Stewardship of Synthetic Auxins in 2,4-D and Dicamba–resistant Crops and Mitigation of Off-target Movement

by

Frances Baker Browne

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Approved by

Steve Li, Chair, Assistant Professor of Crop, Soil, and Environmental Sciences
Scott McElroy, Professor of Crop, Soil, and Environmental Sciences
Yucheng Feng, Professor of Crop, Soil, and Environmental Sciences
Andrew Price, Plant Physiologist of United States Department of Agriculture

Abstract

A series of greenhouse, field, and laboratory experiments were conducted to evaluate preemergence (PRE) and postemergence (POST) herbicides for Palmer amaranth (*Amaranthus palmeri* L.) control in addition to assess the potential for dicamba to move off-target to sensitive soybean (*Glycine max* L.) through tank contamination and volatility routes. Cotton (*Gossypium hirsutum* L.) residual herbicides acetochlor, diuron, fomesafen, fluridone, and pendimethalin were found to provide the greatest Palmer amaranth control when applied at highest use rates and activated with 1.91, 0, 0.64, and 1.27 cm of water, respectively. Furthermore, fomesafen combinations with acetochlor, diuron, fluridone, and prometryn were not shown to significantly impact cotton yield when applied up to 2x use rates as compared to the nontreated control. Field experiments in a non-crop setting indicate sequential applications of dicamba + glyphosate followed by (fb) glufosinate and 2,4-D + glufosinate fb glufosinate at seven day intervals have potential to effectively control Palmer amaranth escapes that have exceeded heights recommended for chemical control. However, control was variable among years and timely applications of POST herbicides remain the best approach. Greenhouse experiments suggest glufosinate severely impacted Palmer amaranth photosynthesis with up to 90% reductions in CO_2 assimilation. Furthermore, applications of dicamba + glyphosate seven days before glufosinate could reduce regrowth as compared to the reverse sequence or tank mixture. A replicated field study and sprayer survey indicate triple rinse with water was is sufficient for dicamba removal from equipment following applications and remaining contaminants (concentrations <1.25 mg L^{-1}) were not shown to reduce soybean yields. A field study where dicamba was applied at 0.56, 5.59, 56.42, 559.17, 5591.75, and 11183.51 g ae ha^{-1} to soil pans placed under sealed low tunnels covering two rows of soybeans for 48 hours resulted in visual injury for all dosages, ranging from 1 to 45% across all dosages and site-years. However, no significant soybean yield reductions were observed over three site-years as compared to the nontreated control. These results can provide guidelines for row crop producers to practice proper stewardship of synthetic auxins and aid to preserve technology for future use.

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DAT	Days after treatment
DAIT	Days after initial treatment
FB	Followed by
PRE	Preemergence
POST	Postemergence
WAP	Weeks after planting

Chapter 1

Literature Review

1.1. Cotton and soybean production and herbicide resistance traits

1.1.1. Cotton production

Cotton (*Gossypium hirsutum* L.) is one of the most preferred textiles in production and a major cash crop, accounting for 25% of total fiber use globally (USDA 2019). The US ranks as the third leading producer of cotton and the top exporter (USDA 2020a). Annual revenues generated from the cotton industry and related services exceed $21 billion and provides jobs to 125,000 people (USDA 2019). Jobs range from preparation and management of the crop from emergence to harvest in addition to textile processing. In total, over 5 million hectares of cotton were farmed in the US during 2019, producing over 12 million bales each weighing 217 kg and grossing over $6 billion (USDA 2020b). Included in those stats were 218,531 hectares planted in Alabama which yielded nearly 260 tons and produced over $331 million (USDA 2020c). Approximately 98% of all cotton grown in the US during 2019 contained herbicide resistance traits, indicating widespread adoption (USDA 2020b).

As a textile, cotton is primarily grown in more temperate and tropical regions of the globe and produced in more than 77 countries worldwide (USDA 2019d). Cotton production dates back 2,000 years in India and several hundred for the US and other countries (Martin et al. 2006). Domestication of cotton was a parallel process among humans on several continents from relatively diverse wild ancestors (Wendel et al. 2009; Wendel and Grover 2015; Hutchinson 1951). Although nearly 50 species are classified in the genus *Gossypium* L., very few lint-bearing species are cultivated for mass production including *G. barbadense* L. (Egyptian cotton), *G. herbaceum* L. (Levant cotton), and *G. arboretum* (tree cotton) (Wendell et al. 2009; Wendell and Grover 2015). Today, the market is dominated by *G. Barbadense* L. and *G. hirsutum* L. (Hutchinson 1951; Lee 1984). The *Gossypium* genus is classified within the Malvaceae family and thought to have originated in Africa (Wendell et al. 2009). Primitive ancestors of cotton were perennial vines that were cultivated to become the four species used in production today. Origins of cultivated cotton plants are likely scattered from Africa to Central and South America (Wendell et al. 2009).

Cotton is produced in the temperate zones of the globe with mean annual temperatures of 16°C and adequate light and rainfall. Cotton production is more geographically restricted in comparison to soybean production. Although cotton is a perennial plant, it is managed as an annual crop in the US. Plants usually grow to 60 to 150 cm tall and produce a long taproot capable of growing 2.5 cm per day (Martin et al. 2006). Solitaire leaves with palmate venation and several lobes are born on a petiole with 2 buds located at the base. One bud produces vegetative growth and the other gives rise to a fruiting branch. Typically, flowers are produced every 6 days once bloom is initiated. Flower buds are frequently referred to as squares and develop around 30 days after cotton emergence. Cotton has an indeterminate fruiting pattern and bloom can extend for 7 to 8 weeks. However, fruiting periods can vary as cotton plants compensate for environmental stresses such as pathogens, fertility, and drought. Cotton is a self-pollinated plant but out-crossing can occur. Cotton fiber growth is initiated at first bloom and followed by fiber lengthening at a rapid pace, completed in 15 to 25 days. Lint quality is often based on length, strength, and fineness and up to 9 seeds can be produced in a single boll. The perennial nature of cotton can warrant applications of plant growth regulators to limit growth throughout the season and increase harvest efficiency. Due to its indeterminate growth habit and perennial nature, cotton must be terminated at maturity prior to harvest (Martin et al. 2006).

1.1.2 Soybean production

The US is the top producer of soybean (*Glycine max* L,) and second highest exporter (USDA 2020d). Soybean planted area in 2019 averaged over 30 million hectares which yielded a total of 3.19 tons (USDA 2020b). Total soybean area farmed in Alabama during 2019 was 107,242 hectares which yielded an average of 2,229 kg ha^{-1} and contributed to a total value of $86 million (USDA 2020c). An estimated 94% of all soybeans planted in the US during 2019 had biotech-derived herbicide resistance traits, indicating majority of producers are seeking transgenic technology as means of weed control (USDA 2020b).

Soybean is considered one of the oldest cultivated crops with origins in Southeast Asia and documented cultivation in China around 1100 BC (Hymowitz 2008). Cultivated varieties were derived from a wild type known as *Glycine ussuriensis* Regel and Maack (Martin et al. 2006). Soybeans are considered a high value crop as seeds can be used as an oil, livestock and fishery feed, protein for human consumption, as well as biofuel. This crop is arguably one of the most important crops grown globally due to its protein meal and vegetable oil. The season-average farm price for soybeans growing in the US for 2019 is $8.59 per bushel (USDA 2020c).

Day length is of particular importance for soybean growth and likely the limiting factor for production in different geographical areas (Martin et al. 2006). Soybeans can be cultivated in nearly all types of soil. Following germination and emergence of cotyledons, soybeans will produce two true leaves that are opposite and unifoliate and later-developing leaves will all be trifoliate. Flowering is typically initiated 6 to 8 weeks following emergence. However, this time period can vary depending on the maturity level of the variety. Soybean varieties are either classified as determinate or indeterminate depending on flowering habit. Determinate plants stop vegetative growth once pods develop at the terminal end of the main stem. The majority of soybeans grown in the southern US are determinate varieties due to extended nocturnal periods (Martin et al. 2006). Indeterminate varieties will continue to produce vegetative structures at the same time that flowering is initiated and continue to do so for a certain period of their reproductive stage. Flowers may appear white or purple and pods are long and slender. Similar to cotton, soybeans are self-pollinated and pollinating insects or other sex plants are not needed; however, some natural cross-pollination can occur. Eventually, soybeans develop into erect plants 60 to 120 cm tall and branching creates a relatively bushy appearance. Varieties are also grouped by maturity groups. Typically, group IV and lower maturity groups are indeterminate soybean varieties and group V or later are determinate. Once maturity has been reached, plants will naturally defoliate once seeds have reached 20% moisture. However, some producers will use defoliants to speed up defoliation and aid in harvest (Martin et al. 2006).

Similar to cotton, one of the first major advancements in soybean genetics was commercialization of the glyphosate resistance traits. In 2017, dicamba resistant soybeans (Roundup Ready® Xtend Crop System, Monsanto Co., St. Louis, Mo, 63167) hit the market and were later followed by 2,4-D resistant traits (Enlist® Weed Control System, Corteva Agriscience, Indianapolis, IN, 46268). Glyphosate resistant weeds remain a great challenge for soybean and cotton production alike and new herbicide tolerance traits will extend chemical management options. Producers frequently rotate between soybeans and cotton as growing soybeans for consecutive years is strongly discouraged (Martin et al. 2006). Therefore, herbicides must be compatible for both crops to avoid injury if cotton and soybeans are in rotation. Fortunately, herbicide resistance traits for glyphosate, glufosinate, 2,4-D and dicamba are registered for both crops (Martin et al. 2006; Egan et al. 2014; Kniss 2018b).

Herbicides are commonly used in both cotton and soybean production for weed control. Common programs include herbicides applied preplant burndown, preplant incorporated, preemergence (PRE) at planting, postemergence (POST), and POST-directed (Martin et al. 2006). Burndown programs used in both soybeans and cotton frequently utilize glyphosate and paraquat to kill emerged weeds prior to planting, especially in conservation tillage systems. Common PRE herbicides used in cotton production include fluridone, fomesafen, acetochlor, diuron, fluometuron, norflurizone, clomazone, prometryn, penidmethalin, trifluralin and S-metolachlor. POST herbicides and/or POST-directed herbicides applied in cotton include fluometuron, oxyfluorfen, pyrithiobac, quizalofop, sethoxydim, clethodim, fluazifop, and MSMA/DSMA (Martin et al. 2006). Several of the herbicides listed above provide selective control of grasses and are safe on broadleaf crops. Crops with resistance to glyphosate, glufosinate, 2,4-D, and dicamba are also available and add additional platforms for chemical control (Martin et al. 2006).

Soybean preplant and PRE herbicides frequently include trifluralin, metribuzin, pendimethalin, clomazone, dimethenamid, and alachlor. Soybean POST options include bentazon, chlorimuron, imazamox, sethoxydim, and flumiclorac (Martin et al. 2006). Transgenic cotton and soybean crops with resistance to glyphosate, glufosinate, 2,4-D and dicamba will also allow POST applications of those herbicides. Some herbicides listed above are commonly sold as premixtures. Tillage and cultivation may also be used in conjunction with chemical control options to further suppress weeds (Martin et al. 2006).

Weed pressure is a major limiting factor for both cotton and soybean production. No doubt, Palmer amaranth is one of the most troublesome weeds to manage in the southeast. The competitive nature of Palmer amaranth combined with the evolution of herbicide resistant biotypes can make control nearly impossible. New 2,4-D and dicamba technologies will expand POST herbicide options for cotton and soybeans. However, additional control measures will be required to delay the onset of resistance development and mitigation of off target movement will be imperative to extend the commercial life of these technologies.

1.1.3 Herbicide resistance traits in cotton and soybean

One of the greatest challenges in management of Palmer amaranth is the season long interference with the crop (Ward et al. 2013). PRE herbicides alone are not enough to sufficiently control Palmer amaranth due to extended emergence periods. The highest level of weed interference usually occurs in the first few weeks of crop production (MacRae et al. 2013). Palmer amaranth established later in the season are less likely to reduce yield due to the crop's ability to create a wide canopy (MacRae et al. 2013; Rowland et al. 1999; Ward et al. 2013). However, weeds established later in the season can still replenish the seed bank and need to be controlled. Before herbicide resistant crops were commercialized, control was limited to few herbicide families (Martin et al. 2006).

Herbicide resistant crops have been developed through either modification of the target enzyme to render it less sensitive to the specific herbicide or introduction of a new enzyme that can readily detoxify the herbicide (Davey et al. 2010). Techniques used to accomplish these methods are either selected through traditional breeding or gene transfer. Transfer of genetic material can be achieved through a bacteria known as *Agrobacterium spp.* or through microprojectile methods (Davey et al. 2010). Although phenotypical herbicide resistance may be apparent, commercial development requires vigorous testing to ensure crop safety. Both acceptable tolerance to an herbicide and crop performance must reach specific standards before commercial distribution (Martin et al. 2006).

One of the first herbicide resistance genes used in cotton conferred resistance to Bromoxynil, a nitrile herbicide used to control broadleaf weeds (Martin et al. 2006). The weak competitive ability of cotton early in the season can allow weeds to emerge and compete for limited resources. Before 1996, POST herbicide options were not available for use in cotton without the potential for interference with maturity or yield (Guthrie and York 1989; Wilcut et al. 1995). Therefore, commercialization of cotton with resistance to a selective POST herbicide had great potential as a weed management strategy. The mode of action of bromoxynil is inhibition of photosystem II (Shaner 2014). Applications over the top of cotton provided producers with an additional option to control troublesome weeds during the growing season. The bromoxynil resistance trait in cotton was introduced in 1995 but was quickly phased out and research efforts were directed towards development of the next big resistance trait, the Roundup Ready® gene (Monsanto, St. Louis, MO63167). Nonetheless, commercialization of bromoxynil-resistant cotton demonstrated the advantage of introducing herbicide resistance traits to crops and optimizing use of existing herbicide chemistries.

Resistance to ALS-inhibitors is available in some soybean varieties marketed as STS-Tolerant Technology™ (Dupont, Wilmington, DE 19805). The technology was first introduced in the mid 1990's. These varieties allow use of sulfonylureas without causing crop injury. Initial varieties contained a single ALS1 gene. However, newer soybean varieties released in 2015 by DuPont Pioneer contain two ALS-inhibitor resistance genes. Soybeans with this technology were developed through seed mutagenesis to confer resistance to chlorimuron and thifensulfuron (Sebastian et al. 1989). Although ALS-resistant weeds have spread across majority of the US, use of resistant soybeans allows double cropping with other crops such as wheat or rice where sulfonylureas are one of limited herbicides available for use and would otherwise damage soybeans. Double cropping with crops such as wheat may reduce Palmer amaranth populations through competition and shading and use of these varieties could be warranted where additional control methods are needed.

Resistance to glyphosate is conferred in majority of transgenic crops through expression of the *cp4 epsps* gene (Green 2018). Glyphosate is a broad spectrum, non-selective herbicide that inhibits biosynthesis of aromatic amino acids (Steinrücken and Armhein 1980). Transgenic varieties are marketed under the trade name Roundup Ready® (Monsanto Co., St. Louis, Mo., 63167) in both soybeans and cotton. The *cp4 epsps* gene was isolated from a bacterium known as *Agrobacterium*, originally discovered in runoff from a glyphosate manufacturing site (Pallett 2018). The gene confers resistance by reducing the binding affinity for glyphosate (Saroha et al. 1998). Other mechanisms such as detoxification of glyphosate and overexpression of the EPSPS enzyme have also been explored (Vats 2015; Nafzinger et al. 1985; Saroha 1998). Early attempts to develop glyphosate resistant crops were focused on overexpression of EPSPS. However, commercially acceptable levels of resistance were difficult to achieve. Sales of the original Roundup Ready® gene was initiated in 1996 in GTS 40-3-2 soybeans (Nandula 2019). Soybeans were the first commodity released for this technology. However, other commodities such as corn, cotton, and canola are also engineered with glyphosate resistance. Additional genes have been identified with similar resistance and multiple varieties produced by different companies are available for each commodity (Duke 2018).

Ease of use and feasibility resulted in rapid adoption of glyphosate resistance technology by producers. Adoption rates exceeded 90% just 10 years after the commercial introduction of glyphosate-resistant soybean and cotton followed similar trends by 2014 (Duke 2018). Following a change in trait designation, a new round of genetics was released for soybeans in

2009 as MON89788 (Monsanto, St. Louis, MO 36167; USDA 2019c). The original Roundup Ready ® trait was later removed from the soybean market in 2015. These technologies are widespread across North America and South America. Commercial varieties are available with the stand alone Roundup Ready ® trait. However, other varieties with stacked traits conferring resistance to glyphosate, glufosinate, and dicamba/2,4-D are also available and optimize POST herbicide options. Additional traits are available that are not marketed at Roundup Ready® in cotton and soybean that also confer resistance to glyphosate (Duke 2018). Although glyphosate resistant weeds have created immense challenges for crop production, the glyphosate resistance trait still dominates the market for herbicide resistant crops.

Glufosinate resistance in soybeans and cotton are conferred by the *pat* and *bar* genes under the trade name LibertyLink® (BASF, Florham Park, NJ 07932). The *pat* gene was isolated from *Streptomyces viridochromogenesa* and the *bar* gene was isolated from *Streptomyces hygroscopicus* (Dröge et al. 1992; Thompson et al. 1987). The *pat* and *bar* genes encode for two homologous phosphinothricin acetyltransferases which inactivate glufosinate through acetylation (Dröge et al. 1992). Transgenic crops with glufosinate resistance were engineered via *Agrobacterium* gene transfer (Broer et al. 1989). The mode of action of glufosinate is inhibition of glutamate synthase, the enzyme responsible for ammonia assimilation. However, only the L-enantiomer has herbicidal activity. Also commonly known as phophinothricin, glufosinate is the only herbicide with this unique mode of action. Herbicide trade names include Liberty® (Bayer CropScience LP, Research Triangle Park, NC, 27709) and Basta® (BASF, Florham Park, NJ 07932). The only weed species with reported resistance in the US is Italian ryegrass (Loliom perenne ssp. Multiflorum) (Heap 2019). Therefore, glufosinate is an effective herbicide option for glyphosate resistant weeds such as Palmer amaranth. However, glufosinate is a contact herbicide so adequate coverage is needed for control. Weed height at the time of application also has a large influence on the herbicide efficacy. Palmer amaranth control is significantly reduced in plants taller than 8 cm (Culpepper et al. 2010; Coetzer et al. 2002). Therefore, timely applications are required for optimal use.

Glutamate synthase is a primary component of nitrogen metabolism and combines glutamine and ammonia to form glutamate. Downstream effects following glufosinate exposure include ammonia toxicity in the plant, degradation of pH gradients, and rapid defoliation. In a study by Coetzer and Al-Khatib (2001), ammonia concentrations in Palmer amaranth treated with 410 g ha^{-1} were 22 and 53 times higher than nontreated plants 6 and 24 hours after application, respectively. Furthermore, photosynthesis was inhibited by 31% just 2 hours after application (Coetzer and Al-Khatib 2001). Buildup of ammonia in the plant is known to decouple photophosphorylation and binds to the oxygen-evolving complex of PSII (Krogman et al. 1959; Izawa 1977). Ammonia may also induce the PSII light harvesting complex to enter an oxidized state, resulting in lipid peroxidation and membrane destruction (Hess 2000; Lea and Ridley 1989). The Liberty Link® technology is available for both cotton and soybean as a standalone trait or in varieties with stacked herbicide-resistance traits such as glyphosate, 2,4-D, and dicamba. A new trait in soybeans known as LLGT27® was introduced to the market in 2019 which provides tolerance to glufosinate, glyphosate, and a new HPPD-inhibitor that is pending registration by the EPA (BASF, Florham Park, NJ 07932; Beckie et al. 2019). This technology may extend chemical control by adding an additional mode of action to herbicides that can be used in soybean production.

Discovery of glyphosate-resistant Palmer amaranth has been a major threat to the sustainability of glyphosate-resistant crops. Therefore, in response to the rapid spread of resistant populations, the herbicide industry focused efforts on development of crops with resistance to other existing modes of action. Soybean and cotton varieties with resistance to synthetic auxins are the latest technology to enter the market. Synthetic auxins mimic the natural auxins in a plant such as indole-3-acetic acid (Tan et al. 2007). Observable symptomology resulted from synthetic auxins includes twisting of stems, crinkling of leaves, and leaf cupping (Kniss 2018; Egan and Mortensen 2012; Egan et al. 2014). Dicamba resistance is conferred by the dicamba monooxygenase (*dmo*) gene and marketed under the trade name Roundup Ready 2 Xtend® (Bayer, Kansas City, MO 64120). The gene was isolated from a bacterium known as *Stenotrophomonas maltophila* and readily degrades dicamba to nontoxic 3,6-dichlorosalycylic acid and formaldehyde through oxidative demethylation (Behrens et al. 2007). This system was fully available to the public in 2017. However, these technologies are currently only available in the US. Bayer Cropsciences markets the dicamba tolerant varieties under the name XtendFlex® (Bayer CropScience LP, Research Triangle Park, NC 27709). Transgenic traits in commercial crop varieties confer resistance to dicamba, glufosinate, and glyphosate.

Crop resistance to 2,4-D was co-engineered by Monsanto Co. (Monsanto Co. St. Louis, MO 63167) and Corteva Agriscience under the trade name Enlist™ (Corteva Agriscience, Wilmington, DE 19805) . The Enlist™ weed control system provides soybean and cotton resistance to 2,4-D conferred by the *tfdA* gene isolated from soil bacteria *Sphingobium herbicidivorans* and *Delftia acidovorans* (Nandula 2019). These bacteria can rapidly degrade 2,4-D to nontoxic dichlorophenol through a two-step dioxygenase reaction (Peterson et al. 2016). Commercial soybean varieties were late to the market in comparison to dicamba resistant varieties due to regulatory delay. Similar to the Xtend® technology, Enlist™ varieties have stacked traits conferring resistance to 2,4-D, glufosinate, and glyphosate. Soybeans with these stacked traits are marketed under the trade name Enlist E3® and are available for several different maturity levels. It is important to note that Enlist® crops are sensitive to dicamba and Xtend® crops are sensitive to 2,4-D. Therefore, herbicides cannot be interchanged in these systems.

1.2. Palmer amaranth biology, herbicide resistance, and crop interference

1.2.1. Palmer amaranth biology and competitive ability

Geographic distribution of Palmer amaranth across the US is remarkable, spanning to majority of the country in less than 20 years (Ward et al. 2013). Palmer amaranth is native to North America. However, spread was likely hastened by agricultural advancements and human interference. Specimens collected from California, Arizona, New Mexico, and Texas date back to the late 1800's and early 1900's (Sauer 1957; Ward et al. 2013). A few decades later, specimens were collected in Oklahoma and documented throughout the south by 1940. Palmer amaranth is one of 75 species classified within the *Amaranthus* genus. Ten of those species belong to their own subgroup of dioecious plants known only to inhabit North America (Steckel 2007; Ward et al. 2013). Ironically, the greek origin of the name *Amaranthus* is "amarantos", the name of a mythical unfading flower. Proper understanding Palmer amaranth biology is needed to develop strategies for control.

