen + *S*-metolachlor (Prefix® herbicide) on two weed sizes (10- and 30-cm). All mixtures of glufosinate plus a fomesafen product, regardless of weed size, resulted in ≥96% control of PPO inhibitor-susceptible Palmer amaranth. All mixtures provided ≥90% control of prickly sida, however, antagonism was identified for glufosinate (451 g ai ha^{-1}) + Flexstar herbicide, glufosinate (451 g ai ha^{-1}) + Reflex herbicide, and glufosinate (595 g ai ha^{-1}) + Reflex herbicide at the 10-cm weed size. The addition of a fomesafen product to glufosinate had a negligible effect on control of barnyardgrass and tended to improve control of large crabgrass when compared to glufosinate alone, applied to 30 cm weeds. Most of the interactions between glufosinate and fomesafen were additive and it did not appear that one herbicide was negatively affecting the activity of the other. A premix of fomesafen + *S*-metolachlor tended to provide better POST activity than either Reflex or Flexstar herbicides, whether alone, or in mixture with glufosinate. The improvement when Prefix herbicide was used may partially be explained by droplet size; fomesafen + *S*-metolachlor produced the smallest Dv_{50} (245 µm), compared to 289 and 303 µm, for Flexstar and Reflex herbicides, respectively. The trends in droplet size observed with the fomesafen products alone held true when compared as mixtures with glufosinate. Smaller droplet sizes tend to improve efficacy of contact herbicides such as glufosinate and fomesafen. Therefore, Prefix herbicide would be the preferred partner for glufosinate to maximize weed efficacy and has the added benefit of multiple sites of action for residual weed control.

Nomenclature: fomesafen; glufosinate; *S*-metolachlor; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; large crabgrass, *Digitaria sanguinalis* L.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; prickly sida, *Sida spinosa* L.

Key words: Antagonism, barnyardgrass, glufosinate + fomesafen, glufosinate + fomesafen + S-metolachlor, Palmer amaranth, herbicide interactions

Research into the confirmation and control of many glyphosate-resistant Palmer amaranth populations determined many protoporphyrinogen oxidase (PPO)-inhibiting herbicides, such as fomesafen, still provided excellent control (Chahal et al. 2017; Nandula et al. 2012). As a result, adoption of PPO-inhibiting herbicides into weed management programs became a common recommendation in glyphosate-resistant soybean (Owen and Zelaya 2005). In glufosinate-resistant soybean, a PPO-inhibitor, such as fomesafen, can be mixed with glufosinate to provide multiple effective sites of action POST to reduce the likelihood of evolving herbicide resistance (Norsworthy et al. 2012).

Prior to the widespread identification of PPO inhibitor-resistant (Salas et al. 2017; Varanasi et al. 2018; Heap 2018), recommendations for LibertyLink soybean systems across the Midsouth included an early POST application of glufosinate plus a fomesafen-containing product, such as Flexstar or Prefix herbicide (Scott et al. 2017). Glufosinate + fomesafen is effective on Palmer amaranth and other broadleaf weeds, but may not achieve the same levels of control on grass species (Culpepper et al. 2000; Scott et al. 2017). Culpepper et al. (2000) showed the addition of fomesafen to glufosinate either increased, or did not change, control of many grass and broadleaf weeds [e.g., broadleaf signalgrass (*Urochloa platyphylla* Griseb.) and common lambsquarters (*Chenopodium album* L.)], however, the levels of control on the grass weeds were not always acceptable. Beyers et al. (2002) reported foxtail biomass was greater when lactofen, another PPO-inhibiting herbicide, was added to glufosinate, indicating possible antagonism. However, no differences between visual control were detected and no herbicide interaction analysis was conducted.

Colby’s method (Colby 1967) is a common procedure used to investigate herbicide interactions, and is well suited for evaluating mixtures of many products in the field. Glufosinate and fomesafen are both considered contact-type herbicides (vs. systemic or translocated herbicides); however, mixtures of two contact herbicides can still result in antagonism. Zhang et al. (1995) compiled herbicide interaction results from 479 previously published cases and determined that an antagonistic interaction was just as likely for a combination of herbicides with similar transport mechanisms (i.e., both contact herbicides) as a mixture of a systemic and a contact herbicide.

Many fomesafen products are commercially available. Both Reflex® and Flexstar® herbicides contain the sodium salt formulations of fomesafen and are recommended for use PRE and POST in soybean. Prefix® herbicide also contains the sodium salt of fomesafen and is a premix of fomesafen and *S*-metolachlor. Reflex has a slightly higher concentration of the sodium salt of fomesafen than Flexstar (240 g L^{-1} vs. 226 g L^{-1}), includes a small amount of a preservative (1,2-benzisothiazolin-3-one), and is generally recommended as a PRE in soybean, whereas Flexstar is typically sprayed POST (Scott et al. 2017). The formulations vary between Flexstar, Prefix, and Reflex herbicides, and a change in the adjuvant component of the formulation can impact herbicide efficacy (Kudsk and Mathiassen 2004). Furthermore, Nalewaja and Matysiak (1992) demonstrated identification of herbicide interactions can be dependent upon the formulation of the herbicides used.

When considering applications of contact herbicides, such as glufosinate and fomesafen, droplet size is an important consideration for maximizing efficacy. Glufosinate is known to perform better when applied with nozzles with a medium to coarse droplet designation compared to an ultra-coarse (Etheridge et al. 2001; Meyer et al. 2015a). Applications of fomesafen and lactofen appear to be less sensitive to changes in droplet size compared to glufosinate, as the impact of nozzle selection seems appears to depend on species for the PPO-inhibiting herbicides

(Berger et al. 2014; Sikkema et al. 2008). The formulation itself can also influence the droplet size for different herbicide products containing the same active ingredient (Mueller and Womac 1997). Thus, a change in droplet size as a result of mixing two herbicides could influence any potential antagonistic interactions.

Weed size influences efficacy of both glufosinate and PPO-inhibiting herbicides, with control declining when weeds are taller (Lee and Oliver 1982; Steckel et al. 1997; Wilson 2005). Antagonism can more easily be identified on large weeds compared to small weeds (Burke et al. 2002; Miller et al. 2015), typically because larger weeds are more likely to survive the application. The objective of this experiment was to determine the impact of weed size and fomesafen product on herbicide interactions between glufosinate and fomesafen. Of primary interest was the impact various formulations of fomesafen may have on the droplet size and subsequent identification of herbicide interactions with glufosinate.

Materials and Methods

Field experiments were conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR to evaluate the interaction between glufosinate and various fomesafen-containing products for control of small (10-cm) and large (30-cm) grass and broadleaf weeds. Plots 3.9 by 9.1 m were established on a Sharkey clay (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept), with a pH 6.5, and 1.7% organic matter. Each treatment was replicated four times in a given year. HBK 4950 LibertyLink soybean was planted at a rate of 313,000 seeds ha^{-1} on June 17, 2015 and June 10, 2016. Plots were furrow irrigated to soil saturation as needed throughout the growing season. Fertilizer and lime were applied based on a soil test and according to University of Arkansas recommendations.

Twelve herbicide treatments were applied at two weed sizes, heights of approximately 10 and 30-cm (Table 1). For continuity, the two application timings will be referred to as 10 and 30-cm weeds. Soybean stages were V4-V5 at the first application and V7-V8 at the second application in both years. Glufosinate (Liberty herbicide, Bayer CropScience, Research Triangle Park, NC) was applied alone at two rates (450 and 595 g ai ha^{-1}) and in combination with two formulations of fomesafen (Flexstar herbicide and Reflex herbicide, Syngenta Crop Protection LLC., Greensboro, NC), and one premix of fomesafen + *S*-metolachlor (Prefix Herbicide, Syngenta Crop Protection LLC., Greensboro, NC). Additionally, a nonionic surfactant (NIS) at 0.25% (v v^{-1}) (Induce, Helena Chemical Company, Collierville, TN) was added to all treatments that contained Flexstar or Reflex herbicides, as recommended by the product labels (Anonymous 2016a, 2016b). Any reference to Flexstar or Reflex herbicides alone or in mixture refers to a solution with NIS. Herbicide rates were selected based on those recommended on the product labels, and were not rates that resulted in equal amounts of fomesafen being applied (Table 2).

Applications were made at 10:00 AM on July 16 and 5:00 PM on July 28 in 2015. In-field assessments at the time of application recorded a temperature of 32 C and 75% relative humidity for the first application and 36 C with 74% relative humidity for the second application in 2015. In 2016, applications were made at 7:30 AM on July 7 and 2:00 PM July 19. Temperatures were 29 and 25 C with a relative humidity of 75 and 49% for the first and second applications in 2016, respectively. A CO_2-pressurized backpack sprayer was used to make all herbicide applications calibrated to deliver 140 L ha^{-1} spray volume at 276 kPa at 4.8 km hr^{-1} through nozzles spaced 51 cm apart. The boom was equipped with Turbo TeeJet (TT) 110015 nozzles. (TeeJet Technologies, Springfield, Illinois). One day following the application of the herbicide treatments, all plots received an application of *S*-metolachlor (Dual Magnum®, Syngenta Crop Protection LLC., Greensboro, NC), except for those that already received an application as part of the experimental treatment.

Weed control ratings were collected 4 weeks after treatment (WAT) for barnyardgrass, Palmer amaranth, prickly sida, and large crabgrass. The Palmer amaranth population was a glyphosate- and acetolactate synthase-inhibitor-resistant population and was still sensitive to PPO-inhibiting herbicides, including fomesafen. Weed control was visually evaluated on a scale of 0 (no control) to 100% (complete death of all plants) relative to the nontreated check. Weed height and density data were collected 4 WAT. At the end of the season, plots were machine harvested and yield data collected. Soybean yields from each plot were corrected to 13% moisture.

Colby's method (Colby 1967) was used to evaluate data for herbicide mixture interactions. Colby's method uses an equation to calculate an Expected Value (E) as shown in Equation 1,

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

where E is the expected level of control of two herbicides mixed together, and variables X and Y represent the level of control provided by each herbicide applied individually. The observed value for the mixture was compared to the E calculated for that mixture using a two-sided t-test ($\alpha = 0.05$). If the t-test was significant, and E was greater than the observed value for a given mixture, it was deemed antagonistic. When E was less than the observed value, the mixture was considered synergistic and when no difference between E and observed was identified, additive. Some mixtures included three herbicides, one being *S*-metolachlor as part of a premix of fomesafen + *S*-metolachlor. As *S*-metolachlor is considered to have no POST activity, the calculation of E for the mixture proceeded as if the premix was a single product.

A low-speed wind tunnel located at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE was used to analyze the droplet spectra for herbicide treatments used in the field experiment. The wind tunnel was equipped with a Sympatec Helos Vario KR particle-size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) which utilized a laser and R7 lens with a particle size detection range from 18 to 3,500 μm. The nozzle was attached 30-cm from the laser and width of the nozzle plume was moved across the laser via a linear actuator. The tunnel was set to produce a wind speed of 24 km h^{-1} to mitigate spatial sampling bias, and each herbicide treatment was replicated three times. The volume median diameter (D_{v50}) was determined for each treatment as well as the D_{v10}, D_{v90}, relative span (RS), and the percentage of fine droplets. The D_{v50} is the droplet diameter below which 50% of the liquid volume is contained in droplets smaller than that value and the D_{v10} and D_{v90} are similar values for 10% and 90% of the volume, respectively. The percentage of fine droplets in this experiment was considered a fraction of the total volume of the spray containing droplets with a diameter <150 μm ($\%_{vol}$ fines). The range in droplet sizes is typically described with the relative span (RS) calculated using Equation 2.

$$\mathrm{RS} = (D_{\mathrm{v}90} - D_{\mathrm{v}10})\ D_{\mathrm{v}50}^{-1} \qquad [2]$$

Data were subject to an analysis of variance (ANOVA) using JMP 13 (SAS Institute Inc., Cary, NC), and means were separated using Fisher's protected last significant difference (LSD) ($\alpha = 0.05$). The statistical model included replication and year as random effects (Table 3). Both Colby's method the ANOVA were used to interpret the data. The results from the ANOVA were used to directly compare a mixture to its component herbicides, whereas Colby's method is comparing the mixture to the calculated Expected value for that mixture (e.g., Table 2). The experimental design for the particle-size data did not include a blocking factor and a more-conservative Tukey adjustment ($\alpha = 0.05$) was used to identify differences between the means.

Results and Discussion

Palmer amaranth. All treatments of glufosinate and fomesafen-products alone or in combination provided greater than 96% and 88% control 4 weeks after treatment (WAT) of 10 and 30-cm Palmer amaranth, respectively (Table 2). Seven treatments, all of which were mixtures of glufosinate plus a fomesafen product, provided 100% Palmer amaranth control, but as a result, the control, height reduction, and density reduction data did not meet the assumptions of ANOVA. Therefore, no ANOVA was conducted for these data. Instead, means for all treatments are presented in Table 2 and include a standard error for reference. The Palmer amaranth population evaluated in this study was sensitive to PPO-inhibiting herbicides, and all three fomesafen products alone resulted in ≥93% control.

Prickly sida. In general, fomesafen was not effective at controlling prickly sida, whereas all treatments that contained glufosinate provided ≥87% control 4 WAT (Table 4). No fomesafen product alone applied to small (~10-cm) prickly sida provided more than 40% control, 42% height reduction, or 51% density reduction 4 WAT. Control with glufosinate at 451 g ai ha^{-1} was 87% when applied to large (~30-cm) prickly sida, and all mixtures of glufosinate plus a fomesafen product provided ≥90% control of prickly sida.

Antagonism was noted for three treatments applied to small prickly sida: glufosinate (451 g ai ha^{-1}) + Flexstar herbicide, glufosinate (451 g ai ha^{-1}) + Reflex herbicide, and glufosinate (595 g ai ha^{-1}) + Reflex herbicide (Table 4). Although the mixtures of glufosinate plus a premix of fomesafen + *S*-metolachlor did not provide greater control than other mixtures, all observed values for glufosinate + fomesafen + *S*-metolachlor were greater than, or equal to, expected values (i.e., no antagonism was identified). The premix of fomesafen + *S*-metolachlor tended to provide superior control compared to the other fomesafen products, whether alone or in mixture, indicating the premix may be the best product to utilize when applying fomesafen in regards to resistance management.

Barnyardgrass. Fomesafen products alone only provided suppression of 10- and 30-cm tall barnyardgrass at 4 WAT (28 to 46% control), while glufosinate alone controlled barnyardgrass 84 to 96% (Table 5). No differences in control were observed between any mixtures of glufosinate plus a fomesafen product and the equivalent rate of glufosinate alone. Two treatments, glufosinate at 451 g ai ha^{-1} + Flexstar herbicide, and glufosinate at 451 g ai ha^{-1} + Reflex herbicide, showed significantly greater height reduction on 10-cm tall barnyardgrass compared to glufosinate alone, but was not reflected in the control ratings. As such, fomesafen does not appear to antagonize glufosinate activity on barnyardgrass, regardless of weed size. Only one case of antagonism was identified across all parameters. Height reduction for glufosinate (451 g ai ha^{-1}) + Flexstar on 30-cm barnyardgrass was antagonistic, but control and density reduction for the same treatment were considered additive.

Large crabgrass. Stark reductions in large crabgrass control were observed when the same treatment was applied to 30-cm large crabgrass, compared to 10-cm (Table 6). For example, large crabgrass control with glufosinate at 595 g ai ha^{-1} was 95% 4 WAT, whereas the same treatment only provided 67% control when the application was made to 30-cm large crabgrass.

Monks and Schultheis (1998) also reported the ability to control large crabgrass is diminished after it begins to form adventitious roots at the stem internodes.

Large crabgrass evaluations generally responded positively when a fomesafen product was added to glufosinate. Control with glufosinate (595 g ai ha^{-1}) + fomesafen + *S*-metolachlor was 82% compared to 70% with glufosinate alone. When applied to 10-cm large crabgrass, a synergistic response was detected for height reduction for all mixtures of glufosinate plus a fomesafen product. However, neither percent control nor density reduction detected a synergistic response on 10-cm large crabgrass. These findings are likely due to the overall high levels of control (≥94%) and density reduction (96%) that were observed when applications were made to 10-cm weeds. Height assessments were only collected on plants that survived the application, and it is possible the addition of fomesafen to glufosinate inhibited the survivors' ability to regrow new tissue compared to glufosinate alone.

Droplet Parameters. The droplet spectra analysis (Table 7) provides some insight to the differing performance between fomesafen products and herbicide treatments on weed control. Of the fomesafen products alone, the premix of fomesafen + *S*-metolachlor generally provided superior control to Reflex herbicide and was either equal to, or greater than, Flexstar herbicide. This generalization correlates with the droplet data, where the premix of fomesafen + *S*-metolachlor, Flexstar herbicide, and Reflex herbicide had D_{v50} values of 245, 289, and 303 μm, respectively.

Mixtures of glufosinate and fomesafen-containing products produced similar or significantly smaller droplets in comparison to the components alone. For example, glufosinate (451 g ai ha^{-1}) plus Flexstar herbicide had a volume median diameter (D_{v50}) of 283 μm which was equivalent to Flexstar herbicide alone (289 µm) and less than glufosinate alone (296 µm), although this subtle difference may not be sufficient for a biological response. A premix of fomesafen + *S*-metolachlor produced the smallest droplets (D_{v50}=245 µm) and was equal to mixtures with glufosinate that included the premix, as well as glufosinate + *S*-metolachlor.

In general, treatments that produced the smallest D_{v50} also produced the greatest number of fine droplets ($\%_{vol}$ fines), although mixtures of glufosinate + Reflex herbicide had $\%_{vol}$ fines equal to that of the treatments that contained *S*-metolachlor (either in Prefix or Dual herbicides). It is possible for one treatment to produce a similar number of percent fines as another and have a smaller D_{v50}. The relative span (RS) of a treatment is a unitless index that represents the range or spread in droplet sizes for a given treatment and can explain discrepancies between D_{v50} and $\%_{vol}$ fines. For example, glufosinate (451 g ai ha^{-1}) + Reflex herbicide produced a D_{v50}=270 µm, $\%_{vol}$ fines=16.8, and RS=1.30, compared to glufosinate (451 g ai ha^{-1}) + fomesafen + *S*-metolachlor with a D_{v50}=241 µm, $\%_{vol}$ fines=16.8, and RS=1.07. In regards to efficacy, an ideal mixture of two contact herbicides would result in a smaller D_{v50}, larger $\%_{vol}$ fines, and a narrow relative span, indicating the mixture is producing smaller and more uniform droplets than either of its components.

Although the droplet data may explain some of the differences observed between the fomesafen products on weed control, it is not the only contributing factor. For example, Flexstar herbicide had a droplet spectra closer to that of Reflex than the fomesafen + *S*-metolachlor premix. Thus, differences between Reflex and Flexstar may also be associated with the adjuvant system in each product. When considering Prefix herbicide, *S*-metolachlor unlikely impacted efficacy, as all plots that did not already have an application of *S*-metolachlor had it applied 24 h later. In addition to the droplet size, the most likely explanation for the differences between fomesafen products is the adjuvant system associated with the product itself (Kudsk and Mathiassen 2004). Although few differences were observed between fomesafen products when they were mixed with glufosinate, the premix of fomesafen + *S*-metolachlor appears to be the better mixture partner with glufosinate in regards to optimizing spray droplet parameters for efficacy, and has the added benefit of already including *S*-metolachlor. If spray drift is a concern, large droplets with a smaller $\%_{vol}$ fines is preferred, and Flexstar herbicide would be a better mix partner than a premix of fomesafen + *S*-metolachlor in such cases.

Grain Yield. Overall, soybean yield was greatest for treatments that provided superior control of all species. The interaction between herbicide and weed size and the main effect of weed size was not significant in the ANOVA model at $\alpha = 0.050$ ($p=0.0730$ and 0.2973 for the interaction and weed size main effect, respectively). Only the main effect of herbicide was interpreted for grain yield (Table 8). Overall, the presence of glufosinate was the most important factor for maximizing grain yield. The treatment of glufosinate alone (451 g ai ha^{-1}) produced 3286 kg ha^{-1} of soybean, averaged over weed sizes, was not different from any of the mixtures with fomesafen products. The lowest yields were obtained from treatments composed of only a fomesafen-product. No differences in yield were identified between Flexstar, Prefix, and Reflex herbicides, averaged over weed size. The lowest yields from the fomesafen-only treatments were likely due to the intense grass pressure (Table 1) and lack control of barnyardgrass, large crabgrass, and prickly sida with those treatments. One interesting difference was obtained with mixtures of Flexstar herbicide with the high and low rates of glufosinate; a significantly greater yield (3,340 kg ha^{-1}) was obtained when glufosinate at 595 g ai ha^{-1} was mixed with Flexstar herbicide (264 g ai ha^{-1} fomesafen) compared to the mixture with the low rate (451 g ai ha^{-1}) of glufosinate (3,170 kg ha^{-1}). The improved yield from the higher rate of glufosinate in mixture highlights the importance of using full herbicide rates, even in mixtures (it should be noted that both 451 and 595 g ai ha^{-1} are labeled use rates).

Practical Implications

Fomesafen does not appear to be interacting negatively with activity of glufosinate on grass species. In fact, the addition of a fomesafen product to glufosinate appeared to improve control of large crabgrass compared to glufosinate alone. Even so, the highest rates of both herbicides should be used to maximize control and reduce the likelihood of yield loss. No severe cases of antagonism or synergism were identified in this experiment, although the identification of an herbicide interaction does depend on weed size and parameter investigated. Overall, the results from these experiments agree with the findings of Culpepper et al. (2000), that the addition of fomesafen does not antagonize the efficacy of glufosinate.

Even though the prevalence of PPO-inhibitor resistant (PPO-resistant) Palmer amaranth populations across the Midsouth may diminish applications of currently labeled PPO-inhibiting herbicides, chemical companies are bringing new PPO-inhibitors to market (Armel et al. 2018). Furthermore, Umphres et al. (2018) determined soil-applied PPO-inhibiting herbicides, including fomesafen, still have activity on a PPO-resistant Palmer amaranth population. Even if fomesafen lost some of its utility as a POST herbicide on PPO-inhibitor resistant populations, it may still provide value as a POST residual option, in addition to other species it may control POST.

Because of the presence of Palmer amaranth populations resistant to two or more sites of action (e.g., ALS-, EPSPS-, PPO-inhibitor; ALS and EPSPS; EPSPS- and PPO-inhibitor) (Heap 2018; Schwartz-Lazaro 2017), fomesafen may still be of some value in soybean production. Fomesafen does not appear to severely antagonize the activity of glufosinate on grass or broadleaf weeds and can improve control when mixed with glufosinate, compared to glufosinate alone. Furthermore, the residual activity of fomesafen on PPO-resistant Palmer amaranth may reduce the selection pressure on very long chain fatty acid synthesis-inhibitors (e.g., *S*-metolachlor) which are frequently applied PRE and POST in soybean.

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Appendix

Table 1. Weed sizes and densities of four weed species at both herbicide application timings evaluated in 2015 and 2016 in Keiser, AR.

	2015			2016		
	Height			Height		
Species	First[a]	Second	Density	First	Second	Density
	-------cm-------		plants m^{-2}	-------cm-------		plants m^{-2}
Palmer amaranth	13	25	1	9	22	8
Prickly Sida	5	18	6	3	12	1
Barnyardgrass	10	35	9	10	26	20
Large crabgrass	10	31	2	9	22	3

[a] First and second application timing, to approximately 10 and 30-cm weeds, respectively

Table 2. The effect of weed size and mixtures of glufosinate plus fomesafen-containing products on Palmer amaranth control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab]

Product name	Common name	Rate	Size	Control		Height reduction		Density reduction	
		g ai ha^{-1}	cm	%	SE	%	SE	%	SE
Liberty	Glufosinate	451	10	99	1	90	7	97	2
			30	89	3	86	14	96	4
Liberty	Glufosinate	595	10	99	1	92	8	93	6
			30	95	2	79	14	85	12
Flexstar	Fomesafen	264	10	98	3	74	17	86	12
			30	93	3	54	18	76	19
Reflex	Fomesafen	280	10	98	1	73	14	92	4
			30	93	3	63	14	93	3
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	10	97	2	75	16	95	4
			30	95	2	60	16	90	4
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	10	99	1	90	8	98	2
			30	96	1	87	12	90	10
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	10	100	0	100	0	100	0
			30	100	0	100	0	100	0
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	10	100	0	100	0	100	0
			30	96	2	87	13	96	4
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	10	100	0	100	0	100	0
			30	98	2	87	11	90	10
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	10	100	0	100	0	100	0
			30	97	2	89	7	79	19
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	10	100	0	100	0	100	0
			30	100	0	100	0	100	0
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	10	99	1	88	10	95	4
			30	93	2	93	8	98	2

[a] Data did not meet the assumptions of ANOVA and are reported as means followed by the standard error (SE) of the mean.

[b] Height and density reduction is expressed as a percent of the nontreated control.

Table 3. Variance components estimates obtained from the ANOVA for barnyardgrass, Palmer amaranth, prickly sida, large crabgrass control, hiehgt reduction, and density reduction, and soybean yield.

	Barnyardgrass			Palmer amaranth			Prickly sida			Large crabgrass			
Model effect	4 WAT	Height redn	Density redn	4 WAT	Height redn	Density redn	4 WAT	Height redn	Density redn	4 WAT	Height redn	Density redn	Soybean yield
	---% of total---												
Year	47.0	<0.1	25.3	<0.1	<0.1	0.5	7.5	13.7	1.9	<0.1	3.6	23.4	49.9
Rep(Year)	5.6	1.8	3.9	0.6	2.9	3.3	<0.1	1.5	2.2	1.5	2.4	2.9	6.2
Residual	47.4	98.2	70.8	99.4	97.1	96.1	92.5	84.9	95.9	98.4	93.9	73.7	43.9
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0

Abbreviation: Redn, reduction; WAT, weeks after treatment

Table 4. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on prickly sida control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab]

Product name	Common name	Rate	Size	Control[c]				Height Reduction				Density reduction			
				Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
		g ai ha^{-1}	cm	%		%		%		%		%		%	
Liberty	Glufosinate	451	10	97				75				95			
			30	87				67				82			
Liberty	Glufosinate	595	10	99				88				97			
			30	90				85				83			
Flexstar	Fomesafen	264	10	36				32				39			
			30	30				34				24			
Reflex	Fomesafen	280	10	28				17				46			
			30	21				24				22			
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	10	40				35				51			
			30	25				42				30			
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	10	92	NS	98	*	86	NS	88	NS	93	NS	98	NS
			30	92	NS	90	NS	73	NS	78	NS	83	NS	86	NS
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	10	98	NS	99	NS	95	NS	91	NS	97	NS	99	NS
			30	94	NS	93	NS	87	NS	90	NS	84	NS	86	NS
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	10	93	NS	97	*	85	NS	79	NS	94	NS	97	NS
			30	95	NS	89	NS	74	NS	76	NS	87	NS	85	NS
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	10	93	NS	99	*	95	NS	90	NS	88	NS	98	*
			30	94	NS	92	NS	86	NS	88	NS	84	NS	87	NS
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	10	98	NS	98	NS	94	^	84	NS	92	NS	97	NS
			30	90	NS	90	NS	71	NS	81	NS	87	NS	88	NS
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	10	99	NS	99	NS	93	NS	92	NS	97	NS	99	NS
			30	94	NS	92	NS	89	NS	91	NS	93	NS	88	NS

Table 4. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on prickly sida control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab] (Cont.)

				Control[c]				Height Reduction				Density reduction			
Product name	Common name	Rate	Size	Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
		g ai ha^{-1}	cm	%		%		%		%		%			
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	10	95	NS			85	NS			96	NS		
			30	90	NS			62	NS			75	NS		
	LSD			9				13				13			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant

[b] Height and density reduction is expressed as a percent of the nontreated control.