Palmer amaranth is an erect annual weed and regularly reported to grow over 2 m tall (Parker 1972; Culpepper et al. 2006). Some reports of exceptionally tall plants exist at 4.6 m with stems ranging in diameter up to 15 cm (Zollinger 2015; Norsworthy et al. 2008; Parker 1972). Females have been reported to grow 11% larger than males (Keeley et al. 1987). Research suggests females invest more resources into stem growth resulting in greater height and total dry weight whereas males were noted for higher leaf area and dry weight (Korres et al. 2017). Rapid growth complicates management as the window for chemical control may be short. According to Sellers et al. (2003), Palmer amaranth only has a 2 week window for herbicide applications after germination under optimal conditions before heights exceed those recommended for chemical control. More rapid growth is known to occur early in the growing season compared to later emerging weeds (Sellers et al. 2003). Growth rates for Palmer amaranth plants have been recorded up to 3.5 cm per day (Horak and Loughlin 2000). The competitive ability of Palmer amaranth is likely a function of the growth rate and plants can quickly grow taller than target crops. Growth rates of Palmer amaranth exceed those of other troublesome pigweeds in the southeast (Horak and Loughlin 2000). Palmer amaranth regrowth following mechanical or chemical injury is of particular concern. Browne et al. (unpublished manuscript - see Chapter 3) conducted a greenhouse experiment where Palmer amaranth leaves were removed and 78% of initial foliage biomass was restored 21 days later. Furthermore, a study by Sosnoskie et al. (2014) severed Palmer amaranth stems 3 cm above the soil level and plants recovered to produce 28,000 seeds per plant. These data suggest Palmer amaranth can recover from severe injury in a relatively short amount of time.

An extensive root structure also aids in competitive abilities. Palmer amaranth root structure is comprised of a deep taproot with an extensive network of finer, fibrous roots. Wright et al. (1999) reported Palmer amaranth roots have 5 times the number of primary roots and more than 3 times the number of total roots as compared to soybeans. Furthermore, Palmer amaranth root length dwarfed that of soybean by 5-fold (Wright et al. 1999). Mycorrhizal relationships have not been observed for Palmer amaranth and root complexity may compensate for this disadvantage (Tester et al. 1987; Wright et al. 1999). Root strength appears to give Palmer amaranth an advantage over some crops. Compared to soybean, Palmer amaranth roots have been shown to grow through highly compacted soils more readily (Place et al. 2008). This ability could grant Palmer amaranth access to a greater level of nutrients and moisture that target crops cannot reach in addition to providing increased stability for subsequent growth.

Rapid herbicide resistance evolution observed in Palmer amaranth populations is likely due to its dioecious habit. Male and female flowers exist on separate plants, thereby forcing outcrossing and increasing genetic variability (Franssen et al. 2001). Female flowers are not showy and contain bracts, tepals, and seed capsules. Bracts arise at the base of flowers and become sharp to the touch at maturity (Culpepper et al. 2010). Male flowers are composed of shorter bracts along with tepals and anthers. Flowers cluster together to form long terminal spikes up to 30 cm in diameter during September and October (Bond and Oliver 2006). Differentiation of male and female flowers is difficult before reaching maturity. The texture of the reproductive spikes is usually the most distinctive characteristic involved in determining whether a Palmer amaranth plant is male or female. Spikes on male plants will feel much softer to the touch as compared to the sharp bracts on female flowers (Bond and Oliver 2006). Further complicating the evolution of herbicide resistance, interspecific breeding has been verified between common waterhemp (*Amaranthus rudis* J.D. Sauer) and Palmer amaranth (Franssen et al. 2001). Facultative apomixis has also been suggested where isolated Palmer amaranth females produced viable seeds in the absence of males (Ribeiro et al. 2013). If true, this could impact management strategies aimed at manipulating the male to female ratio in a population.

Under favorable conditions, female plants have been documented to produce up to 1 million seeds which can become viable 2 to 3 weeks after flowering (Keeley et al. 1987). Higher seed production is directly correlated with greater plant biomass, time of emergence, and distance from the crop (Korres et al. 2018; Clay et al. 2005; Webster and Grey 2015). MacRae et al. (2013) reported Palmer amaranth seed production was reduced 77% in plants established in cotton at the 17-leaf stage as opposed to the 3-leaf stage. Palmer amaranth seeds are relatively small in size (1 to 2 mm), smooth, round to disc-shaped, and easily dispersed (Sauer 1955). Research indicates smaller seeds are more likely to become buried than larger weed seeds (Korres et al. 2018; Westerman et al. 2009). Seed burial advantages could lead to increased numbers in the soil seed bank and likely influence germination rates. Dispersal can occur through several diverse mechanisms associated with animals, irrigation, equipment, tillage, and rainfall (Norsworthy et al. 2014). The number of dispersal agents capable of moving seed are likely related to the haste of colonization throughout the United States.

Seeds are relatively persistent in soil seedbanks which is directly proportional to burial depth (Sosnoskie et al. 2013; Korres et al. 2018). After 36 months, Sosnoskie et al. (2013) found that 15% of Palmer amaranth seeds were viable at depths of 10 cm. Some degree of sunlight is required for germination and majority of seeds emerge when located in the upper 2 inches of soil (Keeley et al. 1987; Jha et al. 2010). For example, a 23% increase in germination has been observed under full sunlight conditions as compared to low levels of light (Jha et al. 2010). These data suggest Palmer amaranth seeds need to be located in soil relatively close to the surface for germination. However, seeds located at deeper burial depths could remain viable and agricultural practices such as tillage could move them to the surface where germination can occur. Germination has been shown to initiate as soon as soils reach 18°C and numbers gradually increase as temperature increases with maximum germination occurring at 32-38°C (Keeley et al. 1987). Furthermore, higher germination rates are observed with alternating temperatures as compared to constant temperatures (Guo and Al-Khatib 2003; Steckel et al. 2004). Complete germination of Palmer amaranth has been shown to occur in less than one day (Steckel et al. 2004). Extended germination periods for Palmer amaranth present one of the greatest challenges as germination can begin as early as March and continue through October (Keeley et al. 1987). For many agronomic crops such as cotton and soybean, this encompasses majority of the growing season from emergence to harvest.

The small diameter of Palmer amaranth pollen granules (around 31 µm in size) combined with a low density of 1,435 kg m^{-3} allow particles to travel large distances (Sosnoskie et al. 2009; Sosnoskie et al. 2012). Palmer amaranth pollen enzymatic activity reductions have been observed just 30 minutes after anthesis, indicating reduced viability (Sosnoskie et al. 2012). While pollen grain dispersal distances up to 300 m have been reported, those that travel long distances may be less likely to fertilize an ovule during the optimal timeframe (Sosnoskie et al. 2012). Nonetheless, long distance dispersal of pollen granules likely has a major influence on the spread of herbicide resistance and management strategies directed at reducing transport could be useful.

Photosynthesis by Palmer amaranth is carried out through the C_4 pathway and carbon can be fixed at a higher rate than C_3 plants such as cotton and soybeans (Horak and Loughin 2000). The photosynthetic rate of Palmer amaranth is 81µm CO_2 m^{-2} s^{-1} at optimum temperatures. In comparison, photosynthetic rates of C_3 plants such as cotton and soybean rarely exceed 20 µm CO_2 m^{-2} s^{-1} and 25 µm CO_2 m^{-2} s^{-1}, respectively. (Ehleringer 1983; Wullschleger and Oosterhuis 1990; Reddy et al. 1991; Hutmacher and Krieg 1983; Ma et al. 1995). Furthermore, research shows C_4 plants are more capable of adapting to lower levels of light than C_3 plants, thereby limiting the constraints associated with shading (Regnier and Harrison 1993; Stoller and Myers 1989; Jha et al. 2008). In order to further optimize the amount of sunlight received by the plant, Palmer amaranth leaves are capable of solar tracking meaning leaves can be oriented perpendicular to the sun during the day (Ehleringer and Forseth 1980). Photosynthetic flux increases up to 38% have been reported for plants capable of solar tracking compared those plants with fixed leaf positions (Ehleringer 1985). Solar tracking could provide Palmer amaranth with a competitive advantage over plants that cannot adapt to the movement of the sun.

Drought tolerance may also increase competitiveness of Palmer amaranth. According to Ehleringer (1985), plants adapt to drought conditions by either developing mechanisms to survive or increasing productivity during extended drought periods. Palmer amaranth can exploit osmotic adjustments to prevent leaves from wilting during mild drought conditions and growth can continue during low leaf water potentials. Through these adjustments, stomata can stay open and carbon fixation can continue (Ehleringer 1983;1985).

Allelopathy has been reported to play a role in Palmer amaranth interference with nearby plants. In a study by Menges (1987), Palmer amaranth residue incorporated into the soil resulted in growth reductions of carrot (*Daucus carota* L. var. *sativa*) and onion (*Allium cepa* L.) 49% and 68%, respectively. Inhibition of growth and germination of other Palmer amaranth plants in addition to several grass crops has also been observed (Dafaallah et al. 2018; Menges 1988). Various studies suggest interference with nearby plants may be a result of volatile organic compound emmisions from Palmer amaranth (Menges 1987, 1988; Bradow and Connick 1987). These compounds have been identified as 2-heptanone and 2-heptanol and have been shown to inhibit root and shoot growth of cotton when emitted from cover crop residues (Connick et al. 1987; Bradow 1993). Allelopathic compounds could explain some of the interference associated with early-season competition in cotton.

Arguably one of the most successful weeds, the competitive abilities of Palmer amaranth are attributed to high fecundity, extended germination, aggressive growth, prolific seed production, extensive root system, and allelopathy (Ward et al. 2013; Keeley et al. 1987). Evolution of herbicide resistance in Palmer amaranth is inevitable. However, some practices could delay the onset of resistance. For example, a study by Montgomery et al. (2017) demonstrated the use of a cover crop with delayed termination in soybean production could increase the number of days needed for Palmer amaranth to reach 10 cm in height. This could allow timely herbicide applications and reduce early-season interference with the main crop. Knowledge of Palmer amaranth biology, phenology, and population dynamics could lead to the discovery characteristics to be exploited for weed control. Successful management in the future will likely require a variety of strategies combining physical, chemical, and cultural approaches.

1.2.2. Herbicide resistant palmer amaranth

The Weed Science Society of America refers to herbicide resistance as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA 1998). Evolution of herbicide resistance in Palmer amaranth populations is a significant concern for crop production in the southeastern US. The number of herbicide chemistries capable of controlling Palmer amaranth is rapidly declining and resistance to 8 different modes of action has been documented (Heap 2019). Moreover, some populations have been identified with resistance to more than one mode of action. Resistance to the 5-enolpyrovyk shikimate-3-phosphate synthase (EPSPS) inhibitor, acetolactate synthase (ALS) inhibitors, microtubule inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidate (PPO) inhibitors, 4-hydroxyphenylpiruvate dioxygenase (HPPD) inhibitors, long chain fatty acid synthesis inhibitors, and synthetic auxins have now been identified for Palmer amaranth (Heap

2019). Chemical control options are quickly being depleted by rapid evolution of resistance much faster than they are being developed.

Dinitroaniline resistant biotypes were one of the first cases of resistance in Palmer amaranth discovered, initially reported in 1989 (Gossett et al. 1992). According to Gossett et al. (1992), trifluralin resistance was confirmed at eight different locations in South Carolina. Additionally, herbicides benefin, isopropalin, pendimethalin, ethalfluralin, and oryzalin provided less than 75% control, suggesting cross-resistance (Gossett et al. 1992). Due to limited POST herbicide control options during this time frame, trifluralin and pendimethaline were used repeatedly for nearly a quarter of a century in South Carolina cotton fields for residual Palmer amaranth control (Ward et al. 2013). These data demonstrate the negative effects related to overuse of one herbicide mode of action.

Cross-resistance to ALS-inhibiting herbicides is fairly common across the US. Acetolactate synthase catalyzes the first step in formation of the branched-chain amino acids valine, leucine, and isoleucine (Shaner 2014; Heap 2019). Inability to synthesize these amino acids inhibits plant growth and ultimately results in plant death. First commercialized in 1982, this group of herbicides was a large advancement for chemical weed control. Compared to other herbicides on the market at that time, relatively small quantities were required for efficient weed control making their use economically and environmentally appealing. Herbicides families with this mode of action include sulfonyl ureas, imidazolinones, pyrimidinylthiobenzoates, triazolopyrimidines, and sulfonylaminocarbonyltriazolinones (Heap 2019). The first case of resistance to this mode of action was in prickly lettuce (*Lactuca serriola* L.) in 1986 (Mallory-Smith et al. 1990). Since its discovery, resistance to ALS-inhibitors has now been reported for 162 weed species (Heap 2019). Previous studies have indicated mutations in the target enzyme were responsible for such resistance in *Amaranthus* species (Sprague 1997). However, recent genetic sequencing indicate both mechanisms for resistance to ALS-inhibitors can exist in the same population and the frequency of such populations is on the rise (Nakka et al. 2017). Eight different sites on the ALS loci have potential to confer ALS resistance (Yu and Powles 2014a; Tranel et al. 2018). The ALS-gene sequence is highly variable in nature. Therefore, immense selection pressure placed on a population in the form of an ALS-inhibitor can rapidly select for resistant biotypes. As a result, ALS-inhibiting herbicides are no longer viable options for control of Palmer amaranth in several locations and other modes of action will be required for chemical management.

Resistance to PSII herbicides in Palmer amaranth was confirmed in 1993 in Texas (Heap 2019). Discovery of resistant populations in Kansas, Georgia, and Nebraska ensued in the following year (Heap 2019). Herbicides with this mode of action are frequently used for PRE and POST-directed applications in agronomic crops. PSII inhibitors block electron flow by binding to the D1 proteins in the photosynthetic pathway (Shaner 2014). Ultimately, CO_2 can no longer be fixed and energy production is inhibited. Not only is photosynthesis depleted but formation of highly reactive oxygen species results in destruction of cell membranes which leads to plant death (Shaner 2014).

Perhaps the most alarming challenge in crop production to date has been the spread of glyphosate resistant weeds. Crops with resistance to glyphosate were commercialized in 1996 (Padgette et al. 1996). However, the herbicide has been on the market since the mid-1970's. Glyphosate is a non-selective broad spectrum herbicide, therefore its use increased drastically once it could be applied directly over crops (Culpepper and York 1998). This ability decreased the total volume of herbicide use, especially for residual herbicides (Culpepper and York 1998).

Advancement of GMO crops is paralleled by the switch to some version of a conservation tillage program, revolutionizing weed control methods both physically and chemically (Brookes and Barfoot 2010). Therefore, crop production under conversation tillage systems has a higher reliance on herbicides for weed control and glyphosate applications allowed an effective and economical approach (Culpepper and York 1998, 1999; Wilcut et al. 1996).

The dramatic shift in crop management practices caused significant changes in the weed spectrum to be controlled. Adoption of different physical and chemical measures inadvertently selected for the survival of the most tolerant weed species (Culpepper et al. 2004; Buhler et al. 1994; Culpepper et al. 2005; Culpepper 2006). Given that Palmer amaranth is one of the most problematic weeds in crop production, multiple applications of glyphosate were often used as the only method for control (Culpepper et al. 2010). Palmer amaranth was previously well controlled with glyphosate. However, overuse led to unprecedented and rapid evolution of glyphosate resistant Palmer amaranth biotypes (Culpepper et al. 2006). The first case of herbicide resistance was confirmed in Georgia in 2006, just 10 years after commercialization of resistant crops (Culpepper et al. 2006). In the Georgia population, the mechanism of resistance was attributed to gene amplification of EPSPS (Gaines et al. 2010).

Target-site mechanisms of resistance to glyphosate that involve mutations of EPSPS have been attributed to the amino acid position Proline106 which is located in a highly conserved region (Baerson et al. 2002). Mutation of the target enzyme usually results in reduced sensitivity to the herbicide through inhibition of the binding site or overexpression of the enzyme (Powles and Yu 2010). Reports by Gaines et al. (2010) suggest 160 times more EPSPS gene copies can occur in resistant Palmer amaranth versus susceptible plants. Furthermore, those copies can be independent of glyphosate exposure (Gaines et al. 2010). Protein levels and enzymatic activity of

EPSPS increase with the number of gene copies; therefore, additional copies of the enzyme gene in resistant plants is likely to result in higher resistance levels (Gaines et al. 2010). Ultimately, the more copies of the EPSPS gene, the higher dosages required to kill the plant. Low levels pf nontarget-site resistance in the form of glyphosate metabolism has also been documented in Palmer amaranth; however, metabolism rates were low (<10%) (Domingues-Valenzuela et al. 2017).

Palmer amaranth resistance to PPO-inhibiting herbicides is a recent phenomenon and a large concern for cotton and soybean production in the US. In response to glyphosate-resistant Palmer amaranth, Sosnoskie and Culpepper (2014) reported a 10-fold increase in PPO-inhibitor usage in Georgia. Palmer amaranth resistance to PPO-inhibitors has not been reported in Alabama or Georgia as of yet (Heap 2019). However, increased usage of herbicides with this mode of action place will added selection pressure on Palmer amaranth populations. Palmer amaranth nontarget-site resistance to fomesafen has been confirmed in Arkansas in 2011 (Salas et al. 2016). However, nontarget-site resistance to PPO-inhibitors has been also been confirmed in Palmer amaranth populations (Varanasi et al. 2018). Data reported by Varanasi et al. (2018) suggest metabolic detoxification is the non-target site mechanism of resistance of Palmer amaranth to PPO-inhibitors, confirmed with glutathione-S-transferase inhibitors. Target-site resistance to PPO-inhibitors has been attributed to amino acid substitutions and/or deletions that confer broad-spectrum resistance to this family of herbicides and may be present in the same population (Varanasi et al. 2018). Since the first discovery, accessions associated with resistance to PPO-inhibitors have been detected in 62% of the farming counties in Arkansas (Heap 2019). Fomesafen is frequently applied both PRE and POST for broadleaf control in some agronomic crops. Furthermore, PPO-inhibiting herbicides are often used for management of troublesome glyphosate resistant weeds. Therefore, weed resistance may have serious implications for crop production in the southeast.

Herbicides that prevent synthesis of very long chain fatty acids are widely used in soybean and cotton production. Specifically, S-metolachlor is frequently applied both PRE and POST to overlap residual Palmer amaranth control throughout the growing season. Until recently, this mode of action appeared to remain a viable option for control. However, Brabham et al. (2019) observed inadequate control levels in Arkansas in 2016. Following greenhouse and field trials, low levels of resistance to S-metolachlor were confirmed (Brabham et al. 2019). Data indicated glutathione-s-transferases were a plausible mechanism of resistance. Cross resistance to other long chain fatty acids were not observed; however, reduced sensitivity was noted (Brabham et al. 2019). Given the high use rates of this mode of action in agronomic crops, there is an increased likelihood of selecting for resistant populations.

Palmer amaranth populations with multiple resistance to glyphosate, atrazine, mesotrione, chlorsulfuron, and 2,4-D has been documented in Kansas (Kumar et al. 2019; Heap 2019). Three-way resistance to glyphosate, ALS inhibitors, and atrazine has also been confirmed in Michigan (Kohrt et al. 2017). According to Heap (2019), Palmer amaranth populations with multiple resistance are not uncommon. However, new synthetic auxin technology is now being heavily relied on for control of glyphosate resistance and research conducted by Kumar et al. (2019) suggest the mechanism exists for resistance development to synthetic auxins. For example, Tehranchian et al. (2017) exposed susceptible Palmer amaranth populations to sublethal doses of dicamba over three generations and the lethal dose required for 50% mortality (LD_{50}) increased over 2.5-fold from 111 g ha^{-1} to 309 g ha^{-1}. The same methodology was applied using 2,4-D and LD_{50} values were also reduced 2-fold and 25% of the third generation survived the full labeled rate of 2,4-D at 1120 g ha^{-1} (Tehranchian et al. 2017). These data suggest the same mechanism for resistance is likely for both 2,4-D and dicamba.

In cotton and soybean production systems, 2,4-D and dicamba applications were not permitted throughout the growing season until 2017 when formulations for use in transgenic crops were commercialized. Previously, these herbicides were primarily used in burndown applications before Palmer amaranth has emerged. Therefore, 2,4-D and dicamba herbicides have only been applied while Palmer amaranth was actively growing for 3 years in cotton and soybean systems. Based on the research described by Tehranchian et al. (2017), reduced sensitivity of Palmer amaranth to synthetic auxins could occur in as little as three generations. Labels for new 2,4-D and dicamba formulations limit applications to 2 per season (Anonymous 2019b,c; 2018b). Therefore, only 6 applications should have been made during the summer months thus far when Palmer amaranth is actively growing. Multiple applications in upcoming years may result in widespread development of resistance. Metabolism-based herbicide resistance has been associated with several small-seeded weed species and overuse of certain pesticides will likely have evolutionary consequences (Yu and Powles 2014b). This mechanism is likely responsible for a large portion of multiple-resistance in weed species. Proper stewardship of new synthetic auxin technologies and incorporation of multiple modes of action into herbicide programs will be imperative to extend their viability as Palmer amaranth control options.

Herbicide resistant Palmer amaranth populations are often overlooked in early years of existence. Low levels of resistance in a population are often not enough to cause economic loss and therefore fields are considered susceptible (Sosnoskie et al. 2012). However, the rate of gene flow in Palmer amaranth is high and fields can become resistant in just 2 years (Sosnoskie et al.

2012; Norsworthy et al. 2014). Once resistance is discovered, Palmer amaranth plants have usually exceeded heights able to be controlled effectively through other modes of action and fields may be abandoned (Salas et al. 2016; Sosnoskie et al 2012). Biological qualities such as high fecundity, dioecious nature, and prolonged emergence enhance Palmer amaranth's ability to adapt to selection pressure placed upon populations. Consequently, *Amaranthus* species have the highest incidence of herbicide resistance compared to other problematic weeds in the US. Considering the rate of gene flow possible in Palmer amaranth, this information poses a great threat to new technology and may create additional challenges for crop production in future years.

1.2.3. Palmer amaranth interference with cotton and soybean

Weed emergence timing is one of the most important factors implicated in crop losses (Kropff et al. 1992). The concept associated with weed emergence in a crop setting is the critical period of weed control. This period is defined as the amount of time in which a crop can tolerate weed competition before yield loss occurs (Knezevic et al. 2002). Widespread reports of increased crop yield losses exist in the literature when Palmer amaranth emerges at the same time as crops (Massinga et al. 2001; Bensch et al. 2003; Keeley et al. 1987; Fast et al. 2009). Furthermore, Palmer amaranth is known to germinate at similar timings as cotton and soybean (Bell et al. 2015; Fast et al. 2009).

The critical weed free period for soybeans based on 5% yield loss is between emergence and V3 stages (Van Acker et al. 1993). Compared to other crops, the critical period for weed control in soybeans is short. Soybeans are sensitive to early-season Palmer amaranth competition and densities as low as 8 plants m^{-1} row can result in yield reductions up to 78% (Bensch et al. 2003: Rowland et al. 1999). Palmer amaranth densities of 10 plant m^{-2} have also been reported to reduce soybean yields up to 68% through season-long interference (Klingaman and Oliver 1994). Other studies report 14 to 48% yield reductions at Palmer amaranth densities as low as 2 plants m^{-1} row (Klingman and Oliver 1994; Dieleman et al. 1996). Although the studies previously mentioned place a large amount of focus on Palmer amaranth densities, some data suggests pigweed time of emergence is more influential on soybean yield loss (Dieleman et al. 1995; 1996). Bensch et al. (2003) reported no yield reductions when Palmer amaranth emergence was delayed 19 and 38 days after soybean emergence. Dieleman et al. (1995) estimated yield losses of 16.4% when pigweed emerged at the same time as soybean and 0.5% when pigweed emergence occurred 20 days later. Nonetheless, soybean emergence later in the season can be problematic for harvest and can replenish the seed bank leading to larger populations needed to be controlled in subsequent years.

Cotton is also susceptible to weed-crop competition by Palmer amaranth. Biomass of Palmer amaranth plants grown with cotton exceed that of any other weeds by 25% (Askew and Wilcut 2002). Similar to other crops, cotton is most sensitive to early-season weed competition. A study by Morgan et al. (2001) showed Palmer amaranth densities of 1 to 10 plants per 9.1 m of row can reduce crop canopy 45% ten weeks after emergence. Higher densities implemented in that study were also shown to reduce cotton biomass by 50% eight weeks after emergence (Morgan et al. 2001). This response not only may have implications on crop growth and development but can also limit the crop's ability to shade out weedy competitors.

The extent of cotton yield reductions are directly related the Palmer amaranth time of emergence (Webster and Grey 2015). Cotton yield reductions of 92% and 67% have been reported for densities as low as 0.9 and 0.42 plants m^{-2} (Rowland 1999; Webster and Grey 2015). Palmer amaranth densities of 8 plants per m row are capable of decreasing cotton yield 79 and 91% when weed and crop emergence occur simultaneously (Bensch et al. 2003; Massinga et al. 2001). Furthermore, Rowland et al. (1999) found that yield reductions of 10.7% and 11% can occur with each increase of 1 Palmer amaranth per row of cotton when established at the two-leaf stage. Similarly, MacRae et al. (2008) observed a cotton yield reduction of 0.9% with every Palmer amaranth increase for 1 m^2 when established at the three and nine-leaf stages. These reports suggest herbicide applications can be warranted with as little as 1 to 2 Palmer amaranth per 10 m row (Rowland et al. 1999). Alternatively, MacRae et al. (2013) did not observe any yield effect when Palmer amaranth plants were transplanted next to cotton at the 12 and 17 leaf stages even though the period of competition extended 80 or more days into the season. Although yield reductions may not occur with late-season Palmer amaranth competition, escapes can produce enough seed to replenish the seed bank and can reduce harvest efficacy.

The duration of interference is another major factor of cotton yield response to Palmer amaranth. A study by Fast et al. (2009) studied different durations of Palmer amaranth interference in cotton at 7 day increments. Results from their study showed a gradual lint yield loss of 0 up to 3% when Palmer amaranth was allowed to compete for 21 days. However, rapid increases in yield reductions were observed following the three week period from 3% at 21 days after emergence to 77% at 63 days after emergence. The duration of Palmer amaranth growth was also correlated to biomass and therefore, there appeared to be a direct relationship with Palmer amaranth biomass and cotton lint yield. Interestingly enough, majority of Palmer amaranth biomass increase was observed 21 to 63 days after emergence when the interference with cotton is highest. The ultimate conclusion was the critical period of weed control for glyphosate-resistant cotton was 19 days after emergence based on a 2.7% yield loss threshold. Cotton can tolerate some degree of weed pressure before an economical yield loss occurs. Therefore, economic thresholds are more useful when determining critical periods of weed removal. These data demonstrate the need for residual herbicides applied at planting to allow cotton to establish in the absence of weed competition.

Although a cotton yield response to late-season competition with Palmer amaranth is often not observed, weed presence can indirectly interfere with cotton yields through reduction of cotton quality and mechanical harvesting efficiency. According to Smith et al. (2000), 697 Palmer amaranth plants ha^{-1} (.07 plants m^{-2}) with stem diameters greater than 4 cm increased harvest time by 2 to 4 fold due to blockages from weed residues. Mechanical harvest efficiency was reduced significantly at as little as 0.3 plants m^{-2}. Furthermore, Palmer amaranth at densities of 3,260 plants ha^{-1} was responsible for 15% of all plant trash detected in cotton harvested. Although weeds that emerge later in the season may not interfere directly with crop yields, their presence can still negatively impact harvest and quality.

A “zero-tolerance threshold” has been recommended for Palmer amaranth by several researchers. Norsworthy et al. (2014) studied the spatial movement of palmer amaranth in previously uninfested fields. In the first year, cotton yield reductions were not observed as plants had only spread to 0.56% of the field. However, plants spread to cover 20% of the testing area in the second year and 95-100% of the area in the third year which led to complete crop loss due to yield response and equipment failure from high densities. These data demonstrate the rate at which Palmer amaranth can spread. Herbicide failure could go unnoticed the first year and populations can increase to levels that impede crop harvest and diminish yields in as little as 2 years. Researchers frequently recommend use of preemergence herbicides in combination with postemergence herbicides to reduce the pressure for postemergence herbicides to control Palmer amaranth and also reduce the number of seeds reaching the soil seedbank. Other strategies such as use of cover crops or optimizing canopy cover may also reduce Palmer amaranth emergence (Jha et al. 2010; Jha and Norsworthy 2009; Steckel et al. 2004; Whitaker et al. 2010). However, Bell et al. (2015) placed more importance on effective preemergence herbicide application as opposed to crop density.

Best management practices for delay of resistance development have been recommended by Norsworthy et al. (2012). The first recommendation is to gain proper knowledge regarding the biology of the weed to be controlled. Strategies can be used to exploit certain weed characteristics such as emergence patterns, reproduction biology, seed/pollen dispersal, and seed persistence. The second recommendation is the use of chemical and nonchemical means of weed control to reduce the number of seeds to reach the soil seedbank (Lindsay et al. 2017; Norsworthy et al. 2012). This tactic is of particular importance for management of Palmer amaranth due to its high fecundity. Computer modeling conducted by Lindsay et al. (2017) indicates that reduction of the number of seeds in the soil seedbank may be a more efficient approach to Palmer amaranth management. Mode of action usage is dependent on herbicide labeling for different crops. Therefore, Northworthy et al. (2012) also suggested the use of crop rotation with different herbicide resistance traits as a strategy for mitigation of herbicide resistance development. Other recommendations include planting weed-free crop seed, planting into clean fields, routine field scouting, exploiting crop competition for weed control, preventing transfer of weed propagules to adjacent fields, management of weed seeds at harvest, and use of multiple modes of action in chemical weed control programs.

1.3. Residual herbicides applied preemergence in cotton for palmer amaranth control

1.3.1. Herbicide physiology

Several herbicides exist on the market for residual weed control in cotton production. The spread of herbicide-resistant Palmer amaranth has increased the need for PRE herbicides as several POST herbicide options have been rendered ineffective. Herbicide effectiveness is often dependent on certain soil characteristics such as texture and organic matter content (Shaner 2014). Soils containing a high percentage of sand is considered coarse. Examples include sand, loamy sand, and sandy loam. Alternatively, high clay content in soils indicates a finer texture and examples include silty clay loam, clay loam, and clay. Organic matter fractions often influence herbicide efficacy through adsorption (Martin 2006). Clay particles are negatively charged and positively charged herbicides may be tightly bound to soil colloids and unavailable for plant uptake. Conversely, negatively charged herbicides are more susceptible to leaching. The degree of attraction to soil particle can also be influenced by soil pH. However, herbicide labels usually contain a warning if this is a concern (Shaner 2014; Martin 2006).

Fomesafen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide) is commonly used for control of many troublesome broadleaf weeds (Shaner 2014). The mechanism of action of fomesafen is PPO-inhibition which leads to the accumulation of a chlorophyll precursor, protoporphyrin IX (Shaner 2014). Light absorbed by the precursor causes the molecule to enter a triplet state that can interact with molecular oxygen and result in the production of reactive oxygen species. Ultimately, plant death results from lipid peroxidation and destruction of membranes. Fomesafen is readily absorbed by leaves and roots and translocation is minimal. Symptoms usually appear in the form of chlorosis and necrosis 1 to 3 days after application (Shaner 2014). Use rates range for different soil textures due to differences of adsorption in soils with varying organic matter (Anonymous 2019a; Shaner 2014). Solubility of the fomesafen parent acid in water is relatively low at 50 mg L^{-1} at 25°C. However, the solubility of the sodium formulation in which it is applied is 600,000 mg L^{-1} at 25°C and only moderately adsorbed by soil particles (Shaner 2014). These combined properties allow high mobility in soil. Volatilization is not a concern for fomesafen loss. The average half-life of fomesafen is relatively long and persistence in soil can extend up to 1 year after application. The average half-life reported for fomesafen is 100 days (Shaner 2014).

Pendimethalin (N-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitrobenzeneamine) is a soil herbicide commonly used in several cropping systems (Shaner 2014). The herbicide family that penidmenthalin belongs to is the dinitroanilines. The mode of action of dinitroanilines is inhibition of the microtubule protein known as tubelin which is needed to complete mitosis during cell division. Sensitive plants can no longer produce new tissues and plant death results. Low solubility of only 0.275 mg/L in water at 25°C and strong adsorption decrease the propensity to leach in soils (Shaner 2014). Loss of pendimethalin is most likely due to microbial degradation under favorable conditions. However, pendimethalin is moderately volatile with a vapor pressure of 1.25×10^{-3} Pa at 25°C and preplant incorporation is often recommended on the label (Anonymous 2018a; Shaner 2014). Pendimethalin does not persist in the soil as long as some of the other herbicides mentioned in this section, as it has a half-life of 44 days. Incorporation into the soil may extend the amount of time the herbicide remains in the ground by limiting dissipation.

S-metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide) has a moderate to high solubility of 488 mg/L in water at 25°C and moderately adsorbed to soil particles. Herbicidal activity is attributed to inhibition of very long chain fatty acid synthesis (Shaner 2014). Emerging shoots are the main site of absorption, although a small amount of root absorption is possible. Most sensitive plants fail to emerge from the soil. Typical symptoms in both grass and broadleaf plants are malformed or rolled leaves. Broadleaf leaves often show a heart-shaped appearance if injured. Loss due to volatilization is relatively low. However, photodegradation and microbial degradation is a concern (Shaner 2014). S-metolachlor is frequently applied in both PRE and POST herbicides programs in cotton and soybean to provide residual control of weeds throughout the growing season.

Diuron (3-[2,4-dichlorophenyl]-1,1-dimethylurea) is another soil herbicide commonly selected by producers due to its economical appeal. This herbicide is in the substituted urea family and is commonly used as a soil herbicide in many different cropping systems. Similar to fomesafen, the solubility of diuron is low at 42 g/L 25°C and adsorption is moderate (Shaner 2014). The combination of solubility and adsorption properties makes diuron moderately leachable. The average half-life of diuron is 90 days; however, high rates can persist in the soil up to 1 year (Shaner 2014). The mechanism of action is PSII inhibition at site A. Diuron binds to the Q_B binding site on the D1 protein of PSII and blocks the flow of electrons. Carbon dioxide fixation ceases and ATP and NADPH can no longer be produced (Shaner 2014; Heap 2019). The backflow of electrons induces the chlorophyll molecule to enter a triplet state and react with molecular oxygen which results in lipid peroxidation and membrane destruction. Absorption mainly occurs in plant roots and less by leaves and stems. Translocation occurs rapidly in the xylem following application. Vapor pressure of diuron is 9.2×10^{-6} Pa at 25°C and volatilization is not a significant concern. Microbial degradation is the primary mechanism of loss in the field (Shaner 2014).

Acetochlor (2-chloro-N-ethoxymethyl-.N-[2-ethyl-6-methylphenyl]acetamide) is marketed as a PRE, preplant incorporated, and/or POST herbicide with activity on annual grasses and broadleaf weeds (Shaner 2014). Herbicidal activity is attributed to inhibition of the biosynthesis of long chain fatty acids (Heap 2019). Symptomology is similar to those observed with S-metolachlor discussed above. Emerging plant shoots and seedling roots are the primary sites of absorption in the plant. While translocation can occur, it is irrelevant as the primary target is emerging seedlings. Photodegradation is not a large concern for acetochlor. Majority of degradation occurs as a result of microbial activity. Moderate solubility of 223 mg/L in water at 25°C and moderate adsorption decrease the propensity for acetochlor to leach. However, this can be dependent on the soil where it is applied (Shaner 2014).

Fluridone (1-methyl-3-phenyl-5[3-trifluorromethylphenyl]) has existed on the market since 1986 for aquatic vegetation management. However, it was recently introduced for use in cotton as a pre herbicide in 2016 (EPA 2016). The product claims a wide spectrum of weed control but places emphasis on Palmer amaranth management. The mode of action of fluridone is inhibition of carotenoid biosynthesis in the plant through inhibition of the enzyme phytoene desaturase (Shaner 2014). Carotenoids are responsible for quenching excess energy from oxygen singlets. In the absence of carotenoids, reactive oxygen species will cause lipid peroxidation and membrane destruction, resulting in plant death. Fluridone is absorbed by plant shoots and roots and readily translocated. Symptomology is slow to appear at 7-10 days after application and sensitive plants often exhibit bleaching on leaves. The propensity of fluridone to leach in soils is low due to a low solubility of 12 mg/L in water at 25°C. However, low adsorption and slow leaching has been observed in a laboratory setting (Shaner 2014). Volatility is not a concern and microbial activity is responsible for majority of loss. The half-life of fluridone is around 90 days in soil.

Majority of soil herbicides used in cotton production have low risk for leaching and are relatively stable in soils. Addition of soil-applied residual herbicides is necessary to give cotton a competitive advantage over weeds emerging early in the season. Furthermore, the development and spread of herbicide resistance in Palmer amaranth can make management difficult later in the season and residual herbicides can add an additional level of control. PRE applications can reduce the pressure for POST herbicides to manage Palmer amaranth populations throughout the growing season. Herbicide best management practices include incorporation of multiple modes of action into chemical weed control programs to delay the evolution and spread of herbicide resistance (Norsworthy et al. 2012). Limited modes of action are available for POST weed control in cotton; therefore, incorporation of PRE herbicides into herbicide programs provides a niche where additional modes of action can be added to a program.

1.3.2. Palmer amaranth control following residual herbicide applications

Palmer amaranth's ability to outcompete cotton is a major threat to the industry for yield quantity, quality, and harvest efficiency (MacRae et al. 2013; MacRae et al. 2008; Rowland et al. 1999; Smith et al. 2000). Three major factors drive crop-weed competition. The first is weed emergence relative to crop emergence. The first plant to establish itself in a given area will have a competitive advantage in terms of resource availability. Greater yield losses are often observed when weeds become established before or simultaneously with the target crop (Rajcan and Swanton 2001; Buchanan and Burns 1970; Van Heemst 1985). Weed thresholds for crops are often much lower during crop establishment than later in the season (Cardina et al. 1995; Coble and Mortensen 1992). Weed thresholds are often defined as the number of weeds to cause 10 to 20% yield loss (Oliver 1988). The second major factor is weed density and duration of interference (Dunan 1995; Heemst 1985). The third major factor in weed crop competition is the weed species involved (Swanton et al. 2015; Heemst 1985). However; all three factors are interrelated. Ultimately, crop response will depend on how many weeds infested the field at a specific time, for a certain duration, and the individual competitive ability of the weed species.

The critical period of weed control is defined as of the amount of time a crop is tolerable to weed competition before yield loss occurs (Knezevic et al. 2002). This concept relies on the ability of a producer to effectively remove the weed interfering with the crop. Unfortunately, Palmer amaranth removal is often difficult through chemical means as plants can quickly exceed recommended heights for optimal control (Horak and Loughlin 2000; Culpepper et al. 2010). Effective management will require multiple modes of action in the form of PRE and POST herbicides. Applications of PRE herbicides can allow cotton to emerge and gain a competitive advantage over weeds emerging later in the season and potentially extend the time required to Palmer amaranth to grow and development. Research in the literature indicates PRE applications of residual herbicides is necessary to adequately control glyphosate resistant Palmer amaranth (Culpepepr et al. 2006). Furthermore, research conducted by Busi et al. (2019) indicate rotations and mixtures of residual PRE herbicides with different modes of action can delay the onset of herbicide resistance development.

Fomesafen is a diphenyl ether herbicide first registered for use in cotton in 2006. Herbicidal activity is attributed to PPO-inhibition. Field use rates vary based on soil texture with 280 to 420 g ha^{-1} labeled for coarse textured soils and 280 g ha^{-1} for medium or fine textured soils (Anonymous 2019a). Control up of 93 to 99% fourteen days after cotton establishment has been observed for Palmer amaranth in Georgia (Whitaker et al. 2011; Whitaker et al. 2010). Fomesafen usage by producers in Georgia were reported to increase 10-fold following discovery of glyphosate-resistant Palmer amaranth. Furthermore, fomesafen has been identified as one of the most efficacious residual herbicides available for control of ALS and glyphosate resistant palmer amaranth in cotton (Whitaker et al. 2011).

Although high levels of rainfall during cotton emergence can lead to significant injury, the benefit of controlling glyphosate-resistant Palmer amaranth outweighs the risk (Main et al. 2012; Culpepper and Steckel 2010; Kichler et al. 2010). Cotton foliar necrosis can occur with sufficient rainfall in fomesafen-treated soil. In a study by Main et al. (2012), yield loss of 23 and 25% were observed when fomesafen was applied at 2x and 3x rates. However, this was only observed in 1of 5 testing sites across 5 states and heavy rainfall was associated (Main et al. 2012). Other studies suggest crop safety up to 1,120 g ha^{-1} in sandy loam soils (Li et al. 2018). However, tolerance could be subject to change in soils with low organic matter and high sand content.

Several other PRE herbicides have been well evaluated for their utility in Palmer amaranth management. Houston et al. (2019) tested a series of PRE herbicides commonly used in corn, cotton, and soybean production to evaluate products for their ability to control Palmer amaranth resistant to PPO-inhibitors. Diuron applied at 841 g ha^{-1}, fomesafen at 280 and 560 g ha^{-1}, acetochlor at 1,261 g ha^{-1}, and S-metolachlor at 1,389 g ha^{-1} were shown to reduce Palmer amaranth density by 83%, 68%, 81%, 77%, and 80% twenty eight days after treatment, respectively (Houston et al. 2019). These data suggest that Palmer amaranth population numbers could be reduced with the addition of a PRE herbicide. However, control ratings for the herbicides mentioned in this study only ranged from 65 to 76% twenty eight days after treatment. Fomesafen efficacy was likely reduced due to the presence of PPO-inhibitor resistance within Palmer amaranth populations (Houston et al. 2019).

Some studies have shown combinations of PRE herbicides can be more effective than when applied alone. Residual herbicides are frequently applied as tank mixtures at planting for enhanced weed control. According to Cahoon et al. (2015), aceotchlor applied at 1,260 g ha^{-1} has been shown to control Palmer amaranth 67 and 82% in North Carolina and Georgia 18 to 23 days after planting cotton. Control was increased 6 and 17% with combinations of acetochlor and fomesafen (200 g ha^{-1}) (Cahoon et al. 2015). Braswell et al. (2016) observed 93% Palmer amaranth control or better following preemergence applications of acetochlor plus diuron, fomesafen plus diuron, and fluridone at the 2-leaf cotton stage. The University of Georgia extension service recommends several PRE herbicide mixtures for control of glyphosate resistant Palmer amaranth such as fluridone plus fomesafen, fluridone plus acetochlor, diuron plus warrant, fomesafen plus diuron, and fomesafen plus reflex (Culpepper and Vance 2019).

While effective Palmer amaranth control has been observed with several PRE herbicides, cotton tolerance is a major factor to consider when choosing a program. Herbicide performance often varies in different soil types. Factors that influence crop tolerance to residual herbicides include pH, solubility of the herbicide, soil moisture, and percentage of organic matter (Main et al. 2012; Taylor-Lovell et al. 2001; Reiling et al. 2006; Li et al. 2018). Residual herbicide labels indicate application rates for soil applied herbicides often depend on the texture of soils where it is applied (Anonymous 2019a, Anonymous 2018a; Anonymous 2015).

1.3.3. Residual herbicide activation

Periods of high rainfall can be a concern for crop injury from residual herbicides applied preemergence. However, activation through irrigation is often required for adequate weed control through incorporation into the soil (Whitaker et al. 2011; Walker and Roberts 1975). The main factors implicated in soil herbicide activation are water requirements to enter soil solution, redistribution among the soil profile, and availability to the plant (Walker 1971). Soil type, pH, organic matter, and moisture availability are often limiting factors for activity and length of efficacy of soil-applied herbicides (Riar et al. 2012).

Chemical properties such as water solubility, soil adsorption, and microbial degradation can also affect how long herbicides are available in the soil solution. Soil herbicides must reach their target zone through either tillage, irrigation or rainfall (Walker 1970; Riar et al. 2012; Hager et al. 2011). Irrigation is often relied on to move residual herbicides into the soil profile with minimal soil disturbance (Smith et al. 2016). Soil herbicide efficacy and safety relies on the premise that majority of the herbicide applied is adsorbed to soil particles and a small amount is available to weed structures for absorption (Fast et al. 2009). Hence, herbicide properties implicated in availability and persistence are water solubility, sorption, and herbicide half-life.