[c] A “^” indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 5. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on barnyardgrass control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab]

Product name	Common name	Rate	Size	Control[c]				Height reduction				Density reduction			
				Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
		g ai ha^{-1}	cm	%		%		%		%		%		%	
Liberty	Glufosinate	451	10	95				68				95			
			30	84				73				90			
Liberty	Glufosinate	595	10	96				88				96			
			30	88				73				91			
Flexstar	Fomesafen	264	10	36				14				56			
			30	42				17				44			
Reflex	Fomesafen	280	10	28				17				56			
			30	34				23				41			
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	10	41				18				53			
			30	46				16				39			
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	10	95	NS	97	NS	84	∧	83	NS	95	NS	98	NS
			30	85	NS	91	NS	69	NS	77	*	87	NS	94	NS
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	10	98	NS	97	NS	94	NS	91	NS	97	NS	97	NS
			30	90	∧	92	NS	69	NS	76	NS	87	NS	95	NS
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	10	96	NS	97	NS	84	∧	75	NS	97	NS	97	NS
			30	86	NS	89	NS	66	NS	78	NS	86	NS	95	NS
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	10	98	NS	97	NS	95	NS	91	NS	99	NS	98	NS
			30	88	NS	92	NS	79	NS	79	NS	91	NS	95	NS
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	10	99	NS	97	NS	81	NS	75	NS	98	NS	98	NS
			30	89	NS	91	NS	63	NS	76	NS	89	NS	94	NS
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	10	99	NS	98	NS	91	NS	91	NS	98	NS	98	NS
			30	91	∧	93	NS	77	NS	77	NS	86	NS	94	NS

Table 5. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on barnyardgrass control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab] (Cont.)

Product				Control[c]				Height reduction				Density reduction			
name	Common name	Rate	Size	Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	10	96	NS			86	^			97	NS		
			30	88	NS			65	NS	35		86	NS		
	LSD			6				15				14			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant

[b] Height and density reduction is expressed as a percent of the nontreated control

[c] A "^" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 6. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on large crabgrass control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab]

				Control[c]				Height Reduction				Density reduction			
Product name	Common name	Rate	Size	Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
		g ai ha^{-1}	cm	%		%		%		%		%		%	
Liberty	Glufosinate	451	10	95				53				93			
			30	67				52				51			
Liberty	Glufosinate	595	10	98				51				95			
			30	70				53				46			
Flexstar	Fomesafen	264	10	41				15				32			
			30	22				14				24			
Reflex	Fomesafen	280	10	34				10				40			
			30	13				14				28			
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	10	44				9				38			
			30	20				18				16			
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	10	95	NS	98	NS	85	∧	54	*	98	NS	97	NS
			30	76	∧	75	NS	59	NS	58	NS	54	NS	61	NS
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	10	97	NS	99	NS	79	∧	58	*	98	NS	96	NS
			30	81	∧	76	NS	65	NS	60	NS	50	NS	60	NS
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	10	94	NS	96	NS	85	∧	58	*	96	NS	95	NS
			30	77	∧	73	*	52	NS	58	NS	51	NS	61	NS
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	10	96	NS	99	NS	72	∧	56	NS	97	NS	97	NS
			30	81	∧	75	*	61	NS	59	NS	47	NS	61	NS
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	10	98	NS	97	NS	98	∧	57	*	99	NS	96	NS
			30	78	∧	74	NS	61	NS	60	NS	51	NS	55	NS
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	10	95	NS	99	NS	88	∧	55	*	97	NS	97	NS
			30	82	∧	76	*	59	NS	57	NS	59	NS	54	NS

Table 6. The effect of weed size and mixtures of glufosinate plus fomesafen-containing product on large crabgrass control, height reduction, and density reduction at 4 weeks after treatment in 2015 and 2016 in Keiser, AR.[ab] (Cont.)

				Control[c]				Height Reduction				Density reduction			
Product name	Common name	Rate	Size	Obs		Exp[d]	p	Obs		Exp	p	Obs		Exp	p
		g ai ha^{-1}	cm	%		%		%		%		%		%	
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	10	97	NS			58	NS			97	NS		
			30	70	NS			42	NS			48	NS		
	LSD			6				15				14			

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant

[b] Height and density reduction is expressed as a percent of the nontreated control

[c] A “^” indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 7. Spray characteristics of various herbicide combinations for glufosinate, three fomesafen products, and various mixtures used in the field experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm.

Product name	Herbicide treatment	Rate	Droplet spectra parameters[a]				
			D_{v10}	D_{v50}	D_{v90}	Relative span[b]	<150 µm
		g ai ha^{-1}	----------------µm----------------			-	$\%_{vol}$
	Water		143 a	307 a	488 a	1.12 de	11.1 de
Liberty	Glufosinate	451	136 ab	296 bc	478 ab	1.15 cde	12.4 bc
Liberty	Glufosinate	595	126 cd	280 de	470 ab	1.23 abc	14.9 ab
Flexstar	Fomesafen	264	141 a	289 cd	462 b	1.11 de	11.7 ab
Reflex	Fomesafen	280	140 ab	303 ab	483 ab	1.13 cde	11.8 bc
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	122 cd	245 g	393 c	1.10 de	17.1 de
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	131 bc	283 d	470 ab	1.20 bcd	14.0 a
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	126 cd	280 de	485 a	1.28 ab	15.1 cd
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	120 d	270 ef	472 ab	1.30 a	16.8 ab
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	122 d	267 f	462 b	1.27 ab	16.6 ab
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	124 cd	241 g	382 c	1.07 e	16.8 ab
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	122 cd	241 g	383 c	1.08 e	17.2 de
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	121 d	238 g	374 c	1.06 e	17.5 e

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] Relative span is a unitless index of the uniformity of droplet size distribution. Smaller values represent more uniformity in droplet size distribution.

Table 8. The effect of mixtures of glufosinate plus fomesafen-containing product on soybean grain yield, averaged over application timing in 2015 and 2016 in Keiser, AR.

Product name	Herbicide Treatment	Rate	Yield[a]
		g ai ha^{-1}	kg ha^{-1}
Liberty	Glufosinate	451	3286 ab
Liberty	Glufosinate	595	3253 ab
Flexstar	Fomesafen	264	2814 c
Reflex	Fomesafen	280	2810 c
Prefix	Fomesafen + *S*-metolachlor	266 + 1189	2934 c
Liberty + Flexstar	Glufosinate + fomesafen	451 + 264	3174 b
Liberty + Flexstar	Glufosinate + fomesafen	595 + 264	3337 a
Liberty + Reflex	Glufosinate + fomesafen	451 + 280	3285 ab
Liberty + Reflex	Glufosinate + fomesafen	595 + 280	3287 ab
Liberty + Prefix	Glufosinate + fomesafen + *S*-metolachlor	451 + 266 + 1189	3239 ab
Liberty + Prefix	Glufosinate + fomesafen + S-metolachlor	595 + 266 + 1189	3324 a
Liberty + Dual Magnum	Glufosinate + *S*-metolachlor	451 + 1389	3233 ab

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

Chapter 6

Overcoming antagonism in mixtures of glufosinate + glyphosate and glufosinate + clethodim on grasses

Proper management of glufosinate and the LibertyLink® and Glytol® LibertyLink technology is needed to mitigate the likelihood of resistance evolution. Antagonism can result from mixtures of herbicides that can be utilized in these technologies. These experiments investigated the impact of herbicide rates and weed species on herbicide antagonism. Two experiments were conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR, in 2015 and 2016 that included four grass weed species: barnyardgrass, broadleaf signalgrass, johnsongrass, and large crabgrass. The experiments evaluated mixtures of glufosinate + clethodim and glufosinate + glyphosate. Herbicide interactions were evaluated using Colby's method. Weed control and biomass data were collected 4 weeks after the herbicide application. Furthermore, herbicide treatments were evaluated in a low-speed wind tunnel to determine if changes in droplet spectra were associated with identification of herbicide interactions. Antagonism was identified for both glufosinate + glyphosate mixtures and glufosinate + clethodim mixtures, but the instances of antagonism were dependent on the herbicide rates and the grass weed species. For barnyardgrass and large crabgrass, glufosinate + glyphosate was antagonistic at all rates evaluated. When large crabgrass was evaluated, some mixtures (e.g., 595 g ha^{-1} glufosinate + 76 g ha^{-1} clethodim) had a significant reduction in control relative to one of the herbicides applied alone. Glufosinate (451 and 595 g ai ha^{-1}) + glyphosate (867 and 1735 g ae ha^{-1}) was anatagonistic at all four possible rate combinations for broadleaf signalgrass control. Fewer instances of antagonism were observed for seedling johnsongrass control than for other species, but certain treatments were identified as antagonistic (e.g., glufosinate at 451 g ai ha^{-1} +

clethodim at 76 g ai ha^{-1}). Overall, antagonism was less likely and greater control were observed when the highest rates of both herbicides in a given mixture were used. The addition of glyphosate or clethodim to glufosinate can increase the volume median diameter and decrease the percentage volume of fines, compared to glufosinate alone. The droplet spectra analyses indicate that the glufosinate performance may be negatively impacted by the addition of glyphosate or clethodim, but changes in droplet size is not likely the primary cause of antagonism.

Nomenclature: clethodim; glyphosate; glufosinate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; broadleaf signalgrass, *Urochloa platyphylla* (Griseb.) Nash; johnsongrass, *Sorghum halepense* (L.) Pers; large crabgrass, *Digitaria sanguinalis* L.

Key words: Antagonism, glufosinate plus clethodim, glufosinate plus glyphosate, herbicide interactions

Glufosinate can be applied POST in crops with a glufosinate-resistance trait, including: LibertyLink® soybean (*Glycine max* [L.] Merr.) and cotton (*Gossypium hirsutum* L.), Enlist™ soybean and cotton, and Bollgard® II XtendFlex® cotton. Glufosinate will control a broad spectrum of grass and broadleaf weeds. With the commercialization of Enlist and Bollgard II XtendFlex varieties, compounded by the increased occurrence of glyphosate-resistant weeds, POST applications of glufosinate will likely increase dramatically in the coming years. As a single application of glufosinate is not always enough to control emerged grasses, a detailed investigation on the performance of glufosinate in mixtures on common, hard-to-control grass weeds in the midsouthern U.S. is needed. Four common and troublesome grass weeds in this region include barnyardgrass, broadleaf signalgrass, large crabgrass, and johnsongrass.

Barnyardgrass has been reported as one of the ten most troublesome weeds in the midsouthern U.S., and is one of the most common weeds in Arkansas soybean fields (Webster 2013). Many herbicides (e.g., glyphosate, clethodim, sethoxydim, and quizalofop) are available in soybean and cotton that have been reported to provide adequate control of barnyardgrass (Jordan 1995; Scott et al. 2015; Sikkema et al. 2005; Vidrane et al. 1995); however, these herbicides must be managed appropriately to minimize the risk of evolving further resistance. This consideration is important because barnyardgrass has been positively identified as resistant to nine sites of action (SOA) globally, seven of those in southern U.S., with several instances of multiple resistance (Heap 2018).

Prior to the introduction of glyphosate, large crabgrass was a considerable pest in row crops such as soybean. Glyphosate provides 99% control of large crabgrass (Culpepper et al. 2000), allowing for effective control in glyphosate-resistant crops. However, large crabgrass has remained a troublesome weed in specialty crops, such as snap bean (*Phaseolus vulgaris* L.)

(Aguyoh and Masiunas 2003) and watermelon (*Citrullus lanatus* Thunb.) (Monks and Schultheis 1998). Large crabgrass becomes considerably more difficult to control after it begins to form adventitious roots at the stem internodes (Monks and Schultheis 1998). Large crabgrass with resistance to various acetolactate synthase-, acetyl CoA carboxylase-, and photosystem II-inhibiting herbicides has been documented (Heap 2018).

Although no cases of herbicide-resistant broadleaf signalgrass have been identified, this weed remains one of the most common and troublesome weeds in midsouthern U.S. agricultural crops (Webster 2012; 2013). Control of broadleaf signalgrass with POST applications of glufosinate was less than control with glyphosate but, when a PRE was used in combination with glufosinate POST, control was equal to that of a PRE followed by glyphosate (Culpepper et al. 2000). Glyphosate alone provides ≥ 90% control of broadleaf signalgrass (Culpepper et al. 2000; Scott et al. 2015) and the extensive adoption of glyphosate-resistant crops shifted common agricultural weed species away from glyphosate-sensitive species (Reddy and Norsworthy 2010). However, broadleaf signalgrass has persisted as a common agricultural weed, thus, populations are continuously exposed to various herbicides on a large number of acres.

Johnsongrass was a major threat to crop production in the U.S. before the commercialization of glyphosate-resistant (GR) crops. Introduction of glyphosate in the mid-1970s provided more-effective control of johnsongrass than tillage, and glyphosate provided even better control when it was applied POST in GR crops (Johnson et al. 2003). Unfortunately, glyphosate-resistant johnsongrass was identified in 2007 in Arkansas, 2008 in Mississippi, and 2010 in Louisiana (Heap 2018; Riar et al. 2011), and johnsongrass could again become a challenging weed to control in midsouthern U.S. agriculture.

Although glufosinate has broad-spectrum activity, it is not as effective for johnsongrass control as systemic herbicides such as glyphosate (Johnson et al. 2003). Glufosinate is likely not as effective as glyphosate because glufosinate has limited translocation to the rootstocks. Sequential applications of glufosinate and mixtures with clethodim improved control over a single application of glufosinate alone (Johnson et al. 2014a; Johnson and Norsworthy 2014). To manage severe infestations or escapes POST, sequential applications of glufosinate plus clethodim were effective at controlling small (15 cm) johnsongrass (Meyer et al. 2015b).

Multiple applications of glufosinate or glufosinate plus another effective grass herbicide is typically needed to control a number of troublesome grass weeds. Unfortunately, some mixtures containing glufosinate have been reported as antagonistic, meaning the benefit of applying two effective SOAs may not provide the control that would be expected. Colby (1967) defined antagonism as a result of applying two herbicides in combination which is less than what would be expected based on how the individual herbicides perform alone. Gardner et al. (2006) determined glufosinate antagonized the activity of clethodim on a mixed population of annual grass species [e.g., large crabgrass and fall panicum (*Panicum dichotomiflorum* Michx.)]. Antagonism has been observed between glufosinate and clethodim on goosegrass (*Eleusine indica* L.) (Burke et al. 2005) and glyphosate plus glufosinate on giant foxtail (*Setaria faberi* Herrm.) (Bethke et al. 2013). However, Eytcheson et al. (2015) did not identify antagonism of glufosinate plus clethodim on barnyardgrass, indicating identification of antagonism may be dependent upon the species and specific mixtures evaluated.

Although the effect of droplet size on herbicide efficacy has been documented, little research has been conducted to evaluate if droplet size could influence herbicide interactions. The efficacy of contact herbicides, such as glufosinate, is more dependent upon the droplet size and resultant coverage of the application than for systemic herbicides (Etheridge et al. 2001; Meyer et al. 2015a) and application parameters have the potential to drastically affect the efficacy of glufosinate (Meyer et al. 2015a; 2016a; 2016b). Thus, an additive to a glufosinate solution, such as another herbicide, that alters the droplet spectra has the potential to influence the efficacy of glufosinate as part of the mixture.

Herbicide recommendations that result in antagonism between two herbicides are not an effective resistance management strategy (Norsworthy et al. 2012). Interactions between glufosinate, glyphosate, and clethodim are not well-documented on barnyardgrass and other common grass weeds in the midsouthern U.S., so research is needed to determine if antagonism is occurring with these applications. The objectives of the present experiments were to: 1) evaluate mixtures of glufosinate for herbicide interactions at field use rates; 2) determine if increasing the rate of herbicides in mixture mitigates antagonism; and 3) determine if instances of antagonism vary by the grass species evaluated.

Materials and Methods

Two experiments were conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas on a Leaf silt loam. Plot sizes were 2.4 by 9.1 m and the entire experimental area was disked and field cultivated prior to planting. At the time of trial establishment, johnsongrass seed was sown in two rows spaced 1.5 m apart by filling a planter unit for one row on a Hege 500 (Hege Equipment Inc., Colwich, KS, USA) and making two passes across the plots in each replication (i.e., perpendicular to the spray direction). In addition, 1 L volumes of each barnyardgrass, broadleaf signalgrass, and large crabgrass seed were broadcast across the experimental area. The field contained a native population of broadleaf signalgrass and barnyardgrass. Planting occurred June 24, 2015 and June 9, 2016 for both experiments. In both experiments, herbicide applications were made with a CO_2-pressurized backpack sprayer calibrated to deliver 143 L ha $^{-1}$ spray volume at 276 kPa at 4.8 km h^{-1} through nozzles spaced 51 cm apart. The boom was equipped with Turbo TeeJet (TT) 110015 nozzles (TeeJet Technologies, Springfield, Illinois). Weed sizes at the time of herbicide application were recorded and are listed in Table 1.

In Experiment 1, glufosinate (Liberty herbicide, Bayer CropScience, Research Triangle Park, NC) was applied alone at 451, 595, and 738 g ai ha^{-1} alone and in combination with various rates (76, 136 and 204 g ai ha^{-1}) of clethodim (Select Max® herbicide, Syngenta Crop Protection LLC., Greensboro, NC). Additionally, *S*-metolachlor (Dual Magnum®, Syngenta Crop Protection LLC., Greensboro, NC) was included in mixture for some treatments. A nontreated check was included for comparison (Table 2). Treatments containing clethodim included 1.0 % v v^{-1} of Agridex (Helena Chemical Company, Collierville, TN), a crop oil concentrate (COC), unless *S*-metolachlor was included as a part of the mixture because the herbicide label does not recommend *S*-metolachlor in mixture with COC (Anonymous 2015). Following application of the herbicide treatments, all plots that did not receive *S*-metolachlor as part of the experimental treatment received an application of *S*-metolachlor within 24 h. Treatments were applied at 9:00 A.M. on July 24, 2015, and 8:00 A.M. on July 7, 2016. Air temperature was 25 C and 27 C, relative humidity was 59 and 70%, and wind speed was 3 and 2 km h^{-1} in 2015 and 2016, respectively, based on in-field observations.

In Experiment 2, various rates of glufosinate, clethodim, and glyphosate (Roundup Powermax II® herbicide, Monsanto Company, St. Louis, MO) alone, and combinations of glufosinate plus clethodim or glyphosate, were applied as herbicide treatments (Table 3). Similar to Experiment 1, the entire experimental area received an application of *S*-metolachlor 24 h after treatment application to minimize further weed emergence. Treatments were applied at 2:00 P.M. on July 24, 2015, and 6:30 A.M. on July 7, 2016. Air temperature was 27 C and 26 C, relative humidity was 51 and 75%, and wind speed was 1 and 3 km h^{-1} in 2015 and 2016, respectively, based on in-field observations.

Weed control ratings were collected 4 weeks after treatment (WAT) in both experiments for barnyardgrass, broadleaf signalgrass, johnsongrass, and large crabgrass. Weed control was visually evaluated on a scale of 0 (no control) to 100% (complete death of all plants) relative to the nontreated check. Weed biomass was collected by species within 3 d of the 4 WAT assessment. For barnyardgrass, broadleaf signalgrass, and large crabgrass, biomass was collected from a 1 m^{-2} quadrat in each plot. Johnsongrass biomass was collected from 1 m-row in each plot, as johnsongrass was sown with a planter as previously described. Following collection, biomass was dried at 40 C for 7 d and weighed to determine dry biomass relative to the nontreated check.

Droplet-size spectra for each herbicide treatment were analyzed in a low-speed wind tunnel at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE. Droplet spectra were determined using a Sympatec Helos Vario KR particle-size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) equipped with an R7 lens capable of detecting particle sizes in a range from 18 to 3,500 µm as described by Creech et al. (2015) and Henry et al. (2014). The laser was positioned 30-cm from the tip of the nozzle, and a linear actuator moved the width of the nozzle plume across the laser. Droplet spectra were analyzed with a wind speed of 24 km h^{-1} to mitigate spatial sampling bias. Each herbicide treatment in Experiment 1 and Experiment 2 was analyzed in the wind tunnel and replicated three times. The same formulated products used in the field experiments were used for particle-size analysis. Spray parameters determined from the droplet spectra analysis included the D_{v10}, D_{v50} D_{v90}, relative span (RS), and the percentage of driftable fines. D_{v10} is the droplet diameter below which 10% of the liquid volume is contained in droplets smaller than that value. D_{v50} and D_{v90} are similar parameters for 50% and 90% of the volume, respectively. For simplicity of reporting, the percentage of driftable fines was classified in this study as the percentage of the volume containing droplets with a diameter <150 µm ($\%_{vol}$ fines). The RS is a parameter describing the range of droplet sizes of the spray plume calculated using Equation 1.

$$\mathrm{RS} = (D_{\mathrm{v90}} - D_{\mathrm{v10}})\ D_{\mathrm{v50}}^{-1} \qquad [1]$$

Data were subject to an analysis of variance (ANOVA) using JMP 13 (SAS Institute Inc., Cary, NC), and means were separated using Fisher's protected least significant difference (LSD) test ($\alpha = 0.05$). The ANOVA conducted in JMP Pro 13 included replication and year as random effects (variance components estimates are reported in Table 4). The results from the ANOVA and Colby's method were used to interpret the data and evaluate the mixtures. Colby's method was used to evaluate for the herbicide interaction (e.g., antagonism) and the ANOVA was used to determine if the mixtures provided control that was different from the component herbicides. These analyses could demonstrate that a mixture may be additive based on Colby's method, but may not necessarily provide better control that the component herbicides alone (e.g., Table 2). A natural-log transformation of biomass weight was used to improve normality when needed. ANOVA was conducted on the transformed values and values were back-transformed for discussion and reporting. For the particle-size analysis, a completely randomized design was used, and a more-conservative Tukey adjustment ($\alpha = 0.05$) was used to identify differences between means.

Herbicide mixture interactions were identified using Colby’s method (Colby 1967), where an Expected value (E) is calculated using Equation 2,

$$E = (X + Y) - (XY)/100 \qquad [2]$$

where E is the expected level of control of a given species when two herbicides are applied in a mixture, and variables X and Y represent the level of control of a given weed species provided by each herbicide applied individually. The observed and expected values were compared using a two-sided t-test ($\alpha = 0.05$). If E was significantly greater than the observed value for a given mixture, it was deemed antagonistic. When a mixture included three herbicides with one herbicide that had no POST activity (i.e., *S*-metolachlor) the calculation for the expected value (Equation 1) used the values from the two herbicides that had POST activity.

Results and Discussion

Barnyardgrass

Experiment 1. Control and biomass reduction of barnyardgrass with glufosinate, clethodim, and mixtures of glufosinate plus clethodim was >88% for all treatments (Table 2). Antagonism was identified only for glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} (biomass reduction) and glufosinate at 595 g ha^{-1} plus clethodim at 76 g ha^{-1} (percent control). As all mixtures provided >90% control, glufosinate plus clethodim may be an acceptable mixture for controlling barnyardgrass when the density of large plants (18 to 25 cm tall) is low (1 to 1.5 plants m^{-2}). However, it should be noted that both glufosinate at ≥ 595 g ha^{-1} and clethodim at ≥ 136 g ha^{-1} provided a high level of control alone (≥94% control). The addition of another herbicide with POST activity on barnyardgrass may not be needed for acceptable control and may be better suited as a follow-up application at a later time.

The addition of *S*-metolachlor to glufosinate at 451 g ha^{-1} improved control from 88% to 94% despite *S*-metolachlor having no measurable POST activity. It should also be noted that all treatments that did not contain *S*-metolachlor received the same rate of *S*-metolachlor 24 h after treatments, applied primarily to prevent further emergence. However, the application of *S*-metolachlor within 24 h would likely mitigate any physiological synergy that could occur, meaning any improvements in control are likely due to the adjuvants contained in the formulated product of *S*-metolachlor, or a reduction in droplet size of the treatment application which tends to improve glufosinate efficacy (Meyer et al. 2015a; Etheridge et al. 2001).

Experiment 2. Similar to Experiment 1, antagonism on barnyardgrass was identified for the mixture of the low rates of glufosinate plus clethodim (451 + 76 g ha^{-1}, respectively) for the biomass reduction assessment, as well as for percent control (Table 3). Antagonism was identified for all mixtures of glufosinate plus glyphosate for percent control evaluations. For biomass reduction, the mixtures of glufosinate at 451 g ha^{-1} plus glyphosate at 867 and 1,735 g ae ha^{-1} were also antagonistic. When glyphosate at 1,735 g ha^{-1} was applied with glufosinate at 451 g ha^{-1}, the biomass reduction was significantly less (91%) than glyphosate alone (99%). Although the differences between mixtures and individual components can be subtle, having survivors of glufosinate + glyphosate application could lead to the evolution of herbicide resistance to either, or even both, herbicides.

Broadleaf Signalgrass

Experiment 1. The response of broadleaf signalgrass to the various rate structures of glufosinate plus clethodim mixtures supports the concept of increasing the rate of the systemic herbicide in a mixture to help mitigate antagonism. Glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} was antagonistic for both percent control (91% observed vs. 99% expected) and biomass (Table 5). Increasing the rate of clethodim from 76 to 204 g ha^{-1} increased control numerically to 95% but mitigated antagonism. Increasing the rate of clethodim in mixture with glufosinate may improve clethodim uptake and translocation, thereby increasing control. In contrast, mixtures utilizing the high rate of glufosinate (738 g ha^{-1}) were antagonistic.

Even though the addition of *S*-metolachlor to glufosinate at 451 g ha^{-1} improved barnyardgrass control in Experiment 1, it did not impact broadleaf signalgrass control. Interestingly, the mixture of glufosinate plus clethodim plus *S*-metolachlor did have improved control over glufosinate plus *S*-metolachlor. Control with glufosinate plus clethodim plus *S*-metolachlor was less than clethodim alone (76 g ha^{-1}) (86% compared to 93% control for the mixture and clethodim alone, respectively). The results from broadleaf signalgrass and barnyardgrass suggest the response of control to additional herbicides, even *S*-metolachlor that has no measurable POST activity, is dependent upon the species being evaluated.

Experiment 2. A clear indication of the impact of rate structure on observed antagonism was present with broadleaf signalgrass control and biomass in Experiment 2. When either glufosinate at 451 or 595 g ha^{-1} was mixed with glyphosate at 867 g ha^{-1}, a reduction in control occurred for the mixture compared to glyphosate alone (Table 6). When the glyphosate rate was increased to 1,735 g ha^{-1}, antagonism was still present but control or biomass reduction was no longer less than glyphosate alone. Therefore, using a high rate of glyphosate (the systemic herbicide in the mixture), may be of value when the mixture is needed to control a broad weed spectrum present in a given field, despite the fact the mixture is considered antagonistic on some species within the field.

For the glufosinate plus clethodim mixtures in Experiment 2, the only mixture that had less control than one of its components (i.e., clethodim) was glufosinate at 595 g ha^{-1} plus clethodim at 136 g ha^{-1} (84% control vs. 92% with clethodim alone). Based on the control values for the mixtures evaluated, glyphosate at 1,735 g ha^{-1} plus glufosinate at 451 or 595 g ha^{-1} and glufosinate at 451 g ha^{-1} plus clethodim at 136 g ha^{-1} provided the greatest control and did not have a reduction in control relative to one of the components in the mixture.

Seedling Johnsongrass

Experiment 1. The combination of glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} had improved control and biomass reduction over either component of the mixture alone (Table 7). This rate structure (451 + 76 g ha^{-1}) was identified as antagonistic for broadleaf signalgrass (both assessments) and for barnyardgrass biomass reduction; however, the combination also had greater barnyardgrass control than either component. These results demonstrate some of limitations of evaluating various rate structures of the same herbicides on different species using Colby's method and the difficulty of drawing broad conclusions from those results.

As was observed with barnyardgrass, glufosinate plus *S*-metolachlor had greater control and biomass reduction than glufosinate alone (Table 7). Glufosinate at 451 g ha^{-1} provided 81% control of seedling johnsongrass 4 WAT and the addition of *S*-metolachlor increased control to 90%. Similarly, glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} plus *S*-metolachlor at 1389 g ha^{-1} had greater control over both glufosinate alone (81%) and clethodim alone (85%).

Experiment 2. No antagonism was identified for mixtures of glufosinate plus glyphosate (Table 8). Whenever glyphosate was applied to johnsongrass, whether alone or in a mixture, control of johnsongrass was ≥98%. Although glyphosate was very effective at controlling johnsongrass, glyphosate-resistant populations have been identified in the midsouthern U.S. (Riar et al. 2011), and other herbicides or mixtures will need to be utilized for effective control.

Antagonism was identified for various combinations of glufosinate plus clethodim for seedling johnsongrass biomass reduction (Table 8). For example, glufosinate at 595 g ha^{-1} plus clethodim at 76 g ha^{-1} had an observed value for biomass reduction of 93% compared to an expected value of 99%. These results do conflict with the results from the same combinations also found in Experiment 1 (Table 7), but the discrepancy may be explained by taller average plant heights of johnsongrass, particularly in 2015 (33 cm and 41 cm in height for Experiments 1 and 2 in 2015, respectively). Although direct comparisons cannot be made between experiments, this may suggest the importance of weed size on the identification of herbicide interactions (Miller et al. 2015).