Water and other components make up the soil solution in areas with adequate moisture available for plant uptake. However, under dry conditions less soil solution is available for plant uptake and herbicides can lose their efficacy. For this reason, PRE herbicide applications often fail for lack of adequate rainfall or irrigation (Whitaker et al. 2011; Smith et al. 2016). Dry soil has an affinity for water and once field capacity is reached, gravity will then move the soil solution (including herbicides not bound to colloids) down into the soil profile. In general, 0.5” of rainfall is recommended for soil herbicide activation on herbicide labels (Anonymous 2015; Anonymous 2018a,e; Anonymous 2019d). This number is usually assumed for the amount of water needed to move herbicide 1 to 2 inches into the soil profile where weeds are germinating. However, water volume for activation may be frequently over or underestimated as herbicides often differ in solubility and sorption properties (Shaner 2014). Furthermore, initial moisture levels of soil often vary and drier soils will require additional water to reach the same amount of soil solution. Alternatively, excessive rainfall can dilute herbicide applications and cause leaching away from the desired target, resulting in decreased herbicide efficacy, crop injury, or water contamination (Jhala 2017).

Contrary to other herbicides labels for PRE herbicides such as diuron, pendimethalin, acetochlor, and fluridone, only 0.25 inches of rainfall is recommended after fomesafen applications for sufficient weed control (Anonymous 2019a,d; Anonymous 2018a,e; Anonymous 2015). The information from the herbicide label is in agreement with research in the literature. A study conducted by Smith et al. (2016) found that fomesafen applied at 280 g ha^{-1} reduced Palmer amaranth biomass below 3% of the nontreated control regardless of irrigation amount in sandy loam soil. Alternatively, the study found irrigation levels to be a significant factor for acetochlor and S-metolachlor activation when applied at 1,267 g ha^{-1} 1,424 g ha^{-1}. Acetochlor and S-metolachlor have moderate solubility at 223 mg/L and 488 mg/L in water at 25°C (Smith et al. 2016; Shaner 2014). In comparison, fomesafen in the sodium salt formulation has a solubility of 600,000 mg/L and is likely more mobile in soils than acetochlor and S-metolachlor, requiring less irrigation (Shaner et al. 2014). Smith et al. (2016) found that Palmer amaranth control increased as irrigation levels increased following S-metolachlor treatments. Palmer amaranth control in plots treated with S-metolachlor were highest when irrigated by 6.4 and 12.7 mm of water with biomasses 4 and 2% of the NTC (Smith et al. 2016). Plots that received 0 mm of rainfall following S-metolachlor applications were 61% of the NTC. Acetochlor efficacy was also related to irrigation level; however, differences were not significant past 3.2 mm of irrigation (Smith et al. 2016). These results could be representative of solubility levels, indicating moisture requirements may not be as high for acetochlor as compared to S-metolachlor. These data support claims made that fomesafen can provide the most consistent control of Palmer amaranth even without occurrence of a rainfall event (Smith et al. 2016; Baumann et al. 1998; Everman et al. 2009; Whitaker et al. 2010).

Soil-binding properties can also influence the persistence of soil herbicides. Pendimethalin and trifluralin are less likely to leach and more likely to bind to soil colloids due to their relatively low water solubility in comparison to other herbicides and high adsorption coefficients (Jha 2017; Shaner 2014). The solubility of pendimethalin and trifluralin is 0.275 and 0.3 mg/L in water at 25°C (Shaner 2014). The chemical properties of these herbicides cause them to become relatively immobile in soil.

Time intervals between preemergence herbicide application and activation event can interfere with the level of weed control achieved (Wright et al. 1995; Barnes and Oliver 2004). Delayed activation of preemergence herbicides can increase the risk of reduced herbicide performance. Some studies indicate delayed activation may have a larger effect on herbicide efficacy than soil texture (Barnes and Oliver 2004; Wright et al. 1995; Walker and Roberts 1975). Often dryland cotton production does not receive adequate rainfall for optimum herbicide efficacy and PRE herbicides can sometimes fail. Research suggests a 7 day delay in a rainfall event would be acceptable for fomesafen and acetochlor efficacy (Smith et al. 2016). Solubility and persistence of several soil herbicides may allow reductions in irrigation needed for activation and should be evaluated on individual cases.

1.4. Postemergence herbicides in cotton and soybean for palmer amaranth control

1.4.1. Herbicide physiology

Transgenic cotton and soybean varieties resistant to glyphosate and glufosinate have been available on the market several years. Recently, varieties with stacked resistance to the aforementioned herbicides in addition to 2,4-D or dicamba have been commercialized and will provide an additional platform for weed control. Combinations of the three herbicides can control a wide range of weed species including those resistant to existing modes of action.

Glyphosate (N-[phosphonomethyl]glycine) is a non-selective, foliar applied herbicide used in a variety of crops. It is often applied preplant burndown, POST, and/or POST-directed (Shaner 2014). Glyphosate is highly soluble at 15,700 mg/L in water at 25°C as the parent acid form. However, solubility can vary depending on the salt formulation (Shaner 2014). Rapid adsorption to soil can limit residual activity of glyphosate and reduce mobility. Microbial degradation is the primary mechanism for degradation in the soil and the typical field half-life is 47 days (Wauchope et al. 1992; Shaner 2014). Losses due to volatilization are considered negligible. Following patent expiration under the Roundup Ready® trade name, several companies began manufacturing their own glyphosate products and large numbers of generic products exist on the market today. The herbicidal activity of glyphosate is due to inhibition of enolpyruvyl shikimate-3-phosphate (EPSP) synthase which prevents biosynthesis of the branched chain amino acids valine, leucine, and isoleucine (Shaner 2014). Symptoms appear in 4-7 days and sensitive plants will become chlorotic and foliage will often turn red to purple. Glyphosate is readily translocated in plants making it a viable control option for several perennial weeds.

Application of glufosinate (2-amino-4-[hydroxymethylphosphinul]butanoic acid) was limited to burndown and noncrop areas prior to the development of transgenic crops with resistance. The mode of action of glufosinate is inhibition of glutamine synthesis, the primary enzyme associate with nitrogen assimilation (Shaner 2014). Symptoms appear in 3-5 days and sensitive plants exhibit chlorosis and wilting. Plants may become defoliated very quickly after application. Glufosinate is considered a contact herbicide so adequate coverage is required for optimal control. Although glufosinate is weakly adsorbed to the soil, activity is limited due to rapid microbial degradation. Similarly, glufosinate is highly soluble in water (1,370,000 mg/L). However, rapid microbial degradation also limits leaching and glufosinate is rarely detected at soil depths deeper than 15 cm (Shaner 2014). Ultimately, the high level of microbial activity leads to a short half-life of 7 days (Wauchope et al. 1992).

Synthetic auxins 2,4-D ([2,4-dichlorophenoxy] acetic acid) and dicamba (3,6-dichloro-2-methoxybenzoic acid) can now be applied postemergence over the top of transgenic crops with respective resistance for selective control of broadleaf weeds (Kniss 2018). Synthetic auxins mimic natural plant hormones such as indole-3-acetic acid and disrupt several growth processes in sensitive plants. Extremely low doses can result in observable symptomology such as epinasty of stems and leaves, leaf cupping or curling, and stem elongation (Egan et al. 2014). Although symptoms can appear several hours after application, complete plant death usually occurs 2 to 4 weeks later. Dicamba and 2,4-D are readily absorbed in both roots and shoots and rapidly translocated in the phloem of the plant (Shaner 2014). Crops with respective transgenic traits are not resistant to both chemistries and applications will be dependent on the variety selected.

Prior to the engineering of crops with resistance to 2,4-D or dicamba, applications of these herbicides were limited to preplant burndown in cotton and soybeans. Volatility and off-target movement have been large concerns for both herbicides due to their relatively high vapor pressures (Kniss 2018; Egan et al. 2014; Egan and Mortensen 2012). The herbicide family to which 2,4-D belongs is the phenoxyacetic acids. The pure form of 2,4-D is relatively nonvolatile and forms a dry crystalline solid (Shaner 2014). This pure form is not readily soluble in water with solubility of 44,558 mg L^{-1} (Jervais et al. 2008). Therefore, other formulations are manufactured to a form that can be dispersed and effectively applied in a field setting. As the parent acid form, the vapor pressure of 2,4-D is 1.4 x 10^{-7} mm Hg (25°C) (Shaner 2014). However, vapor pressure can be described as a function of the formulation and can vary.

Amine salt formulations of 2,4-D are widely used and when placed in water, readily dissociate into the parent acid and the amine portion. The parent acid has a negative charge and hard water can cause precipitates to form and clog sprayer equipment. Therefore, water softeners are often used in tank mixtures (Shaner 2014). In addition to amine formulations, ester formulations were also manufactured. However, volatility concerns have limited their use. Research supports manufacturer claims that formulations with a choline salt attached are significantly less volatile than the amine and ester formulations, with the ester formulation being the most volatile (Sosnoskie et al. 2015). Soil adsorption for 2,4-D is relatively low and the herbicide is expected to have moderate mobility. However, rapid microbial degradation usually prevents leaching and the herbicide is not likely to be a groundwater concern (Shaner 2014). For this reason, persistence is not usually an issue as the half-life of 2,4-D is only 6.2 days (Wauchope et al. 1992; Shaner 2014). However, plant back intervals have been a concern for both 2,4-D and dicamba when used as a preplant burndown and the subsequent crop does not have the respective herbicide resistance gene. Thompson et al. (2007) observed 11 and 13% soybean injury when 560 g ha^{-1} of 2,4-D and 280 g ha^{-1} dicamba were applied 7 days before planting. Injury was shown to decrease with increased plant back intervals (Thompson et al. 2007). These data indicate caution is needed when incorporating these herbicides into burndown programs when transgenic crops with resistance to 2,4-D or dicamba are not used.

Similar to 2,4-D, dicamba is also sold as different formulations and physiological properties are likely to vary for each. Dicamba is a member of the benzoic acid herbicide family but has a similar mode of action as 2,4-D. The parent acid has a vapor pressure of 4.5 x 10^{-3} pa (25°C) (Strachan et al. 2010; Shaner 2014). The solubility of dicamba is higher than 2,4-D at 4500 mg L^{-1} in water at 25°C. Moderate mobility in soil has been noted. However, microbial degradation reduces the risk for leaching. Field persistence of dicamba is longer than 2,4-D at 14 days (Shaner 2014). For this reason, dicamba is commonly applied with tank mixtures of residual soil herbicides for PRE applications. However, sole applications of dicamba appled preemergent are not recommended as sufficient weed control is not often achieved (Cahoon et al. 2015).

1.4.2. Palmer amaranth control following 2,4-D, dicamba, and glufosinate applications

Lack of sufficient rainfall can cause PRE herbicides to fail in the field. Rainfall is required to move residual herbicides into the rooting zone where they can be intercepted by the germinating plant (Whitaker et al. 2011). In such a scenario, the pressure to control Palmer amaranth escapes through POST applications increases. In cotton and soybean, herbicides known to control glyphosate-resistant Palmer amaranth is limited and only a few modes of action are used. Further complicating control, Palmer amaranth can quickly exceed optimal heights recommended for herbicide applications. Adverse weather conditions among other complications can make timely applications of postemergence herbicides impossible and producers may have to resort to hand weeding or field abandonment.

Recommendations have been provided by Culpepper and Vance (2019) for POST herbicide programs utilizing sequential applications to manage glyphosate-resistant Palmer amaranth in cotton. For LibertyLink® (BASF, Florham Park, NJ 07934) and Roundup Ready® systems (Monsanto Co., St. Louis, MO 63167), the first POST application recommendations include glufosinate plus glyphosate plus a residual herbicide such as S-metolachlor or acetochlor or glufosinate plus S-metolachlor or acetochlor of pyrithiobac (Culpepper and Vance (2019).

Incorporation of residual herbicides in initial POST applications can help to overlap control before Palmer amaranth emerges. The sequential post application recommended is glufosinate + S-metolachlor or acetochlor 15 days after the initial postemergence application (Culpepper and Vance 2019). Dicamba and 2,4-D resistant cotton will allow addition programs for Palmer amaranth control. Recommendations for dicamba-resistant crops include sequential POST applications of dicamba plus glyphosate spaced 15 days apart. Cotton with resistance to 2,4-D is recommended to receive POST applications of 2,4-D choline plus glyphosate or 2,4-D choline plus glufosinate. Layby applications of diuron plus MSMA or glyphosate plus diuron may also provide late-season control (Culpepper and Vance 2019).

Glyphosate resistant Palmer amaranth control recommendations in soybeans also exist. Steckel et al. (2019) recommends POST applications of lactofen, glufosinate, fomesafen, or acifluorfen for control of Palmer amaranth. Similar to cotton, 2,4-d and dicamba resistant soybean varieties will allow those options as well throughout the season. Of particular concern is the discovery of PPO resistant Palmer amaranth populations (Heap 2019). Lactofen, acifluorfen, and fomesafen are all PPO-inhibitors and may lose their efficacy in the southeast if PPO-resistant Palmer amaranth continues to spread.

While weed competition is most injurious to crop yields early in the cropping season, late season emergence can make harvest difficult and mature plants can replenish the seedbank to be controlled in subsequent years (Smith et al. 2000). Depending on cotton varieties in production, POST herbicide options for control of broadleaf weeds in cotton include glyphosate, bromoxynil, pyrithiobac, glufosinate, 2,4-D and dicamba. While some of these options can control Palmer amaranth, applications must be made before weeds reach 10 cm (Culpepper et al. 2010). Improper timing of herbicide applications can allow escapes to quickly exceed optimal height for control and replenish the soil seed bank if allowed to grow to maturity. A study by Vann et al. (2017a) observed a linear relationship between Palmer amaranth control and the amount of time passed before the first postemergence application of glufosinate plus dicamba applied at 880 and 560 g ha^{-1}. Specifically, control decreased from 99%, 96%, 89%, 75%, and 73% with delays at 7 day increments (Vann et al. 2017a). Additionally, Palmer amaranth height increased to 7, 20, 33, 53, and 71 cm for each of those time intervals (Vann et al. 2017a). These data stress the importance of timely applications and demonstrate how quickly Palmer amaranth plants can exceed optimal heights for control. Removing Palmer amaranth by hand is laborious and expensive, indicating a need for additional options. According to Sosnokie and Culpepper (2014), glyphosate-resistant Palmer amaranth drove hand weed costs up to \$57 ha^{-1} in 2010 and 52% of the cotton crop in GA was hand weeded due to inability to achieve adequate control through herbicide applications.

Glyphosate resistant Palmer amaranth has created immense pressure for residual herbicides, glufosinate, dicamba, and 2,4-D for control in cotton and soybeans. Glufosinate has been widely accepted as an alternative to glyphosate to control resistant weeds. While PPO-inhibiting herbicides have been shown to effectively control Palmer amaranth, concern for resistance development in Palmer amaranth populations is increasing (Cahoon et al. 2014: Salas et al. 2016). New synthetic auxin technology provides additional options for control, allowing over the top applications of glyphosate, glufosinate, and dicamba/2,4-D. Furthermore, some researchers suggest strategic POST applications of these herbicides could rescue Palmer amaranth infested fields (Cahoon et al. 2015; Merchant et al. 2013).

Glufosinate based herbicide programs in cotton have been shown to effectively control Palmer amaranth (Culpepper et al. 2009; Whitaker et al. 2011; Everman et al. 2007). However, this level of control is only consistently achieved when applications are made to Palmer amaranth less than 10 cm tall (Coetzer et al. 2002; Culpepper et al. 2010). Aggressive growth and adverse weather conditions can make timely applications difficult. Results from Coetzer et al. (2002) indicate Palmer amaranth control with glufosinate applications is reduced as plant height increases. Because herbicides provide inadequate control of Palmer amaranth at taller heights, rapid growth can quickly lead to unmanageable populations.

Sufficient Palmer amaranth control is not often achieved with a single application. Merchant et al. (2013) only observed 74% Palmer amaranth control with one application of glufosinate at 431 ga ha^{-1}. Coetzer et al. (2002) only observed Palmer amarnath control greater than 80% when glufosinate was applied sequentially at 410 g ha^{-1}. Palmer amaranth biomass in plots treated with a single application of glufosinate at the same rate did not differ from the nontreated control. Cahoon et al. (2015) reported sequential applications of dicamba at 560 g ha^{-1} are up to 11 to 25% more effective than when applied at one timing. Vann et al. (2017b) only achieved 84% and 81% control 14 days after a second application with sequential applications spaced 12 days apart of glufosinate and dicamba applied alone at 880 and 560 g ha^{-1}. The initial applications were made to Palmer amaranth with average heights of 16 cm tall indicating several plants exceeded optimal height for herbicide efficacy.

Herbicide mixtures of glyphosate or glufosinate plus 2,4-D have been shown to increase control of several troublesome weeds (Beckie 2011; Robinson et al. 2012). A study by Vann et al. (2017b) revealed sequential applications of glufosinate applied at 880 g ha^{-1} only reduced Palmer amaranth 84% fourteen days after the second post application. The addition of dicamba in the initial treatment at 560 g ha^{-1} increased control by 11%. Sequential applications of tank mixtures of dicamba plus glufosinate resulted in 97% control with the rates mentioned above.

Similar results were observed by Cahoon et al. (2015) where sequential applications of glufosinate at 560 g ha^{-1} only resulted in 75% control 4-5 weeks after initial application. Applications of glufosinate followed by glufosinate and dicamba (560 g ha^{-1}) tank mixtures resulted in a 9% increase and 16 to 17% when the order was reversed or tank mixtures applied in both applications. A study by Merchant et al (2014) showed applications of 2,4-D alone at 1,060 g ha^{-1} can control 20 cm tall Palmer amaranth 80% twenty days after application. Control levels were increased to 97% with the addition of glufosinate at 431 g ha^{-1} in a tank mixture. Dicamba was applied in a similar manner and applications of dicamba at 1,120 g ha^{-1} only resulted in 83% control 20 days after treatment compared to 94% control when glufosinate was tank mixed. In a study by Vann et al. (2017b), sequential applications of tank mixtures of dicamba plus glufosinate were shown to completely control Palmer amaranth 14 days after the second post application. However, initial application was made to plants under 10 cm tall, a height at which glufosinate alone has been shown to control Palmer amaranth (Culpepper et al. 2006).

Several studies in the literature suggest enhanced weed control may be achieved with tank mixtures of synthetic auxins and glufosinate (Steckel et al. 2006; Vann et al. 2017a; Vann et al. 2017b; Voth et al. 2012). Alternatively, antagonism of dicamba and glufosinate tank mixtures has also been reported (Bitha et al. 2012; Browne et al. unpublished manuscript). Regardless of possibility of antagonism, current Xtendimax®, Fexapam®, and Engenia® labels restrict tank mixing dicamba with glufosinate due to volatility concerns (Anonymous 2018; 2019a,b,c). However, Enlist One label will allow for tank mixtures of 2,4-D with glufosinate (Anonymous 2019b). Studies discussed above indicate systems utilizing combinations of glufosinate and synthetic auxins are more effective for Palmer amaranth control than when applied alone.

The mechanisms for development of Palmer amaranth resistance to synthetic auxins has been confirmed (Vieira 2019). Custom applications exploiting time and plant metabolism could enhance control of Palmer amaranth and delay resistance development. New 2,4-D and dicamba technology will add additional tools to the herbicide portfolio used to control Palmer amaranth during the growing season. However, proper stewardship will be required to prevent off-target movement to sensitive crops.

1.5. Dicamba off-target movement

1.5.1. New dicamba formulatinos

Following commercial release of dicamba technology, reports of crop injury son sensitive soybeans have dominated the news (Hager 2017). Low dosages of dicamba are capable of inducing a plant response injury resulted is easily distinguishable between other modes of action and can appear just hours after exposure (Egan et al. 2014; Andersen et al. 2004). The most obvious symptoms of synthetic auxin exposure include twisting or epinasty of stems and crinkling/cupping of leaves (Egan et al. 2014). Crop injury reports began several decades ago, long before commercial release of dicamba resistant crops as dicamba was first commercialized on the late 1960’s (Behrens and Lueschen 1979). However, new transgenic crops with resistance to dicamba allow applications throughout the growing season when sensitive crops are growing simultaneously. As a result, large numbers of complaints of off-target movement has ensued. Primary sources of dicamba off-target movement have been identified as contamination of sprayer equipment, volatilization, or particle drift (Egan et al. 2014; Egan and Mortensen 2012; Mortensen et al. 2012).

New formulations of dicamba were developed to minimize off target movement. Monsato commercialized a diglycolamine (DGA) salt of dicamba with a built-in pH modifier (VaporGrip®) for use on resistant crops known as Xtendimax® (Monsanto Co., St. Louis, MO 63167) (Anonymous 2018b). Concurrently, BASF introduced N,N-bis-(-aminopropyl) methylamine salt of dicamba (BAMPA) marketed as the trade name Engenia® and Corteva commercialized another DGA product known as FeXpan® which also includes the VaporGrip® technology (Corteva Agriscience, Indianapolis, IN 46268) (Anonymous 2018c,d).

In an attempt to reduce off-target movement, dicamba formulations for use on transgenic crops has several label requirements enforced by federal law (Anonymous 2018b,c,b). Dicamba labels required record keeping for two years and must be recorded no later than 72 hours after application. Applications are only permitted one hour after sunrise until two hours before sunset to minimize the number of applications during temperature inversions. Labels also have buffer requirements of 33.5 m from downwind edges of the field and specifically prohibit applications near sensitive crops. Specific wind speeds of 4.8 to 16.1 km per hour are required during applications. Temperature inversions are often associated with the absence of wind and higher wind speeds may enhance the risk for particle drift. Tank mixtures not specified on the label are also prohibited, specifically products that have ammonia salts. Addition of pH neutralizing buffers are also recommended when pH of the spray mixture is reduced below 5. Addition of a drift reduction agent is required for specific tank mixtures and only approved nozzles are to be used for application per website recommendations on herbicide labels. Sprayer cleanout using a triple rinse procedure is also required following use (Anonymous 2018b,c,d).

Crop injury assessments are commonly conducted through visual injury estimates, investigating foliar residue retention, and evaluating yield responses (Byrd et al. 2016; Egan et al. 2014; Egan and Mortensen 2012; Andersen et al. 2014). A visual injury scale was developed by Behrens and Lueschen (1979) to evaluate visual injury resulted from pant growth regulator herbicides and researchers commonly utilize similar scaling for evaluation (Andersen et al. 2004; Foster and Griffin 2018; Egan and Mortensen 2012). Soybeans are commonly selected as indicator plants for dicamba off-target movement due to high sensitivity (Egan et al. 2014).

1.5.2. Soybean response to dicamba

Soybean injury to dicamba has been of particular focus in the media. Although 2,4-D usage is likely similar to dicamba following release of transgenic crops, soybeans are far more sensitive to dicamba as a opposed to 2,4-D (Egan et al. 2014). In 2017, injured soybean acreage was estimated at nearly 3.6 million acres (Bradley 2017). In 2018, estimates were reduced to 1.1 million acres (Bradley 2018). Such high numbers indicate off-target movement is still a large concern for this technology and proper stewardship will be mandatory for its survival.