It is important to reiterate that both Experiment 1 and 2 evaluated a population consisting of only seedling johnsongrass. The trials were initiated in fields that did not have a native population of johnsongrass, and the johnsongrass evaluated was easily identified as plants sown into rows using a planter. Single applications of glufosinate at ≥ 595 g ha^{-1} or clethodim at ≥136 g ha^{-1} provided >90% control of seedling johnsongrass in both experiments, whereas both Johnson et al. (2014b) and Meyer et al. (2015) reported that two applications of glufosinate were needed for effective control when rhizomatous johnsongrass is present.

Large Crabgrass

Experiment 1. Mixtures were antagonistic for large crabgrass control, with the exception of glufosinate at 738 g ha^{-1} plus clethodim at 76 g ha^{-1} (Table 9). Even more concerning is when clethodim was applied at 76 or 136 g ha^{-1} with glufosinate 451 or 595 g ha^{-1}, a reduction in control was observed compared to clethodim alone (Table 9). The observed value for control with glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} was 82% and the expected value was 99%, indicating a considerable deviation from the expected response. Increasing the rate of clethodim from 76 to 136 g ha^{-1} in mixture with glufosinate at 451 g ha^{-1} increased control from 82% to 93%, although the mixture was still antagonistic.

Experiment 2. Rate structures of both glufosinate plus glyphosate and glufosinate plus clethodim mixtures were antagonistic for percent control and biomass reduction of large crabgrass (Table 10). A reduction in control was also observed for glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1} and glufosinate at 595 g ha^{-1} plus clethodim at 76 or 136 g ha^{-1} compared to the appropriate rate of clethodim alone. Glufosinate at 451 g ha^{-1} plus clethodim at 136 g ha^{-1} proved to be superior with 93% control, whereas the other glufosinate plus clethodim combinations only provided ≤83% control. These results may be explained by examining the ratio of glufosinate:clethodim in the mixtures. If glufosinate is limiting the activity of the systemic herbicide, a higher amount of clethodim relative to glufosinate should improve control. The ratios of glufosinate:clethodim were 5.9 for 451 + 76 g ha^{-1}, 4.4 for 595 + 136 g ha^{-1} and 3.3 for 451 + 136 g ha^{-1}; the treatment with the lowest glufosinate:clethodim ratio also had the greatest control. Although it is considered a systemic herbicide, only a fraction (≤20%) of clethodim that is absorbed is translocated out of the treated leaf when applied alone (Nandula et al. 2007), meaning slight reductions in translocation may have a profound impact on clethodim efficacy. Furthermore, adjuvant selection and the addition of contact herbicides (i.e., bromoxynil) are both known to have an impact on uptake and transport of clethodim in barnyardgrass (Culpepper et al. 1999).

Combinations of glufosinate plus glyphosate had ≥95% control and ≥90% biomass reduction, although none of them had improved control over the appropriate rate of glyphosate alone. Unfortunately, applications of mixtures will be needed in most farmer fields to control a broad spectrum of weeds, with some species resistant to glyphosate. These results suggest if glufosinate is to be applied to a field with large crabgrass, glyphosate should be added to glufosinate instead of clethodim, if the crop technology allows (e.g., Glytol LibertyLink cotton). In the case of large crabgrass, glufosinate plus glyphosate may be better than glufosinate plus clethodim from a resistance management perspective simply because the performance of the mixture is less likely to be reduced relative to the systemic herbicide alone across a range of rates.

Droplet Spectra Analysis

A possible explanation for the improved control of glufosinate at 451 g ha^{-1} plus *S*-metolachlor at 1,389 g ha^{-1} over glufosinate alone for barnyardgrass and johnsongrass control is the effect the addition of *S*-metolachlor has on the droplet spectra compared to the same rate of glufosinate alone. However, if a reduction in droplet size was improving efficacy, it would be expected to positively impact efficacy on all grass species, and no differences were observed between glufosinate and glufosinate + *S*-metolachlor for broadleaf signalgrass and large crabgrass control. The addition of *S*-metolachlor reduced the D_{v50} of the droplet spectra from 296 μm to 238 μm and increased the percentage of volume containing fine droplets ($\%_{vol}$ <150 μm) from 12.4 to 17.4% (Table 11). Smaller droplet size and increased percent fines has been documented to increase coverage and improve weed control with glufosinate (Etheridge et al. 2001; Meyer et al. 2015a).

The effect of the addition of clethodim to glufosinate on the droplet spectra is dependent upon the rate of both herbicides used in the mixture. When the low rate of glufosinate (451 g ha^{-1}) is considered, the addition of clethodim at 76 g ha^{-1} reduces the D_{v50} from 296 to 276 µm and when more clethodim is added, the D_{v50} is further reduced (Table 11). In contrast, glufosinate at 738 g ha^{-1} has a D_{v50} of 277 µm and a $\%_{vol}$ fines of 15.9%. When clethodim at 76 g ha^{-1} is added to this rate of glufosinate, D_{v50} does not change but $\%_{vol}$ fines is reduced to 11.6%. A change in $\%_{vol}$ fines without a change in D_{v50} is explained by a change in relative span of the droplet size spectra, meaning the distribution of droplet sizes for the application is more focused around the D_{v50}. Ultimately, the slight differences in droplet size spectra for glufosinate plus clethodim mixtures is unlikely to fully account for the antagonism observed in the field.

The addition of glyphosate to glufosinate also had variable effect on droplet size depending on the rates of both herbicides in the mixture. When glyphosate at 867 g ha^{-1} was added to both rates of glufosinate, no change in D_{v50}, RS, or $\%_{vol}$ fines was observed (Table 12). When glyphosate at 1,735 g ha^{-1} was added to the higher rate of glufosinate (595 g ha^{1}), an increase in D_{v50} was observed and $\%_{vol}$ fines decreased from 14.9 to 10.3%. It should be noted that droplet size of a mixture is affected by the formulation of a given herbicide and any adjuvants in the system (Holloway et al. 2000; Mueller and Womac 1997). An increase in D_{v50} and a decrease in $\%_{vol}$ fines is not favorable for glufosinate efficacy, and may impact the performance of the mixture. However, as glufosinate + glyphosate was generally antagonistic across rate structures, the impact of the change in droplet spectra is likely minimal and the antagonism is more likely a result of limited translocation of glyphosate (Besançon et al. 2018; Bethke et al. 2013; Meyer et al. 2018).

The impact the addition of one herbicide has on the droplet spectra of another is dependent on a variety of factors and the conditions the mixture was applied. These experiments were applied with a TT nozzle at 143 L ha^{-1}. Additionally, mixtures of glufosinate plus clethodim always had COC, and a change in adjuvant will impact the droplet spectra (Spanoghe et al. 2007) and may alter the interaction occurring in the mixture. Different products of the same herbicide can differ in droplet spectra (Mueller and Womac 1997) meaning the individual product is also likely to influence the droplet spectra of the mixture.

Practical Implications

Antagonism was observed for both mixtures of glufosinate plus glyphosate and glufosinate plus clethodim, which was dependent upon the rate and species evaluated. Increasing the rate of either herbicide in mixture increases control and decreases the likelihood of identifying antagonism using Colby's method. It is generally accepted that contact herbicides inhibit the activity of systemic herbicides (Bethke et al. 2013; Chuah et al. 2008; Fish et al. 2015; Norsworthy et al. 2010). This research suggests if glufosinate is going to be mixed with glyphosate or clethodim, a high labeled rate of either glyphosate or clethodim should be used. Glufosinate has previously been reported to antagonize clethodim (Burke et al. 2005) and glyphosate (Bethke et al. 2013). Furthermore, the identification of antagonism is not only dependent upon species evaluated, but also the conditions of a given experiment.

For glufosinate at 451 g ha^{-1} plus clethodim at 76 g ha^{-1}, an improvement in control was observed over the individual herbicides for barnyardgrass and johnsongrass control, whereas a reduction was observed for large crabgrass and no difference for broadleaf signalgrass. The inconsistencies in the performance of this mixture across species over the products alone, coupled with the identification of antagonism for various assessments suggest this specific rate combination should not be utilized in a situation where many grass species are present in a given field. For example, if large crabgrass is present in a field, glufosinate alone does not provide sufficient control and the addition of clethodim may be warranted. Although data from these experiments lead to the conclusion that the mixture of glufosinate plus clethodim is antagonistic, the improvement in control over glufosinate alone for some species and overall high levels of control for the higher use rates indicate this mixture may be more beneficial than glufosinate alone. Thus, when mixtures are needed to improve control or broaden the weed control spectrum, high rates of the individual herbicides should be utilized to minimize the risk for evolving resistance.

Although clethodim at 76 g ha^{-1} is a labeled rate, performance of the herbicide at this rate was not consistent across Experiment 1 and 2 for either broadleaf signalgrass, johnsongrass, or large crabgrass control. Therefore, the recommended use rate for clethodim alone or in mixture should be at least 136 g ha^{-1}. For control of barnyardgrass, broadleaf signalgrass, seedling johnsongrass, and large crabgrass, the optimum mixture would depend on the trait technology used. For instance, in a LibertyLink soybean system, glufosinate should be applied at 595 g ha^{-1} with clethodim at 136 g ha^{-1}, and in Glytol/LL cotton, glufosinate should be applied at 595 g ha^{-1} with glyphosate at 1,735 g ha^{-1}.

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Appendix

Table 1. Weed sizes and densities of four grass weeds at the time of herbicide application evaluated in Experiment 1 and Experiment 2 in 2015 and 2016.

	Experiment 1				Experiment 2			
	2015		2016		2015		2016	
Species	Height	Density	Height	Density	Height	Density	Height	Density
	cm	plants m^{-2}	Cm	plants m^{-2}	cm	plants m^{-2}	Cm	plants m^{-2}
Barnyardgrass	18	1.5	25	1	33	2	24	1.5
Broadleaf signalgrass	19	8	23	8	27	20	25	12
Johnsongrass	33	12[a]	37	7[a]	41	15[a]	41	9[a]
Large crabgrass	20	1.5	25	1	18	1	17	1

[a] Plant density is in plants m-row.

Table 2. Effect of glufosinate alone and in combinations with clethodim on observed and expected control 4 WAT and aboveground biomass of barnyardgrass in Experiment 1 at Fayetteville, AR.[a]

		Control			Biomass reduction[b]		
Herbicide treatment	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	%	%		%	%	
Nontreated					0		
Glufosinate	451	88			89		
Glufosinate	595	94			96		
Glufosinate	738	97			99		
Clethodim	76	91			90		
Clethodim	136	97			95		
Clethodim	204	99			97		
Glufosinate + clethodim	451 + 76	96 ∧	99	NS	92 NS	99	*
Glufosinate + clethodim	451 + 136	96 NS	99	NS	96 NS	99	NS
Glufosinate + clethodim	451 + 204	96 NS	99	NS	98 NS	99	NS
Glufosinate + clethodim	595 + 76	93 NS	99	*	97 NS	100	NS
Glufosinate + clethodim	595 + 136	96 NS	100	NS	97 NS	100	NS
Glufosinate + clethodim	595 + 204	100 NS	100	NS	99 NS	100	NS
Glufosinate + clethodim	738 + 76	98 NS	99	NS	98 NS	100	NS
Glufosinate + clethodim	738 + 136	100 NS	100	NS	99 NS	100	NS
Glufosinate + clethodim	738 + 204	95 NS	100	NS	98 NS	100	NS
Glufosinate + *S*-metolachlor	451 + 1389	94 ∧			95 ∧		
Glufosinate + clethodim + *S*-metolachlor	451 + 76 + 1389	93 NS	99	NS	97	99	NS
Glufosinate + clethodim + *S*-metolachlor	451 + 136 + 1389	99 NS	100	NS	98	100	NS

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

Table 3. Effect of glufosinate alone and in combinations with glyphosate or clethodim on observed and expected control 4 WAT and aboveground biomass of barnyardgrass in Experiment 2 at Fayetteville, AR.[a]

		Control			Biomass reduction[b]		
Herbicide treatment	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	%	%		%	%	
Nontreated					0		
Glufosinate	451	84			83		
Glufosinate	595	91			92		
Glyphosate	867[e]	99			96		
Glyphosate	1735[e]	99			99		
Clethodim	76	88			81		
Clethodim	136	95			94		
Glufosinate + glyphosate	451 + 867[e]	95 NS	100	*	91 NS	99	*
Glufosinate + glyphosate	451 + 1735[e]	95 NS	100	*	91 ∨	100	*
Glufosinate + glyphosate	595 + 867[e]	97 NS	100	*	95 NS	99	NS
Glufosinate + glyphosate	595 + 1735[e]	96 NS	100	*	94 NS	100	NS
Glufosinate + clethodim	451 + 76	94 ∧	98	*	93 ∧	97	NS
Glufosinate + clethodim	451 + 136	96 NS	99	NS	98 NS	99	NS
Glufosinate + clethodim	595 + 76	93 NS	99	NS	94 NS	98	NS
Glufosinate + clethodim	595 + 136	94 NS	100	*	94 NS	100	NS

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-}

Table 4. Variance components estimates obtained from the ANOVA for barnyardgrass, johnsongrass, broadleaf signalgrass, and large crabgrass control and biomass reduction from Experiments 1 and 2[a].

		Barnyardgrass		Johnsongrass		Broadleaf signalgrass		Large crabgrass	
Experiment	Model effect	4 WAT	Biomass reduction	4 WAT	Biomass reduction	4 WAT	Biomass reduction	4 WAT	Biomass reduction
		% of total							
Experiment 1	Rep(Year)	4.3	3.5	3.7	1.0	<0.1	<0.1	16.7	2.6
	Year	25.2	11.9	33.7	17.3	53.8	6.8	19.8	6.7
	Residual	70.5	84.7	62.6	81.7	46.2	93.2	63.4	90.6
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Experiment 2	Rep(Year)	<0.1	<0.1	<0.1	0.6	0.3	5.9	<0.1	0.7
	Year	26.6	5.0	40.8	1.8	27.1	26.4	10.4	7.6
	Residual	73.4	95.0	59.2	97.6	72.6	67.8	89.6	91.7
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Abbreviation: WAT, weeks after treatment.

Table 5. Effect of glufosinate alone and in combinations with clethodim on observed and expected control 4 WAT and aboveground biomass of broadleaf signalgrass in Experiment 1 at Fayetteville, AR.[a]

		Control			Biomass reduction[b]		
Herbicide treatment	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	%	%		%	%	
Nontreated					0		
Glufosinate	451	87			90		
Glufosinate	595	87			93		
Glufosinate	738	95			92		
Clethodim	76	93			87		
Clethodim	136	95			96		
Clethodim	204	97			98		
Glufosinate + clethodim	451 + 76	91 NS	99	*	94 NS	98	*
Glufosinate + clethodim	451 + 136	91 NS	99	NS	98 NS	100	*
Glufosinate + clethodim	451 + 204	95 NS	99	NS	97 NS	100	NS
Glufosinate + clethodim	595 + 76	91 NS	99	*	98 ∧	99	NS
Glufosinate + clethodim	595 + 136	88 ∨	99	*	94 NS	100	*
Glufosinate + clethodim	595 + 204	98 NS	99	NS	97 NS	100	NS
Glufosinate + clethodim	738 + 76	96 NS	100	NS	90 NS	99	*
Glufosinate + clethodim	738 + 136	99 NS	100	NS	95 NS	100	*
Glufosinate + clethodim	738 + 204	96 NS	100	NS	98 NS	100	*
Glufosinate + *S*-metolachlor	451 + 1389	87 NS			84 ∨		
Glufosinate + clethodim + *S*-metolachlor	451 + 76 + 1389	86 ∨	99	*	90 NS	97	NS
Glufosinate + clethodim + *S*-metolachlor	451 + 136 + 1389	96 NS	99	NS	95 NS	99	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

Table 6. Effect of glufosinate alone and in combinations with glyphosate or clethodim on observed and expected control 4 WAT and aboveground biomass of broadleaf signalgrass in Experiment 2 at Fayetteville, AR.[a]

		Control				Biomass reduction[b]			
Herbicide treatment	Rate	Obs[c]		Exp	p[d]	Obs[c]		Exp	p[d]
	g ai ha^{-1}	%		%		%		%	
Nontreated						0			
Glufosinate	451	86				91			
Glufosinate	595	86				93			
Glyphosate	867[e]	99				97			
Glyphosate	1735[e]	100				97			
Clethodim	76	91				82			
Clethodim	136	92				92			
Glufosinate + glyphosate	451 + 867[e]	92	∨	100	*	88	∨	100	*
Glufosinate + glyphosate	451 + 1735[e]	96	NS	100	*	97	NS	100	NS
Glufosinate + glyphosate	595 + 867[e]	93	∨	100	*	91	∨	100	NS
Glufosinate + glyphosate	595 + 1735[e]	95	NS	100	*	95	NS	100	*
Glufosinate + clethodim	451 + 76	90	NS	99	*	90	NS	98	*
Glufosinate + clethodim	451 + 136	93	NS	99	NS	98	∧	100	NS
Glufosinate + clethodim	595 + 76	87	NS	99	*	96	NS	98	NS
Glufosinate + clethodim	595 + 136	84	∨	99	*	94	NS	99.6	NS

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}

Table 7. Effect of glufosinate alone and in combinations with clethodim on observed and expected control 4 WAT and aboveground biomass of johnsongrass in Experiment 1 at Fayetteville, AR.[a]

		Control			Biomass reduction[b]		
Herbicide treatment	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	%	%		%	%	
Nontreated							
Glufosinate	451	81			81		
Glufosinate	595	92			95		
Glufosinate	738	98			99		
Clethodim	76	85			89		
Clethodim	136	96			95		
Clethodim	204	99			99		
Glufosinate + clethodim	451 + 76	93 ∧	97	NS	96 ∧	97	NS
Glufosinate + clethodim	451 + 136	96 NS	99	NS	94 NS	99	NS
Glufosinate + clethodim	451 + 204	99 NS	100	NS	99 NS	99	NS
Glufosinate + clethodim	595 + 76	94 NS	98	NS	96 NS	99	NS
Glufosinate + clethodim	595 + 136	97 NS	99	NS	99 NS	99	NS
Glufosinate + clethodim	595 + 204	100 NS	100	NS	97 NS	100	NS
Glufosinate + clethodim	738 + 76	99 NS	99	NS	100 NS	100	NS
Glufosinate + clethodim	738 + 136	98 NS	99	NS	99 NS	100	NS
Glufosinate + clethodim	738 + 204	98 NS	100	NS	99 NS	100	NS
Glufosinate + *S*-metolachlor	451 + 1389	90 ∧			93 ∧		
Glufosinate + clethodim + *S*-metolachlor	451 + 76 + 1389	95 ∧	98	NS	93 NS	99	*
Glufosinate + clethodim + *S*-metolachlor	451 + 136 + 1389	99 NS	99	NS	97 NS	99	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∧" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

Table 8. Effect of glufosinate alone and in combinations with glyphosate or clethodim on observed and expected control 4 WAT and aboveground biomass of johnsongrass in Experiment 2 at Fayetteville, AR.[a]

		Control				Biomass reduction[b]			
Herbicide treatment	Rate	Obs[c]		Exp	p[d]	Obs[c]		Exp	p[d]
	g ai ha^{-1}	%		%		%		%	
Nontreated									
Glufosinate	451	79				78			
Glufosinate	595	90				94			
Glyphosate	867[e]	99				99			
Glyphosate	1735[e]	100				100			
Clethodim	76	88				87			
Clethodim	136	92				96			
Glufosinate + glyphosate	451 + 867[e]	99	NS	100	NS	97	NS	100	NS
Glufosinate + glyphosate	451 + 1735[e]	98	NS	100	NS	97	NS	100	NS
Glufosinate + glyphosate	595 + 867[e]	99	NS	100	NS	99	NS	100	NS
Glufosinate + glyphosate	595 + 1735[e]	99	NS	100	NS	99	NS	100	NS
Glufosinate + clethodim	451 + 76	91	NS	97	*	94	^	96	NS
Glufosinate + clethodim	451 + 136	94	NS	98	NS	96	NS	99	*
Glufosinate + clethodim	595 + 76	94	NS	99	NS	93	NS	99	*
Glufosinate + clethodim	595 + 136	96	NS	99	NS	96	NS	100	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "^" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}

Table 9. Effect of glufosinate alone and in combinations with clethodim on observed and expected control 4 WAT and aboveground biomass of large crabgrass in Experiment 1 at Fayetteville, AR.[a]

		Control			Biomass reduction[b]		
Herbicide treatment	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	%	%		%	%	
Nontreated					0		
Glufosinate	451	87			74		
Glufosinate	595	87			79		
Glufosinate	738	92			79		
Clethodim	76	94			93		
Clethodim	136	98			91		
Clethodim	204	98			98		
Glufosinate + clethodim	451 + 76	82 ∨	99	*	77 ∨	98	*
Glufosinate + clethodim	451 + 136	93 ∨	100	*	87 NS	98	*
Glufosinate + clethodim	451 + 204	94 NS	100	*	94 NS	99	*
Glufosinate + clethodim	595 + 76	85 ∨	99	*	89 NS	99	*
Glufosinate + clethodim	595 + 136	83 ∨	99	*	83 ∨	97	*
Glufosinate + clethodim	595 + 204	94 NS	100	*	91 NS	99	*
Glufosinate + clethodim	738 + 76	95 NS	100	NS	92 NS	98	NS
Glufosinate + clethodim	738 + 136	95 NS	100	*	93 NS	98	NS
Glufosinate + clethodim	738 + 204	94 NS	100	*	93 NS	99	NS
Glufosinate + *S*-metolachlor	451 + 1389	88 NS			81 NS		
Glufosinate + clethodim + *S*-metolachlor	451 + 76 + 1389	88 ∨	99	*	90 NS	97	*
Glufosinate + clethodim + *S*-metolachlor	451 + 136 + 1389	95 NS	100	*	91 NS	99	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone, based on the ANOVA and Fisher's protected LSD ($\alpha = 0.05$). NS indicates the mixture was similar to both of the herbicides alone.

[d] A "*" denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby's equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

Table 10. Effect of glufosinate alone and in combinations with glyphosate or clethodim on observed and expected control 4 WAT and aboveground biomass of large crabgrass in Experiment 2[a]

		Control			Biomass Reduction[b]		
Common name	Rate	Obs[c]	Exp	p[d]	Obs[c]	Exp	p[d]
	g ai ha^{-1}	-%-	-%-		-%-	-%-	
Nontreated					0		
Glufosinate	451	86			84		
Glufosinate	595	86			83		
Glyphosate	867[e]	98			96		
Glyphosate	1735[e]	100			100		
Clethodim	76	94			86		
Clethodim	136	97			95		
Glufosinate + glyphosate	451 + 867[e]	95 NS	100	*	93 NS	99	*
Glufosinate + glyphosate	451 + 1735[e]	98 NS	100	*	95 NS	100	*
Glufosinate + glyphosate	595 + 867[e]	95 NS	100	*	90 NS	99	*
Glufosinate + glyphosate	595 + 1735[e]	97 NS	100	*	95 NS	100	*
Glufosinate + clethodim	451 + 76	82 ∨	99	*	81 NS	98	*
Glufosinate + clethodim	451 + 136	93 NS	99	*	90 NS	99	*
Glufosinate + clethodim	595 + 76	83 ∨	99	*	84 NS	97	*
Glufosinate + clethodim	595 + 136	81 ∨	100	*	88 ∨	99	*

[a] Abbreviation: Obs, observed value; Exp, expected value; NS, not significant; WAT, weeks after treatment.

[b] Biomass is expressed as a percent of the nontreated control.

[c] A “∨” indicates a mixture that provided significantly less control compared to at least one of the herbicides alone, based on the ANOVA and Fisher’s protected LSD ($\alpha = 0.05$). NS indicates the mixture was similar to both of the herbicides alone.

[d] A “*” denotes significant antagonism based on a two-sided t-test between observed and expected values. Expected values are based on Colby’s equation [$E = (X + Y) - (XY)/100$]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}

Table 11. Spray characteristics of various herbicide combinations in Experiment 1 including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm when applied using a TT 110015 nozzle at 276 kPa.

		Droplet spectra parameters[a]				
Herbicide treatment	Rate	D_{v10}	D_{v50}	D_{v90}	Relative span[b]	<150 µm
	g ai ha^{-1}	µm	µm	µm		$\%_{vol}$
Water		143 a	307 a	488 a	1.12 cd	11.1 i
Glufosinate	451	136 abcde	296 b	478 a	1.15 c	12.4 efghi
Glufosinate	595	126 fghi	280 c	470 a	1.23 b	14.9 abcde
Glufosinate	738	122 hi	277 c	476 a	1.28 a	15.9 abcd
Clethodim	76	132 bcdefg	257 d	397 cd	1.03 fg	13.8 cdefghi
Clethodim	136	130 defghi	256 d	401 cd	1.06 ef	14.5 bcdefg
Clethodim	204	130 efghi	254 de	399 cd	1.06 ef	14.6 bcdefg
Glufosinate + clethodim	451 + 76	139 abcd	276 c	429 b	1.05 efg	12.0 fghi
Glufosinate + clethodim	451 + 136	138 abcde	262 d	401 cd	1.00 g	12.5 efghi
Glufosinate + clethodim	451 + 204	132 bcdefgh	255 de	394 cd	1.02 fg	13.9 bcdefgh
Glufosinate + clethodim	595 + 76	140 abc	276 c	429 b	1.05 efg	11.9 ghi
Glufosinate + clethodim	595 + 136	137 abcde	263 d	402 cd	1.00 g	12.6 efghi
Glufosinate + clethodim	595 + 204	132 cdefgh	254 de	393 cd	1.03 fg	14.2 bcdefgh
Glufosinate + clethodim	738 + 76	141 ab	280 c	439 b	1.06 ef	11.6 hi
Glufosinate + clethodim	738 + 136	135 abcdef	262 d	406 c	1.03 efg	13.2 defghi
Glufosinate + clethodim	738 + 204	129 efghi	255 de	399 cd	1.06 ef	14.7 abcdef
Glufosinate + *S*-metolachlor	451 + 1389	122 i	238 f	370 e	1.04 efg	17.4 a
Glufosinate + clethodim + *S*-metolachlor	451 + 76 + 1389	124 ghi	245 ef	387 cde	1.08 de	16.6 ab
Glufosinate + clethodim + *S*-metolachlor	451 + 136 + 1389	125 ghi	244 ef	384 de	1.06 ef	16.3 abc

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment ($\alpha = 0.05$).

[b] Relative span is a unitless index of the uniformity of droplet size distribution. Smaller values represent more uniformity in droplet size distribution.

Table 12. Spray characteristics of various herbicide combinations in Experiment 2 including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm when applied using a TT 110015 nozzle at 276 kPa.

		Droplet spectra parameters[a]									
Herbicide treatment	Rate	D_{v10}		D_{v50}		D_{v90}		Relative span[b]		<150 µm	
	g ai ha^{-1}	---------------------µm---------------------								$\%_{vol}$	
Water		143	Ab	307	a	488	ab	1.12	cde	11.1	de
Glufosinate	451	136	Bcd	296	b	478	abc	1.15	bc	12.4	cd
Glufosinate	595	126	E	280	de	470	c	1.23	a	14.9	a
Glyphosate	867[c]	144	Ab	285	cde	451	de	1.07	defg	11.0	de
Glyphosate	1735[c]	137	Bcd	290	bc	467	cd	1.14	bcd	12.5	bcd
Clethodim	76	132	Cde	257	f	397	g	1.03	gh	13.8	abc
Clethodim	136	130	De	256	f	401	g	1.06	efgh	14.5	ab
Glufosinate + glyphosate	451 + 867[c]	133	Cde	287	bcd	478	abc	1.20	ab	13.3	abc
Glufosinate + glyphosate	451 + 1735[c]	139	Abc	277	e	442	ef	1.09	cdefg	12.1	cde
Glufosinate + glyphosate	595 + 867[c]	124	E	280	de	475	bc	1.25	a	15.3	a
Glufosinate + glyphosate	595 + 1735[c]	148	A	311	a	494	a	1.11	cdef	10.3	e
Glufosinate + clethodim	451 + 76	139	Abc	276	e	429	f	1.05	fgh	12.0	cde
Glufosinate + clethodim	451 + 136	138	Bcd	262	f	401	g	1.00	h	12.5	bcd
Glufosinate + clethodim	595 + 76	140	Abc	276	e	429	f	1.05	fgh	11.9	cde
Glufosinate + clethodim	595 + 136	137	Bcd	263	f	402	g	1.00	h	12.6	bcd

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] Relative span is a unitless index of the uniformity of droplet size distribution. Smaller values represent more uniformity in droplet size distribution.

[c] Rate is in g ae ha^{-1}.

Chapter 7

Do nozzle selection and spray volume affect performance of glufosinate-containing mixtures?

Adjustments to spray volume and droplet size can have a dramatic effect on the performance of some herbicides (e.g., glufosinate) and minimal impact on others (e.g., glyphosate). Herbicide mixtures that can be applied in multiple herbicide-resistant soybean or cotton technologies may also be susceptible to changes in spray volume or droplet size if glufosinate is a component of the mixture. Two experiments were conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR to understand the impact of spray volume and droplet size on herbicide interactions that may occur with glufosinate. Clethodim, fomesafen, and glyphosate, were applied alone and in combination with glufosinate at two spray volumes (47 and 140 L ha^{-1}) in a Spray Volume Experiment. In the Nozzle Selection Experiment, clethodim, dicamba, fomesafen, and glyphosate were applied alone and in combination with glufosinate with three nozzle types. Colby's method was used to test for herbicide interactions for the various mixtures for percent control, height, and density reduction of barnyardgrass, Palmer amaranth, and prickly sida at 4 weeks after application. Although glufosinate alone performed better applied at 140 L ha^{-1} than 47 L ha^{-1} on all species, changing spray volume did not affect control for mixtures of glufosinate + glyphosate on barnyardgrass or prickly sida. When applied at 140 L ha^{-1}, glufosinate + glyphosate was antagonistic and provided significantly less control (85%) than glyphosate alone (93%). Glufosinate + clethodim was also antagonistic for barnyardgrass control at both spray volumes, providing 79% control of barnyardgrass, and did not differ from clethodim alone. In the Nozzle Experiment, smaller droplet sizes generally improved control with fomesafen and glufosinate, but droplet size had no impact on efficacy of dicamba,

glyphosate, or clethodim. Glufosinate + clethodim was antagonistic for barnyardgrass control applied with all nozzle types, but control was greater for the mixture applied with a TADF nozzle (coarse droplet) compared to a TTI nozzle (ultra-coarse droplet). Nozzle selection did not impact barnyardgrass control with glufosinate + glyphosate. The mixture was considered antagonistic when applied with all three nozzles. Spray volume and nozzle selection did not have a clear impact on glufosinate-containing mixtures. However, these results generally support that if glufosinate is applied in mixture, application variables should be optimized for glufosinate to improve the likelihood that control is maximized across a diverse weed spectrum.

Nomenclature: clethodim; dicamba; fomesafen; glufosinate; glyphosate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; prickly sida, *Sida spinosa* L.

Key words: Antagonism; glufosinate mixtures; herbicide interactions; nozzle selection; spray volume.

Application of herbicides in mixtures is a common practice with several recognized benefits, including improving efficiency of a weed control program, increasing spectrum of control, and reducing the likelihood of evolution of herbicide resistance (Hatzios and Penner 1985; Norsworthy et al. 2012). When two herbicides are co-applied, they will either act independently of one another (additive effect), interact to enhance (synergistic effect), or decrease (antagonistic effect) the performance of the mixture. Investigations into herbicide interactions understandably control for application parameters such as droplet size or spray volume, which can have a dramatic impact on herbicide efficacy (Creech et al. 2015b, 2016; Etheridge et al. 2001; McKinlay et al. 1974; Meyer et al. 2015b, 2016; Ramsdale and Messersmith 2001). Thus, altering recommended nozzle type or spray volume may affect the interaction and efficacy of herbicide mixtures.

Nozzle requirements on 2,4-D and dicamba herbicide labels (Anonymous 2017; 2018) increase the likelihood that mixtures with herbicides such as glufosinate will be applied at larger droplet sizes than recommended (e.g., Anonymous 2016) and could compromise efficacy. Droplet size impacts weed efficacy due to a variety of factors. For contact herbicides, improvements in leaf retention and spray coverage tend to be associated with increases in efficacy from the use of smaller droplet sizes (Knoche 1994; McKinlay et al. 1972; Ramsdale and Messersmith 2001). The ability of a leaf to retain larger droplets is more difficult for grass weeds with a vertical leaf architecture than for broadleaf weeds that typically have horizontal leaf surfaces (Etheridge et al. 2001; McKinlay et al. 1974). Droplet size is also correlated with spray coverage, with smaller droplets achieving more coverage than larger droplets (Meyer et al. 2016; Ramsdale and Messersmith 2001). The effect of droplet size on efficacy of systemic herbicides is less straightforward, where optimum droplet size tends to be more dependent upon the specific herbicide and weed species in question (Creech et al. 2016; Feng et al. 2003; Knoche 1994; Meyer et al. 2015b).

The impact of carrier volume on herbicide efficacy is also dependent upon herbicides applied and target weed species (Creech et al. 2015b; Knoche 1994; Meyer et al. 2016). At spray volumes typical for commercial ground applicators (~140 L ha^{-1}), a decrease in spray volume tends to reduce herbicide efficacy (Knoche 1994). Creech et al. (2015b) investigated spray volumes between 47 and 281 L ha^{-1} and determined efficacy was maximized for glyphosate at 70 L ha^{-1}, glufosinate at 140 L ha^{-1}, and lactofen at 197 L ha^{-1}. In the same study, few differences in fluazifop-P efficacy were observed among carrier volumes. The aforementioned publications have offered an understanding of the interaction between weed species and spray volume on performance of single herbicides, few papers have investigated how the performance of herbicide mixtures could be affected by spray volume.

Glufosinate and other contact-type herbicides tend to have improved performance when applications are made with smaller droplet sizes (Etheridge et al. 2001). Additionally, glufosinate efficacy is maximized with droplet sizes of medium to coarse when applied to most weedy species, compared to extremely coarse or ultra-coarse droplet sizes (Creech et al. 2016; Meyer et al. 2015b). In crop technologies with multiple herbicide-resistant traits (i.e., Enlist™ or Bollgard® II Xtendflex® cotton), it is possible glufosinate could be mixed with 2,4-D, dicamba, or other herbicides requiring the use of nozzles that produce larger droplets. Alternatively, sprayer operators that alternate between dicamba or 2,4-D applications and glufosinate may not take the time to switch nozzles on the sprayer if not specifically required to do so. Both situations could result in applications of glufosinate using droplet sizes that are not optimum for one or more of the herbicides in mixture. The objective of these experiments was to determine if nozzle selection and spray volume could affect antagonism of glufosinate-containing mixtures.

Materials and Methods

Experiments were conducted in the field and in the laboratory to determine the effects of nozzle selection and spray volume on the performance of herbicide mixtures. Two field experiments were established in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, Arkansas. No crop was planted in either experiment. At the time of trial establishment, the experimental area was disked and field cultivated to prepare an optimum seedbed for weed emergence. The soil in the experimental area was a Sharkey clay (very fine, montmorillonitic, nonacid, thermic, Vertic Haplaquept) with 22% sand, 25% silt, and 53% clay, a pH of 6.6, and 1.7% organic matter. Individual plots were 3.6 m wide by 10.7 m long.

Both field experiments used randomized complete block designs with a factorial treatment structure consisting of two factors. Each experiment was repeated twice, and a given experiment contained four replications of each treatment. In the first experiment (Spray Volume Experiment) the two factors were herbicide treatment (seven levels) and spray volume (two levels). The herbicide treatments were standard rates of clethodim, fomesafen, glufosinate, glyphosate, and all two-way mixtures with glufosinate. Specific herbicide products and their use rates are listed in Table 1. The two spray volumes evaluated were 47 and 140 L ha^{-1}. TeeJet (TeeJet Technologies, Springfield, Illinois) Turbo TeeJet (TT) 110015 nozzles were used for application of all herbicide treatments. Spray volume was altered from 47 to 140 L ha^{-1} by reducing speed of the application from 15 km h^{-1} to 5 km h^{-1}, thereby keeping the other application parameters (i.e., nozzle size) constant. A MudMaster multiboom tractor sprayer

(Bowman Manufacturing Co., Inc. Newport, Arkansas) was used to make the herbicide applications calibrated at 276 kPa with 48 cm nozzle spacing.

In the second experiment (Nozzle Experiment) the two factors were herbicide solution (9 levels) and nozzle type (3 levels). The herbicide treatments (solutions) were standard use rates of clethodim, dicamba, fomesafen, glufosinate, glyphosate, and all two-way mixtures with glufosinate (Table 1). The three nozzle types evaluated were TeeJet Turbo TeeJet (TT), TeeJet Turbo TeeJet Induction (TTI), and Greenleaf TurboDrop DualFan (TADF) (Greenleaf Technologies, Covington, LA). All nozzles were 11015 nozzle size and treatments were applied at 344 kPa and 5.4 km hr^{-1} using a MudMaster multiboom tractor sprayer. A higher pressure was used in the Nozzle Experiment than in the Spray Volume Experiment because the manufacturer of the TADF nozzle recommends applications at a minimum of 344 kPa, compared to a range of 100-620 kPa for the TT and 100-690 kPa for the TTI nozzles.

In the Spray Volume Experiment, treatments were applied at 2:00 PM on July 29, 2015, and 3:00 PM on July 18, 2016. Air temperature was 35 C and 34 C, relative humidity was 77% and 48%, and wind speed was 3 km hr^{-1} and 1 km hr^{-1} in 2015 and 2016, respectively. In the Nozzle Experiment, treatments were applied at 6:00 PM on July 29, 2015, and 9:00 AM on July 19, 2016. Air temperature was 35 C and 31 C, relative humidity was 77% and 69%, and wind speed was 3 km hr^{-1} and 0 km hr^{-1} in 2015 and 2016, respectively. Treatments were applied to large, actively growing weeds in both experiments (Table 2). Twenty-four hours after treatments were applied, all plots in both experiments received an application of *S*-metolachlor at 1068 g ai ha^{-1} to prevent new weed emergence.

Control of barnyardgrass, Palmer amaranth, and prickly sida was assessed in both experiments 4 weeks after treatment (WAT). Percentage weed control was visually assessed relative to nontreated plots included in each experiment, with 0% representing no control and 100% indicating plant death**.** At the time of the visual assessment, weed heights and densities of each species were collected. Heights of three individuals were taken for each species, in each plot. Weed densities were assessed by counting individuals of a given species in two, 1-m^2 quadrats per plot. If one or fewer individuals of a given species were counted in a quadrat, all individuals in the plot were counted. Weed height and density data are presented as percent height or density reductions, relative to the nontreated check. Thus, 100% density reduction is equivalent to 100% control of a given species. Representing height and densities as percentages also enables the use of Colby's method on these data.

A laboratory experiment was conducted at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE, to collect droplet spectra for all field treatments. A low-speed wind tunnel was equipped with a laser (Sympatec HELOS-VARIO/KR particle size analyzer, Sympatec GmbH, Clausthal-Zellerfeld, Germany) and an R7 lens enabling the measurement of spray droplets from 18 to 3500 μm in size. Spray particles were measured via laser diffraction as the nozzle plume passes across the laser as described by Creech et al. (2015a) and Henry et al. (2014). The nozzle was positioned 30-cm upwind of the laser and a linear actuator moved the width of the nozzle plume across the laser. Droplet spectra of each treatment were replicated three times. The same products used in the field experiment were also used for particle size analysis. From the particle size data, various parameters used to describe the droplet spectra could be determined including the D_{v10}, D_{v50} D_{v90}, relative span, and the percent fines. The D_{v50} is the median droplet size as a function of the total volume of all droplets instead of the number of droplets. Spray classifications reported in the text are correspond to classifications by American Society of Agricultural and Biological Engineers (ASABE) Standard

572.1 (ASABE 2009). ASABE spray classifications are based off the D_{v50}. The D_{v10} and D_{v90} are similar parameters to the D_{v50} for 10% and 90% of the spray volume, respectively. In these experiments, the number of fine droplets were considered those with a diameter less than 150 μm ($\%_{vol}$ fines), again represented as a fraction of the total volume. To represent the range in droplet sizes in a spectrum, a relative span (RS) was determined using Equation 1.

$$RS = (D_{v90} - D_{v10})\ D_{v50}^{\ -1} \qquad \text{(Equation 1)}$$

Field data were analyzed with ANOVA in JMP Pro 13 with year and block included as random effects (Table 3), and treatment means were separated using Fisher's protected LSD (α = 0.05). If a herbicide had no POST activity on a given species (e.g., clethodim applied to Palmer amaranth), it was removed from analysis for that species. In addition to the ANOVA, Colby's method (Colby 1967) was used to test for herbicide interactions for the glufosinate mixtures. Results from the ANOVA were used to compare mixtures directly to their component herbicides. Colby's method determined if the mixture was antagonistic, additive, or synergistic by comparing the mixture to the calculated Expected value. Results from both analyses on a given species are presented together in a single table (e.g., Table 4). For the particle-size analysis, no run or blocking factors were included in the model (completely randomized design) and a Tukey adjustment (α = 0.05) was used to separate means.

Results and Discussion

Palmer Amaranth. *Spray Volume Experiment.* Glufosinate and fomesafen, two contact herbicides, had lower Palmer amaranth control, height reduction, and density reduction at 47 L ha^{-1} compared to 140 L ha^{-1} (Table 4). Even though the Palmer amaranth population evaluated in both experiments was considered glyphosate-resistant, glyphosate provided some control and was not affected by spray volume for any parameter (e.g., 34 and 36% control for 47 L ha^{-1} and

140 L ha^{-1}, respectively). When glufosinate was applied in mixture with glyphosate, percent control was also greater at the higher spray volume, which was also seen with glufosinate alone.

Antagonism was identified for height reduction and density reduction provided by glufosinate + fomesafen at both spray volumes. For percent Palmer amaranth control with glufosinate + fomesafen, antagonism was identified only at 140 L ha^{-1}; however, the observed value for glufosinate + fomesafen at 140 L ha^{-1} was still 94% (expected value=99%). Therefore, a high level of Palmer amaranth control was observed for glufosinate + fomesafen if applied at an optimum spray volume (140 L ha^{-1}), despite the occurrence of antagonism. This result generally agrees with Culpepper et al. (2000) who reported the addition of fomesafen to glufosinate increased control of most species and made no impact on others compared to glufosinate alone. No differences were observed between glufosinate or fomesafen alone and the combination of the two herbicides, indicating that a mixture of glufosinate + fomesafen was not necessary for adequate control of Palmer amaranth in these experiments.

Particle size analysis determined that for glufosinate and fomesafen, D_{v50} was smaller at 47 L ha^{-1} than at 140 L ha^{-1} (Table 5). Contact herbicides tend to perform better at smaller droplet sizes, but also at higher spray volumes (Creech et al. 2015a, 2016; Knoche 1994; Meyer et al. 2015a, 2016). In this experiment, it is likely that relatively small differences in droplet size are not as important as a large change in spray volume for glufosinate and fomesafen efficacy. For example, D_{v50} for glufosinate at 47 L ha^{-1} was 253 µm compared to 280 µm at 140 L ha^{-1}. Changes in nozzle type that result in differences in control from contact herbicides typically require much larger changes (>100 µm) in D_{v50} (Creech et al. 2016; Etheridge et al. 2001; Meyer et al. 2015b).

Glufosinate + glyphosate had a smaller D_{v50} applied at 140 L ha^{-1} (257 µm) compared to 47 L ha^{-1} (269 µm). The smaller droplet size may favor glufosinate activity when in mixture with glyphosate, at the higher spray volume. However, the percentage of fine droplets ($\%_{vol}$ fines) for glufosinate + glyphosate was 16.9$\%_{vol}$ at 47 L ha^{-1} compared to 14.1$\%_{vol}$ at 140 L ha^{-1}. The greater droplet size and $\%_{vol}$ fines is a function of a larger relative span (RS) for glufosinate + glyphosate at 47 L ha^{-1} (RS=1.29) compared to 140 L ha^{-1} (RS=1.04), meaning the treatment also has a larger $\%_{vol}$ of larger droplets at the lower spray volume. Ultimately, the differences in droplet size caused from changing spray volumes is likely negligible compared to the changes in coverage at the two spray volumes.

Nozzle Experiment. Glufosinate alone provided ≥94% control of Palmer amaranth as long as it was applied with a TT or TADF nozzle (Table 6). When glufosinate was applied with a TTI nozzle, control declined to 84%, compared to 95% with the TT. The effect of nozzle on Palmer amaranth control is likely a direct function of droplet size, as glufosinate applied with a TT nozzle produced coarse droplets (D_{v50}=269 µm), compared to ultra-coarse with a TTI nozzle (D_{v50}=686 µm) (Table 7).

Overall, Palmer amaranth control, height reduction, and density reduction with mixtures was not greater than the respective herbicide components alone. One exception was for glufosinate + glyphosate with a TTI nozzle, where all three parameters were significantly improved over both glufosinate and glyphosate alone. Control with glufosinate + glyphosate was not different among the three nozzle types, whereas control with glufosinate alone was significantly less with ultra-coarse droplets compared to coarse.

For some glufosinate-containing mixtures, it appeared nozzle selection was less important to maximize efficacy compared to glufosinate alone. However, control with glufosinate + dicamba was lower with the TTI nozzle (86%) compared to either the TT or TADF nozzles (92 and 95%, respectively). Additionally, glufosinate + dicamba applied with a TTI nozzle was antagonistic for percent control, height reduction, and density reduction. As a clear pattern was not apparent, a nozzle should be selected that is most likely to maximize efficacy of glufosinate if the mixture and nozzle combination is allowed by the herbicide labels.

Prickly Sida. *Spray Volume Experiment.* Both mixtures of glufosinate + glyphosate and glufosinate + fomesafen were antagonistic for prickly sida control at both spray volumes (Table 8). Control was lower when glufosinate + fomesafen was applied at 47 L ha^{-1} compared to 140 L ha^{-1}. These results suggest mixtures should be applied with application variables that result in the best control or are most likely to provide the greatest control. In the case of glufosinate + fomesafen, both are contact herbicides that tend to perform better applied at greater spray volumes (Creech et al. 2015a; Knoche 1994).

Nozzle Experiment. As was observed with Palmer amaranth control, prickly sida control with glufosinate was greatest (92%) with the TT nozzle (coarse droplets) compared with the TTI nozzle (77%) (ultra-coarse droplets) (Table 9). Only height reduction from fomesafen followed the trend of improved control at smaller droplets, although fomesafen alone provided marginal control of prickly sida, regardless of droplet size. Antagonism was identified for glufosinate + dicamba, applied with either the TTI or TADF nozzles. Even though control, height reduction, and density reduction for glufosinate + dicamba were not different among nozzles, all three parameters were numerically greater for the TT nozzle with the smallest droplet size (D_{v50}=268, 306, 682 μm for TT, TADF, and TTI nozzles, respectively) (Table 7). Glufosinate performs better at medium to coarse droplet sizes. Thus, when glufosinate is applied in combination with a systemic herbicide, using medium to coarse droplets may provide improved control over applications with larger droplet sizes.

Barnyardgrass. *Spray Volume Experiment.* In contrast to the broadleaf weeds, glyphosate alone (140 L ha^{-1}) provided the greatest control of barnyardgrass of all treatments (Table 10). The level of control with glyphosate alone (≥92% for both spray volumes) highlights the herbicide's versatility and the notion that glyphosate still has a key role in multiple herbicide trait technologies (e.g., Enlist and Bollgard II Xtendflex). Glufosinate alone controlled barnyardgrass only 78% at 140 L ha^{-1}, and the mixture of glufosinate + glyphosate was antagonistic at both spray volumes and for all three response parameters (percent control, height reduction, and density reduction). At 140 L ha^{-1}, glufosinate + glyphosate provided less control (85%) than glyphosate alone (93%), and the trend was similar for density reduction, implying that glyphosate>glufosinate + glyphosate > glufosinate for barnyardgrass control. As glyphosate alone is no longer an option for many growers across the Midsouth, glufosinate + glyphosate may be the preferred POST option if weed management decisions are driven by Palmer amaranth but large barnyardgrass is also present in the field.

Glufosinate + clethodim was also antagonistic at both spray volumes. Observed control for glufosinate + clethodim at 140 L ha^{-1} was 79%, compared to an expected value of 95%. No differences in control were observed between spray volumes for glufosinate + clethodim. At 47 L ha^{-1}, control with glufosinate + clethodim (79%) was greater than control with glufosinate alone (69%) but was not different from clethodim (76%). When glufosinate and clethodim were applied in a mixture at 140 L ha^{-1}, a spray volume preferable for glufosinate, the mixture did not differ from either glufosinate or clethodim alone. The clethodim rate used in this study (76 g ai ha^{-1}) is the low end of the labeled use rate, and clethodim may be applied up to 292 g ai ha^{-1} in a single application. The results from this study indicate that higher rates of clethodim may be needed for adequate control of large grass weeds and may reduce the likelihood of antagonism if higher rates are mixed with glufosinate.

Nozzle Experiment. As observed with both Palmer amaranth and prickly sida, barnyardgrass control with glufosinate declined from 87% applied with a TT nozzle to 68% applied with a TTI (Table 11). Glyphosate efficacy was not impacted by nozzle selection (all response parameters), and neither was percent control of glyphosate + glufosinate. However, density reduction was greater when glufosinate + glyphosate was applied with a TT nozzle compared to a TTI, once again indicating the value of applying glufosinate-mixtures using optimum spray droplets. The $D_{v50}$for glufosinate + glyphosate was 239 µm with the TT nozzle and 610 µm with the TTI nozzle.

Glufosinate + glyphosate mixtures applied with TT, TADF, and TTI nozzles were antagonistic for all parameters. Also, height and density reduction of all glufosinate + glyphosate treatments were less than glyphosate alone. When observed values for a mixture are less than one of the component herbicides alone, which serious cause for concern is in regards to resistance management, implying the mixture should not be used on that species. The antagonism observed between glyphosate and glufosinate is attributed to reduced uptake and translocation of glyphosate (Besançon et al. 2018). As glufosinate, a fast acting, contact herbicide, inhibits uptake and translocation of glyphosate, it is also likely mixtures glufosinate + clethodim are responding the same way.

When glufosinate + clethodim was applied with TT, TADF, or TTI nozzles, antagonism was identified for all parameters, except for density reduction with a TTI nozzle. For example, the observed level of barnyardgrass control with glufosinate + clethodim with a TT nozzle was 79%, compared to an expected value of 96%. Although glyphosate alone provided considerably more control than clethodim alone, mixtures of glufosinate + glyphosate and glufosinate + clethodim did not differ for any response parameter when compared within a given nozzle type, as a result of antagonism. Thus, if a grower wishes to apply another herbicide with glufosinate to broaden activity to other grass weeds, selecting clethodim over glyphosate will not likely have any impact on barnyardgrass control.

Fomesafen alone provided negligible (≤ 20%) control of barnyardgrass. When applied in mixture, fomesafen did not have any measurable effect on barnyardgrass control for all nozzles (i.e., no antagonism was identified and no difference in control compared to glufosinate alone). Protoporphyrinogen oxidase (PPO)-inhibitor resistant weeds (e.g., Palmer amaranth) are present throughout the U.S. (Heap 2018) and fomesafen had marginal activity POST on the other weeds in this study (prickly sida and barnyardgrass). However, fomesafen may still be useful as a soil applied herbicide (Umphres et al. 2018) or as an additional POST option to glufosinate for other weeds in glufosinate-resistant soybean (Culpepper et al. 2000).

Practical Implications

As was expected, weed control with glufosinate and fomesafen was greater at smaller droplet sizes (Creech et al. 2016; Etheridge et al. 2001; Meyer et al. 2015a) and greater spray volumes (Creech et al 2015; Knoche 1994; Meyer et al. 2016). Thus, glufosinate and fomesafen should not be applied at 47 L ha^{-1} or with ultra-coarse droplets. The effects of spray volume and droplet size on systemic herbicides, such as glyphosate, remain unclear. In these experiments, glyphosate was typically unaffected by changes in spray volume and droplet size. Creech et al. (2015) observed no clear trends in grain Amaranth (*Amaranthus hypochondriacus* L.) dry weight reduction with glyphosate across spray volumes (47-281 L ha^{-1}) but saw an increase in efficacy with higher spray volumes in corn. As for droplet size, Meyer et al. (2015b) observed that barnyardgrass biomass in plots treated with glyphosate was consistently lower with AIXR nozzles than TTI nozzles (D_{v50}=465 vs 788 um for AIXR and TTI nozzles, respectively), whereas others observed improved glyphosate activity with larger droplets (Creech et al. 2015; Feng et al. 2003).

The prevalence of glyphosate-resistant weeds diminishes the likelihood glyphosate will be applied alone and will almost certainly be applied in mixture with glufosinate or other herbicides. Control with glufosinate-containing mixtures in the current experiments were less likely to be affected by changes in spray volume and droplet size than glufosinate alone. The impact of spray volume or droplet size on glufosinate-mixtures depended on the specific mixture and weed species evaluated. These data suggest that application parameters such as spray volume and droplet size should still be optimized for glufosinate regardless of the mix partner or targeted weed species.

Antagonistic mixtures, glyphosate-resistant weeds, nozzle requirements, and grower preferences to apply at lower spray volumes, all present a challenge for managing weeds in crop technologies with a glufosinate-resistant trait. If glufosinate was applied at 140 L ha^{-1} with a TT nozzle, no other herbicide or mixture provided greater Palmer amaranth or prickly sida control. However, when glufosinate was applied to a tall (~22 cm), dense (~35 plants m^{-2}) population of barnyardgrass, control was improved when glyphosate was added to glufosinate in the Spray Volume Experiment. Glufosinate alone can provide equivalent control to glyphosate when applied to small weeds, depending on the species (Culpepper et al. 2000). Thus, the recommended treatment for a field infested with small barnyardgrass, Palmer amaranth, and prickly sida is glufosinate alone applied at 140 L ha^{-1} with coarse spray droplets. The addition of a residual herbicide, such as *S*-metolachlor, dimethanamid-P, or acetochlor to glufosinate, is a better resistance-management tactic than glufosinate alone (Norsworthy et al. 2012). In an Enlist or Bollgard II Xtendflex cotton system, the glufosinate application could be followed with a second POST application of glyphosate plus the appropriate synthetic auxin herbicide. If large barnyardgrass, or other difficult-to-control weeds are present, sequential applications may be required for adequate control (Culpepper et al. 2000; Meyer et al. 2015a).

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Appendix

Table 1. Herbicide information for all products used in the experiments [a]

Herbicide common name	Herbicide Trade Name	Rate	Manufacturer	Address	Website	Adjuvant[b]
		g ai ha^{-1}				
Clethodim	Select Max	76	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	COC
Dicamba	Clarity	560[c]	BASF Corporation	Research Triangle Park, NC	www.basf.com	NIS
Fomesafen	Flexstar	264	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	NIS
Glufosinate	Liberty	594	Bayer CropScience LP	Research Triangle Park, NC	www.bayercropscienceus.com	
Glyphosate	Roundup Powermax	867[c]	Monsanto Company	St. Louis, MO	www.monsanto.com	
S-metolachlor	Dual Magnum	1068	Syngenta Crop Protection LLC	Greensboro, NC	www.syngenta.com	

[a] Abbreviations: NIS, nonionic surfactant (Helena Chemical Company, Collierville, TN); COC, crop oil concentrate (Helena Chemical Company, Collierville, TN).

[b] Adjuvant rates: NIS, 0.25% v v^{-1}; COC, 1% v v^{-1}.

[c] Rate is in g ae ha^{-1}

Table 2. Weed sizes and densities of weeds present at herbicide application evaluated in both Experiments in 2015 and 2016.

	Spray Volume Experiment				Nozzle Experiment			
	2015		2016		2015		2016	
Species	Height	Density	Height	Density	Height	Density	Height	Density
	cm	plants m^{-2}	cm	plants m^{-2}	cm	plants m^{-2}	cm	plants m^{-2}
Barnyardgrass	23	31	20	39	24	19	15	42
Palmer amaranth	24	2	21	2	24	3	13	6
Prickly sida	14	2	12	10	17	12	12	7

Table 3. Variance components estimates obtained from the ANOVA for barnyardgrass, Palmer amaranth, and prickly sida control, height reduction, and biomass reduction from the Spray Volume and Nozzle Experiments[a].

		Barnyardgrass			Palmer amaranth			Prickly sida		
Experiment	Model effect	4 WAT	Height reduction	Density reduction	4 WAT	Height reduction	Density reduction	4 WAT	Height reduction	Density reduction
		---% of total---								
Spray Volume	Rep(Year)	1.4	0.0	<0.1	<0.1	5.5	0.7	1.7	0.8	5.3
	Year	26.6	87.0	87.8	14.4	15.3	<0.1	<0.1	<0.1	<0.1
	Residual	72.0	12.9	12.2	85.6	79.2	99.3	98.3	99.2	94.7
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nozzle	Rep(Year)	3.7	0.0	<0.1	0.8	4.4	5.8	<0.1	<0.1	<0.1
	Year	28.6	11.1	22.2	46.3	<0.1	<0.1	54.5	70.3	1.9
	Residual	67.7	88.9	77.8	52.9	95.6	94.2	45.5	29.7	98.0
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Abbreviation: WAT, weeks after treatment.