Correlation investigations between dicamba dosage, soybean visual injury, and yield loss are widespread in the literature (Egan et al. 2014; Johnson et al. 2012; Egan and Mortensen 2012; Wax et al. 1969). Dicamba dosages as low as 0.028 g ha^{-1} have been reported to cause observable symptomology on sensitive soybeans (Soloman and Bradley 2014; Kelley and Riechers 2007; Griffin et al. 2013; Kniss 2018). However, the lowest dosages predicted to cause yield loss have varied across the literature and the lowest dose was reported at 0.15 g ae ha^{-1} (Soltani et al. 2016; Kniss 2018; Robinson et al. 2013). Of particular concern, a rate of 5.6 g ae ha^{-1} has been implicated in particle drift estimates which is much higher than the lowest dosage expected to reduce soybean yield (Egan et al. 2014). Another study by Anderson et al. (2004) reported 14% yield reductions when dicamba was applied at 5.6 g ae ha^{-1} at the V3 growth stage.

These studies suggest dicamba rates high enough to induce a plant response are capable of moving off-target and mitigation will be need to reduce risks.

Soybean yield responses to dicamba are highly variable in the literature. For example dicamba dosages reported to cause at least 5% yield loss has varied from 0.16 to 47 g ae ha^{-1} when exposed at the vegetative stage (Auch and Arnold 1978; Soltani et al. 2016; Kniss 2018). Often, dicamba injured plants exhibit damage to the apical meristem. Consequently, lower leaf axillary buds are aborted and lateral branching is stimulated (Andersen et al. 2004; Wax et al. 1969; Weidenhamer et al. 1989). High variability in yield response to different dicamba dosages could be a result of this phenomena. One factor that is well highlighted in the literature is the effect of crop stage at the time of application.

Injury resulted from dicamba exposure is far more pronounced in soybeans at vegetative stages compared to reproductive stages (Robinson et al. 2013; Auch and Arnold 1978; Egan and Mortensen 2012; Egan et al. 2014; Kniss 2018). However, younger plants can compensate for some injury and yield loss is a larger concern when soybean are exposed at reproductive stages (Kniss 2018; Egan et al. 2014). In a meta-analysis by Kniss (2018), dicamba dosages applied to soybeans at V1 to V3 stages to cause 5 % yield loss ranged from 1.6 to 97 g ha^{-1} and 1.2 to 47 g ha^{-1} for V4 to V7 stage exposure. Conversely, dosages associated with 5% yield loss at R2 stage were much closer in range and relatively lower at 0.15 to 14 g ha^{-1} (Kniss 2018). Kelley et al. (2005) reported a 12% increase in dicamba injury when 0.56 g ha^{-1} was applied to vegetative stages compared to reproductive stages. Soybeans may be more sensitive at reproductive stages due to limited time for recovery.

The variable response of soybeans to dicamba at early stages may be dependent on the plant's ability to regrow following injury, a response that relies on favorable conditions. Soybean vegetative growth is reduced as plants approach flowering and may not produce enough new tissue to compensate for injury accrued (Martin et al. 2006). Soloman and Bradley (2014) reported dicamba applied to V3 soybeans at 28, 2.8, 0.28, and 0.028 resulted in visual injury of 44, 32, 28, and 21% two weeks after treatment. When the soybeans were rated again two weeks later, visual injury was 12, 9, 9, and 10%, suggesting plants recovered (Soloman and Bradley 2014). This observation is further confirmed by the lack of yield response at maturity (Soloman and Bradley 2014). Alternatively, visual injury of soybeans only varied 1 to 4% between ratings when the same dosages were applied to R2 soybeans and yield reductions of 2 to 67% were observed compared to the nontreated control (Soloman and Bradley 2014). Other studies confirm soybeans at the R2 stage are more sensitive to dicamba than other growth stages (Scholtes et al. 2019; Wax et al. 1969). The higher injury observed for vegetative exposure timings may be indicative of increased translocation of dicamba to actively growing tissue as opposed to reproductive stages. Although leaf malformations may not be as pronounced when exposure occurs during reproductive stages, other meristematic regions that influence pod development may be directly affected (Jones et al. 2019a). Ultimately, these data indicate crop stage must be factored into assessments of drift events.

Soybean injury has been tightly correlated with the dose of dicamba applied (Egan and Mortensen 2012; Egan et al. 2014; Kniss 2018). However, yield loss is much more difficult to predict based on visual injury (Egan et al. 2014; Kniss 2018). Kniss (2018) suggests dose prediction from visual injury is nearly impossible when the source of exposure is not known. The vast majority of research in the literature regarding soybean dose-response to dicamba is useful in anticipating the potential effect of exposure but has limited use in terms of diagnosing the source and dose of dicamba implicated in off-target assessments. Al-khatib and Peterson (1999) noted visual injury always exceeds yield reductions and predictions will be difficult. Attempts have been made by researchers to correlate visual injury resulted from synthetic auxins to yield (Kniss 2018; Egan et al. 2014). However, pooled data from 11 dicamba studies suggest those types of analyses are subjective and the timing of exposure is critical information for predictions (Kniss 2018). For example, analyses conducted by Kniss (2018) suggest that visual injury lower than 30% and 12% is not likely to result in yield reductions of high magnitude at vegetative and reproductive stages, respectivelty. However, visual injury of less than 11% has been associated with 5% yield loss when exposure occurs at reproductive stages.

Additional concerns have been raised regarding soybean populations produced from parents exposed to a dicamba drift events. Pod malformations have been reported following dicamba exposure at reproductive stages (Auch and Arnold 1978; Weidenhamer et al. 1989). Furthermore, 13, 46, and 50% germination reductions have been observed by seed collected from parent plants exposed to dicamba at 30 g ha^{-1} during pod filling stages (Thompson and Egli 1973; Auch and Arnold 1978). Leaf malformations in offspring has also been reported for dosages of 8.75 to 560 g ha^{-1} (Thompson and Egli 1973; Auch and Arnold 1978). Recent research conducted by Jones et al. (2019a) indicate drift events imposed on soybeans at the R1 to R6 stages can have negative impacts on offspring and pod malformation could be an early sign this may occur. Drift simulation studies often place emphasis on yield as that is the most direct consequence to exposure. However, seed production fields may require additional attention to protect subsequent soybean generations from synthetic auxin damage.

Although often not statistically significant, some data even suggest hormesis responses with low dosages of synthetic auxins further complicating predictions (Auch and Arnold 1978; Robinson et al. 2013; Weidenhamer et al. 1989). Increased soybeans yields over 102% compared to the nontreated check have been reported in several studies investigating soybean responses to sublethal doses of dicamba (Auch and Arnold 1978; Robinson et al. 2013; Weidenhamer et al. 1989). However, results have not been sufficient to conclude sublethal dosages of dicamba will consistently result in a hormesis response. Plant growth regulator herbicides inhibit apical dominance and axillary buds will form new branches and reproductive structures. This could explain the variable responses and may allow for soybeans to compensate for some degree of injury.

Further limiting yield prediction from visual injury, dose-response evaluations are usually conducted at a single known timing. Variability in yield response is often greater when exposure occurs at vegetative stages as opposed to reproductive stages, potentially due to increased time for plant recover (Egan et al. 2014; Kniss 2018). In reality, off-target exposure could result from tank contamination, particle drift, volatility, or a mixture of exposures (Sciumbato et AL. 2004a,b). Moreover, exposure could occur several times during a season for different durations. While data suggests visual injury is not a good indicator of yield loss, the low dosages of dicamba capable of causing injury are concerning. Egan et al. (2014) estimates more significant yield losses are most likely to occur as a result from misapplication or physical drift as opposed to volatilization.

1.5.3. Particle drift of dicamba

Spray drift is defined as the movement of airborne spray droplets of the spray solution downwind of the designated area after aerial or ground applications (Stephenson et al. 2006). Manufacturers of dicamba have taken several precautions to reduce physical drift including label requirements enforced by law and new formulations with potential to reduce off-target movement (Anonyms 2018 b,c,d). However, physical drift of dicamba has been well documented in the literature and identified as one of the major routes of off-target exposure (Egan et al. 2014, Egan and Mortensen 2012; Robinson et al. 2013; Kniss 2018). Research suggests up to 16% of the original spray solution of dicamba can migrate from the original site of application (Maybank et al. 1978; Wolf et al. 1993). Other studies show 0.1 to 9% of pesticide applications are deposited 2 m from target area and deposition rate decreases exponentially as distance from the original swath increases (Carlsen et al. 2006). Fritz et al. (2018) examined in-swath deposition of spray mixtures and deposition in the target area ranged from 32.5 to 94.2%. Variability was likely a result of different wind speeds. However, one could assume all deposition unaccounted for moved away from the target area. New dicamba formulations are known to decrease drift rates. However, even small amounts could induce a crop response and successive applications could have an additive effect on the amount of injury (Maybank et al. 1978). Furthermore, nozzle selection, boom height, and wind speeds are likely to interfere with the amount of product to reach its target (Wolf et al. 1993).

Droplet size has been identified as a main factor implicated in off-target movement of pesticides (Antuniassi et al. 2016). Larger droplets are less likely to drift than smaller droplet sizes. The current Xtendimax® label approves 36 different nozzles for use with specified minimum and maximum operating pressures (Anonymous, 2018b). The Engenia® label references 29 approved nozzles (Anonymous 2018b.c.d). Majority of approved nozzles include air induction nozzles designed to produce a greater proportion of larger droplet sizes as opposed to drift able fines (McGinty et al. 2016). All nozzles produce a spectrum of droplet sizes and are most often characterized by the mean diameter droplet size (Nuyttens et al. 2007;Arituniassi et al. 2018). Furthermore, the design of nozzles influences the distribution of droplet sizes. Most nozzles have a one or two orifices where the spray mixtures exits by force of pressure. The orifice most often causes the spray mixture to be excreted as a flat fan or a thin stream (Butts et al. 2018; Alves et al. 2017). Air induction nozzles have a pressure-reducing chamber inside and incorporate air into the spray droplets (Arituniassi et al. 2018). A study by Alves et al. (2017) examined Extended Range (XR), Turbo TeeJet (TT), Air-induction Extended Range (AIXR), and Turbo TeeJet Induction (TTI) nozzles for dicamba spray drift potential. The volumetric mean diameter spray droplets for the XR, TT, AIXR, and TTI nozzles were 172, 248, 372, and 774 µm and driftable fines (<100µm) were 19%, 7%, 2%, and 0.3% of the droplet spectrum. The lowest amount of dicamba drift was observed for TTI nozzles as compared to the others (Alves et al. 2017). These data suggest utilization of air induction nozzles and new dicamba formulations can significantly reduce herbicide drift. However, the combinations alone cannot completely eliminate the risk. Furthermore, research conducted by Butts et al. (2018) suggests minor clogs of the air inclusion ports in air induction nozzles can have negative effects on the droplet spectrum and proper maintenance of nozzles is required to ensure drift is minimized.

Tank mixtures of glyphosate and dicamba may have an influence on droplet size as well. A study by Alves et al. (2017) examined the drift potential of such mixtures using wind tunnels. Droplet sizes were reduced 8.9 and 6.8% for TTI and AIXR nozzles when dicamba was combined with glyphosate as opposed to dicamba alone at a pressure of 76 kPa. This combination is frequently used in applications on respective transgenic crops to expand the weed control spectrum. Furthermore, the combination may have other influences on off-target movement such as volatility (Mueller et al. 2019b).

Adjuvants can also influence droplet size. Jones et al. (2007) found that mean droplet size was increased and driftable fines were reduced when glyphosate was applied with polysaccharide adjuvants marketed for drift reduction of pesticides. Studies suggest surface tension, viscosity, density, and evapotranspiration rates are major factors involved in the physical movement of sprayed liquids and polymer materials can reduce drift (De Schampheleire et al. 2009). Most drift control agents thicken the spray solution and reduce the production of driftable fines in the droplet spectrum. Some manufacturers claim the drift reduction adjuvants produce fewer droplets smaller than 150 µm (Bissell et al. 2019). In attempts to limit particle dift, dicamba herbicide labels require addition of approved drift control adjuvants for specific tank mixtures (Anonymous b,c,d).

Adjuvants marketed for drift reduction can be composed of a variety of materials. One group of adjuvants include polyacrylamides composed of several acrylamine monomers. These adjuvants thicken spray mixtures by forming gels or linear structures and readily absorb water allowing adequate flow (Bissell et al. 2019). Polysaccharides are another group of DRAs and are usually derived from vegetable or seed oils. Repeating monosaccharides comprise these products joined by glycosidic bonds forming both linear and branched structures that can also modify the flow of a spray mixture and reduce the number of fine droplets (Bissell et al. 2019). Some data suggests emulsion adjuvants and oil-based products used in conjunction with air induction nozzles may actually increase the number of fine droplets produced (Miller and Ellis 2000; Costa et al. 2018). This observation is possibly a result of the ballistic behavior of air filled droplets on the intended surface (McArtney and Obermiller 2008). The large air-filled droplets produced by air induction nozzles may fracture into several smaller droplets during applications. Some DRAs may also be subject to shearing within the pump in a sprayer system. A study by Bissell et al. (2019) demonstrated reduced efficacy of polyacrylamide adjuvants following 50 circulations as compared to polysaccharide products such as guar gum.

Pulse-width modulation sprayer systems have also been developed to deliver more precise pesticide applications by standardizing flow. Conventional sprayers use pressure as a form of delivery and parameters such as flowrate, droplet size, droplet velocity, and spray patterns can be influenced by changes in pressure. According to Giles et al. (2002), spray drift reductions are directly related to retaining droplet velocity. The longer droplets are in the air, the more likely they will be displaced by wind or other forces. Pulse width modulation allows an alternative to modifying pressure to change flow and droplet size (Giles and Comino 1990; Giles 1997). An electronically actuated solenoid positioned at the nozzle inlet is used to influence flow rate. Giles et al. (2002) showed droplet velocity is directly related to flow rate in conventional pressure systems and was decreased 50% when flow rate was reduced by 1/3. Alternatively, changes in droplet velocity were much less significant in response to flow rate in pulse width modulation systems (Giles et al. 2002). These data suggest the use of pulsed spray systems could be more effective than pressure regulated systems in reducing physical drift.

A rough estimate of dosage correlated to dicamba particle drift is 5.6 g ha^{-1}, assuming an initial application rate at 560 g ha^{-1} (Egan et al. 2014; Egan and Mortensen 2012; Wang and Rautman 2008; Brown et al 2004). According to a meta-analysis conducted in 2014, the most severe case of soybean yield loss in response to this dosage of dicamba was 8.7% (Egan et al. 2014). The major route of transport of synthetic auxin droplets is wind. Therefore, the degree of movement outside of the target field is likely dependent on wind speed. According to Jones et al. (2019b), visual injury on soybean resulted from dicamba exposure has been detected up to 152 m from the original application area. However, the distance to 5% yield reductions was only 42 m. These data indicate visual injury much likely to occur at greater distances than those at which yield responses are likely to occur.

Arguments have been made that physical drift assessments where the same carrier volume is used to deliver diluted spray mixtures are not an adequate representation of spray drift that occurs in the field (Smith et al. 2017). Banks and Scroeder (2002) suggested the use of constant carrier rates could result in underestimation of crop injury to synthetic auxins. Their solution to this problem was the use of variable carrier volumes that were proportional to the herbicide dosage. In this study, cotton yield reductions in response to another synthetic auxin, 2,4-D, were higher when applied using variable carrier volumes as compared to constant carrier volumes. Similarly, Smith et al. (2017) studied the effect of carrier volumes on cotton response to 2,4-D and dicamba at rates of 18.7 and 37.4 g ha^{-1}, respectively. Higher cotton injury was observed for dicamba applications using variable rates as compared to constant rates. For example, when applied at first square, cotton injury of 16 to 24% was recorded for variable carrier rates (Smith et al. 2017). Alternatively, constant carrier volumes only resulted in 3 to 11% injury. Yield response to dicamba at the 6th leaf stage and first square followed the same trends where yield was reduced 30 and 41 % when applied at variable rates and 13% and 19% when applied with constant carrier volumes. Cotton response to 2,4-D was similar but exaggerated due to increased sensitivity. When applied at first square, 2,4-D applied at variable rates resulted in 39 to 60% injury whereas constant rates only resulted in 19 to 36% injury. Yield response followed suit with 81% and 97% reductions at variable carrier volumes as opposed to 68 and 89% when applied using constant carrier volumes at the 6th leaf stage and first square, respectively (Smith et al. 2017). These data suggest particle drift assessments may need to be adjusted to different carrier volumes for more accurate predictions of crop response to synthetic auxins.

Barriers of crops that are not sensitive to synthetic auxin drift such as corn have been suggested as a solution. A study by Vieira et al. (2018) demonstrated how corn barriers could reduce off-target deposition distance 7-fold when applications were made using non-air induction nozzles and 10-fold with air inclusion nozzles. This approach could potentially capture majority of spray particles traveling by wind from the intended site. However, sensitive plant selection should be based on height. A study by Van de Zande et al. (2000) used grass strip barriers 1.2 m wide to capture spray drift and plants with height at or greater than the nozzle height reduced particle drift 80 to 90% whereas plants shorter than the nozzles only reduced drift by 50%. This strategy may be useful in areas of high dicamba usage to reduce particle drift.

1.5.4. Volatility of dicamba

Vapor pressures are often used as a measurement of potential for a substance to volatilize and values often change with formulation (Shaner 2014). Dicamba vapor pressure is much higher in the parent acid form as compared to the newer salt formulations (Hartzler 2017). Volatility has been assessed through several approaches in greenhouse and bioassay experiments (Mueller et al. 2019a; Mueller et al. 2013; Behrens and Leschen 1979). Some studies have measured volatility directly through air concentrations collected in polyurethane foams with high volume air samplers (Mueller et al. 2019a; Mueller et al. 2019b). Other studies have examined observable symptomology as a result of experimental treatments that attempted to separate volatility and physical drift (Sosnoskie et al. 2015; Sciumbato et al. 2004a, Sciumbato et al. 2004b).

Reports of volatility for all available dicamba formulations are widespread in the literature (Mueller et al. 2019a; Egan and Mortensen 2012, Bish and Bradley 2017, Behrens and Lueschen 1979; Mueller et al. 2013; Sciumbato et al. 2004a; Sciumbat et al. 2004b; Mueller et al. 2019b; Wright et al. 2012). Dicamba is characterized as a weak acid and therefore the parent herbicide can easily become protonated or deprotonated, a characteristic likely implicated in volatility (MacInnes 2017). Research suggests temperature, humidity, and formulation are major factors involved in volatility of dicamba (Mueller and Steckel 2019; Mueller et al. 2013). Mueller et al. (2013) also found that time of day can effect dicamba volatility, likely reflective of environmental factors listed above. Findings suggested the highest dicamba concentrations in the air are detected following applications made at midday.

Dicamba formulation is perhaps the most important aspect involved in off-target movement. Higher dicamba volatility has been observed for the DMA formulations as compared to the DGA salt (Mueller et al. 2013; Egan and Mortensen 2012). Mueller et al. (2013) observed a 50% increase in dicamba volatility of DMA as compared to DGA when assessed using high volume air samplers. In a bio-assay study by Egan and Mortensen (2012), a 94% reduction in off-target movement was reported when the DGA salt was applied plots versus the DMA salt. However, applications of the DMA salt still resulted in observable symptomology 20 m from a treated area of only 335 m^2, indicating the need for sufficient downwind buffers (Egan and Mortensen 2012). These data also suggest that applications to larger areas may result in a higher magnitude of injury downwind.

Another main driving factor for dicamba volatility is pH (Mueller and Steckel 2019). Lower pH tends to favor disassociation of dicamba molecules in solution (Hemminghaus et al. 2017; MacInnes 2017). The latest dicamba herbicide labels place importance on maintaining pH levels at 5.0 for spray mixtures (Anonymous 2018b,c,d). Different dicamba formulations have been shown to have varying influences on the pH of spray solutions. For example, Mueller et al.

(2019) observed consistent increases of pH when BAPMA formulations of dicamba were added to water and variable responses from addition of the DGA salt formulation.

Tank mixtures of dicamba and ammonium sulfate or urea ammonium nitrate is strictly prohibited by new dicamba formulation labels due to increases in volatility (Mueller et al. 2019; Anonymous 2018b). Ammonium sulfate is commonly used in herbicide solutions with glyphosate to reduce hard water interference (Mueller et al. 2006; Jordan et al. 1997; Thelen et al. 1995). Glyphosate is often referenced as an efficient chelator and hard-water cations can cause antagonism (Thelen 1995; Bromilow et al. 1993). Glyphosate is sold as different salt formulations. The most common formulations include either dimethylamine, isopropulamine, trimesium, and potassium salts. Data suggests the isopropylamine salt formulations have a higher pH than potassium salt formulations (Mueller et al. 2019b). Interestingly enough, Mueller et al. (2019b) only observed a pH decrease of 0.7 when ams was added to water. The decrease in pH was much smaller in comparison to the addition of glyphosate-K which resulted in pH decreases of 1 to 2.1 units (Mueller et al. 2019b). Glyphosate is frequently added to tank mixtures of synthetic auxins to control a wider spectrum of weeds. The pH response of spray mixtures including glyphosate are variable when tank mixed with different dicamba formulations (Mueller et al. 2019b). Low pH increases of 0.2 to 0.3 units has been documented when glyphosate-K is added to DGA plus vaporgrip spray solutions with final pH range of 4.8 to 4.9 (Mueller et al. 2019B). Although the shifts are minimal, the final pH of the spray solution was below the recommended pH of 5 per herbicide label. Shifts in pH of higher magnitude were noted for other formulations of dicamba. Water quality was not found to have a major influence on pH therefore it is not a significant factor for volatility (Mueller et al. 2019b). The research conducted by Mueller et al. (2019b) suggest the volatility observed in dicamba mixtures with AMS is not a result of pH reductions and likely another unidentified factor is involved (Mueller et al. 2019b). Furthermore, if pH was the main influential factor on volatility, higher volatility would be observed in glyphosate and dicamba tank mixtures as opposed to AMS. Mueller et al. (2019b) suggested additional drivers of volatility could be cation interactions or environmental conditions. No doubt, a better understanding of the mechanism of volatility is needed.

Temperature is another major factor implicated in dicamba volatility. According to Mueller et al. (2019a), dicamba volatility should not occur at temperatures of 15°C or lower. In this study, high volume air samplers and humidomes were used to assess dicamba volatility in a greenhouse setting (Mueller et al. 2019b). Results indicate dicamba volatility increases as temperatures increased. Furthermore, 2.9 to 9.3 times more dicamba was recovered when glyphosate was added to DGA plus vaporgrip tank mixtures as opposed to DGA plus vaporgrip alone (Mueller et al. 2019b). This observation may be a result of a pH decrease following the addition of glyphosate. Egan and Mortensen (2012) also observed a significant correlation between temperature and volatility. Humidity has also been implicated in volatility concerns (Behrens and Lueschen 1979). According to Behrens and Lueschen (1979), soybean injury was increased 12% as a result of DMA vapors at relative humidity levels of 70-75% as compared to 85 to 95%. Other studies in the literature suggest less adsorption to soil occurs when humidity is increased resulting in higher volatility (Mcwhorter and Gebhardt 1988). Temperature inversions are also of particular concern when applying dicamba. For this reason, dicamba labels prohibit applications during nighttime hours (Anonymous 2018b,c,d). The labels suggest the presence of fog as an indicator but warn that inversions can occur in the absence of fog. Inversions are said to dissipate at winds greater than 4.8 km per hour and at sunrise when surface temperatures increase. Applications are permitted one hour after sunrise and two hours before sunset in an attempt to limit negative impacts of inversions (Anonymous 2018b,c,d).

Although a large amount of concern has been raised for dicamba volatility, the estimated dosage of exposure at 0.56 g ha^{-1} has resulted in minimal yield loss regardless of soybean stage at the time of exposure (Egan et al. 2014; Kniss 2018). However, observable symptomology can occur. Some research suggests volatility can occur up to 3 days after applications (Behrehns and Lueschen 1979). Academic studies in recent years have used low tunnel experiments to assess dicamba volatility (WSSA 2018). In these types of studies, treated soil pans are placed under a tunnel that covers sensitive plants. One low tunnel experiment discussed in a WSSA dicamba workshop (2018) demonstrated how DGA dicamba formulation plus AMS can increase soybean visual injury over 2-fold as compared to DGA alone. This type of investigation could be useful in separation of volatility effects from particle drift.

1.5.5. Sprayer contamination with dicamba residues

New dicamba formulations have labeling requirements of a triple rinse cleanout procedure following application. While three rinses can exponentially reduce pesticide concentrations in the tank, small amounts of dicamba can result in observable symptomology on sensitive plants (Osbourne et al. 2015; Boerboom 2004). Dicamba is not as water soluble as other commonly used pesticides such as glyphosate and residues are much more likely to remain in sprayers and care must be taken for proper removal (Steckel et al. 2010).

Dicamba and other synthetic auxins are known to be difficult to remove from spray equipment (Boerboom 2004; Cundiff et al. 2017). These herbicides have the potential to remain in the spray tank, hoses, measuring equipment, nozzles, etc. The lowest rate of dicamba predicted to cause soybean injury is 0.005% (Soloman and Bradley 2014). Assuming a sprayer is calibrated for 140 L ha^{-1}, only 0.377 g of dicamba (1.08 ml Xtendimax®) would be required to contaminate a sprayer with a tank capacity of 1,892.5 L sprayer at the 0.005% level. Based on these calculations 94 mls of the original spray solution would be sufficient to contaminate the next sprayer load and possibly result in soybean injury (concentration of 0.2 mg L^{-1}). However, the lowest dosage expected to cause soybean yield loss is 0.03% of the use rate 560 g ha^{-1} (Kniss 2018; Soltani et al. 2016; Griffin et al. 2013; Auch and Arnold 1978). Therefore, higher concentrations may be tolerated by crops before yield loss is likely to occur.

Boerboom (2004) performed a sprayer cleanout test and found dicamba in all water samples following a cleanout protocol with ammonia and water. The concentrations detected were low. However, the levels detected were similar rates as those known to induce plant responses on other studies (Kniss 2018; Egan et al. 2014). Osborne et al. (2015) also detected dicamba in sprayer rinsates following a triple rinse protocol. Initial rinses removed 90-95% of dicamba and less than 5% remained by the third rinse. However, average dicamba concentrations of .41 mg L^{-1} were detected after three rinses with water, a rate equivalent to 0.057 g ai ha^{-1} in a sprayer delivering 140 L ha^{-1}. Soloman and Bradley observed 10% injury on soybeans following dicamba applications of 0.028 g ha^{-1}. Therefore, triple rinse with water following dicamba applications may not be sufficient to remove residues capable of inducing crop injury.

Off-target movement has been known to occur from sources other than volatility, tank contamination, or particle drift. Plant growth regulator accumulation in the atmosphere has been suggested. Hill et al. (2002) detected dicamba residues in Canada rainfall. This is of particular concern considering the increase in use rates following commercialization of resistant crops. Tuduri et al. (2006) conducted a similar study in Canada where dicamba air concentrations were detected as well as precipitation deposition. If these herbicides are capable of collecting in the atmosphere and deposited as rainfall, avoidance of exposure will be nearly impossible without resistance genes.

Widespread reports of dicamba drift has increased adoption rates of similar technology as a strategy of protection from crop injury (Egan et al. 2014; Mortensen et al. 2012). However, this strategy only applies to crops with the technology available. Several other sensitive crops will still be grown during the time of in-season synthetic auxin applications. Patterns of crop injury reports are indicative of an on-going trend and threats of off-target movement are not likely to be resolve any time soon. The two greatest challenges of weed management in crop product today are prevention of off-target movement of herbicides and minimizing herbicide resistance development in weed populations. Palmer amaranth herbicide resistance is widespread across the US and quickly depleting available options for control. Research is needed to evaluate optimal herbicide applications for Palmer amaranth control and to assess risks associated with increased usage of synthetic auxins.

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Chapter 2

Evaluation of Residual Herbicides for Variety Tolerance and Water Activation Requirements in Cotton[1]

2.1. Abstract

In order to evaluate residual herbicide performance at different irrigation rates for activation and assess cotton safety to several tank mixtures, greenhouse and field experiments were conducted. Acetochlor, diuron, fomesafen, fluridone, and pendimethalin were applied to pots with moist sandy loam soil and 0.25 g Palmer amaranth seed in a greenhouse setting at 1262, 841, 280, 231, and 1066 g ha^{-1}, respectively. Irrigation of 0, 0.64, 1.27, and 1.91 cm was applied immediately following herbicide applications. Palmer amaranth germination and biomass were assessed 2 wks later. Acetochlor, fomesafen, fluridone, and pendimethalin performance was significantly impacted by irrigation rate with greatest Palmer amaranth germination reductions recorded at 1.91, 0, 0.64, and 1.27 cm water, respectively. With exception of pendimethalin that required 1.27 cm of irrigation to be activated, all other herbicides resulted in significantly reduced Palmer amaranth germination regardless of irrigation rate as compared to the nontreated control. Field studies conducted at three locations in Alabama during 2017 were used to evaluate tolerance of four cotton varieties to fomesafen combinations with acetochlor, diuron, fluridone, and prometryn at one and two times the highest labeled rates. Cotton varieties responded similarly to

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[1] Submitted and under review in Weed Technology

Authors: Frances B Browne, Xiao Li, Katilyn J Price, Ryan Langemeier, J Scott McElroy, Yucheng Feng, Andrew Price herbicide combinations. Cotton height was reduced at one location by 24% 3 weeks after planting (WAP) for applications of fomesafen + prometryn at the 2x rate; however, plants recovered by 7 WAP. No other treatment tested resulted in reduced seedling biomass, height, stand, or yield at any location as compared to the nontreated control. These results suggest cotton safety to the residual herbicide combinations tested in this study up to the 2x rate and activation requirements should be considered when selecting residual herbicide programs in sandy soil for optimal performance.

2.2. Introduction

The addition of residual herbicides into weed management programs can provide flexibility for timely postemergence (POST) applications as weed control can be extended several weeks into the growing season. Residual herbicide efficacy is dependent on incorporation through mechanical means or overhead irrigation into the soil profile where weed seeds germinate (Whitaker et al. 2011; Walker and Roberts 1975). Herbicides with lower solubility may require a higher volume of water to dissolve into the soil solution and migrate through the soil profile to the target zone where they can be available for weed absorption (Smith et al. 2016). Alternatively, excessive rainfall can lead to leaching of residual herbicides away from the target zone, resulting in reduced efficacy (Jhala 2017; Stewart et al. 2012).

Excellent Palmer amaranth control can be achieved through residual herbicide applications when properly activated. Acetochlor, diuron, fomesafen, fluridone, and pendimethalin are commonly applied PRE in cotton production systems in the southeastern United States. Braswell et al. (2016) reported 93% or greater Palmer amaranth control at the 2-leaf cotton stage following applications of acetochlor plus diuron, fomesafen plus diuron, and fluridone. Whitaker et al. (2011) observed 91%, 99%, and 82% Palmer amaranth control 20 days after applications of diuron (1120 g ai ha^{-1}), fomesafen (280 g ai ha^{-1}), and pendimethalin (1064 g ai ha^{-1}), respectively, at a location that received 13 cm of rainfall in the first 10 days. Alternatively, the same treatments applied at locations receiving less than 5 cm of rainfall in the first 10 days resulted in Palmer amaranth control of 55 to 86%, 74 to 89%, and 49 to 73% 20 days after applications of diuron, fomesafen, and pendimethalin, respectively (Whitaker et al. 2011). These data indicate residual herbicides can provide excellent Palmer amaranth control however, adequate rainfall or irrigation is required to ensure efficacy. Smith et al. (2016) observed Palmer amaranth biomass reductions of 97% or greater following fomesafen applications at 280 g ai ha^{-1} regardless of irrigation rate in sandy loam soil. However, irrigation level influenced herbicide efficacy for acetochlor and *S*-metolachlor when applied at 1,267 and 1,424 g ai ha^{-1}, respectively. Precise activation amounts are needed to achieve optimal weed control, reduce irrigation inputs, and predict crop and weed responses when adverse weather conditions occur.

Injury of cotton seedlings from residual herbicides is a concern for producers which is more common in cool, moist soil where cotton development may be delayed (Askew et al. 2002). Occasionally, heavy rainfall in soils with high sand content can cause residual herbicides to move through the soil profile to the region where cotton roots develop, leading to stunting and injury (Kleifield et al. 1988). For example, Main et al (2012) observed 8% cotton stunting when fomesafen PRE was applied at 280 and 420 g ha^{-1} in Georgia where 4 cm of rainfall was received two days after herbicide application, as well as 12 and 23% stand loss at a North Carolina site where over 5 cm of rainfall was received within 5 days after herbicide applications. Research suggests cotton can recover from injury resulted from fomesafen applications (Li et al. 2018a; Chandler and Savage 1980). However, yield may be reduced when stand loss occurs and recovery is likely dependent on cotton vigor (Main et al. 2012; Schrage et al. 2012). Alternatively, some researchers report minimal to no injury following preemergence applications of residual herbicides in a variety of soils with adequate rainfall for activation (Cahoon et al. 2015b; Riar et al. 2011; Faircloth et al. 2001). Cahoon et al. (2015a) considered cotton injury acceptable following applications of microencapsulated acetochlor alone as well as in combination with fomesafen and diuron with less than 10% growth reductions and no yield response. The inconsistency of cotton tolerance to residual herbicides warrants further investigation for responses in different soil types in the Southeast. Newly commercialized cotton varieties may vary in crop response to fomesafen-based mixtures in different environments and will require further evaluation due to different growth habits. Therefore, more information is needed regarding accurate activation of soil herbicides and their potential for crop injury. The objectives of this study were to assess residual herbicide efficacy following activation with different rates of irrigation and evaluate crop safety of commercial cotton varieties to fomesafen-based treatments.

2.3. Materials and methods

2.3.1. Greenhouse experiment

An experiment was conducted during May and June 2019 in a greenhouse at Auburn University in Auburn, AL to evaluate the influence of irrigation rate on residual herbicide control of Palmer amaranth. Surface horizon soil collected from Escambia County, AL (31° 8' 29.652" N 87° 2' 52.296" W, soil texture can be found in Table 2.1.) was air dried and sieved to removed debris and large clumps for use in this study. Round plastic pots with a diameter of 10 cm and a depth of 8 cm were filled with 200 g of soil. Then, a uniform mixture of 0.25 g Palmer amaranth seed (~500 seeds) collected from Henry County, AL and 50 g soil were spread in each pot to ensure even seed distribution in the upper layer. Prior to herbicide applications, 0.25 cm irrigation was applied to moisten soil and Palmer seeds. Herbicide treatments (Table 2.2.) were applied on May 30, 2019 with a CO_2 pressurized sprayer equipped with two TT110025 nozzles (TeeJet Technologies, Wheaton, IL) was calibrated for 187 L ha^{-1} volume output.

Immediately following herbicide applications, pots were placed under different irrigation regimes. Irrigation was applied slowly through overhead nozzles at a rate of 0.32 cm every 30 minutes to accumulate 0, 0.64, 1.27, 1.91 cm of water in each pot. Individual pots were then sealed with plastic wrap for 14 days to prevent desiccation and minimize additional irrigation inputs that can cause herbicide leaching. Palmer seedlings were allowed to grow with only natural daylight and daily temperatures in the greenhouse ranged between 20 and 31°C. At 14 d after treatment (DAT), the number of Palmer amaranth seedlings in each pot was recorded, then above and below ground structures in each pot were weighted.

The experimental design was a six by four factorial. Factor one was residual herbicide treatment (6 levels) and factor two was irrigation rate (4 levels). Each combination of herbicide treatment x irrigation rate had 4 replications (pots) and experiment was repeated twice. Pots were blocked in the greenhouse by irrigation rate for activation. Data were analyzed with PROC GLIMMIX in SAS® 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513). Data were pooled across two experiment runs. Herbicide treatment and irrigation rate were considered fixed effects and experiment run was considered a random variable. Means comparisons were generated with Tukey's Honest Significant Difference test at $P = 0.1$. Weed scientists suggest a zero-tolerance threshold is necessary for Palmer amaranth management (Norsworthy et al. 2014). Cotton has a low tolerance to Palmer amaranth competition early in the season and aggressive measures are needed to reduce the potential for yield loss (Fast et al. 2009;

Morgan et al. 2001; Rowland et al. 1999; MacRae et al. 2013). Therefore, a lower degree of protection was chosen to assess any potential for residual herbicide efficacy to be maximized and reduce the number of Palmer amaranth survivors, providing cotton with a competitive advantage.

2.3.2. Field experiments

Field experiments were conducted at the Brewton Agricultural Research Unit in Escambia County (31° 8' 29.652" N 87° 2' 52.296" W), Wiregrass Research and Extension Center in Henry County (31°21'17.1"N 85°19'35.3"W), and E.V. Smith Research Center in Macon County (32°29'45.6"N 85°53'25.2"W) in Alabama in 2017. Surface horizon soil types and textures can be found in Table 2.1.

Cotton varieties included Deltapine1538B2XF, Deltapine1646BSXF (Deltapine®, Monsanto Co., St. Louis, MO), Phytogen444WRF, and Phytogen490 W3FE (PhytoGen Cottonseed®, Dow AgroSciences, Indianapolis, IN). Cotton was planted at 107,639 seeds per hectare using standard production practice on May 11, 10, and 30 of 2017, then harvested on November 6, October 19, and November 6 in 2017 for Escambia, Henry, and Macon counties, respectively. The experimental design was a randomized complete block in a factorial arrangement replicated four times. Factor one was herbicide treatment (8 levels) and factor two was cotton variety (4 levels). Plot size was 3.6 m wide by 7.6 m long with four rows of cotton. Eight tank-mixtures of soil herbicides were applied PRE at one and two times the highest labeled rates for cotton (Table 2.2.). A CO_2 backpack sprayer equipped with six TT110025 nozzles (TeeJet Technologies, Wheaton, IL) was calibrated for 187 L ha^{-1} and used for all applications. All herbicide treatments were applied immediately after planting within the same day and activated by timely rainfall the next 2-3 days. Standard cotton management practices were followed throughout the season and all plots including NTC were maintained weed free using handweeding and postemergence application of glufosinate + *S*-metolachlor.

Cotton visual injury was estimated on a scale of 0 (no injury) to 100 (complete mortality) and recorded in addition to seedling biomass at 3 wks after planting (WAP). At 3 and 7 WAP, cotton stands were evaluated by counting all plants in two 1-m-long stands randomly selected from the two center rows. Cotton heights were recorded from 10 randomly selected plants in the two center rows of the plots. Seed cotton yield was collected at each location from the two center rows and averaged for statistical analysis.

All data collected were converted to a percentage of nontreated check (NTC) prior to statistical analysis, then subjected to a mixed model analysis of variance through PROC GLIMMIX in SAS® 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513). Treatment, location and variety were considered fixed effects, while block was a random effect, and all interactions were considered. If the interaction was significant, data was analyzed and presented by the fixed effect. Means comparisons were generated using Tukey's honest significant different test with $P = 0.05$.

2.4. Results and discussion

2.4.1. Greenhouse experiment

An interaction was observed between residual herbicide treatment and irrigation rate for Palmer amaranth germination and biomass. Therefore, data was analyzed separately for each herbicide treatment. Further analysis indicated the main effects of both residual herbicide treatment and irrigation level were significant for Palmer amaranth germination and biomass.

Palmer amaranth germination in the NTC decreased as irrigation rate increased with 59, 41, 37, and 34 individuals found in pots received 0, 0.64, 1.27, and 1.91 cm of water, respectively (Table 2.3.). Seedling biomass also reflected germination decreases with increased irrigation, ranging from 2.3 to 0.63 g pot^{-1} (Table 2.4.). These data suggest Palmer amaranth germination and growth may be reduced in water saturated soils, other weeds such as crabgrass (*Digitaria ciliaris*) and annual grasses may be more problematic in that situation (personal observation). However, the soil used in this study was air dried, sieved, and damp prior to irrigation rates and may not be fully representative of a field scenario.

Acetochlor, fomesafen, fluridone, and pendimethalin were significantly impacted by activation irrigation rate (Tables 2.3. and 2.4.). Diuron performance was similar for all irrigation rates tested with germination ranging from 9 to 17 Palmer amaranth seedlings in each treated pot. Applications of acetochlor resulted in the lowest Palmer amaranth germination of 4.88 plants 14 DAT when activated with 1.91 cm of water. However, biomass in acetochlor-treated pots did not differ across irrigation rates. Palmer amaranth germination and biomass increased as irrigation rate increased in fomesafen-treated pots, suggesting possible herbicide leaching. However, seedlings did not exceed 6 individuals or biomass of 0.03 g per pot for all irrigation rates tested (Tables 2.3. and 2.4.). Similarly, Smith et al. (2016) did not observe an effect of irrigation rates of 0 to 12.7 mm following fomesafen applications at 280 g ha^{-1} on Palmer amaranth biomass production which ranged from 0 to 3% of the NTC 35 DAT. Fluridone-treated pots that did not receive activation irrigation had at least 16 more Palmer amaranth seedlings compared to those that received irrigation which ranged from 4 to 12 individuals pot^{-1}. Pendimethalin required at least 1.27 cm of water for activation in this study as the average number of seedlings in pots activated with 0 and 0.64 cm water were 55 and 48 while those receiving 1.27 cm and 1.91 cm of water were 18 and 28. These data suggest fluridone and pendimethalin may have higher irrigation requirements than acetochlor, fomesafen, and diuron due to lower water solubility. However, pendimethalin performance was poor regardless of activation amounts. Pendimethalin control of Palmer amaranth in the literature is variable and not recommended as a sole PRE herbicide treatment (Whitaker et al. 2011). Weed scientists frequently recommend including residual herbicides with at least two modes of action in weed management programs to enhance Palmer amaranth control (Culpepper 2019; Steckel 2019). Cahoon et al (2015a) observed at least 16% higher Palmer amaranth control 20 days after combinations of pendimethalin (1100 g ai ha^{-1}) with diuron (560 g ai ha^{-1}) or fomesafen (175 and 280 g ai ha^{-1}) were applied as compared to pendimethalin alone when adequate rainfall was received. However, too little or excessive rainfall following residual herbicide applications can lead to reduced efficacy of soil herbicides (Whitaker et al. 2011; Culpepper et al. 2007).

Contrary to other herbicides labels for preemergence herbicides that recommend 1.27 cm of irrigation for activation such as diuron, pendimethalin, acetochlor, and fluridone, only 0.64 cm is recommended for fomesafen applications for sufficient weed control which is consistent with the findings of this study (Anonymous 2019; Anonymous 2018a; Anonymous 2018b; Anonymous 2015). The solubility of fomesafen in the sodium salt formulation (Reflex®) is 600,000 mg/L in water at 25C. In comparison, solubility figures for acetochlor, diuron, fluridone, and pendimethalin are 223, 42, 12, and 0.275 mg/L water at 25°C (Shaner 2014). The large difference in herbicide solubility may explain fomesafen performance even in the absence of activation irrigation whereas less soluble herbicides such as fluridone and pendimethalin required at least 0.64 and 1.27 cm of irrigation for activation. These data suggest solubility is a major factor for residual herbicide activation. Additional studies may be warranted in different soil types with varying organic matter.

2.4.2. Field experiments

Field sites in 2017 received adequate rainfall for residual herbicide activation with 14.61, 7.57, and 7.31 cm recorded in the 2 week period following applications for Escambia, Henry, and Macon Counties, respectfully. Location by treatment interactions occurred for cotton height 3 and 7 WAP (Table 2.5.), and cotton stand 3 WAP (Table 2.6.). No treatment by variety interactions were observed for biomass, height, or stand at any observation timing; therefore, data were combined across varieties and are presented for each location.

Early-season cotton injury did not exceed 10% regardless of herbicide treatment or cotton variety (data not shown). These results are consistent with other research where minimal to no cotton injury was observed following residual herbicide applications (Cahoon et al. 2015b; Riar et al. 2011; Faircloth et al. 2001). None of the residual herbicide treatments at 1x or 2x rates resulted in significantly reduced cotton seedling biomass 3 WAP or cotton stand at 3 or 7 WAP compared to the NTC. The 2x rate of fomesafen (562 g ha^{-1}) + prometryn (4480 g ha^{-1}) resulted in 24% cotton height reduction compared to the NTC at 3 WAP in Macon County potentially due to a rainfall on the same day herbicides were applied; however, plants recovered by 7 WAP. Results from the greenhouse section of this study suggest fomesafen is highly mobile in soils and the rainfall event in close proximity after the herbicide application may have caused herbicide leaching to germinating cotton. Several studies have reported the propensity of fomesafen to leach in sandier soils with low organic matter and cotton response is directly related to rainfall or irrigation (Li et al. 2018b; Weber et al. 1993; Costa et al. 2015; Main et al. 2012). Cotton injury up to 13% has been observed 28 DAT following prometryn applications at 800 g ha^{-1} in sandy soils (Keeling and Abernathy 1989). No other herbicide treatment tested in this study significantly impacted cotton height at any location.