Table 4. Palmer amaranth control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by spray volume in the Spray Volume Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Spray volume	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	L ha^{-1}	%		%		%		%		%		%	
Glufosinate	595	47	85				80				83			
		140	96				91				92			
Glyphosate	867[e]	47	34				9				4			
		140	36				13				9			
Fomesafen	264	47	77				73				83			
		140	92				82				91			
Clethodim	76	47	0				0				0			
		140	0				0				0			
Glufosinate + glyphosate	595 + 867[e]	47	85	NS	90	NS	82	NS	82	NS	84	NS	83	NS
		140	93	NS	97	NS	97	NS	92	NS	96	NS	92	NS
Glufosinate + fomesafen	595 + 264	47	89	NS	96	NS	81	NS	95	*	87	NS	97	*
		140	94	NS	99	*	86	NS	97	*	85	NS	99	*
Glufosinate + clethodim	595 + 76	47	88	NS			87	^			87	NS		
		140	93	NS			86	NS			89	NS		
LSD			7				8				8			

[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A "^" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percentage of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 5. Spray characteristics of various herbicide combinations at two spray volumes used in the Spray Volume Experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm.

			Droplet spectra parameters[a]				
Herbicide	Rate	Spray volume	D_{v10}	D_{v50}	D_{v90}	Relative span[b]	<150 µm
	g ai ha^{-1}	L ha^{-1}	---------------------µm---------------------			-	$\%_{vol}$
Glufosinate	595	47	111 h	253 gh	444 c	1.32 ab	19.7 a
		140	126 def	280 bc	470 ab	1.23 bc	14.9 cde
Glyphosate	867[e]	47	129 cde	282 b	473 a	1.22 bc	14.2 de
		140	122 efg	275 bcd	471 ab	1.27 abc	16.1 bcd
Fomesafen	264	47	132 cd	275 bcd	454 bc	1.17 cd	13.7 def
		140	146 a	295 a	470 ab	1.10 de	10.8 g
Clethodim	76	47	128 cdef	255 fgh	403 d	1.08 de	15.0 cde
		140	142 ab	285 ab	454 bc	1.10 de	11.5 fg
Glufosinate + glyphosate	595 + 867[e]	47	119 fgh	269 cde	467 ab	1.29 ab	16.9 bc
		140	131 cd	257 fgh	398 d	1.04 e	14.1 de
Glufosinate + fomesafen	595 + 264	47	114 gh	262 efg	467 ab	1.35 a	18.5 ab
		140	124 def	274 bcd	465 ab	1.24 bc	15.5 cde
Glufosinate + clethodim	595 + 76	47	129 cde	251 h	394 d	1.06 e	15.1 cde
		140	125 def	277 bc	468 ab	1.24 bc	15.4 cde

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] Relative span is a unitless index of the uniformity of droplet size distribution. Smaller values represent more uniformity in droplet size distribution.

[c] Rate is in g ae ha^{-1}.

Table 6. Palmer amaranth control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle[d]	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
	g ai ha^{-1}		%		%		%		%		%		%	
Glufosinate	595	TT	95				88				90			
		TADF	94				92				92			
		TTI	84				79				80			
Glyphosate	867[f]	TT	24				18				7			
		TADF	23				14				4			
		TTI	21				12				6			
Dicamba	560[f]	TT	85				85				85			
		TADF	86				82				84			
		TTI	86				78				79			
Fomesafen	264	TT	91				92				92			
		TADF	89				89				89			
		TTI	83				77				80			
Clethodim	76	TT	0				0				0			
		TADF	0				0				0			
		TTI	0				0				0			
Glufosinate + glyphosate	595 + 867[f]	TT	90	NS	96	NS	86	NS	90	NS	88	NS	91	NS
		TADF	93	NS	95	NS	91	NS	92	NS	89	NS	92	NS
		TTI	92	^	88	NS	88	^	81	NS	92	^	81	SYN
Glufosinate + dicamba	595 + 560[f]	TT	92	NS	99	NS	92	NS	98	NS	93	NS	98	NS
		TADF	95	NS	99	NS	95	NS	98	NS	97	NS	99	NS
		TTI	86	NS	98	*	77	NS	95	*	88	^	96	*
Glufosinate + fomesafen	595 + 264	TT	92	NS	99	NS	87	NS	99	*	90	NS	99	*
		TADF	91	NS	99	*	88	NS	99	*	93	NS	99	*
		TTI	89	NS	97	*	91	^	95	NS	94	^	95	NS

Table 6. Palmer amaranth control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016. (Cont.)

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle[d]	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
			%		%		%		%		%		%	
Glufosinate + clethodim	595 + 76	TT	88	∨			85	NS			87	NS		
		TADF	93	NS			84	NS			87	NS		
		TTI	89	NS			85	NS			91	∧		
LSD			6				9				8			

[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percent of the nontreated control.

[d] TT, TeeJet Turbo TeeJet 110015 nozzle; TTI, TeeJet Turbo TeeJet Induction 110015 nozzle; TADF, Greenleaf TurboDrop DualFan 110015 nozzle

[e] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[f] Rate is in g acid equivalent ha^{-1}.

Table 7. Spray characteristics of various herbicide combinations for the herbicide treatment and nozzle combinations used in the Nozzle Experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm.

			Droplet spectra parameters[a]				
Herbicide	Rate	Nozzle[b]	D_{v10}	D_{v50}	D_{v90}	Relative span[c]	<150 µm
	g ai ha^{-1}		µm			-	$\%_{vol}$
Water		TT	137 lmn	295 k	478 g	1.16 def	12.5 d
		TADF	194 fg	383 f	584 d	1.02 ij	4.8 jk
		TTI	403 a	765 a	1083 a	0.89 mn	0.5 l
Glufosinate	595	TT	120 p	269 l	451 ghi	1.23 abc	16.7 ab
		TADF	155 ij	324 hij	540 ef	1.19 bcdef	9.1 fgh
		TTI	352 bcd	686 bcd	1023 b	0.98 jk	0.6 l
Glyphosate	867[f]	TT	127 nop	275 l	463 gh	1.22 abcd	14.9 bc
		TADF	168 h	349 g	563 de	1.14 efg	7.3 hi
		TTI	363 b	699 b	1007 b	0.92 klm	0.5 l
Dicamba	560[f]	TT	133 mno	277 l	455 ghi	1.16 cdef	13.5 cd
		TADF	182 g	371 f	583 d	1.08 ghi	6.0 ij
		TTI	402 a	751 a	1068 a	0.89 lmn	0.5 l
Fomesafen	264	TT	129 mnop	264 l	426 i	1.13 fgh	14.7 bc
		TADF	160 hi	340 gh	547 ef	1.14 efg	8.5 gh
		TTI	362 b	694 bc	1012 b	0.94 klm	0.5 l
Clethodim	76	TT	124 op	267 l	446 hi	1.21 abcde	16.1 ab
		TADF	146 jkl	316 ij	535 ef	1.23 abc	10.6 ef
		TTI	348 cde	694 bc	1018 b	0.96 jk	0.7 l
Glufosinate + glyphosate	595 + 867[f]	TT	122 op	239 m	377 j	1.06 hi	17.2 a
		TADF	206 f	383 f	584 d	0.99 jk	3.7 k
		TTI	339 de	610 e	839 c	0.82 n	0.7 l
Glufosinate + dicamba	595 + 560[f]	TT	122 op	268 l	454 ghi	1.24 ab	16.3 ab
		TADF	140 klm	306 jk	527 f	1.26 a	11.5 de
		TTI	338 e	682 bcd	994 b	0.96 jkl	0.8 l

Table 7. Spray characteristics of various herbicide combinations for the herbicide treatment and nozzle combinations used in the Nozzle Experiment including D_{v10}, D_{v50}, D_{v90}, relative span, and % of the volume ($\%_{vol}$) containing droplets with diameters <150µm. (Cont.)

			Droplet spectra parameters[a]									
Herbicide	Rate	Nozzle[b]	D_{v10}		D_{v50}		D_{v90}		Relative span[c]		<150 µm	
	g ai ha^{-1}		---------------------µm---------------------						-		$\%_{vol}$	
Glufosinate + fomesafen	595 + 264	TT	125	op	265	l	442	hi	1.20	abcdef	15.7	ab
		TADF	153	ijk	328	hi	536	ef	1.17	bcdef	9.5	efg
		TTI	345	cde	677	cd	997	b	0.96	jk	0.7	l
Glufosinate + clethodim	595 + 76	TT	127	nop	265	l	434	hi	1.16	def	15.0	bc
		TADF	153	ijk	324	hij	531	ef	1.17	bcdef	9.5	efg
		TTI	353	bc	669	d	996	b	0.96	jk	0.4	l

[a] Means followed by the same letter within a column are not statistically different according to Fisher's protected LSD with a Tukey adjustment (α = 0.05).

[b] TT, TeeJet Turbo TeeJet 110015 nozzle; TTI, TeeJet Turbo TeeJet Induction 110015 nozzle; TADF, Greenleaf TurboDrop DualFan 110015 nozzle

[c] Relative span is a unitless index of the uniformity of droplet size distribution. Smaller values represent more uniformity in droplet size distribution

[d] Rate is in g acid equivalent ha^{-1}.

Table 8. Prickly sida control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by spray volume in the Spray Volume Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Spray volume	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	L ha^{-1}	%		%		%		%		%		%	
Glufosinate	595	47	86				51				77			
		140	93				83				87			
Glyphosate	867[e]	47	86				62				82			
		140	90				69				82			
Fomesafen	264	47	57				41				58			
		140	66				48				67			
Clethodim	76	47	0				0				0			
		140	0				0				0			
Glufosinate + glyphosate	595 + 867[e]	47	91	NS	98	*	88	^	82	NS	93	^	95	NS
		140	95	NS	99	*	87	NS	94	*	88	NS	97	*
Glufosinate + fomesafen	595 + 264	47	85	NS	94	*	74	^	71	NS	88	^	90	NS
		140	94	NS	97	*	85	NS	91	*	90	NS	95	*
Glufosinate + clethodim	595 + 76	47	93	^			60	^			76	NS		
		140	94	NS			86	NS			91	NS		
LSD			6				9				6			

[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A “^” indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 9. Prickly sida control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle[d]	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
	g ai ha^{-1}		%		%		%		%		%		%	
Glufosinate	595	TT	92				92				96			
		TADF	89				87				97			
		TTI	83				77				88			
Glyphosate	867[f]	TT	77				63				64			
		TADF	71				63				70			
		TTI	74				65				61			
Dicamba	560[f]	TT	65				47				62			
		TADF	65				45				63			
		TTI	64				47				58			
Fomesafen	264	TT	62				34				51			
		TADF	60				41				53			
		TTI	56				21				51			
Clethodim	76	TT	0				0				0			
		TADF	0				0				0			
		TTI	0				0				0			
Glufosinate + glyphosate	595 + 867[f]	TT	91	NS	97	NS	76	∨	95	*	94	NS	98	*
		TADF	89	NS	96	NS	80	NS	96	NS	93	NS	97	NS
		TTI	89	NS	96	NS	77	NS	90	*	86	NS	96	*
Glufosinate + dicamba	595 + 560[f]	TT	87	NS	96	NS	79	∨	95	NS	95	NS	95	NS
		TADF	83	NS	96	*	77	NS	91	NS	92	NS	98	NS
		TTI	83	NS	94	*	72	NS	86	NS	93	NS	95	NS
Glufosinate + fomesafen	595 + 264	TT	92	NS	97	NS	89	NS	93	NS	96	NS	98	NS
		TADF	88	NS	96	NS	84	NS	92	NS	96	NS	98	NS
		TTI	89	NS	93	NS	79	NS	81	NS	91	NS	94	NS

Table 9. Prickly sida control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016. (Cont.)

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
	g ai ha^{-1}		%		%		%		%		%		%	
Glufosinate + clethodim	595 + 76	TT	89	NS			90	NS			94	NS		
		TADF	79	NS			86	NS			93	NS		
		TTI	79	NS			74	NS			83	NS		
LSD			8				11				7			

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[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone based on the LSD. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percent of the nontreated control.

[d] TT, TeeJet Turbo TeeJet 110015 nozzle; TTI, TeeJet Turbo TeeJet Induction 110015 nozzle; TADF, Greenleaf TurboDrop DualFan 110015 nozzle

[e] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[f] Rate is in g acid equivalent ha^{-1}.

Table 10. Barnyardgrass control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by spray volume in the Spray Volume Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Spray volume	Obs		Exp	p[d]	Obs		Exp	p[d]	Obs		Exp	p[d]
	g ai ha^{-1}	L ha^{-1}	%		%		%		%		%		%	
Glufosinate	595	47	69				58				58			
		140	78				71				70			
Glyphosate	867[e]	47	92				87				87			
		140	93				83				85			
Fomesafen	264	47	18				13				13			
		140	31				20				18			
Clethodim	76	47	78				64				71			
		140	76				64				75			
Glufosinate + glyphosate	595 + 867[e]	47	86	NS	98	*	64	∨	93	*	72	∨	93	*
		140	85	∨	98	*	72	∨	93	*	77	∨	95	*
Glufosinate + fomesafen	595 + 264	47	69	NS	75	NS	58	NS	62	NS	66	∧	62	NS
		140	78	NS	85	NS	68	NS	76	NS	73	NS	74	NS
Glufosinate + clethodim	595 + 76	47	79	NS	93	*	71	NS	82	NS	72	NS	86	*
		140	79	NS	95	*	73	NS	88	*	76	NS	91	NS
LSD			7				8				7			

[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percent of the nontreated control.

[d] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[e] Rate is in g acid equivalent ha^{-1}.

Table 11. Barnyardgrass control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016.

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle[d]	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
	g ai ha^{-1}		%		%		%		%		%		%	
Glufosinate	595	TT	87				70				71			
		TADF	86				65				68			
		TTI	68				50				48			
Glyphosate	867[f]	TT	93				82				84			
		TADF	93				82				84			
		TTI	87				79				81			
Dicamba	560[f]	TT	0				0				0			
		TADF	0				0				0			
		TTI	0				0				0			
Fomesafen	264	TT	18				16				11			
		TADF	20				12				9			
		TTI	19				12				15			
Clethodim	76	TT	65				52				35			
		TADF	63				44				45			
		TTI	61				48				45			
Glufosinate + glyphosate	595 + 867[f]	TT	86	NS	99	*	60	∨	93	*	71	∨	94	*
		TADF	88	NS	99	*	65	∨	93	*	63	∨	93	*
		TTI	80	NS	96	*	61	∨	91	*	58	∨	89	*
Glufosinate + dicamba	595 + 560[f]	TT	83	NS			62	NS			66	NS		
		TADF	80	NS			57	NS			62	NS		
		TTI	70	NS			49	NS			51	NS		
Glufosinate + fomesafen	595 + 264	TT	82	NS	89	NS	61	NS	74	NS	64	NS	74	NS
		TADF	81	NS	89	NS	58	NS	69	NS	62	NS	70	NS
		TTI	71	NS	74	NS	41	NS	56	NS	52	NS	55	NS

Table 11. Barnyardgrass control, height reduction, and density reduction assessed 4 weeks after application for various herbicide treatments as affected by 3 nozzle types in the Nozzle Experiment at Keiser, AR, in 2015 and 2016. (Cont.)

			Control[ab]				Height reduction[abc]				Density reduction[abc]			
Herbicide	Rate	Nozzle[d]	Obs		Exp	p[e]	Obs		Exp	p[e]	Obs		Exp	p[e]
	g ai ha^{-1}		%		%		%		%		%		%	
Glufosinate + clethodim	595 + 76	TT	79	NS	96	*	60	∨	85	*	62	NS	80	*
		TADF	80	NS	95	*	59	NS	80	*	61	NS	81	*
		TTI	74	NS	87	*	52	NS	74	*	61	∧	71	NS
LSD			9				10				12			

[a] Abbreviation: Exp, expected value; LSD, least significant difference; NS, not significant; Obs, observed value; p, p-value.

[b] A "∧" indicates a mixture that provided significantly greater control than both herbicides alone based on the LSD. A "∨" indicates a mixture that provided significantly less control compared to at least one of the herbicides alone. NS indicates the mixture was similar to both of the herbicides alone.

[c] Height reduction and density reduction are expressed as a percent of the nontreated control.

[d] TT, TeeJet Turbo TeeJet 110015 nozzle; TTI, TeeJet Turbo TeeJet Induction 110015 nozzle; TADF, Greenleaf TurboDrop DualFan 110015 nozzle

[e] 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]. Expected values can only be calculated when two herbicides in the mixture have POST activity on the species.

[f] Rate is in g acid equivalent ha^{-1}.

Chapter 8

Uptake, translocation, and metabolism of glyphosate, glufosinate, and dicamba as potential mechanisms for antagonism of mixtures on *Echinochloa crus-galli* and *Amaranthus palmeri*

Barnyardgrass and Palmer amaranth are two herbicide resistance-prone species prevalent in most cropping systems in the midsouthern United States. Prior field experiments have determined mixtures of glyphosate + glufosinate and glyphosate + dicamba are antagonistic when applied to barnyardgrass, raising the concern of evolving glyphosate or glufosinate-resistance. Fewer instances of antagonism have been reported with these herbicides on Palmer amaranth. Potential mechanisms for the herbicide antagonism observed in the field were investigated using ^{14}C-labeled herbicide absorption, translocation, and metabolism experiments. Three ^{14}C-labeled herbicides, ^{14}C-glyphosate, ^{14}C-glufosinate, and ^{14}C-dicamba, were utilized in different experiments where one experiment focused on an individual ^{14}C-labeled herbicide applied alone and in mixture with other non-radiolabeled herbicides. Results showed applying a solution of glufosinate + glyphosate reduced uptake of ^{14}C-glyphosate in barnyardgrass by 10% of the total applied compared to glyphosate alone (22% and 32% of applied ^{14}C-glyphosate, respectively). A similar reduction in ^{14}C-glyphosate was observed in Palmer amaranth. Furthermore, glyphosate + glufosinate reduced translocation of ^{14}C-glyphosate in barnyardgrass from the treated leaf, with 12% of applied radioactivity moving from the treated leaf for glyphosate alone compared to 4% for the mixture. Applying glyphosate with dicamba also reduced ^{14}C-glyphosate uptake by 9 and 4% of the total in Palmer amaranth and barnyardgrass, respectively. In the ^{14}C-glufosinate experiment, barnyardgrass absorbed 49% of the applied ^{14}C-glufosinate compared to only 29% when applied in mixture with cold glyphosate. These findings lead to the suggestion that altered absorption or translocation of both glufosinate and glyphosate

in mixture could be the source of antagonism observed in the field. In the metabolism experiments, no glyphosate metabolism was observed in either species. Both species metabolized glufosinate, with Palmer amaranth metabolizing 63% of absorbed glufosinate and barnyardgrass metabolizing 53% at 48 h after application. Barnyardgrass rapidly metabolized dicamba, with only 6% of absorbed radioactivity remaining as the parent compound, whereas Palmer amaranth only metabolized 4% of the dicamba that was absorbed. When glufosinate was applied with dicamba, dicamba metabolism was further limited in both species. These results indicate that mixing herbicides can impact absorption, transport, and metabolism of one, or both, herbicides in mixture and have important implications in regards to resistance management.

Nomenclature: clethodim; glufosinate; glyphosate; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Palmer amaranth, *Amaranthus palmeri* S. Wats.

Key words: ^{14}C-labeled herbicides; antagonism, herbicide metabolism, herbicide translocation; herbicide uptake

Enlist™ and Bollgard II ® XtendFlex™ technologies allow for postemergence (POST) applications of both glufosinate and glyphosate plus an appropriate auxinic herbicide (2,4-D or dicamba, respectively). To control a broad spectrum of weeds, including grass, broadleaf, and glyphosate-resistant species, POST mixtures of multiple herbicides will be needed in these technologies. However, numerous greenhouse and field experiments have documented antagonism with certain combinations of these herbicides including: glyphosate + glufosinate (Besançon et al. 2018; Bethke et al. 2013) and glyphosate + dicamba (Flint and Barrett 1989b; Meyer et al. 2015; Meyer et al. 2017b; Ou et al. 2018; O'Sullivan and O'Donovan 1980) Evaluation of herbicide interactions can produce mixed results. The interaction can vary by species (O'Sullivan and O'Donovan 1980), rates used (Flint and Barrett 1989b), weed size (Flint and Barrett 1989a), and individual herbicide product (Flint and Barrett 1989b; Kudsk and Mathiassen 2004).

The mixture of glyphosate + glufosinate is generally accepted to be antagonistic (Bethke et al. 2013; Chuah at al. 2008; Kudsk and Mathiassen 2004). Antagonism was observed between glyphosate and glufosinate in goosegrass [*Eleusine indica* (L.) Gaertn.] at various rates below recommended field doses, although the mixture of the two herbicides generally produced control greater than levels observed with the individual herbicides alone (Chuah et al. 2008). The antagonism between glyphosate and glufosinate is generally attributed to the fast action of glufosinate inducing plant injury (Chuah et al. 2008; Bethke et al. 2013). Bethke et al. (2013) suggested the rapid activity of glufosinate may reduce the ability of the plant to translocate glyphosate. Besançon et al. (2018) recently identified reduced uptake and transport of glyphosate as potential mechanisms for antagonism using reduced rates (glyphosate at 110 and 220 g ae ha^{-1} and glufosinate at 20 or 40 g ae ha^{-1}) in giant foxtail (*Setaria faberi* Herrm.). In the same study, reduced uptake and transport of glyphosate for glufosinate + glyphosate mixtures were also identified, although to a lesser extent, when evaluated in velvetleaf (*Abutilon theophrasti* Medik.) and common lambsquarters (*Chenopodium album* L.).

Merchant et al. (2013) reported 2,4-D reduced control of glufosinate on Texas millet [*Urochloa texana* (Buckl.) and broadleaf signalgrass [*Urochloa platyphylla* (Nash), whereas dicamba did not affect control of glufosinate. Addition of glufosinate to dicamba or 2,4-D generally improved control on common lambsquarters, common waterhemp (*Amaranthus rudis* Sauer), and Palmer amaranth (Chahal and Johnson, 2012; Craigmyle et al. 2013a; Craigmyle et al. 2013b) although these experiments did not analyze for herbicide interactions.

One aspect of herbicide mixtures lacking investigation is how a mixture may impact the metabolism of one, or both, of the herbicides being mixed. Most weeds do not metabolize glyphosate, and glyphosate metabolism has not been identified as an evolved resistance mechanism in weeds (Duke 2018; Feng et al. 1999). Everman et al. (2009) reported differences in the ability of three species to metabolize glufosinate and suggested metabolism as a source of variable sensitivities among species to glufosinate. Chang and Vanden Born (1971) reported differing dicamba metabolism among various species, (monocots and dicots) and suggested rapid metabolism was responsible for dicamba tolerance in wheat (*Triticum vulgare* L.).

As discussed, reduced uptake and translocation have been cited as herbicide resistance and antagonistic mechanisms in various species. Herbicide uptake and transport experiments are often evaluated using ^{14}C-labeled techniques, as described by Nandula and Vencill (2015). Prior research has shown dicamba, glufosinate, and glyphosate uptake over time varies by species, but most of the uptake and translocation of all three herbicides occurs within the first 24 h following application, with minimal uptake occurring after 48 h (Everman et al. 2009; Grangeot et al. 2006;

Hoss et al. 2003; Lorentz et al. 2014; Ou et al. 2018). Herbicide metabolism studies have utilized ^{14}C-labeled techniques to simplify the extraction and identification of herbicides and their metabolites in plants (Everman et al. 2009; Küpper et al. 2017). The objectives of these experiments were to utilize ^{14}C-labeled herbicides to determine if reduced uptake, reduced translocation, or enhanced metabolism may contribute to antagonism assessed 48 h after application for mixtures containing glufosinate, glyphosate, or dicamba.

Materials and Methods

Barnyardgrass and Palmer amaranth plants were established in a greenhouse at the Bayer Weed Resistance Competence Center in Frankfurt, Germany. Two populations (biotypes) of each species were used. One barnyardgrass biotype was obtained from Azlin Seed Services (Azlin Seed Services, Leland, MS), the same source used to overseed the field experiments in Meyer et al. (2017a; 2017b). A second barnyardgrass biotype was obtained at the time of harvest in 2016 from a rice field in Crittenden County, AR, one of the top soybean producing counties in the state (USDA-NASS 2018). Palmer amaranth seed samples were collected from the Arkansas Agricultural Research and Extension Center in Fayetteville, AR, in 2017, and from a soybean field near Gregory, AR, in 2015. The population from Gregory, AR has confirmed resistance to acetolactate synthase (ALS), enolpyruvylshikimate-3-phosphate synthase (EPSPS), and protoporphyrinogen oxidase inhibitors (Schwartz-Lazaro 2017).

Uptake and Transport Experiments. Radiolabeled (^{14}C-) herbicide solutions were applied to Palmer amaranth and barnyardgrass plants using a procedure modified from Nandula and Vencill (2015). At the 4- to 5-leaf stage, plants were treated with a cold (non-radioactive) herbicide solution in a motorized spray chamber. Following application of the cold solution, a hot (^{14}C-labeled) solution was applied to the second-oldest fully expanded leaf using a micropipette. Hot herbicide solutions contained 1.333 kBq μL^{-1} [80,000 disintegrations per minute (DPM) μL^{-1}] of the ^{14}C-herbicide, and six 0.5-µm droplets spaced evenly apart were applied to each plant. Thus, a total of 240,000 DPM was applied to each plant. Hot solutions were prepared to resemble the cold solution, meaning the hot solution contained the same herbicide products used in cold solutions.

Following application of the hot herbicide solution, plants were incubated in a growth chamber at 28C, 70% humidity, and under continuous light (400 µmol photons m^{-2} s^{-1}) for 48 h. After 48 h in a growth chamber, both treated and nontreated plants were dissected and sectioned into four parts: treated leaf (TL), shoot above treated leaf (ATL), shoot below treated leaf (BTL), and roots. The section of the treated leaf that was spotted with ^{14}C-herbicide was washed in a vial of deionized water prior to further sample processing. The water wash was used to determine the amount of ^{14}C-herbicide remaining on the leaf surface. To assess the efficacy of the washing process, treated leaves were washed 1 h after droplet application, and 83%, 89%, and 88% of the applied ^{14}C-glyphosate, -glufosinate, and -dicamba, respectively were recovered in the wash. Based on the experimental data, 89, 83, 90% of applied radioactivity for ^{14}C-labeled glyphosate, glufosinate, and dicamba, respectively, was recovered in the plant parts and leaf wash after 48 h.

The plant sections were dried at 60 C for three days. After drying, plant samples were combusted in a biological oxidizer (OX-500, Zinsser Analytic GmbH, Frankfurt, Germany) at 900 C for 3 min. CO_2 gas evolved during combustion was trapped in 15 mL of scintillation cocktail (Oxysolve C-400, Zinsser Analytic GmbH, Frankfurt, Germany). The amount of ^{14}C-herbicide in each plant part was determined in a Tri-Carb 2900TR Liquid Scintillation Counter (Packard Instrument Company, Downers Grove, IL 60515). The amount of ^{14}C-herbicide absorbed was determined by summing the quantities in each of the four plant parts: treated leaf (TL), shoot above treated leaf (ATL), shoot below treated leaf (BTL), and roots. The amount of ^{14}C-herbicide retained in each plant part is presented as a fraction of the total radioactivity applied per plant (240,000 DPM).

Three uptake and translocation experiments were conducted, with each experiment focusing on a single ^{14}C-herbicide. One experiment used ^{14}C-glyphosate and focused on those particular treatments and mixtures, another experiment for ^{14}C-glufosinate, and the final one for ^{14}C-dicamba. A list of treatments and the use rates of the commercial herbicide formulations used in all three experiments can be found in Tables 1 and 2. Commercially available formulations of the herbicides were used in both the cold application and spotting solution. The herbicide used were as follows: dicamba (Clarity® herbicide, BASF Corporation, Research Triangle Park, NC), glufosinate (Basta® herbicide, Bayer CropScience Ltd., Monheim, Germany), glyphosate (Roundup PowerMax herbicide (Monsanto Company, St. Lous, MO), and *S*-metolachlor (Dual Gold Herbicide, Syngeta UK Ltd., Cambridge, United Kingdom). The three ^{14}C-herbicides used in each respective experiment were aqueous solutions of ^{14}C-glyphosate ([phosphonomethyl-^{14}C]-glyphosate, 3.7 kBq μL^{-1}, specific activity: 2.04 kBq μg^{-1}, PerkinElmer, Inc., Boston, MA, USA); ^{14}C-glufosinate (1-^{14}C glufosinate hydrochloride, 6.66 kBq μL^{-1}, specific activity: 4.14 MBq mg^{-1}, Bayer CropScience AG, Monheim, Germany); and ^{14}C-dicamba ([ring-U-^{14}C dicamba], 5.66 kBq μL^{-1}, specific activity of 7.951 MBq mg^{-1}, Institute of Isotopes Co. LTD., Budapest, Hungary).

Metabolism Experiments. Metabolism experiments were conducted under similar conditions as the Uptake and Transport Experiments. Additionally, experiments were arranged so that during a given run of the Uptake and Transport Experiment (e.g., ^{14}C-labeled glyphosate), the corresponding run of the Metabolism Experiment was also taking place. Plants were established, treated, and incubated under similar conditions, with the exception that the second and third youngest fully-expanded leaves were spotted with hot solution in the Metabolism Experiment, so that each plant was treated with 480,000 DPM of radioactivity.

As in the Uptake and Transport Experiments, plants were first sprayed with the appropriate herbicide solution in a motorized spray chamber. Following application of the cold solution, two leaves were spotted with a micropipette. Six 0.5-µl droplets of the hot solution was applied to each of the two leaves with 1.333 kBq or 80,000 dpm μl^{-1}. The treated plants were kept in a growth chamber for 48 h following application.

At harvest, the plants were washed in 70% ethanol three times to remove any non-absorbed radiolabeled herbicide. Plants were prepared for high-performance liquid chromatography (HPLC) separation and ^{14}C-herbicide detection using methodology adapted from Küpper et al. (2017). Plant tissue was disrupted in 600 µl of 90:10% of methanol:water with 5-mm stainless steel beads at 30 Hz for 10 min in a Qiagen TissueLyser II (Qiagen N.V., Hilden, Germany). Following disruption, the homogenate was centrifuged at 6000 g for 10 min and the supernatant was extracted and dried under continuous air flow at 55 C in a Biotage Turbovap 96 (Biotage AB, Uppsala, Sweden). The cycle of disruption, centrifugation, extraction, and evaporation was repeated using 90:10% acetonitrile:water solution in the second, and 10:90% methanol:water solution in the third and fourth extractions. The pooled and dried supernatant was then re-suspended in 200 µl of 10% methanol using a shaker and ultrasonic bath (Sonorex Super, Bandelin electronic GmbH and Co., Berlin, Germany) and then filtered through a 0.45-µm low-binding hydrophilic polytetrafluoroethylene (PTFE) mesh for 10 min at 2200 g in the centrifuge. All solvents used were HPLC grade (Sigma-Aldrich, Steinheim, Germany; ≥ 99.9 % HPLC grade).

Non-treated control samples, spiked with the ^{14}C-herbicide being analyzed, were injected just prior to extraction. For glyphosate, the mobile phases consisted of K200 (A) and RG019 (B). Both mobile phases are proprietary eluents available from Pickering Laboratories Incorporated (Mountain View, CA) and required for separation of glyphosate and its primary metabolite Aminomethylphosphonic acid (AMPA) using a Pickering K+ cation exchange column (Anonymous 2006). K200 is a potassium-based eluent and RG019 is a potassium regenerant that equilibrates the cation exchange sites on the column after separation. Solvents were run at a 15 min isocratic run of 100% solvent A, followed by a 2 min run of 100% solvent B. The column was then flushed with 100% solvent A for 8 min. The flow rate was 0.4 mL min^{-1} and column temperature was 55C for glyphosate analysis. Although glyphosate was detected in the glyphosate metabolism experiment, no metabolites were present in any of the treatments (data not shown).

For glufosinate, the mobile phases consisted of 50 mmol ammonium acetate (pH=4.5) (C) and HPLC grade H_20 (D). Solvents were run at a 5 min linear gradient from 85 to 70% solvent D plateauing for 2 min, followed by a 5 min linear gradient returning to 85% solvent D. The column was then flushed with 85% solvent D for 8 min. A Waters ZIC-pHilic 5um LC column (100 x 4.6 mm) was used for glufosinate. The flow rate through the column was 0.6 mL min^{-1} and column temperature was ambient. On average, % recovery was 92% of applied radioactivity.

For dicamba, the mobile phases consisted of 5mM ammonium formate in water (E) and 5mM ammonium formate in methanol (F). Solvents were run for 25 minutes in five stages: 1) 1.5

min linear gradient from 98% solvent E to 60% solvent E; 2) 8.5 min linear gradient from 60% solvent E to 48% solvent E; 3) 7 min linear gradient from 48% solvent E to 10% solvent E; 4) followed by a 2-minute plateau at 10% solvent E; and 5) 6-minute flush of 98% solvent E. A Kinetex (Kinetex GmbH & Co., Walderns, Germany) F5 column (150 x 4.6 mm) was used for the dicamba separations. The flow rate through the column was 0.5 mL min^{-1} and column temperature was ambient. On average, the recovered radioactivity was 89% of the total applied dicamba.

Separation and HPLC identification of the parent ^{14}C-herbicides and their metabolites were performed on a reverse-phase HPLC system (LC Net II/ADC with PU-980 pump unit, LC-980-02 gradient unit and CO-2060 Plus column thermostat; Jasco, Oklahoma City, OK, USA). An in-line radio flow detector (Flowstar LB 513 with YG40-S6Mdetector cell; Berthold Technologies, BadWildbad, Germany) was used for radioactive peak determination. Parent ^{14}C-herbicides and non-radiolabeled reference standards were injected for peak identification. Nonradioactive standards, with known metabolites, were detected with an inline UV–visible spectrophotometer (MD-910; Jasco) to establish retention times. For the Glufosinate Experiment, two primary metabolites were obtained and injected: 2-acetamido-4-methylbutanoic acid (NAG); and 3-methylphosphinico-propanoic acid (MPP). Two other metabolites have been identified in plants treated with glufosinate, 4-methylphosphinyl-2-oxobutanoic acid (PPO) and 4-methylphosphinylbutanoic acid (MPB) (Dröge et al. 1992; Jalaludin et al. 2017) however, the nonradioactive standards of PPO and MPB were not obtainable for these experiments. In the Dicamba Experiment and two major metabolites were injected: 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid (5-OH dicamba); and 3,6-dichlorosalicylic acid (DCSA).

Statistical Analysis. For a given ^{14}C-herbicide, all appropriate treatments were applied to both biotypes of both species. Additionally, both the Uptake and Transport Experiment and Metabolism Experiment were conducted concurrently, meaning plants were treated and harvested on the same day in both experiments. All experiments included four replications of each treatment and every experiment was conducted twice, except for the ^{14}C-glufosinate and -dicamba metabolism experiments, which were conducted three times. All data were averaged over the two (or three) experimental runs.

Initially data for each species were analyzed using an ANOVA with Biotype treated as a fixed effect to determine if the biotype of an individual species had an impact on the results. For all response variables in all six experiments, no higher order term containing Biotype (i.e., interaction) was considered significant ($\alpha = 0.05$) (not shown). Thus, all data were averaged over Biotype and within a given experiment, species were analyzed together with Species as a fixed effect. For example, the final analysis for the ^{14}C-glufosiante metabolism experiment was an ANOVA with a completely randomized, two-factor factorial design with Species (barnyardgrass and Palmer amaranth) and Herbicide (4 treatments) as factors A and B, respectively.

For the Uptake and Transport Experiments, the total absorption data (sum of radioactivity in each of the four plant sections) were treated as a two-factor factorial design with Species and Herbicide as factors. The translocation data (as % of radioactivity applied) were treated as a three-factor split-plot type design with the whole-plot factor being a Herbicide*Species interaction and the sub-plot factor being Plant Section. In the Metabolism Experiments, an ANOVA was conducted on the % of radioactive parent compound remaining in the plant after 48 hours.

All data were analyzed using JMP 13 (SAS Institute Inc., Cary, NC), and means separated using Fisher's protected least significant difference (LSD) (α = 0.05). The statistical model included run as a random effect. Glufosinate metabolite data were analyzed using an ANOVA and means were separated with a LSD (Table 3). The dicamba parent compound was also analyzed using an ANOVA (Table 4). Variance components estimates for all experiments are listed in Table 4. The data for the dicamba metabolites did not follow a normal distribution according to a Shapiro-Wilk test (α =0.05) and contained a large amount of 0's (i.e., non-detects for that specific metabolite). Thus, a zero-inflated Poisson regression analysis was used for these dicamba metabolite data and means were separated according to a Student's t multiple comparisons test (α =0.05). In cases where all treatments for a given species had a mean and standard error of the mean (SE) equal to zero, these data were excluded from the analysis. For DCSA, an analysis was conducted for the Palmer amaranth data, but the model effect for treatment was not significant (p=0.2437) and means are followed by SEs (Table 5).

Results and Discussion

Glyphosate. The uptake of ^{14}C-glyphosate in both Palmer amaranth and barnyardgrass was reduced when glufosinate was added to both rates of glyphosate (Table 1). For barnyardgrass, ^{14}C-glyphosate uptake at 897 g ae ha^{-1} was reduced from 25% to 15% of total applied radioactivity when glufosinate (595 g ai ha^{-1}) was added. Glufosinate also reduced translocation of ^{14}C-glyphosate when mixed. The addition of glufosinate to both rates of glyphosate (897 and 1735 g ae ha^{-1}) resulted in less transport of ^{14}C-glyphosate to the tissue above the treated leaf (ATL), below the treated leaf (BTL) and roots in Palmer amaranth (Table 2). In barnyardgrass, less ^{14}C-glyphosate (11% applied) was identified in the treated leaf (TL) for the mixture of glufosinate + glyphosate (595 + 1735 g ha^{-1}) than the corresponding rate of glyphosate alone

(16% of applied). Additionally, barnyardgrass BTL and roots had less ^{14}C-glyphosate when both rates of glyphosate were applied with glufosinate, compared to either glyphosate alone treatment (897 and 1735 g ha^{-1}).

A reduction in transport to meristematic regions, such as the growing point, was associated with glyphosate-resistance in rigid ryegrass (*Lolium rigidum* Gaud.) (Wakelin et al. 2004). Glyphosate-resistant barnyardgrass was reported in TN in 2017 (Steckel et al. 2017) and preliminary results indicate that a reduction in transport grants a low level of resistance (2- to 4-fold) in these populations (Steckel 2018). Herbicide mixtures that further limit uptake and transport of glyphosate (i.e., mixing with glufosinate) will likely select for higher levels of resistance in populations like those in TN and should be avoided if possible.

More ^{14}C-glyphosate (% of total applied) was recovered from the TL of the glufosinate + glyphosate treatments than from the glyphosate alone treatments in Palmer amaranth (TL retained 29 and 22% of the applied ^{14}C-glyphosate for glyphosate + glufosinate alone, respectively). However, the overall uptake of ^{14}C-glyphosate was still less for the glufosinate + glyphosate mixtures than for glyphosate alone (both rates) (Table 1). The reduced uptake and transport of ^{14}C-glyphosate caused by mixing with glufosinate agrees with the results of Besançon et al. (2018) and supports the hypothesis proposed by Bethke et al. (2013) in that the rapid activity of glufosinate limits translocation of glyphosate.

Glyphosate uptake at 897 g ha^{-1} was reduced in Palmer amaranth and barnyardgrass when dicamba (560 g ha^{-1}) was added. For example, ^{14}C-glyphosate uptake in barnyardgrass decreased from 25 to 21% in mixture with dicamba (Table 1). In Palmer amaranth, the mixture of dicamba + glyphosate (897 g ha^{-1}) caused a reduction in ^{14}C-glyphosate transport ATL (7% of applied) compared to glyphosate alone (11% of applied). Flint and Barrett (1989a) observed that ^{14}C-glyphosate uptake when mixed with 2,4-D or dicamba was rate dependent in field bindweed (*Convolvulus arvensis* L.); glyphosate uptake increased when mixed with either auxin at 280 g ae ha^{-1} but not at 840 g ae ha^{-1}. However, in a similar experiment on johnsongrass (*Sorghum halpense* (L.) Pers.), Flint and Barrett (1989b) observed reduced glyphosate uptake and translocation to the roots when dicamba or 2,4-D was added to glyphosate, thus, the impact one herbicide has on the uptake and transport of another appears to be dependent upon the species.

No metabolism of glyphosate was observed in either Palmer amaranth or barnyardgrass (data not shown). Feng et al. (1999) also reported a lack of glyphosate metabolism in both glyphosate-sensitive and -resistant rigid ryegrass. Thus, antagonism of glyphosate by glufosinate and dicamba appear to be based on changes in glyphosate uptake and transport.

The results from this experiment generally agree with that reported by Flint and Barrett (1989b) and Ou et al. (2018) in that a reduction in uptake and, in some cases, translocation is responsible for antagonism between glyphosate and dicamba**.** Plant response to synthetic auxins is a rapid, complex, and dynamic pathway that induces various physiological changes that could ultimately affect uptake and translocation of glyphosate. Applications of dicamba disrupt natural hormone signaling, stimulate ethylene biosynthesis within hours of application, and is associated with growth inhibition within first 24 h of exposure (Grossman 2010). Herbicide transport via phloem may also be disrupted by changes in abcissic acid and gibberellin levels, both of which are involved with phloem loading and unloading (Lalonde et al. 2003). Another association exists for glyphosate and dicamba that may explain interactions between the two herbicides; glyphosate inhibits tryptophan biosynthesis, a precursor in the biosynthesis of indole acetic acid (Taiz and Zeiger 2004). Therefore, disrupting indole acetic acid activity may indirectly affect what the EPSPS glyphosate will bind to.

Glufosinate. Uptake of ^{14}C-glufosinate in Palmer amaranth was equivalent for glufosinate alone, glufosinate + dicamba, and glufosinate + *S*-metolachlor (Table 1). Palmer amaranth took up 59% of applied radioactivity for glufosinate alone, compared to 31% for the glufosinate + glyphosate mixture. Similarly, in barnyardgrass, the glufosinate + glyphosate treatment only resulted in 29% absorption of ^{14}C-glufosinate, compared to 49% for glufosinate alone. Based on the results of the glyphosate and glufosinate experiments, mixtures of glufosinate + glyphosate reduce uptake of both herbicides in both species, which helps to explain the antagonism observed for the mixture.

More glufosinate was retained in the treated leaf for all mixtures applied to barnyardgrass and only for glufosinate + *S*-metolachlor on Palmer amaranth, compared to glufosinate alone, in each respective species (Table 2). Beriault et al. (1999) observed reduced translocation of glufosinate in sensitive canola (*Brassica napus* L.) compared to a glufosinate-resistant variety, and the authors hypothesized that activity of glufosinate limits its own translocation. Both Everman et al. (2009) and Steckel et al. (1997) described lower amounts of glufosinate translocation in species more sensitive to glufosinate.

Glufosinate metabolism was detected in both species. At 48 h after application when the tissue was harvested, Palmer amaranth had metabolized 64% of the absorbed glufosinate and barnyardgrass 54% (Table 3). The parent compound (glufosinate), 3-methylphosphinylpropionic acid (MPP), and 2-acetamido-4-methylbutanoic acid (NAG) accounted for 79 to 91% of the absorbed radioactivity in the Glufosinate Metabolism Experiment (Table 3). Palmer amaranth is generally considered more sensitive to glufosinate than grass species such as barnyardgrass, but it appears metabolism is not the cause of the difference in sensitivity between these two species. Barnyardgrass absorbed 10% less of the ^{14}C-glufosinate that was applied, compared to Palmer amaranth, and barnyardgrass also retained less glufosinate in the treated leaf. Although glufosinate metabolism appears to differ across species (Everman et al. 2009; Pline et al. 1999), no clear association between sensitivity and ability to metabolize glufosinate exists.

Results of the Glufosinate Metabolism Experiment seem to conflict with conclusions from the Uptake and Transport Experiment. More metabolism of ^{14}C-glufosinate was observed in Palmer amaranth when glufosinate was mixed with glyphosate (68% of absorbed) or *S*-metolachlor (68% of absorbed) compared to glufosinate alone (63% of absorbed). One possible explanation for the higher rate of metabolism in glufosinate + glyphosate mixtures could be the reduction in the amount of glufosinate absorbed (Table 1) is biasing the metabolism results. If less herbicide is taken up by the plant, the more rapidly the plant would be able to deplete the pool of active glufosinate that is present. However, ^{14}C-glufosinate uptake was similar for glufosinate alone and glufosinate + *S*-metolachlor; therefore, a different explanation is needed for this mixture. *S*-metolachlor is detoxified to varying degrees in both crops and weeds via glutathione-S-transferases (GST) (Hatton et al. 1996), a family of enzymes responsible for detoxification of a wide range of xenobiotics. A POST application of *S*-metolachlor could upregulate GSTs and other detoxifying enzymes (e.g., cytochrome P450 systems) that slightly increase metabolism of glufosinate in Palmer amaranth.

The results of the both glufosinate experiments indicate *S*-metolachlor could antagonize the activity of glufosinate. Culpepper et al. (2009) and Steckel et al. (2012) reported the addition of *S*-metolachlor to glufosinate increased injury to cotton (Steckel et al. 2012). Whitaker et al. 2011 reported better Palmer amaranth control 2 weeks after POST in two of four locations with glufosinate + *S*-metolachlor compared to glufosinate alone. Chloroacetamide herbicides, including *S*-metolachlor, inhibit very long chain fatty acid synthesis which ultimately disrupts membrane stability and permeability (Böger 2003). In algae (*Scenedesmus vacuolatus*), herbicidal effects from *S*-metolachlor exposure has been shown to occur within 48 h (Vallotton et al. 2008). Thus, it is possible *S*-metolachlor has some herbicidal activity applied POST that aids other herbicides in causing plant death. Additionally, *S*-metolachlor reduces the droplet spectra when applied with glufosinate, and reducing droplet size has been shown to benefit glufosinate activity (Meyer et al. 2015a; Etheridge et al. 2001).

Dicamba. When ^{14}C-dicamba was applied to Palmer amaranth, the plants absorbed 63% of the applied radioactivity (Table 1). Absorption of ^{14}C-dicamba was greater for the glufosinate + dicamba treatment than dicamba alone, in both species. It is likely the addition of the glufosinate ammonium salt causes a decrease of the solution pH, causing a conversion of the dicamba salt to a free acid and improving dicamba uptake. The effect of the glufosinate ammonium salt is likely similar to the impact of ammonium sulfate, which has been shown to improve efficacy and uptake of dicamba via the mechanism described (Ou et al. 2018; Roskamp et al. 2013; Sterling 1994).

Dicamba translocation differed greatly between Palmer amaranth and barnyardgrass. For dicamba alone, the radioactivity was distributed in Palmer amaranth as 30% ATL, 11% in TL, 17% BTL, and 5% in roots (Table 2). In comparison, 67% of the ^{14}C-dicamba applied was retained in the treated leaf of barnyardgrass. Glufosinate limited the transport of ^{14}C-dicamba when mixed, reducing the amount of ^{14}C-dicamba transported from the treated leaf in Palmer amaranth and barnyardgrass. For example, only 4% of the applied ^{14}C-dicamba was translocated for glufosinate + dicamba, compared to 52% for dicamba alone in Palmer amaranth.

In Palmer amaranth, glyphosate decreased the transport of dicamba to above the treated leaf (Table 2). Ou et al. (2018) also observed a reduction in dicamba transport from the treated leaves of kochia (*Kochia scoparia* L.) as a result of mixing with glyphosate and attributed it to the rapid physiological response induced from dicamba application.

The Dicamba Metabolism Experiment appears to provide one explanation for the differences in sensitivities to dicamba between monocots and dicots. Negligible metabolism (>5%) of the absorbed dicamba occurred in Palmer amaranth (Table 5) with the primary metabolite being DCSA (2.9% for the dicamba alone treatment). In contrast, barnyardgrass metabolized 94% of the applied dicamba in the dicamba alone treatment 48 h after application (Table 5). Chang and Vanden Born (1971) observed rapid metabolism of dicamba in wheat, with differing rates of metabolism in other species. Conversely, Tartary buckwheat [*Fagopvrurn tataricum* (L.) Gaertn.], a species sensitive to dicamba, was only able to metabolize 10% of the parent dicamba at 20 d after application (Chang and Vanden Born 1971). Most of the metabolites found in barnyardgrass were unknown, with the second most common metabolite being 5-OH-dicamba. Dicamba has two primary metabolites typically found in plant tissues: 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid (5-OH-dicamba) and 3,6-dichlorosalicylic acid (DCSA) (Chang and Vanden Born 1971; Guo et al. 2016). Recent evidence suggests the transformation of 3,6-DCSA is the rate-limiting step in dicamba metabolism, and subsequent metabolites (e.g., 3,6-dichlorogentisate) are rapidly formed through a cytochrome P450 monooxygenase system (Li et al. 2018).

Practical Implications

The reported metabolism of glufosinate in both barnyardgrass and Palmer amaranth has important implications for management of herbicide resistance. The LibertyLink technology is based on a metabolic detoxification of glufosinate via the insertion of a phosphinothricin-N-acetyl-transferase gene from the bacterium *Streptomyces viridochromogene* (*pat* gene) (Dröge et al. 1992). Another gene (*bar*), also encodes for phosphinothricin-N-acetyl-transferase but is sourced from *Streptomyces hygroscopicus* and is homologous to the *pat* gene (Vasil et al. 1996). Both the *pat* and *bar* genes have been inserted into plants to confer resistance to glufosinate ammonium, and used extensively as marker for genetic selection (D'Halluin et al. 1995; OECD, 2002; Tan et al., 2006). However, when the *pat* or *bar* genes are used solely as a selectable marker for plant transformation (e.g., WideStrike® Cotton varieties), plants can exhibit lower levels of glufosinate metabolism and crop injury can result from glufosinate applications (Norsworthy et al. 2016; Steckel et al. 2012). As glufosinate metabolism is occurring in both Palmer amaranth and barnyardgrass, extensive use of glufosinate in glufosinate-resistant crop varieties could induce metabolic resistance in weed species. Although selection for enhanced metabolism could be occurring in the field, it should be noted no differences in metabolism were observed between the populations of Palmer amaranth and barnyardgrass evaluated in these experiments.

Similarly, the dicamba-resistant technology (e.g., Roundup Ready 2 Xtend) is also based on metabolic detoxification (Behrens et al. 2007). Although Palmer amaranth metabolized <5% of the applied dicamba in these experiments, these data, and previous reports, demonstrate that differential metabolism is associated with variable dicamba tolerance across species (Chang and Vanden Born 1971). Metabolic resistance is already of great concern in many grass species (Beckie and Tardif 2012) and can result in cross resistances to other herbicide sites of action, as detoxification enzymes often act on a broad range of molecules (Délye et al. 2013). Testing for metabolic-based resistance should be of high priority as weed species continue to adapt and evolve resistance to many different herbicide sites of action.

Herbicide physiology is an intricate series of events that ultimately lead to plant death. Investigating plant responses to herbicide mixtures can aid in understanding herbicides and their plant activity. Changes in uptake, transport, or metabolism do not always agree with the visible result of the application of that mixture. Furthermore, data presented in this paper were collected at one time point (48 h) after application and uptake and transport will continue to change beyond 48 h (Everman et al. 2009; Grangeot et al. 2006; Hoss et al. 2003; Lorentz et al. 2014; Ou et al. 2018). The joint activity of two herbicides in mixture at labeled use rates may be masking the antagonism that appears evident by uptake, transport, and metabolism experiments (Ou et al. 2018). Even so, considerable reductions in herbicide uptake for mixtures such as glufosinate + glyphosate, in which the mixture inhibits uptake of both herbicides, should be of great concern as it relates to mitigating the risk of herbicide resistance.

The primary transport mechanism across the plasma membrane for glufosinate is a proton cotransport mechanism; however, diffusion is also an important uptake mechanism at low pH (<5) when more of the undissociated glufosinate acid is present (Kumaratilake and Preston 2005; Ullrich et al. 1990). Glufosinate uptake and activity is greater in the light than in the dark, when the plant is actively photosynthesizing (Ullrich et al 1990). Unlike glufosinate, glyphosate relies entirely upon passive uptake mechanisms and does not utilize a proton-coupled amino acid carrier to enter the leaf cells. Although glyphosate may exist primarily as an uncharged molecule at room temperature (Peixoto et al. 2015), it is a polar molecule that can form negatively charged zwitterions. Regardless of the form the glyphosate molecule takes, overall plant uptake is typically low compared to the amount applied (Flint and Barrett 1989a, 1989b; Schultz and Burnside 1980). The leaf cuticle and plasma membrane is a considerable barrier for cellular uptake of glyphosate, and surfactants play a critical role in maximizing glyphosate absorption (Riechers et al. 1994).

Plant responses to both glufosinate and glyphosate are rapid, with effects on photosynthesis manifesting only a few hours after application. Glyphosate limits its own translocation by reducing photosynthesis thereby disrupting the source-sink relationship that facilitates phloem transport (Geiger and Bestman 1990). Within a few hours of glyphosate application, photosynthesis and starch accumulation declines. Inhibition of glutamine synthetase by glufosinate causes the rapid accumulation of ammonia, which subsequently inhibits photosynthesis and induces plant death (Coetzer et al. 2001; Kudsk and Mathiassen 2004; Lea et al. 1984). Both glufosinate and glyphosate rely on concentration gradients, photosynthesis, and source-sink relationships to enter the plant and their rapid activity inhibits uptake when applied alone. Thus, it is not surprising that glufosinate and glyphosate interfere with one another in regards to uptake and transport when applied in mixture.

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Appendix

Table 1. Absorption of ^{14}C-glyphosate, ^{14}C-glufosinate, and ^{14}C-dicamba in Palmer amaranth and barnyardgrass averaged over biotype as affected by herbicide treatment and harvested 48 hours after application. Absorption is represented as a percentage of the total radioactivity applied.[ab]

			Species	
^{14}C Herbicide	Treatment	Rate	Palmer amaranth	Barnyardgrass
		g ai ha^{-1}	----------%----------	
Glyphosate	Glyphosate low	897^{c}	43	25
	Glyphosate high	1735^{c}	40	30
	Glyphosate low + glufosinate	897^{c} + 595	33	15
	Glyphosate high + glufosinate	1735^{c} + 595	33	14
	Glyphosate low + dicamba	897^{c} + 560^{c}	34	21
	LSD		-------- 4 ----------	
Glufosinate	Glufosinate	595	59	49
	Glufosinate + glyphosate	595 + 897^{c}	31	29
	Glufosinate + dicamba	595 + 560^{c}	56	48
	Glufosinate + *S*-metolachlor	595 + 1390	56	31
	LSD		-------- 6 ----------	
Dicamba	Dicamba	560^{c}	63	68
	Dicamba + glufosinate	560^{c} + 595	75	77
	Dicamba + glyphosate	560^{c} + 897^{c}	63	74
	LSD		-------- 6 ----------	

[a] Abbreviation: LSD, least significant difference.

[b] Means within a column and across columns can be compared using the Fisher's protected LSD (α =0.05).

[c] Rate is in g ae ha^{-1}.

Table 2. Translocation of ^{14}C-glyphosate, ^{14}C-glufosinate, ^{14}C-dicamba in Palmer amaranth and barnyardgrass averaged over biotype as affected by herbicide treatment shown as a percentage of radioactivity applied collected 48 hours after application.[ab]

^{14}C Herbicide	Treatment	Rate	Species								Across column LSD
			--- Palmer amaranth ---				--- Barnyardgrass ---				
		g ai ha^{-1}	ATL	TL	BTL	R	ATL	TL	BTL	R	
			%								
Glyphosate	Glyphosate low	897[c]	11	22	6	4	2	14	6	4	3
	Glyphosate high	1735[c]	7	25	5	3	2	16	8	4	
	Glyphosate low + glufosinate	897[c] + 595	4	29	0	0	1	11	2	1	
	Glyphosate high + glufosinate	1735[c] + 595	2	30	1	1	1	11	1	1	
	Glyphosate low + dicamba	897[c] + 560[c]	7	17	6	5	1	10	5	4	
	Within column LSD		2								
Glufosinate	Glufosinate	595	2	53	2	1	3	30	9	8	3
	Glufosinate + glyphosate	595 + 897[c]	3	27	1	0	1	23	3	3	
	Glufosinate + dicamba	595 + 560[c]	3	50	2	1	1	36	5	6	
	Glufosiante + S-metolachlor	595 + 1390	1	54	1	1	1	26	2	1	
	Within column LSD		3								
Dicamba	Dicamba	560[c]	30	11	17	5	10	48	8	2	3
	Dicamba + glufosinate	560[c] + 595	3	71	1	0	5	67	5	1	
	Dicamba + glyphosate	560[c] + 897[c]	27	14	16	6	6	58	8	2	
	Within column LSD		3								

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

[b] Means within a column can be compared with the Within Column LSD and means across columns can be compared using the Across Column LSD. LSDs are calculated using Fisher's protected LSD (α =0.05) for a split-plot experimental design.