None of the treatments resulted in significantly reduced yields as compared to the NTC and seed cotton yields ranged from 2770 to 3027 kg ha^{-1}. These data suggest that fomesafen, acetochlor, diuron, prometryn, and fluridone can be applied with acceptable cotton tolerance and a margin of safety of to 2x rates on sandy loam soils. Research suggests cotton can recover from early season injury (>68%) resulted from residual herbicide applications (Li et al. 2018a; Chandler and Savage 1980; Richardson et al. 2007). However, stand loss resulted from residual herbicides applied PRE can negatively impact cotton yields (Main et al. 2012; Schrage et al. 2012).

Previous literature indicates preemergence residual herbicide programs are critical for management of herbicide-resistant Palmer amaranth (Whitaker et al. 2011; Culpepper et al. 2008; Whitaker et al. 2008). Palmer amaranth control and cotton safety remain top concerns for producers when selecting residual herbicides applied at planting. Fomesafen is known to be one of the most effective preemergence herbicides for control of Palmer amaranth (Whitaker et al. 2011; Whitaker et al. 2010). The results of this study demonstrate fomesafen mixtures with acetochlor, diuron, fluridone, and prometryn can be applied without interfering with cotton growth and development up to two times the highest labeled rates in sandy soils. No cotton variety used in this study showed more sensitive to residual herbicide treatments than others. However, the greenhouse study suggests the level of Palmer amaranth control following residual herbicide applications of acetochlor, fomesafen, fluridone, and pendimethalin can be affected by irrigation amounts for activation. Careful consideration of herbicide activation requirements and herbicide mobility in the soil type in which it is applied will be warranted to optimize efficacy.

2.5. Acknowledgements

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Table 2.1. Soil texture, pH, organic matter, and soil types

Location	Soil Texture (%)			Soil pH	Organic matter (%)	Soil type[A]
	Sand	Silt	Clay			
Escambia County	73	20	7	6.2	2.1	Benndale fine sandy loam[B]
Henry County	82	1	17	6.2	1.2	Dothan fine sandy loam[C]
Macon County	72	11	18	6.1	0.9	Kalmia sandy loam[D]

[A] Soil information was provided by Auburn University Soil Testing Laboratory (Auburn AL).

[B] Coarse-loamy, siliceous, semiactive, thermic Typic Paleudults

[C] Fine-loamy, kaolinitic, thermic Plinthic Kandiudults

[D] Fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Typic Hapludults

Table 2.2. Residual herbicide formulations and rates used for field and greenhouse study

Common name	Trade name	Field Rate	Greenhouse Rate	Manufacturer
		g ai ha^{-1}	g ai ha^{-1}	
Acetochlor	Warrant®	1340	1262	Monsanto Co., St. Louis, MO 63167
		2690	-	
Diuron	Direx 4L®	896	841	Drexel Chemical Co., Memphis, TN 38113
		1790	-	
Fomesafen	Reflex®	280	280	Syngenta Crop Protection, Greensboro, NC 27419
		562	-	
Fomesafen + fluridone	Reflex + Brake	210 + 168	-	SePRO Corporation, Carmel, IN 46032
		420 + 336	-	
Fluridone	Brake®	-	231	SePRO Corporation, Carmel, IN 46032
Pendimethalin	Prowl H_2O®	-	1066	BASF Corporation, Research Triangle Park, NC 27709
Prometryn	Caparol®	2240	-	Syngenta Crop Protection, Greensboro, NC 27419
		4480	-	
Nontreated Check				

Table 2.3. Effect of irrigation rate on Palmer amaranth germination at 14 DAT[A]

		Irrigation Rate (cm)[BC]											
Herbicide	Rate	0.00			0.64			1.27			1.91		
	g ai ha^{-1}	Number plants pot^{-1}											
Acetochlor	1262	11	CD	ab	15	B	a	6	BC	ab	5	C	b
Diuron	841	9	CD	a	8	B	a	7.	BC	a	17	ABC	a
Fomesafen	280	0.13	D	b	0.88	B	ab	2.50	C	ab	5.00	C	a
Fluridone	2321	28	BC	a	3	B	b	8	BC	b	12	BC	ab
Pendimethalin	1066	55	AB	a	47	A	ab	18	B	c	28	AB	bc
Nontreated control	-	59	A	a	41	A	ab	37	A	ab	34	A	b

[A] An herbicide treatment by irrigation rate was observed at *P*=0.1 for Palmer amaranth germination; therefore, data were analyzed and presented separately for each herbicide treatment at each irrigation rate.

[B] Means followed by the same upper case letter in a column do not differ significantly at *P*=0.1 based on Tukey's Honest Significant Difference test.

[C] Means followed by the same lower case letter in a row do not differ significantly at *P*=0.1 based on Tukey's Honest Significant Difference test.

Table 2.4. Effect of irrigation rate on Palmer amaranth biomass at 14 DAT[A]

		Irrigation Rate (cm)[BC]											
Herbicide	Rate	0.00			0.64			1.27			1.91		
	g ai ha^{-1}	No. pot^{-1}											
Acetochlor	1262	0.07	B	a	0.13	B	a	0.16	B	a	0.02	B	a
Diuron	841	0.06	B	a	0.07	B	a	0.07	B	a	0.12	B	a
Fomesafen	280	0.01	B	b	0.01	B	b	0.02	B	ab	0.03	B	a
Fluridone	2321	0.46	B	a	0.03	B	b	0.12	B	b	0.10	B	b
Pendimethalin	1066	1.66	A	a	1.20	A	ab	0.36	B	c	0.57	A	bc
Nontreated control	-	2.30	A	a	1.09	A	b	0.90	A	b	0.63	A	b

[A] An herbicide treatment by irrigation rate was observed at the *P*=0.1 level for Palmer amaranth biomass; therefore, data were analyzed and presented separately for each herbicide treatment at each irrigation rate.

[B] Means followed by the same upper case letter in a column do not differ significantly at *P*=0.1 based on Tukey's Honest Significant Difference test.

[C] Means followed by the same lower case letter in a row do not differ significantly at *P*=0.1 based on Tukey's Honest Significant Difference test.

Table 2.5. Cotton stand 3 and 7 WAP as influenced by residual herbicide treatment[A]

Treatment	Rate	3 WAP[BC]			7 WAP[D]
		Escambia	Henry	Macon	
		---% NTC-------------------------------			
Fomesafen + acetochlor	280 + 1340	101 a	98 abc	105 a	97 a
Fomesafen + acetochlor	562 + 2690	99 a	97 abc	90 a	97 a
Fomesafen + diuron	280 + 896	101 a	101 abc	106 a	97 a
Fomesafen + diuron	562 + 1790	98 a	107 ab	87 a	96 a
Fomesafen + prometryn	280 + 2240	105 a	95 c	99 a	100 a
Fomesafen + prometryn	562 + 4480	96 a	106 ab	89 a	93 a
Fomesafen + fluridone	210 + 168	102 a	116 a	100 a	100 a
Fomesafen + fluridone	420 + 336	99 a	91 bc	91 a	91 a
Nontreated	-	100 a	100 abc	100 a	100 a

[A] Means followed by the same letter in a column do not differ significantly based on *P* = 0.05 probability level as determined by Tukey's honest significant difference test.

[B] An herbicide treatment by location interaction was observed 3 WAP at the *P* = 0.05 level; therefore, data were analyzed and presented separately for each location.

[C] No treatment by variety interactions were observed for cotton stand 3 WAP at the *P* = 0.05 level; therefore data were combined across varieties for each location.

[D] No treatment by variety or treatment by location interactions were observed 7 WAP at the *P* = 0.05 level; therefore data were combined across varieties and locations to show treatment effect.

Table 2.6. Cotton height 3 and 7 WAP as influenced by residual herbicide treatment

		3 WAP			7 WAP		
Treatment	Rate	Escambia	Henry	Macon	Escambia	Henry	Macon
				% NTC[ABC]			
Fomesafen + acetochlor	280 + 1340	100 ab	98 a	89 bc	94 b	105 a	102 bc
Fomesafen + acetochlor	562 + 2690	98 b	89 a	103 ab	94 b	94 a	119 abc
Fomesafen + diuron	280 + 896	108 a	97 a	110 a	105 a	103 a	119 abc
Fomesafen + diuron	562 + 1790	99 ab	96 a	95 abc	96 ab	95 a	106 bc
Fomesafen + prometryn	280 + 2240	101 ab	100 a	104 ab	104 a	101 a	146 a
Fomesafen + prometryn	562 + 4480	97 b	95 a	76 c	96 ab	104 a	104 bc
Fomesafen + fluridone	210 + 168	98 ab	92 a	102 ab	100 ab	106 a	95 c
Fomesafen + fluridone	420 + 336	103 ab	93 a	90 bc	102 ab	93 a	129 ab
Nontreated	-	100 ab	100 a	100 ab	100 ab	100 a	100 bc

[A] Means followed by the same letter in a column do not differ significantly based on *P* = 0.05 probability level as determined by Tukey’s honest significant difference test.

[B] An herbicide treatment by location interaction was observed 3 WAP at the P = 0.05 level; therefore, data were analyzed and presented separately for each location.

[C] No treatment by variety interactions were observed for cotton height 3 or 7 WAP at the P = 0.05 level; therefore data were combined across varieties for each location to show treatment effect.

CHAPTER 3

Sequential Applications of Synthetic Auxins and Glufosinate for Escaped Palmer amaranth (*Amaranthus palmeri*) Control[2]

3.1. Abstract

Field and greenhouse studies were conducted to investigate the influence of sequence and timing of synthetic auxins and glufosinate on large Palmer amaranth (*Amaranthus palmeri*) control. Field studies were performed in Henry County, AL where treatments were applied to Palmer amaranth with average heights of 37 and 59 cm in 2018 and 2019, respectively. Sequential applications of 2,4-D/dicamba + glyphosate followed by (fb) glufosinate at full label rates 3 or 7 d after initial treatment (DAIT) were used in addition to the reverse sequence with a 7 d interval. Time intervals of 3 or 7 d between applications did not influence Palmer amaranth control. Palmer amaranth was controlled 100% for applications of dicamba + glyphosate fb glufosinate and 2,4-D + glufosinate fb glufosinate 7 DAIT in 2018. However, herbicide performance was reduced due to extended drought conditions and taller plants in 2019 with up to 23% less visual injury. In order to further investigate Palmer amaranth response to dicamba and

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Authors: Frances B Browne, Xiao Li, Katilyn J Price, Ryan Langemeier, Alvaro Sanz-Saez; J Scott McElroy, Yucheng Feng, Andrew Price

glufosinate applied sequentially, a greenhouse study was conducted in 2019 where physiological measurements were recorded over a 35 day period. Treatments were applied to Palmer amaranth averaging 38 cm tall and included dicamba + glyphosate fb glufosinate 7 DAIT, the reverse sequence, and a single application of dicamba + glufosinate + glyphosate. Glufosinate severely inhibited mid-day photosynthesis as compared to dicamba with up to 90% reductions in CO_2 assimilation 1 DAIT. In general, Palmer amaranth respiration and stomatal conductance were not affected by herbicides in this study. Applications of dicamba + glyphosate fb glufosinate 7 DAIT was the only treatment shown to hinder Palmer amaranth regrowth with a 52% reduction in leaf biomass as compared to the nontreated control. These data suggest Palmer amaranth infested fields are more likely to be rescued with synthetic auxins fb glufosinate than the reverse order but consistent control of large Palmer is not probable.

3.2. Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats) is a pest that remains at the center of row crop management concerns due to rapid growth and constant evolution of herbicide resistance. The dioecious growth habit and high fecundity associated with this weed are major factors responsible for the rate at which herbicide resistant populations have evolved and spread (Korres et al. 2018; Franssen et al. 2001; Ward et al. 2013). Low Palmer amaranth density can lead to yield loss, reduced harvest efficiency, and accumulation of seeds in the soil (Smith et al. 2000; MacRae et al. 2008; Morgan et al. 2001). If producers do not use preemergence herbicides or adequate rainfall does not occur soon after application for activation in dryland systems, escapes will need to be controlled through postemergence (POST) herbicide applications. POST control of Palmer amaranth is time sensitive and adverse weather conditions, unforeseen equipment failure, or inadequate coverage due to poor nozzle selection can allow plants to quickly exceed optimal heights for control. Under such circumstances, producers often seek out rescue herbicide programs to control large escapes and prevent crop loss.

Large plant height at maturity, high water use efficiency, drought tolerance mechanisms, and a C4 photosynthetic pathway provide Palmer amaranth with a competitive advantage over cotton (*Gossypium hirsutum* L.) and soybeans (*Glycine max* L.) which are C3 plants (Horak and Loughlin 2000; Culpepper et al. 2010; Ward et al. 2013; Sellers et al. 2003). Palmer amaranth density can significantly impact cotton and soybean canopy width and further impede crop competitiveness (Klingaman and Oliver 1994; Morgan et al. 2001). Klingaman and Oliver (1994) reported soybean width reductions of 54% twelve weeks after emergence (WAE) with 10 Palmer amaranth per 1 m of crop row. Similarly, Morgan et al. (2001) reported 45% cotton canopy volume reductions 10 WAE with 1 to 10 Palmer amaranth per 9.1 m of row. Significant yield losses of 92 and 78% have been reported for cotton and soybean, respectively, when grown with Palmer amaranth at densities of eight plants per 1 m of row (Rowland et al. 1999; Bensch et al. 2003). Norsworthy et al. (2014) reported, 20,000 Palmer amaranth seed spread in a 1 m^2 area resulted in complete cotton crop failure due to high infestations three years later. A single Palmer amaranth female is capable of producing up to 1 million seeds, indicating one escape could significantly impact cropping systems in a relatively short period of time (Keeley 1987; Norsworthy et al. 2014). These data support the adoption of a zero-tolerance threshold and aggressive measures are needed to prevent Palmer amaranth plants from reaching reproductive maturity.

Increasing infestations of herbicide-resistant Palmer amaranth have driven producers to seek alternative means for control. Recently commercialized transgenic cotton and soybean varieties with tolerance to 2,4-D or dicamba and glufosinate can provide new tools for Palmer amaranth control during the growing season. To date, glufosinate resistance has not been confirmed in Palmer amaranth populations, thus this herbicide remains a viable herbicide option for control when applied according to label recommendations (Heap 2019; Anonymous 2016). Palmer amaranth resistance to auxin herbicides has been reported (Heap 2019; Tehranchian et al. 2017); however, populations appear to be isolated at this point and research suggests both 2,4-D and dicamba remain effective options for control for most of row crop growers in the US (Inman et al. 2016; Merchant et al. 2014; Lawrence et al. 2018).

Aggressive growth of Palmer amaranth combined with adverse weather conditions can complicate POST applications and growers will often resort to rescue herbicide programs (Vann et al. 2017a; Barnett et al. 2013; Corbett et al. 2004; Merchant et al. 2014). Combinations of glufosinate and synthetic auxins have been shown to be more effective on control of larger weeds than when applied alone (Merchant et al. 2013; Craigmyle et al. 2013; Vann et al. 2017a, 2017b; Merchant et al. 2014; Cuvaca et al. 2019). Merchant et al. (2013) observed up to 22%, 17%, and 11% greater Palmer amaranth control 20 d after application when glufosinate at 431 g ai ha^{-1} was tank mixed with 2,4-DB, 2,4-D, or dicamba than when applied alone at 1120, 1064, and 1120 g ai ha^{-1}, respectively. Additionally, sequential applications of postemergence herbicides are more likely to control Palmer amaranth than one-time applications (Coetzer et al. 2002). Coetzer et al. reported 80% and 55% Palmer amaranth population size reductions following sequential applications of glufosinate at 410 and 293 g ai ha^{-1}, respectively, whereas single applications did not reduce population size relative to the nontreated control.

Current dicamba labels do not permit tank mixtures with glufosinate due to volatility concerns (Anonymous 2018a, Anonymous 2018b, Anonymous 2019). Therefore, sequential applications will be required when both herbicides are utilized in a weed management program.

New formulations of 2,4-D choline (Enlist One®, Dow AgroSciences, Indianapolis, IN 46268) will allow tank mixtures with glufosinate which could provide more flexibility in POST herbicide treatments (Anonymous 2019). Control of Palmer amaranth is likely to be influenced by the combination and sequence of synthetic auxins and glufosinate in addition to the time intervals between applications. Therefore, the objective of this study was to evaluate sequential applications of synthetic auxins and glufosinate at different sequences and time intervals for Palmer amaranth control in a rescue scenario.

3.3. Materials and methods

3.3.1. Field Studies

Two non-crop studies were conducted at the Wiregrass Research and Extension Center in Henry County, AL (31°21'17.1"N 85°19'35.3"W) during summers of 2018 and 2019 in irrigated field with high Palmer amaranth pressure. The site included natural and augmented Palmer amaranth populations planted at 10 seeds m^{-2} on June 6 and May 10 in 2018 and 2019, respectively. Prior to seeding, the study area was disked thoroughly to remove existing weeds, then field cultivated to ensure smooth soil surface. Artificial population was established by seeds sourced from peanut and cotton fields in Alabama with known glyphosate and ALS-inhibitor resistance. The experimental design was a randomized complete block with four replications. Plot size was 3.3 m long by 3.3 m wide. Herbicide treatments and rates can be found in Table 3.1 and consisted of combinations of 2,4-D choline (Enlist One®; Dow AgroSciences LLC, Indianapolis, IN 46268) or diglycolamine salt of dicamba (Xtendimax® with Vaporgrip®; Monsanto Co. St. Louis, MO 63167), glufosinate (Liberty® 280 SL; BASF Corporation, Research Triangle Park, NC 27709), and glyphosate (Roundup PowerMax®; Monsanto Co. St. Louis, MO 63167) at 1066, 559, 594, and 1543 g ai ha^{-1}. Initial herbicide applications were performed on July 6 and June 12 in 2018. A blanket application of *S*-metolachlor (Dual Magnum®; Syngenta Crop Protection, LLC, Greensboro, NC 67419) was applied once 7 days after initial herbicides at 1,469 g ai ha^{-1} to limit Palmer amaranth seed germination later in the study, thus confounding ratings and data collection. Herbicides were applied to Palmer amaranth with average height of 37 and 59 cm tall in 2018 and 2019, respectively. All herbicides were applied with a CO_2-pressurized backpack sprayer equipped with four TeeJet nozzles (TeeJet Technologies, Wheaton, IL 60187) delivering 187 L ha^{-1}. Turbo TeeJet induction flat spray tips (TTI110025, TeeJet Technologies, Wheaton, IL 60187) were used for treatments that included synthetic auxins and Turbo TeeJet wide angle flat fan spray tips (TT110025, TeeJet Technologies, Wheaton, IL 60187) were used for glufosinate applications.

Palmer amaranth injury was visually estimated at 14 and 28 DAIT on a scale of 0 (no injury) to 100 (complete mortality). At 35 DAIT, ten Palmer amaranth heights were recorded randomly in each plot by measuring living individuals from the ground to the top of the plant. Palmer amaranth in each plot were cut at ground level 35 DAIT and immediately weighted to determine fresh biomass. Plants were only harvested from the middle area (2.4 x 2.4 m) in each plot to ensure adequate herbicide coverage was received.

Data were subjected to a mixed model analysis of variance through PROC GLIMMIX in SAS 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513). Treatment and year were considered fixed effects, while block was a random effect and all interactions were examined. If treatment by year interactions were observed, data was analyzed separately to show individual effects for each year. Means comparisons were generated using Tukey's Honest Significant (HSD) different test with $P = 0.05$.

3.3.2. Greenhouse Study

In order to better understand Palmer amaranth physiological response to synthetic auxins and glufosinate, an experiment was conducted in a greenhouse with natural daylight at Auburn University in Auburn, AL during June of 2019. Temperatures ranged between 20 and 31°C throughout the course of the study. Glyphosate and ALS-inhibitor resistant Palmer amaranth seed collected from Headland, AL were planted in 4 L pots filled with commercial potting soil (Miracle-Gro® Moisture Control® Potting Mix, The Scotts Company LLC, Marysville, OH 43040). Plants were thinned to two individuals per pot and grown until reaching 30 to 45 cm in height with irrigation and fertilizer applied as needed. One leaf was tagged on each plant, representing a young fully developed leaf (3-4 node position from the tip), two per pot, to ensure the same tissue was analyzed throughout the study.

The experimental design was a randomized complete block with three replications in two separate runs. In addition to a nontreated control, treatments included a tank mixture of dicamba + glufosinate + glyphosate, dicamba + glyphosate fb glufosinate 7 DAIT, and glufosinate fb dicamba + glyphosate 7 DAIT. Reduced herbicide rates relative to the field study were chosen to allow Palmer amaranth survival and enable for physiological measurements up to 35 d after initial treatments. Dicamba, glufosinate, and glyphosate were applied at 186, 198, and 514 g ai ha^{-1}. Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with two flat fan 110025 nozzles delivering 187 L ha^{-1}. Dicamba was applied with Turbo TeeJet induction flat fan nozzles (TTI110025, TeeJet Technologies, Wheaton, IL 60187) and glufosinate with Turbo TeeJet wide angle flat fan nozzles (TT110025, TeeJet Technologies, Wheaton, IL 60187).

Physiological measurements were recorded at 1, 3, 6, 8, 11, 13, and 35 DAIT. Measurements included mid-day photosynthesis, leaf stomatal conductance, and respiration, collected with a LI-6400XT (LI-COR Biosciences, Lincoln, NE, USA 68504). Before each midday photosynthesis measurements, light intensity in the greenhouse was recorded by a photosynthetic photon flux density meter (LI-190; LICOR Biosciences, Lincoln, NE, USA), temperature was monitored by an onsite weather station, and relative humidity was maintained between 60% and 70%. Conditions in the leaf cuvette were then set to match ambient environmental conditions, with the [CO_2] in the cuvette set to match ambient [CO2] (~410 mg L^{-1}). The methodology for respiration measurements was the same but the light intensity was set to 0 μmol mol^{-1}. Photosystem II (PSII) quantum yield was recorded with a portable fluorometer (FluorPen FP 100, Photon Systems Instruments, Albuquerque, NM, USA 87106). Photosynthesis and stomatal conductance measurements were recorded at solar noon (11:00-13:00) while respiration and PSII quantum yield measurements were collected from dark-adapted plants two hours after sunset. At the end of physiological measurements, all Palmer amaranth leaves were removed at the petiole base 14 DAIT, fresh weight was recorded, then leaves were processed through a LI-3100C area meter (LI-COR Biosciences, Lincoln, NE, USA 68504) to determine total leaf area. Palmer amaranth bare stalks were allowed to resume growth until 35 DAIT when photosynthesis, respiration and fluorescence measurements were repeated as indicated above, and leaves were removed at the petiole base once more and weighted.

Each physiological measurement was averaged across the two tagged leaves per pot and data was subjected to a mixed model analysis of variance through PROC GLIMMIX in SAS® 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513) with treatment considered as a fixed effect. Data were pooled across experiment repetitions which was considered a random variable. Scatter plots were generated in Sigmaplot 13.0 (Systat Software, San Jose, CA 95131) and means comparisons were generated using Tukey’s HSD test with P = 0.05.