[c] Rate is in g ae ha^{-1}.

Table 3. Glufosinate metabolism as affected by herbicide treatment in Palmer amaranth and barnyardgrass averaged over biotype 48 hours after application as determined by high-performance liquid chromatography.[ab]

					Glufosinate metabolites			
Species	Treatment	Rate	Glufosinate		MPP + NAG[c]		Unknown[d]	
			%		%		%	
Palmer amaranth	Glufosinate	595	38	c	41	bc	21	a
	Glufosinate + Glyphosate	595 + 897[e]	32	e	53	a	15	bc
	Glufosinate + dicamba	595 + 560[e]	37	cd	42	bc	21	a
	Glufosinate + *S*-metolachlor	595 + 1390	33	de	53	a	14	ab
Barnyardgrass	Glufosinate	595	47	ab	44	b	9	d
	Glufosinate + glyphosate	595 + 897[e]	43	b	40	bc	17	cd
	Glufosinate + dicamba	595 + 560[e]	50	a	35	c	15	bc
	Glufosinate + *S*-metolachlor	595 + 1390	51	a	35	c	14	cd

[a] Abbreviation: MPP, 3-methylphosphinico-propanoic acid; NAG, 2-acetamido-4-methylbutanoic acid.

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

[c] The peaks for MPP and NAG could not be distinguished on the chromatograms and data were combined.

[d] Unknown metabolites could be 4-methylphosphinyl-2-oxobutanoic acid, 4-methylphosphinylbutanoic acid, or others not previously reported.

[e] Rate is in g ae ha^{-1}.

Table 4. Variance components estimates for uptake, transport, and metabolism in the Glyphosate, Glufosinate, and Dicmaba Experiments[a]

	Experiment								
	Glyphosate			Glufosinate			Dicamba		
Model effect	Uptake	Transport	Metabolism[b]	Uptake	Transport	Metabolism	Uptake	Transport	Metabolism
				% of total					
Run	2.995	<0.01	-	<0.01	<0.01	7.3	0.797	<0.01	5.046
Whole-plot error	-	<0.01	-	-	<0.01	-	99.203	<0.01	94.954
Residual	97.005	100	-	100.0	100	92.7	-	100	-
Total	100	100	-	100.0	100	100.0	100	100	100

[a] The Transport ANOVAs were split-plot type designs and contained a model term for whole-plot error. Other ANOVAs were completely randomized designs and contained run as the only random effect.

[b] No metabolism of glyphosate was observed and no formal analysis conducted on these data.

Table 5. Dicamba metabolism as affected by herbicide treatment in Palmer amaranth and barnyardgrass averaged over biotype 48 hours after application as determined by high-performance liquid chromatography.[ab]

					Dicamba metabolites					
Species	Treatment	Rate	Dicamba[b]		5-OH-dic[cd]		DCSA[e]		Unknown[cf]	
		g ai ha^{-1}	-%-		%	SE	%	SE	%	
Palmer amaranth	Dicamba	560^{g}	95.7	b	1.0	0.4	2.9	0.8	0.4	b
	Dicamba + glufosinate	560^{g} + 595	99.9	a	0.0	0.0	0.1	0.1	0.0	d
	Dicamba + glyphosate	560^{g} + 897^{g}	97.5	b	0.3	0.2	2.0	0.7	0.2	c
Barnyardgrass	Dicamba	560^{g}	6.3	d	10.4	a	0.0	0.0	82.0	a
	Dicamba + glufosinate	560^{g} + 595	97.5	ab	0.5	b	0.0	0.0	1.0	b
	Dicamba + glyphosate	560^{g} + 897^{g}	14.6	c	10.0	a	0.0	0.0	75.0	a

[a] Abbreviation: 5-OH-dicamba, 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid; DCSA, 3,6-dichlorosalicylic acid; SE, standard error of the mean.

[b] Means followed by the same letter are not different according to Fisher's protected LSD (α =0.05).

[c] Means followed by the same letter are not different according to a Student's t multiple comparisons test following a zero-inflated Poisson regression analysis

[d] Means followed by a number (standard error of the mean) were excluded from the analysis to improve the model fit for the other species.

[e] Means are followed by the standard error of the mean. A zero-inflated Poisson regression analysis determined there were no differences among the treatment means for Palmer amaranth (p=0.2437).

[f] Unknown metabolites could be 3,6-dichlorogentisate, or others not previously reported.

[g] Rate is in g ae ha^{-1}.

Chapter 9

Impact of antagonistic herbicide mixtures on evolution of resistance in barnyardgrass and Palmer amaranth

Barnyardgrass and glyphosate-resistant Palmer amaranth are common weeds across the midsouthern U.S. and are at a high risk for evolving herbicide resistance. Simulation models were utilized to evaluate the risk of Palmer amaranth evolving resistance to glufosinate and barnyardgrass evolving resistance to either glyphosate or glufosinate, primarily in cotton weed management programs. Glufosinate-resistance (in both species) and glyphosate-resistance (in barnyardgrass) was assumed to be conferred by a completely dominant gene. Cotton herbicide programs consisted of fluometuron + paraquat preemergence (PRE) followed by (fb) a postemergence application every 15 days (POST1 fb POST2 fb POST3) fb MSMA + diuron LAYBY, where POST 1, 2 and 3 differed between management programs. The models simulated weed population dynamics in a given field over 30-year period as a single run, and 1,000 model runs were conducted for a given management program. Scenarios were also investigated to understand the impact of antagonism of herbicide mixtures on resistance evolution, based on data obtained from prior field experiments. When three applications of glyphosate + dicamba (POST1, 2, and 3) were made in continuous cotton, the model predicted a 17-fold increased in a glyphosate-resistance risk for barnyardgrass compared to three applications of glyphosate alone, as a function of antagonism. A program of fluometuron + paraquat PRE fb glufosinate + glyphosate fb glufosinate + glyphosate fb glufosinate + glyphosate fb MSMA + diuron had no risk of either glufosinate- or glyphosate- resistance evolving for barnyardgrass due to rapid depletion of the soil seedbank. However, the same program (glufosinate + glyphosate at POST1, 2, and 3) the model predicted glufosinate-resistance evolving in Palmer amaranth in 40% of the

fields after 30 yr. Even highly robust herbicide programs (e.g., paraquat + fluometuron PRE fb dicamba + glyphosate + acetochlor POST1 fb glufosinate + *S*-metolachlor POST2 fb dicamba + glyphosate POST3 fb MSMA + diuron LAYBY) with only one glufosinate application did not fully mitigate resistance evolution in Palmer amaranth. Sensitivity analyses indicate resistance models output is highly dependent on initial seedbank size, resistant allele mutation rates, and seedbank dynamics, all which vary stochastically in the model. Although the risk of resistance varies with the species in question, resistant-prone weeds should be managed with care by utilizing an integrated weed management approach.

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

Key words: antagonism, herbicide-resistance, simulation modeling, STELLA model, weed seedbank.

Weed management decisions in midsouthern U.S. agriculture are heavily influenced by two weeds: Palmer amaranth and barnyardgrass. Palmer amaranth has evolved resistance to five herbicide sites of action (SOA) in the U.S. including 4-hydroxyphenylpyruvate dioxygenase-, acetolactate synthase (ALS)-, enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, microtubule-, and protoporphyrinogen oxidase (PPO)-inhibitors. Barnyardgrass has also evolved resistance to five SOAs in the U.S.: 1-deoxy-D-xyulose 5-phosphate synthase-, acetyl-CoA carboxylase (ACCase)-, ALS-, cellulose (i.e., quinclorac)-, and photosystem II-inhibitors (Heap 2018). Furthermore, preliminary evidence by Steckel et al. (2017) suggests barnyardgrass populations have evolved resistance to EPSPS inhibitors in TN. The investigation into herbicide resistance has identified a diverse set of mechanisms enabling weeds to survive herbicide applications (Délye et al. 2012). Furthermore, management practices needed to mitigate the risk of resistance evolution have been thoroughly described (Norsworthy et al. 2012).

Despite the breadth of knowledge available on herbicide-resistant weeds and management thereof, problematic weeds, such as Palmer amaranth, continue to evolve resistance to new SOAs. In 2015, PPO-inhibitor-resistant Palmer amaranth was identified (Heap 2018) and has since become widespread across the midsouthern U.S. (Varanasi et al. 2018). Palmer amaranth populations with resistance to multiple SOAs (i.e., ALS-, EPSPS-, and PPO-inhibitor) (Heap 2018; Schwartz-Lazaro 2017) leave soybean and cotton producers with few POST control options.

Glufosinate is being used as an alternative herbicide to control glyphosate-resistant Palmer amaranth in midsouthern U.S. cotton and soybean [*Glycine max* (L.) Merr.] production. Although glufosinate-resistant weeds haven not been confirmed in row crops, Avila-Garcia et al. (2012) identified a glufosinate-resistant Italian ryegrass (*Lolium perenne* L. spp. *multiflorum*) population with a target-site mutation from an orchard in Oregon. Glyphosate-resistance also first evolved in perennial cropping systems under similar use patterns, implying that if glufosinate is managed the same as glyphosate, widespread resistance in problematic weed species is inevitable (Avila-Garcia et al. 2012). Thus, research should be conducted to understand practices that will mitigate the likelihood of evolving resistance to one of the few remaining POST control options for Palmer amaranth in the midsouthern U.S.

Predictive models were utilized by Neve et al. (2011b) and Bagavathiannan et al. (2013) to predict the likelihood of evolving glyphosate-resistance in Palmer amaranth and barnyardgrass, respectively. Bagavathiannan et al. (2014) also adapted the model for barnyardgrass to predict the likelihood of evolving simultaneous resistance to both ACCase and ALS inhibitors. The core of both herbicide resistance models is a life-stage cycle that simulates emergence, growth, reproduction, and seed returns to the soil seedbank for the individual weed species. The model incorporates the genetics and inheritance of a resistant allele into the life-cycle model and then allows for management scenarios to impact the life cycle and inheritance at specific points (e.g., tillage at planting, or a glyphosate application POST). The model was then applied in a Monte Carlo-type analysis in which one run of the model simulated the population dynamics in one field over a 20- or 30-yr period and 1,000 or more runs for each management scenario was used to identify the risk of resistance. The model of the worst-case scenario of five glyphosate-only applications per year in continuous cotton showed Palmer amaranth resistance first evolving within 4 or 5 yr of adopting the system in about 40% of the field simulations (runs). The model correlates well with what was observed in the southern U.S. where glyphosate evolved 4 to 5 yr after the widespread adoption of enhanced, glyphosate-resistant (Roundup Ready Flex) cotton.

An assumption of both simulation models utilized by Neve et al. (2011a) and Bagavathiannan et al. (2013) worth discussing in more detail is that no herbicide interactions (e.g., antagonism) occur when two herbicides are applied in a mixture. Investigating the impact of antagonism is needed to refine the conclusions and implications gleaned from the model simulations. When glufosinate was mixed with glyphosate, antagonism has been reported on various species (Bethke et al. 2013; Chuah at al. 2008; Kudsk and Mathiassen 2004) with the antagonism likely resulting from reduced uptake and transport of glyphosate (Besançon et al. 2018). Glyphosate plus dicamba has also been reported as antagonistic, primarily on grass species (Flint and Barrett 1989; Meyer et al. 2015, 2017a; 2017b; Ou et al. 2018; O'Sullivan and O'Donovan 1980) and also likely results from altered uptake and transport of glyphosate (Flint and Barrett 1989; Ou et al. 2018)

In this paper, both the Palmer amaranth model described by Neve et al. (2011a) and barnyardgrass model described by Bagavathiannan et al. (2013) were utilized to 1) investigate the likelihood of evolving glufosinate-resistance in Palmer amaranth and barnyardgrass; 2) evaluate the impact of antagonism on evolution of both glyphosate and glufosinate-resistance in barnyardgrass; and 3) understand how herbicide programs focused on Palmer amaranth management would impact resistance evolution in barnyardgrass.

Materials and Methods

Predictive herbicide-resistance models were used to assess the risk of evolving resistance to glufosinate in Palmer amaranth and barnyardgrass. The Palmer amaranth model is a modified form of that used by Neve et al. (2011b) to simulate glyphosate-resistance evolution. The barnyardgrass model follows the framework used by Bagavathiannan et al. (2013). Both the barnyardgrass and Palmer amaranth models were implemented in STELLA visual programming language (version 10.3; iSee systems, Lebanon, NH).

The biological parameters were kept the same as those used by Neve et al. (2011b) for Palmer amaranth and Bagavathiannan et el. (2013) for barnyardgrass. Specific model parameters can be found in the appropriate publication for each species. However, a description of certain components of the models is relevant to these experiments. The model categorized the number of individuals that emerge in that particular growing season into defined emergence cohorts. The models separate the emergence into seven cohorts. Barnyardgrass and Palmer amaranth cohorts emerge at biweekly intervals beginning on April 15: cohort 1, emergence prior to planting cotton on May 1; cohort 2, May 1 to May 14; cohort 3, May 15 to May 31; cohort 4, June 1 to June 14; cohort 5, June 15 to June 30; cohort 6, July 1 to July 14; and cohort 7, emergence on or after July 15.

Weed control interventions were timed to correspond with the emergence cohorts: PRE, May 1; POST1, May 15, POST2, June 1; POST3, June 15; LAYBY, July 1. Herbicide or weed control options function by applying the efficacy of an herbicide as the percentage of individuals that do not survive that particular control option, for each cohort. Thus, the models require each management option have an assigned efficacy for all cohorts (Tables 1, 2).

Many biological parameters vary stochastically in the resistance models, particularly those which have a large impact on the model output. Both Neve et al. (2011b) and Bagavathiannan et el. (2013) conducted sensitivity analyses on their respective models, providing an insight into the relative importance of each parameter on model output. For example, both models are highly sensitive to the initial frequency of the resistant allele (R), which varies stochastically between $5x10^{-10}$ and $5x10^{-7}$, with mean of $5x10^{-8}$, in both models. Bagavathiannan et al. (2013) reported that holding the initial frequency of R to $5x10^{-10}$ resulted in a 4% risk of resistance after 15 yr vs. a 47% risk when the initial frequency of R is held at $5x10^{-7}$. Initial seedbank size, mutation rate of R, and annual recruitment (i.e., likelihood that a plant will germinate and survive to produce seed) also have a high impact on the predicted evolution of resistance and are stochastic variables in the models (Bagavathiannan et el. 2013; Neve et al. 2011b).

Glufosinate-resistance in Palmer amaranth was assumed to evolve similarly to glyphosate-resistance, with the exception that glufosinate-resistance is conferred by a one completely dominant gene with a Mendelian pattern of inheritance. Currently, no populations of Palmer amaranth resistant to glufosinate have been confirmed (Heap 2018). The mechanism of glufosinate-resistance was assumed to be completely dominant because it simplifies the model, is considered a reasonable assumption when the mechanism is not known (Bagavathiannan et al. 2013), and the glyphosate-resistance mechanism present in Palmer amaranth is not commonly found in plants (Délye et al. 2013). Evolution of glyphosate-resistance was not modeled for Palmer amaranth because it was assumed all populations of Palmer amaranth were already resistant to glyphosate. Glyphosate-resistant Palmer amaranth is widespread across the U.S. (Heap 2018) and especially prevalent in the midsouthern U.S.

Evolution of glyphosate-resistance in barnyardgrass follows the model by Bagavathiannan et al. (2013), which assessed evolution under continuous glyphosate use in RoundupReady Flex cotton in the midsouthern U.S. Both glufosinate- and glyphosate-resistance in barnyardgrass are also assumed to be endowed by a single gene. Bagavathiannan et al. (2013) made the assumption that glyphosate-resistance would be conferred by a single gene. Previous studies evaluating the risk of glufosinate-resistance suggested that it would evolve similarly to glyphosate-resistance, as both glufosinate- and glyphosate-resistance first appeared in perennial crops under similar use patterns (Avila-Garcia et al. 2012).

Identifying an actual or likely resistance mechanism is an important consideration for the resistance models as it affects efficacy values (dominant vs. recessive allele) and inheritance patterns in a population. A target-site mutation is a mechanism of glyphosate-resistance in junglerice [*Echinochloa colona* (L.) Link.], a closely related species to barnyardgrass (Alarcón-Reverte et al. 2013). Furthermore, assuming a single-trait mechanism of resistance simplifies the model and allows for estimations of inheritance patterns based on Mendelian inheritance patterns. It should be noted that glyphosate-resistant barnyardgrass was identified in TN in 2017 (Steckel et al. 2017) with reduced translocation as proposed resistance mechanism, granting low levels (2-4X) of resistance (Nandula et al. 2018). The current models only considered a resistance trait that was completely dominant and granted high levels of resistance typically observed with target-site resistance mechanisms (i.e., efficacy of 1X rate of glyphosate=0%).

Model Analysis. The model simulates the life-stage cycle for each species, taking into account emergence, growth, reproduction, and seed returns to the soil seedbank. A single run of the model represents the population dynamics of one, 60-ha field over a 30-yr period. For a given scenario, the model is cycled 1,000 times in a Monte Carlo-type analysis, to represent 1,000 individual fields. Resistance to a herbicide was defined when more than 20% of the individuals in the seedbank contained at least one resistant allele (i.e., the Resistance Threshold) (Bagavathiannan et al. 2014). Data are presented as a scatterplot comparing the percentage of fields that evolved resistance over time (30 yr). The % of fields that evolved resistance by yr 30 is also referred to as the resistance frequency. The resistance frequencies for management scenarios are compared to give a relative indication of the risk of evolving resistance.

Cotton herbicide programs were considered in the model made possible through Enlist™, GlyTol® LibertyLink®, and Bollgard II® XtendFlex™ cotton technologies. LibertyLink and RoundupReady 2 Xtend® soybean technologies, as well as RoundupReady® and LibertyLink corn (*Zea mays* L.) technologies, were considered in the model as rotational crops to cotton. The original models considered any herbicide mixture to be additive, and control from a mixture was considered equal to the Expected value calculated from Colby's Equation (Colby 1967). To allow for comparisons of situations considering and not considering antagonism, herbicide efficacies were changed and added to the original list of efficacies described by Bagavathiannan et el. 2013 for barnyardgrass and Neve et al. 2011b for Palmer amaranth). The model then simulated scenarios that both did and did not consider antagonism for specific mixtures (e.g., glufosinate + glyphosate on barnyardgrass) as a component of the overall weed management program.

A majority of the efficacy values for herbicide options were obtained from Bagavathiannan et el. 2013 for barnyardgrass and Neve et al. 2011b for Palmer amaranth. Efficacy values for each herbicide option, including the antagonistic mixtures, are listed in Table 1 for Palmer amaranth model and Table 2 for the barnyardgrass model. The efficacy value for a specific herbicide option functions in the model as a % mortality rate for affected cohorts. Efficacy values for antagonistic herbicide mixtures on Palmer amaranth or barnyardgrass were based on data from field experiments conducted by Meyer et al. (2017a; 2017b; unpublished data) including: glufosinate + clethodim, glufosinate + dicamba, glufosinate + glyphosate, and glyphosate + dicamba. Meyer et al. (unpublished data) evaluated various herbicide mixtures on two weed sizes (10 and 30-cm weeds), to give an indication of how the antagonistic mixtures may perform on various weed sizes. Although the model does not necessarily consider weed size, if POST2 (June 1) corresponded with cohort 3 (emergence from May 15-May 31), a 10 cm weed would approximate the size of a weed on June 15 that emerged with cohort 2, and a 30 cm weed would approximate the size of a weed that emerged with cohort 1. In this way, efficacies from field experiments were used to determine efficacies of herbicide options on specific cohorts.

Management strategies evaluated focused on herbicide programs and crop rotations to understand the impact of herbicide antagonism on evolution of resistance. More diverse weed management programs that include more types of control measures (i.e., mechanical, cultural, etc.) are recommended for effective herbicide-resistance management (Norsworthy et al. 2012). The impact of other types of weed control measures on the risk of evolving resistance in the Palmer amaranth and barnyardgrass models have been investigated previously (Bagavathiannan et al. 2013; 2014; Neve et al. 2011a; 2011b) and are not extensively considered here.

The same herbicide programs and weed management scenarios were evaluated in all three parts of the experiment: glufosinate-resistance in Palmer amaranth, glufosinate-resistance in barnyardgrass, and glyphosate-resistance in barnyardgrass. The scenarios evaluated in the models can be broken up into three sections: basic herbicide programs, diverse herbicide programs, and rotations (both herbicide program and crop rotations). A list of all herbicide programs evaluated for corn, cotton, and soybean can be found in Table 3 and a list of the rotations simulated in the model can be found in Table 4.

The simplest (i.e., Basic) cotton herbicide programs consisted of fluometuron + paraquat PRE followed by (fb) the same herbicide treatments for POST1, POST2, and POST3, fb diuron + MSMA LAYBY. The Diversified Herbicide Programs incorporated variable herbicides and mixtures in the three POST applications, typically with one, or more, residual herbicides. Only two herbicide programs were evaluated for each corn and soybean, as corn and soybean crops were only included as part of a rotation scenario.

Results from the model simulations are presented in graphical format showing the resistance frequency over a 30 yr period (Figure 1 for glufosinate-resistance in Palmer amaranth, Figure 2 for glyphosate-resistance in barnyardgrass, and Figure 3 for glufosinate-resistance in barnyardgrass). Resistance frequency is considered the percentage of fields in which resistance evolved, out of 1,000 fields, after 30 yr. When resistance frequency for one herbicide program is compared to another, a relative risk of evolving resistance for those programs is obtained. The final resistance frequencies (i.e., the frequency after yr 30) for all programs and rotations is also presented in Table 5.

Results and Discussion

Palmer Amaranth. The results of the Palmer amaranth model demonstrate the necessity of an aggressive weed management plan focused on the soil seedbank to effectively manage glyphosate-resistant Palmer amaranth (Figure 1). Although glufosinate-resistance first evolved in a Basic Glytol/LL Program (continuous paraquat + fluometuron PRE fb glufosinate + glyphosate fb glufosinate + glyphosate fb glufosinate + glyphosate fb MSMA + diuron LAYBY) after 4 yr, Rotation 2 (Basic LL cotton to Basic LL soybean to LL corn) had a higher resistance frequency (100%) compared to the Basic Glytol/LL Program (40%) (Figure 1, I).

Resistance frequency is considered the percentage of fields in which resistance evolved, out of 1,000 fields, after 30 yr. The higher resistance frequency in Rotation 2 is a function of the soil seedbank being replenished in the soybean year, corn year, or both (soil seedbank data not shown). The Basic Glytol/LL Program appears to approach an asymptote for resistance frequency around year 10, which is a result of resistance not having evolved in those fields, the soil seedbank dropping to almost 0 seeds m^{-2} by year 10 in those fields, and the assumption that resistant individuals do not migrate from adjacent fields.

For Rotation 2, crop-weed competition is greater with a corn crop than a cotton crop; however, the lack of implementing any weed management strategy after May 1 (POST 1 in corn) allows Palmer amaranth to emerge later in the season and produce seed, particularly after corn harvest (typically mid-August in the Midsouth). The comparison between the Basic Glytol/LL Cotton Program and Rotation 2 illustrates two points:1) rotating crops, but not necessarily herbicide SOAs, does not do much to mitigate risk of resistance and 2) a weakness in a weed management plan (e.g., rotation) will be exploited by Palmer amaranth and replenish the soil seedbank, increasing the risk of resistance.

Comparing the Diversified Glytol/LL Cotton Program I to Diversified Glytol/LL Program II (Figure 1, II) demonstrates the risk of skipping POST3, which some growers in the midsouthern U.S. attempt in order to save on herbicide and application costs (L.T. Barber, personal communication). Including acetochlor in the POST2 and applying MSMA + diuron at LAYBY appears to be a good strategy to balance cost with efficacy of the management plan. However, the risk of escapes from the LAYBY application not controlling all of the individuals shows up in the resistance frequency, where including a POST3 (i.e., glufosinate + glyphosate) reduced the resistance frequency from 93% to 3% in year 30 (Figure 1, II).

Simulations of the four Diversified Xtend programs further demonstrate the importance of various components in the three POST applications. Diversified Xtend Program I with glufosinate alone in POST2 has a resistance frequency of 40% compared to 11% with the Diversified Xtend Program II with glufosinate + *S*-metolachlor in POST2 (i.e., a 72% reduction in resistance risk) (Figure 1, III). Diversified Xtend Program III changed POST3 from glufosinate + glyphosate to dicamba + glyphosate, resulting in the glufosinate-resistance frequency declining from 11% with Diversified Xtend Program II to 3%. Finally, Diversified Xtend Program IV shows how the risk for glufosinate-resistance is reduced almost 10-fold when POST2 is preceded by a POST1 with a residual (i.e., acetochlor), effectively overlapping residual herbicides with the PRE. Although the resistance frequency is < 1% for Diversified Xtend Program IV, it is important to point out that it is not 0%. Additionally, the model does not consider how resistance may evolve to other herbicides in the program (e.g., dicamba). Overall, the Palmer amaranth model demonstrates how a zero-tolerance threshold is needed to mitigate the risk of resistance in highly fecund species such as has been demonstrated previously for Palmer amaranth (Norsworthy et al. 2014).

Barnyardgrass. Using the two barnyardgrass models, an indication of the impact of antagonism on the evolution of resistance was obtained. Antagonism of glyphosate by dicamba increased glyphosate-resistance risk in barnyardgrass 17-fold (1% vs. 17% resistance frequency for the Basic RR and Basic Xtend Program, respectively) (Figure 2). Antagonism of glyphosate by dicamba has been documented in laboratory and field experiments (Flint and Barrett 1989; Meyer et al. 2015; Meyer et al. 2017; Ou et al. 2018; O'Sullivan and O'Donovan 1980). The addition of dicamba to glyphosate has been shown to reduce uptake or transport to the lower parts of the plant depending on species and time assessments (Flint and Barrett 1989; O'Sullivan and O'Donovan 1980; Ou et al. 2018). The glyphosate-resistance barnyardgrass model showed a considerable increase in the risk for resistance when a Basic herbicide program is used consisting of three POST applications of glyphosate + dicamba.

As demonstrated with the resistance frequency of the more robust Diversified programs (0% for all Diversified Programs), utilization of residual herbicides in POST applications and utilizing different mixtures for the POST applications are critical for mitigating the risk of resistance when glyphosate antagonism is present (Figure 2). Based on the results from both models, growers should avoid frequent use of mixtures of glyphosate + dicamba because it has only one effective SOA working on grass species such as barnyardgrass (glyphosate) and glyphosate-resistant broadleaf species (glufosinate on Palmer amaranth), implying the risk of selecting for resistance is high.

Effectively utilizing a technology with multiple herbicide-resistant traits (e.g., Bollgard II XtendFlex Cotton) appeared to be a better management strategy than simply rotating traits from year to year. When a scenario where glufosinate and glyphosate-resistant traits were not stacked but rotated was compared to a continuous program that included mixtures of glufosinate + glyphosate glufosinate-resistance evolved in the rotation (Rotation 1) but not in the continuous Basic Glytol/LL Program (Figure 3). If used effectively, technologies that stack herbicide-resistant traits can be an effective strategy to mitigate the risk of evolving herbicide-resistance (Bagavathiannan et al. 2014; Gressel et al. 2017).

Practical Implications

Comparing the results of a given management program across species provides a unique perspective on the importance of biological characteristics of the weed and the risk of evolving resistance. Palmer amaranth and barnyardgrass have important characteristics factored into the models that help explain how a diverse herbicide program may be enough to mitigate resistance in barnyardgrass, but not Palmer amaranth. For example, the maximum number of seed production allowed per plant is 35,000 for barnyardgrass (Bagavathiannan et al. 2013) and

500,000 for Palmer amaranth (Neve et al. 2011b). Also, annual seedbank mortality, seedling emergence fractions, gene flow for the resistant allele, density dependent fecundity, and other parameters, differ between the Palmer amaranth and barnyardgrass models and help explain the differences in the results of those models from the same management scenarios.

It should be noted that, even though the barnyardgrass model considers a continuous Basic Glytol/LL Program in cotton to be of low risk for glufosinate and glyphosate-resistance, that does not mean that resistance could not evolve in barnyardgrass or other weeds in the system. The risk of evolving glyphosate-resistance under simple management programs was also considered to be nonexistent (Bradshaw et al. 1997; Jasieniuk 1995) and now glyphosate-resistance is documented in over 42 species (Heap 2018). The model is likely underestimating the true risk of resistance in both species. Resistance models simulating for a single trait do not take into account resistance that could evolve to other herbicides in the system (e.g., glufosinate-resistance in Palmer amaranth). Another weakness of the simulation models is they only account for one type of mechanism (i.e., target-site) and do not consider how other types of resistance mechanisms (e.g., low-level metabolic resistance, cross-resistance, etc.) could impact resistance risk. Finally, these models consider the fields in the model runs to be spatially isolated from other resistant fields and do not consider the impact of dispersal mechanisms that may spread resistant seeds across a landscape (Rutledge et al. 2000).

These models also assume that herbicides (both residual and POST) always provide the same level of control for a given application, and do not factor in variations in control that could be due to unfavorable weather conditions (e.g., temperature, humidity, solar radiation, time of day, rainfall, etc.). Glufosinate efficacy in particular can be influenced by light intensity (Kumaratilake and Preston 2005; Norsworthy et al. 2016), relative humidity (Anderson et al.

1993), temperature (Anderson et al. 1993), and time of day (Sellers et al. 2003). A failure from a residual herbicide (i.e., PRE) will also put more pressure on the POST herbicides, both due to larger weed densities and weed sizes. Therefore, the results from these models should be used as a tool for developing robust herbicide programs as a component of an overall weed management plan that incorporates a wide array of control measures to mitigate the likelihood of resistance evolution (Norsworthy et al. 2012). A weed management approach that relies on a single control measure is almost surely to fail, as the results from these models suggest.

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Appendix

Table 1. Efficacy of management options for Palmer amaranth control in corn, cotton, and soybean used in the glufosinate-resistance model[ab].

Management timing	Date	Management option	Emergence timing[c]					
			C1	C2	C3	C4	C5	C6
			SS	SS	SS	SS	SS	SS
			--------------------%--------------------					
PRE	May 1	Glyphosate	5	0	0	0	0	0
		Paraquat	99.9	0	0	0	0	0
		Glufosinate	99	0	0	0	0	0
		Fluometuron	80	97	80	30	0	0
		S-metolachlor	0	9	40	20	0	0
		Atrazine	99.9	99	99	70	20	0
		Metribuzin	80	80	30	0	0	0
		Glyphosate + glufosinate (additive)	99.1	0	0	0	0	0
		Glyphosate + glufosinate (antag)	99	0	0	0	0	0
POST1	May 15	Glyphosate	5	5	0	0	0	0
		Glufosinate	90	99	0	0	0	0
		S-metolachlor	0	0	95	40	20	0
		Acetochlor	0	0	95	40	20	0
		Dicamba	80	90	30	0	0	0
		Atrazine	0	99.9	99	99	70	20
		Glyphosate + glufosinate (additive)	91	99.1	0	0	0	0
		Glyphosate + glufosinate (antag)	85	99	0	0	0	0
POST2	June 1	Glyphosate	5	5	5	0	0	0
		Glufosinate	10	90	99	0	0	0

Table 1. Efficacy of management options for Palmer amaranth control in corn, cotton, and soybean used in the glufosinate-resistance model[ab]. (Cont.)

Management timing	Date	Management option	Emergence timing					
			C1	C2	C3	C4	C5	C6
			SS	SS	SS	SS	SS	SS
			--------------------%--------------------					
POST2	June 1	Clethodim	0	0	0	0	0	0
		S-metolachlor	0	0	0	99	90	60
		Acetochlor	0	0	0	99	90	60
		Fomesafen	90	99.9	95	80	40	0
		Glyphosate + glufosinate (additive)	15	90.5	99.1	0	0	0
		Glyphosate + glufosinate (antag)	5	85	99	0	0	0
POST3	June 15	Glyphosate	5	5	5	5	0	0
		Glufosinate	0	10	90	99	0	0
		Dicamba	0	0	0	80	90	30
		Glyphosate + glufosinate (additive)	0	5	85	99.1	0	0
		Glyphosate + glufosinate (antag)	0	0	80	99	0	0
LAYBY	July 1	MSMA (Dir)	0	0	0	20	99.9	0
		Diuron (Dir)	0	0	0	0	90	99

[a] Abbreviations: antag, antagonistic mixture; C1 to C6, cohort 1 to cohort 6

[b] Efficacies remain the same for the susceptible (SS), heterozygous resistant (RS), and homozygous resistant (RR) genotypes. However, in the glufosinate model, RS and SS genotypes are not affected by glufosinate (the trait is assumed to be completely dominant).

[c] Palmer amaranth emergence timing: C1 (prior to May 1), C2 (May 1 to May 14), C3 (May 15 to May 30), C4 (June 1 to June 14), C5 (June 15 to June 30), C6 (on or after July 1).

Table 2. Efficacy of management options for barnyardgrass control in corn, cotton, and soybean for the glufosinate- and glyphosate-resistance model[ab].

Management timing	Date	Management option	Emergence timing[c]						
			C1	C2	C3	C4	C5	C6	C7
			SS	SS	SS	SS	SS	SS	SS
			----------------------------%----------------------------						
PRE	May 1	Glyphosate	99.99	0.0	0	0	0	0	0
		Paraquat	99.9	0.0	0	0	0	0	0
		Glufosinate	99	0	0	0	0	0	0
		Fluometuron	50	95	75	30	0	0	0
		S-metolachlor	0	95	40	20	0	0	0
		Atrazine	60	90	50	0	0	0	0
		Metribuzin	30	60	30	0	0	0	0
		Glyphosate + glufosinate (additive)	99.9999	0	0	0	0	0	0
		Glyphosate + glufosinate (antag)	99	0	0	0	0	0	0
		Glyphosate + dicamba (antag)	99	0	0	0	0	0	0
POST1	May 15	Glyphosate	99.5	99.99	0	0	0	0	0
		Glufosinate	50	99	0	0	0	0	0
		S-metolachlor	0	0	99	90	60	0	0
		Acetochlor	0	0	99	90	60	0	0
		Dicamba	0	0	0	0	0	0	0
		Atrazine	0	50	90	50	0	0	0
		Glyphosate + glufosinate (additive)	99.75	99.9999	0	0	0	0	0
		Glyphosate + dicamba (antag)	80	99	0	0	0	0	0
		Glyphosate + glufosinate (antag)	80	99	0	0	0	0	0
POST2	June 1	Glyphosate	99	99.5	99.99	0	0	0	0
		Glufosinate	30	60	99	0	0	0	0
		Clethodim	75	97	99	30	0	0	0
		S-metolachlor	0.0	0.0	0.0	99	90	60	0

Table 2. Efficacy of management options for barnyardgrass control in corn, cotton, and soybean for the glufosinate- and glyphosate-resistance model[ab]. (Cont.)

Management timing	Date	Management option	Emergence timing[c]						
			C1	C2	C3	C4	C5	C6	C7
			SS	SS	SS	SS	SS	SS	SS
			----------------------------%----------------------------						
		Acetochlor	0.0	0.0	0.0	99	90	60	0
		Fomesafen	0.0	0.0	0.0	0	0	0	0
		Glyphosate + glufosinate (additive)	99	99.8	99.9999	0	0	0	0
		Glufosinate + clethodim (additive)	83	99	99.99	30	0	0	0
		Glyphosate + dicamba (antag)	50	80	99	0	0	0	0
		Glyphosate + glufosinate (antag)	50	80	99	0	0	0	0
		Glufosinate + clethodim (antag)	50	80	98	30	0	0	0
POST3	June 15	Glyphosate	95	99	99.5	99.99	0	0	0
		Glufosinate	0	30	50	90	0	0	0
		Clethodim	50	75	97	99	30	0	0
		Dicamba	0	0	0	0	0	0	0
		Glyphosate + glufosinate (additive)	95	99.3	99.75	99.9999	0	0	0
		Glyphosate + dicamba (antag)	30	50	80	99	0	0	0
		Glyphosate + glufosinate (antag)	30	50	80	99	0	0	0
LAYBY	July 1	MSMA (Dir)	0	0	0	50	95	0	0
		Diuron (Dir)	0	0	0	0	30	99	90

[a] Abbreviations: C1 to C7, cohort 1 to cohort 7.

[b] Efficacies remain the same for the susceptible (SS), heterozygous resistant (RS), and homozygous resistant (RR) genotypes. However, in the glufosinate model, RS and SS genotypes are not affected by glufosinate and in the glyphosate model, RS and RR genotypes are not affected by glyphosate (the trait is assumed to be completely dominant).

[c] Barnyardgrass emergence timing: C1 (prior to May 1), C2 (May 1 to May 14), C3 (May 15 to May 30), C4 (June 1 to June 14), C5 (June 15 to June 30), C6 (July 1 to July 14), C7 (on or after July 15).

Table 3. Herbicide programs for corn, cotton, and soybean.[ab]

			Application timing				
Program	Crop	Short Description	PRE	POST1	POST2	POST3	LAYBY
1	Cotton	Basic RR Program	Paraquat + fluometuron	Glyphosate	Glyphosate	Glyphosate	MSMA + diuron
2	Cotton	Basic LL program	Paraquat + fluometuron	Glyphosate + dicamba	Glyphosate + dicamba	Glyphosate + dicamba	Glyphosate + dicamba
3	Cotton	Basic Xtend program	Paraquat + fluometuron	Glufosinate	Glufosinate	Glufosinate	MSMA + diuron
4	Cotton	Basic Glytol/LL Program (Additive interaction)	Paraquat + fluometuron	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	MSMA + diuron
5	Cotton	Basic Glytol/LL Program (With antagonism)	Paraquat + fluometuron	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	MSMA + diuron
6	Cotton	Diversified Glytol/LL program I	Paraquat + fluometuron	Glufosinate + *S*-metolachlor	Glufosinate clethodim + acetochlor	None	MSMA + diuron
7	Cotton	Diversified Glytol/Program II	Paraquat + fluometuron	Glufosinate + *S*-metolachlor	Glufosinate clethodim + acetochlor	Glufosinate + glyphosate	MSMA + diuron
8	Cotton	Diversified Xtend program I	Paraquat + fluometuron	Dicamba + glyphosate	Glufosinate	Glufosinate + glyphosate	MSMA + diuron
9	Cotton	Diversified Xtend program II	Paraquat + fluometuron	Dicamba + glyphosate	Glufosinate + *S*-metolachlor	Glufosinate + glyphosate	MSMA + diuron
10	Cotton	Diversified Xtend program III	Paraquat + fluometuron	Dicamba + glyphosate	Glufosinate + *S*-metolachlor	Dicamba + glyphosate	MSMA + diuron

Table 3. Herbicide programs for corn, cotton, and soybean.[ab] (Cont.)

			Application timing				
Program	Crop	Short Description	PRE	POST1	POST2	POST3	LAYBY
11	Cotton	Diversified Xtend program IV	Paraquat + fluometuron	Dicamba + glyphosate + acetochlor	Glufosinate + *S*-metolachlor	Dicamba + glyphosate	MSMA + diuron
12	Soybean	Basic LL Soybean	Paraquat + metribuzin + *S*-metolachlor	None	Glufosinate	Glufosinate	None
13	Soybean	Diversified LL Soybean	Paraquat + metribuzin + *S*-metolachlor	None	Glufosinate + fomesafen + *S*-metolachlor	Glufosinate	None
14	Corn[b]	GR Corn	Glyphosate + *S*-metolachlor + atrazine	Glyphosate + atrazine	None	None	None
15	Corn[b]	LL Corn	Glyphosate + *S*-metolachlor + atrazine	Glufosinate + atrazine	None	None	None

[a] Abbreviations: LL, LibertyLink.

[b] Corn planting occurs April 15, POST 1 May 1.

Table 4. Rotations simulated in the barnyardgrass and Palmer amaranth models.[ab]

Rotation number	Rotation year	Program number	Crop	Description	Herbicide program
1	1	5	Cotton	Basic Glytol/LL Program	Paraquat + fluometuron (PRE) fb glyphosate + glufosinate fb glyphosate + glufosinate fb glyphosate + glufosinate fb MSMA + diuron (LAYBY)
	2	3	Cotton	Basic XTEND Program	Paraquat + fluometuron (PRE) fb glyphosate + dicamba fb glyphosate + dicamba fb glyphosate + dicamba fb MSMA + diuron (LAYBY)
2	1	2	Cotton	Basic LL Program	Paraquat + fluometuron (PRE) fb glufosinate fb glufosinate fb glufosinate fb MSMA + diuron (LAYBY)
	2	Soybean 1	Soybean	Basic LL Soybean	Paraquat + metribuzin + *S*-metolachlor (PRE) fb glufosinate (POST 2) fb glufosinate (POST 3)
	3	Corn 2	Corn[b]	LL Corn	Glyphosate + *S*-metolachlor + atrazine (PRE) fb glufosinate + atrazine (POST 1)
3	1	9	Cotton	Diverse Xtend Program II	Paraquat + fluometuron (PRE) fb dicamba + glyphosate fb glufosinate + *S*-metolachlor fb glyphosate + glufosinate fb MSMA + diuron (LAYBY)
	2	Soybean 2	Soybean	Diverse LL Soybean	Paraquat + metribuzin + *S*-metolachlor (PRE) fb glufosinate + *S*-metolachlor + fomesafen (POST 2) fb glufosinate (POST 3)
	3	Corn 1	Corn[b]	GR Corn	Glyphosate + *S*-metolachlor + atrazine PRE fb glyphosate + atrazine (POST 1)

[a] Abbreviations: LL, LibertyLink

[b] Corn planting occurs April 15, POST 1 May 1.

Table 5. Final glufosinate- and glyphosate-resistance frequencies for all programs and rotations expressed as a % of fields classified as resistant after 30 yr and 1,000 individual runs for Palmer amaranth and barnyardgrass.

		Final resistance frequency			
		Glufosinate		Glyphosate	
Program number	Program description	Palmer amaranth	Barnyardgrass	Palmer amaranth	Barnyardgrass
		---------------------------------%	----------------------------------		
1	Basic RR Program	0	0	.	1
2	Basic LL Program	100	24.2	.	0.0
3	Basic RR Xtend Program	0	0	.	16.6
4	Basic Glytol/LL program	36.7	0	.	0
5	Basic Glytol/LL program (with antagonism)	40	0		0
6	Diversified Glytol program I	93	0	.	0
7	Diversified Glytol program II	3	0	.	0
8	Diversified Xtend program I	40.2	0	..	0
9	Diversified Xtend program II	11.1	0	.	0
10	Diversified Xtend program III	2.7	0	.	0
11	Diversified Xtend program IV	0.3	0	.	0
Rotation 1	Basic Glytol/LL Cotton to Basic Xtend Cotton	51	0.3	.	0
Rotation 2	Basic LL Cotton to Basic LL Soybean Program to Basic LL Corn Program	100	34.1	.	0
Rotation 3	Diverse Xtend Cotton II to Diverse LL Soybean to GR Corn Program	59.7	0	.	0

[a] Abbreviations: LL, LibertyLink

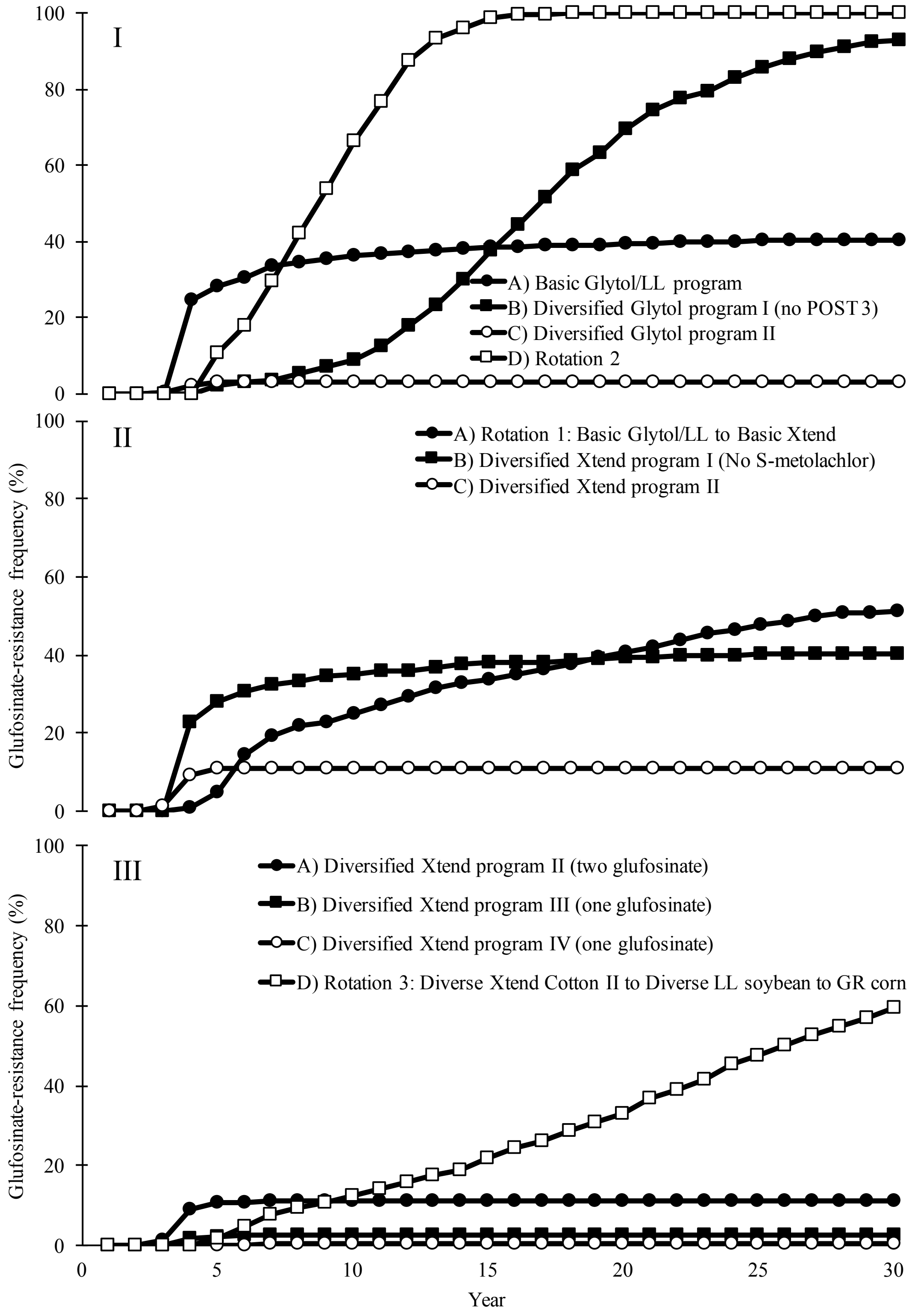

Figure 1. Frequency of Palmer amaranth evolving resistance to glufosinate under various management scenarios including: IA) continuous Basic Glytol/LL Cotton Program; B) continuous Diversified Glytol/LL Program I with no POST3; C) continuous Diversified Glytol/LL Program II; and D) Rotation 2, Basic LL Cotton Program to Basic LL Soybean Program to LL Corn Program. IIA) Rotation 1, Basic LL Cotton Program to Basic Xtend Cotton Program; B) continuous Diversified Xtend Program I with no *S*-metolachlor in POST2; and C) continuous Diversified Xtend Program II with *S*-metolachlor in POST 2. IIIA continuous

Diversified Xtend Cotton Program II with two glufosinate applications; B) continuous Diversified Xtend Cotton Program III with one glufosinate application at POST2; C) continuous Diversified Xtend Cotton Program IV with one glufosinate application at POST2 proceeded by application with acetochlor in POST1; and D) Rotation 3, Diversified Xtend Cotton Program II to Diverse LL Soybean Program to GR Corn Program. Refer to Table 3 for a description of the components of each program and Table 4 for a description of the rotations.

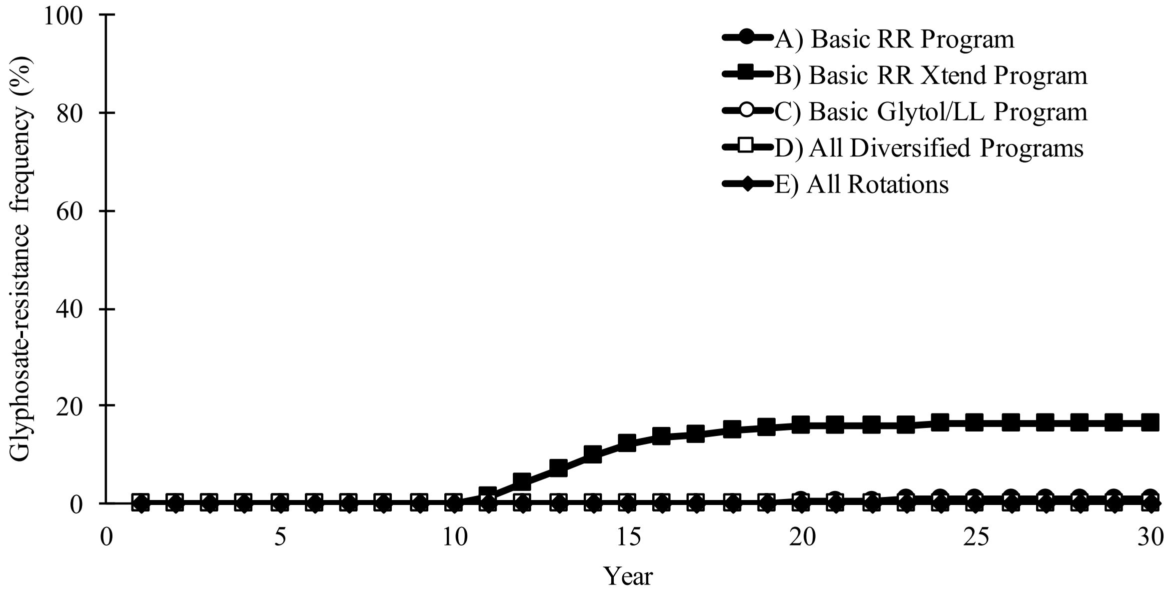

Figure 2. Frequency of barnyardgrass evolving resistance to glyphosate under various management scenarios including: A) continuous Basic RR Cotton Program; B) continuous Basic Xtend Cotton Program; C) continuous Basic Glytol/LL Program I; D) All Diversified Cotton Programs (continuous Diversified Glytol/LL Program I, continuous Diversified Glytol/LL Program II, continuous Diversified Xtend Cotton Program I, continuous Diversified Xtend Cotton Program II, continuous Diversified Xtend Cotton Program IV); and E) all rotations, Rotation 1 (Basic LL Cotton Program to Basic Xtend Cotton Program); Rotation 2 (Basic LL Cotton Program to Basic LL Soybean Program to LL Corn Program); Rotation 3 (Diversified Xtend Cotton Program II to Diverse LL Soybean Program to GR Corn Program). Refer to Table 3 for a description of the components of each program and Table 4 for a description of the rotations.

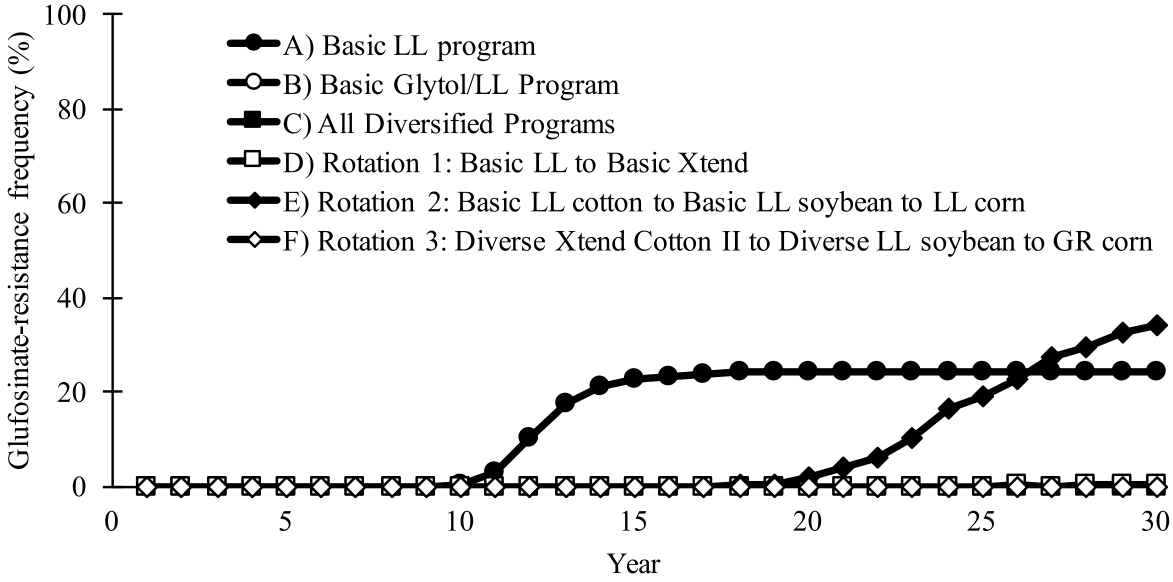

Figure 3. Frequency of barnyardgrass evolving resistance to glufosinate under various management scenarios including: A) continuous Basic LL Cotton Program; B) continuous Basic Glytol/LL Program I; C) All Diversified Cotton Programs (continuous Diversified Glytol/LL Program I, continuous Diversified Glytol/LL Program II, continuous Diversified Xtend Cotton Program I, continuous Diversified Xtend Cotton Program II, continuous Diversified Xtend Cotton Program IV); D) Rotation 1 (Basic LL Cotton Program to Basic Xtend Cotton Program); E) Rotation 2 (Basic LL Cotton Program to Basic LL Soybean Program to LL Corn Program); F) Rotation 3 (Diversified Xtend Cotton Program II to Diverse LL Soybean Program to GR Corn Program). Refer to Table 3 for a description of the components of each program and Table 4 for a description of the rotations.

Chapter 10

General Conclusions

Mitigating the risk of glufosinate-resistance will require optimization of glufosinate applications, recognition of antagonistic herbicide mixtures, and use of an integrated weed management approach expanding beyond use of herbicides. Proper droplet size (medium-coarse) and spray volumes ($\geq$ 141 L ha^{-1}) should be used to maximize the efficacy of glufosinate, even when mixed with other herbicides such as 2,4-D, clethodim, dicamba, fomesafen, and glyphosate. If glufosinate is to be used alone to control large weeds ($\geq$10-cm), two applications of glufosinate at the highest labeled use rate should be made 7-10 days apart to maximize weed control and soybean yield.

To effectively control a broad spectrum of weeds, mixtures will likely be heavily utilized in dicamba- and 2.4-D-resistant crops. Unfortunately, many of the mixtures that may be used can be antagonistic on a variety of species. Glufosinate + glyphosate, glufosinate + clethodim, glyphosate + 2,4-D, and glyphosate + dicamba were all antagonistic when applied to barnyardgrass. Glufosinate reduced uptake and transport of glyphosate and dicamba in barnyardgrass. Additionally, dicamba reduced uptake of glyphosate in barnyardgrass, and is a likely mechanism for the antagonism observed in the field. Based on glyphosate-resistance simulation models in barnyardgrass, antagonism of glyphosate by synthetic auxin herbicides increased the risk of evolving resistance. Although, glufosinate + glyphosate was also determined to be antagonistic in the field, the use of the mixture resulted in minimal risk of resistance, as two effective herbicide SOAs were applied when the mixture was used. These results imply that the stacking of two herbicide-resistant traits in genetically-engineered crops is a preferable herbicide-resistance management strategy rather than annually rotating technologies with either one trait or the other.

The Palmer amaranth resistance model suggests that intense management focused on depleting the soil seedbank is needed to mitigate the risk of evolving glufosinate-resistance. Glufosinate and dicamba uptake and transport experiments suggest that mixtures can reduce the amount of herbicide absorbed by Palmer amaranth. However, experiments were not able to detect antagonism in the field, and Palmer amaranth control with mixtures with two effective SOAs was typically very high (≥90%) even applied to large (> 10-cm tall) plants. To mitigate the risk of resistance in Palmer amaranth, herbicide programs that utilize many effective SOAs (preferably in mixtures that are not antagonistic), applications to small plants (< 10-cm), use of appropriate spray application parameters (nozzle selection, spray volume, etc.), residual herbicides PRE and POST, in addition to other types of control strategies (cultural, mechanical, etc.), are imperative for mitigating resistance evolution.