3.4. Results and discussion

3.4.1. Field study

3.4.1.1. 2,4-D choline-based programs.

A year by treatment interaction was observed for Palmer amaranth control 14 DAIT, height, and biomass for 2,4-D based programs. No interaction was observed for Palmer amaranth control 28 DAIT; therefore, data were combined across years (Table 3.2. and 3.3.). All 2,4-D choline and glufosinate based treatments provided greater than 90% Palmer amaranth control 14 DAIT in 2018 with exception of 2,4-D + glufosinate fb 2,4-D + glyphosate 7 DAIT and glufosinate fb 2,4-D + glyphosate 7 DAIT which provided 84 and 74% control, respectively (Table 3.2.). Although applications of 2,4-D + glyphosate fb glufosinate 7 DAIT provided 97% control 14 DAIT in 2018, only 74% control was observed in 2019 which was significantly lower than other treatments. All other 2,4-D choline and glufosinate based treatments provided greater than 90% control 14 DAIT in 2019.

All 2,4-D and glufosinate combinations where 2,4-D was included in initial applications provided statistically similar control 28 DAIT, ranging from 80 to 93%. In comparison, sequential applications of glufosinate or glufosinate fb 2,4-D + glyphosate resulted in significantly lower control 28 DAIT of 68 and 76%, respectively. Time intervals of 3 and 7 d did not influence the level of control of 2,4-D + glyphosate fb glufosinate at 14 and 28 DAIT. Similar to this study, Merchant et al. (2014) observed poor Palmer amaranth control at cotton layby (79%) with sequential glufosinate applications at 471 g ai ha^{-1} spaced 15 d apart but reported at least 95% control when 2,4-D at 1,120 g ai ha^{-1} was mixed with glufosinate at each application. Craigmyle et al. (2013) also reported 19% higher control of *Amaranthus* sp. 30 to 35 cm tall with sequential applications of 2,4-D + glufosinate at 1.12 and 0.45 kg ai ha^{-1}, respectively, as opposed to glufosinate alone. Data from these studies suggested sequential applications of glufosinate alone or glufosinate applied before 2,4-D are not sufficient for controlling large Palmer amaranth. Greater Palmer control was achieved by either applying 2,4-D before glufosinate, or by applying both 2,4-D and glufosinate in a tank mixture fb either 2,4-D or glufosinate or combination of the two 3 or 7 days later.

With exception of 2,4-D + glufosinate fb glufosinate 7 DAIT which resulted in 100% control in 2018, Palmer amaranth height was similar for all other treatments in 2018 and 2019 (Table 3.3.). All treatments resulted in significantly reduced plant height and biomass relative to the nontreated control for 2018 and 2019. Due to greater Palmer amaranth size variation among individual plots, herbicide treatments did not differ significantly in terms of Palmer amaranth biomass in 2018. Biomass in 2018 ranged from 0 to 2972 kg ha^{-1} for treated plots. Treatments of 2,4-D + glufosinate fb glufosinate and sequential application of 2,4-D and glufosinate tank mixtures resulted in the greatest reductions of biomass and height in 2018 as compared to the nontreated control. Applications of 2,4-D + glyphosate fb glufosinate 3 DAIT and 2,4-D + glufosinate fb 2,4-D + glufosinate produced the lowest Palmer height and biomass, respectively, in 2019. Poor Palmer amaranth control in 2019 was observed in terms of biomass as all treatments resulted in 1,250 kg ha^{-1} or greater biomass, due to extended drought in summer of 2019 and larger size of Palmer at herbicide application.

3.4.1.2. Dicamba-based programs.

No treatment by year interaction was observed for Palmer amaranth control 14 DAIT. However, a treatment by year interaction was observed for control 28 DAIT, height, and biomass at $P = 0.05$. Therefore, data was presented by year for those datasets. Glufosinate applied sequentially provided statistically similar control 14 DAIT as dicamba + glyphosate fb glufosinate 3 or 7 DAIT and dicamba + glyphosate fb dicamba + glyphosate 7 DAIT (Table 3.4.). Palmer amaranth control of these dicamba based programs ranged from 79 to 94%. Glufosinate applied 7 d before dicamba + glyphosate resulted in the lowest Palmer amaranth control 14 DAIT at 77%. Sequential applications of dicamba + glyphosate fb glufosinate provided at least 87% control 14 DAIT regardless of the time interval tested. Treatments of dicamba + glyphosate fb glufosinate 7 DAIT resulted in complete mortality by 28 DAIT whereas the reverse sequence resulted in 70% control in 2018. However, control at 28 DAIT was variable among years and all dicamba based programs performed similarly in 2019. Programs of glufosinate fb glufosinate or glufosinate fb dicamba + glyphosate 7 DAIT did not adequately control Palmer amaranth 28 DAIT in either year with ratings of 78 and 57% in 2018 and 2019, respectively. Dicamba + glyphosate fb either glufosinate 3 DAIT or dicamba + glyphosate again 7 DAIT produced better Palmer control (32 and 34 % respectively) than glufosinate fb glufosinate treatment at 28 DAIT in 2019. Randall et al. (2020) observed less than 90% Palmer amaranth control with sequential glufosinate applications at 660 g ai ha^{-1} spaced 10 to 14 d apart which resulted in 20,000 to 27,000 survivors ha^{-1}. These data suggest multiple herbicide modes of action should be considered to reduce the number of Palmer amaranth escapes and increase control efficacy on large Palmer amaranth as glufosinate alone can be unreliable when targeting larger weeds.

All dicamba programs resulted in significantly reduced Palmer amaranth height as compared to the nontreated control in 2018 and 2019 (Table 3.5.). All herbicide treatments resulted in plants at least 106 cm shorter than those in the nontreated control in 2018 with applications of dicamba + glyphosate fb glufosinate 7 DAIT resulting in complete mortality and no plant height could be measured. The greatest height reductions in 2019 were recorded in plots treated with dicamba + glyphosate fb glufosinate 3 DAIT and dicamba + glyphosate fb dicamba + glyphosate 7 DAIT, which were 100 and 101 cm lower than nontreated control respectively. Biomass reductions produced by dicamba + glyphosate fb glufosinate 7 DAIT did not differ significantly from the other treatments, although total control was observed with this treatment in 2018. All other treatments reduced biomass by at least 10,000 kg ha^{-1} relative to nontreated control in 2018. With exception of glufosinate fb dicamba + glyphosate 7 DAIT, all treatments were lower than 1,000 kg ha^{-1}. The same level of performance was not observed in 2019 and all treatments produced statistically similar amount of biomass ranged from 3,265 to 5,920 kg ha^{-1}. Sequential applications where dicamba + glyphosate was applied before glufosinate resulted in Palmer amaranth biomass of 0 to 403 and 3784 to 5920 kg ha^{-1} in 2018 and 2019, respectively, which were not significantly better than glufosinate sprayed twice or glufosinate fb dicamba + glyphosate. Vann et al. (2017a) demonstrated that salvage programs can be effective in Xtendflex cotton^{TM} (Monsanto Co, St. Louis, MO 63167) with sequential applications of dicamba + glufosinate combined with a layby application of diuron + MSMA which provided 94 to 99% control of Palmer amaranth, ranging in heights of 7 to 71 cm tall at the initial application. However, current labels do not allow dicamba and glufosinate tank mixtures and sequential applications will be the only option to utilize both herbicides in dicamba tolerant crops. These data suggest that dicamba applied before glufosinate may be more effective to control large Palmer amaranth than the reverse sequence. However, glufosinate fb dicamba programs and glufosinate fb glufosinate can still be considered viable options if weather conditions do not permit dicamba to be applied first. Based on data generated from this study, consistent control of large Palmer amaranth is not guaranteed in either dicamba or 2,4-D resistant crops in rescue situations, and herbicide efficacy in rescue situations is subject to plant height and abiotic influences, thus may vary each year.

Although new technology is available allowing broadcast applications of synthetic auxins and glufosinate in cotton and soybeans during the growing season, Palmer amaranth control can be variable among years and locations (Merchant et al. 2013; Merchant et al. 2014). Herbicide efficacy can be significantly impacted by Palmer amaranth size, maturity, and growing conditions (Walker and Oliver 2008; Corbett et al. 2004; Meyer 2019; Cuvaca et al. 2019). Height and biomass of Palmer amaranth in this study was similar in nontreated control plots among years with heights averaging from 133 and 135 cm and biomass of 13,569 and 10,175 kg ha^{-1} at maturity in 2018 and 2019, respectively. However, herbicide performance was highly variable between years as Palmer amaranth heights at initial application ranged from 0 to 39 and 34 to 52 cm for all treatments in 2018 and 2019, respectively. These observations suggest plants received adequate time to reach maturity before collecting height and biomass data. Palmer amaranth height at initial applications was likely the greatest factor to influence the level of control observed. Palmer amaranth heights at initial applications had a greater impact on efficacy of dicamba-based programs than 2,4-D choline-based programs. In 2019, unacceptable weather conditions prior to initial herbicide applications resulted in a substantial delay and allowed for additional Palmer amaranth growth. This occurrence is a frequent challenge for producers and plants can quickly exceed optimal heights for control with growth rates reported up to 5 cm per day (Horak and Loughlin 2000; Culpepper et al. 2010).

Taller, more mature plants with a larger canopy can reduce herbicide coverage of lower leaves, potentially impacting glufosinate efficacy. Glufosinate is a contact herbicide which requires adequate coverage and absorption to be effective (Shaner 2014). For these reasons, glufosinate applications are only recommended for plants smaller than 7.5 cm tall are not as effective on larger Palmer amaranth (Culpepper 2016; York 2017; Everman et al. 2007; Coetzer et al. 2002; Anonymous 2016). Similarly, Cuvaca et al. (2019) observed 7% reductions in dicamba absorption and 15% lower translocation in Palmer amaranth 30 cm tall as compared to 10 cm tall. Dicamba and 2,4-D are systemic herbicides and efficacy is dependent on translocation within the plant (Shaner 2014). Larger plants may have thicker plant cuticles which could reduce herbicide penetration resulting in poor control (Oosterhuis et al. 1991; Coetzer et al. 2002; Culpepper 2016). Furthermore, mature plants have reduced sugar transport which may decrease translocation of some systemic herbicides (Cuvaca et al. 2019; Kirkwood 1999; Lemoine et al. 2013).

Herbicide efficacy in the 2019 experiment may have also been impacted by environmental conditions in this study. Rainfall was more than sufficient during the 2018 study with normal temperatures for June and July. However, extended periods of drought were observed during the 2019 experiment when rainfall did not occur through 24 consecutive days following tillage and seeding Palmer amaranth as artificial population. Overhead irrigation was used in three events, spaced evenly during this time, where 1.27 cm water was applied each time to alleviate drought stress. However, the test area in 2019 still remained dry throughout the study. Palmer amaranth drought tolerance has been widely studied and plants have been known to develop thicker cuticles and slow biosynthetic processes during periods of stress which could interfere with herbicide penetration and activity (Horak and Loughlin 2000). It is reasonable to assume differential treatment efficacy among years was likely due to the combined effects of taller plants and drought conditions in 2019.

3.4.2. Greenhouse study

Glufosinate applications severely inhibited Palmer amaranth photosynthesis as compared to dicamba (Table 3.6.). Treatments in which glufosinate was applied initially reduced mid-day photosynthesis at least 90% 1 DAIT whereas dicamba applied initially only reduced photosynthesis 22%. Photosynthetic rates improved over time and were not significantly different from the nontreated control by 8 DAIT. Dicamba + glyphosate fb glufosinate reduced photosynthesis by 84% compared to nontreated control 8 DAIT whereas glufosinate fb dicamba + glyphosate was similar to the nontreated control at that timing. No treatment differences in photosynthetic rates were observed 13 DAIT or in regenerated tissue 35 DAIT. Quantum yield of PSII followed similar trends as mid-day photosynthesis with the greatest immediate reductions observed for glufosinate applications, whether applied before, after, or tank mixed with dicamba + glyphosate. No statistical differences were observed for quantum yield of PSII for any treatment at and after 13 DAIT, suggesting PSII has returned to normal function. Similar to the mid-day photosynthesis measurements, the only treatment that resulted in reduced PSII quantum yield 11 DAIT was dicamba + glyphosate fb glufosinate 7 DAIT, potentially indicating slower Palmer amaranth recovery from this treatment. .

Leaf stomatal conductance and photosynthesis were variable in the nontreated control at different measurement timings, likely impacted by daily changes in temperature and humidity (Table 3.6.). Treated Palmer amaranth did not result in reduced stomatal conductance relative to the nontreated control with exception of 1 DAIT where reductions of 67, 46, and 89% were observed for three-way mix, dicamba + glyphosate fb glufosinate, and glufosinate fb dicamba + glyphosate treatments, respectively. Similar to mid-day photosynthetic observations 1 DAIT, treatments with glufosinate applied first inhibited stomatal conductance at a greater magnitude as compared to dicamba + glyphosate applied first. Respiration measurements were likely confounded by tissue degradation resulted from glufosinate applications. Glufosinate is a contact herbicide that causes rapid defoliation which may have resulted in an excess of carbon dioxide release as opposed to carbon dioxide flux due to respiration (Shaner 2014). This would explain the more negative respiration values observed 1 DAIT from glufosinate treatments as opposed to dicamba which is a synthetic auxin and does not induce rapid defoliation. Ammonium accumulation and stomatal closure have been suggested to cause rapid photosynthetic inhibition in Palmer amaranth treated with glufosinate in as little as 30 minutes after application (Coetzer and Al.-Khatib 2001). However, the results of this study suggest stomatal conductance was not severely impacted by dicamba + glyphosate and glufosinate applied sequentially or in tank mixtures. The possibility exists that the combination of two herbicides with distinctly different modes of action may have interfered with transpiration processes.

Regrowth of Palmer amaranth is often a major concern associated with glufosinate applications as glufosinate does not prevent weed regrowth (Coetzer et al. 2002; Vann et al. 2017b). All treatments in this study resulted in similar and significantly lower leaf biomass and area 14 DAIT as compared to the nontreated control (Table 3.7.). When plants were allowed to continue growth over a three week period following leaf removal 14 DAIT, the only treatment that resulted in significantly lower leaf biomass from growth was dicamba + glyphosate fb glufosinate 7 DAIT. These data suggest Palmer amaranth recovery may be impaired by application of dicamba + glyphosate fb glufosinate as compared to the reverse sequence or tank mixture, which has valuable implication for Palmer amaranth management in field. Coetzer et al. (2001) showed that less than 2% of glufosinate is translocated outside of the leaf where it was applied. This observation illustrates the need for adequate coverage when applying glufosinate to larger plants with sizable canopy. Such a scenario may explain the lack of control 28 DAIT observed in the field for sequential glufosinate applications by itself, where rapid defoliation occurred but glufosinate was unable to kill large Palmer stem due to lack of coverage, thus allowing plant to regrow and recover from injury.

Incorporation of multiple modes of action into herbicide programs is frequently recommended by weed scientists to reduce Palmer amaranth survivors and delay evolution of herbicide resistance (Norsworthy et al. 2012; Culpepper and Vance 2019; Culpepper et al. 2010). Results of this study suggest multiple applications of POST herbicides with different modes of action may increase control of large Palmer amaranth. Sequential applications of synthetic auxins fb glufosinate are more likely to rescue Palmer amaranth infested fields as opposed to the reverse sequence. Glufosinate alone applied sequentially did not adequately control large Palmer amaranth in either year of this study. Although some combinations of sequential applications of synthetic auxins and glufosinate were effective, herbicide applications to large Palmer amaranth (>10 cm) is not recommended and timely applications of POST herbicides remain the most effective approach. Although results found in this study indicate sequential applications of 2,4-D or dicamba fb glufosinate or tank mixtures of 2,4-D and glufosinate may have potential to rescue infested fields, Palmer amaranth control was not consistent among years with very different growing conditions, suggesting rescue practice should be avoided if possible. If salvage programs are utilized and fail, manual removal is required to limit seed return to the soil seed bank and reduce the risks of herbicide resistance development.

3.5. Acknowledgements

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Table 3.1. Herbicide treatments used in field study[ABC]

POST-1	POST-2	POST-2 Timing DAIT[D]
2,4-D + glyphosate	glufosinate	3
2,4-D + glyphosate	glufosinate	7
2,4-D + glufosinate	glufosinate	7
2,4-D + glufosinate	2,4-D + glufosinate	7
2,4-D + glufosinate	2,4-D + glyphosate	7
Glufosinate	2,4-D + glyphosate	7
Dicamba + glyphosate	glufosinate	3
Dicamba + glyphosate	glufosinate	7
Dicamba + glyphosate	dicamba + glyphosate	7
Glufosinate	dicamba + glyphosate	7
Glufosinate	glufosinate	7

[A] 2,4-D, dicamba, glufosinate and glyphosate were applied at 1066, 559, 594, and 1543 g ai ha^{-1}.

[B] All herbicide treatments were tank mixed with a water conditioning agent/nonionic surfactant blend at 1% v/v (Class Act® Ridion®, Winfield Solutions, LLC, St. Paul, MN 55164).

[C] Dicamba treatments were tank mixed with a drift reduction agent at 0.5% v/v (Intact, Precision Laboratories, LLC, Waukegan, IL 60085).

[D] Abbreviation: DAIT, days after initial treatment.

Table 3.2. Palmer amaranth control as affected by sequential applications of 2,4-D and glufosinate

Treatments[A][B]			Control[D][E] (%)					
			14 DAIT[F]				28 DAIT[G]	
POST-1	POST-2	POST-2 Timing (DAIT)[C]	2018		2019		2018-2019	
2,4-D + glyphosate	glufosinate	3	94	ab	94	a	93	a
2,4-D + glyphosate	glufosinate	7	97	a	74	b	86	ab
2,4-D + glufosinate	glufosinate	7	100	a	95	a	84	ab
2,4-D + glufosinate	2,4-D + glufosinate	7	96	a	96	a	93	a
2,4-D + glufosinate	2,4-D + glyphosate	7	84	bc	93	a	80	abc
Glufosinate	2,4-D + glyphosate	7	74	c	96	a	76	bc
Glufosinate	glufosinate	7	91	ab	97	a	68	c

[A] 2,4-D, glufosinate and glyphosate were applied at 1066, 594, and 1543 g ai ha^{-1}.

[B] All treatments included a water conditioning agent/nonionic surfactant blend at 1% v/v (Class Act® Ridion®, Winfield Solutions, LLC, St. Paul, MN 55164).

[C] Abbreviation: DAIT, days after initial treatment.

[D] Visual injury estimated on a scale of 0% (no injury) to 100% (complete mortality).

[E] Means within a column followed by the same letter do not differ significantly at $P = 0.05$ based on Tukey’s HSD.

[F] A treatment by year interaction was observed for Palmer amaranth control 14 DAIT at $P = 0.05$; therefore, data were analyzed and presented separately for each year.

[G] No treatment by year interaction was observed for Palmer amaranth control 28 DAIT at $P = 0.05$; therefore, data were combined across years to show treatment effects.

Table 3.3. Palmer amaranth height and biomass as affected by sequential applications of 2,4-D and glufosinate[ABCD]

Treatments			Height (cm)				Biomass (kg ha^{-1})			
POST-1	POST-2	POST-2 Timing (DAIT)[E]	2018		2019		2018		2019	
2,4-D + glyphosate	glufosinate	3	27	bc	34	b	1294	b	2136	c
2,4-D + glyphosate	glufosinate	7	14	bc	41	b	284	b	6195	b
2,4-D + glufosinate	glufosinate	7	0	c	45	b	0	b	1587	c
2,4-D + glufosinate	2,4-D + glufosinate	7	12	bc	37	b	122	b	1251	c
2,4-D + glufosinate	2,4-D + glyphosate	7	22	bc	45	b	507	b	1678	c
Glufosinate	2,4-D + glyphosate	7	39	b	46	b	2972	b	3143	bc
Glufosinate	glufosinate	7	21	bc	52	b	903	b	3387	bc
Nontreated control	-	-	133	a	135	a	13569	a	10175	a

[A] 2,4-D, glufosinate, and glyphosate were applied at 1066, 594, and 1543 g ai ha^{-1}.

[B] All treatments included a water conditioning agent/nonionic surfactant blend at 1% v/v (Class Act® Ridion®, Winfield Solutions, LLC, St. Paul, MN 55164).

[C] Palmer amaranth height and above-ground fresh biomass recorded 35 DAIT.

[C] Means within a column followed by the same letter do not differ significantly at *P* = 0.05 based on Tukey's HSD.

[D] A year by treatment interaction was observed for Palmer amaranth height and biomass 35 DAIT at *P* = 0.05; therefore, data were analyzed and presented separately for each year.

[E] Abbreviation: DAIT, days after initial treatment.

Table 3.4. Palmer amaranth control as affected by sequential applications of dicamba and glufosinate

Treatments^{A}			Control (%)^{CD}		
POST-1	POST-2	POST-2 Timing (DAIT)^{B}	14 DAIT^{E}	28 DAIT^{F}	
			2018-2019	2018	2019
Dicamba + glyphosate	glufosinate	3	93 a	85 ab	89 a
Dicamba + glyphosate	glufosinate	7	87 ab	100 a	79 ab
Dicamba + glyphosate	dicamba + glyphosate	7	79 ab	85 ab	91 a
Glufosinate	dicamba + glyphosate	7	77 b	70 b	76 ab
Glufosinate	glufosinate	7	94 a	78 b	57 b

[A] Dicamba, glufosinate, and glyphosate were applied at 599, 594, and 1543 g ai ha^{-1}. All treatments included a water conditioning agent/nonionic surfactant blend at 1% v/v (Class Act® Ridion®, Winfield Solutions, LLC, St. Paul, MN 55164).

[B] Abbreviation: DAIT, days after initial treatment.

[C] Means within a column followed by the same letter do not differ significantly at $P = 0.05$ based on Tukey's HSD.

[D] Visual injury estimated on a scale of 0% (no injury) to 100% (complete mortality).

[E] No treatment by year interaction observed for Palmer amaranth control 14 DAIT; therefore data were combined across years to show treatment effects.

[F] A treatment by year interaction was observed for Palmer amaranth control 28 DAIT at $P = 0.05$; therefore, data were analyzed and presented separately for each year.

Table 3.5. Palmer amaranth height and biomass as affected by sequential applications of dicamba and glufosinate[ABCD]

Treatments			Height (cm)				Biomass (kg ha^{-1})			
POST-1	POST-2	POST-2 Timing (DAIT)[E]	2018		2019		2018		2019	
Dicamba + glyphosate	glufosinate	3	27	b	35	c	403	b	3784	b
Dicamba + glyphosate	glufosinate	7	0	d	40	bc	0	b	5920	ab
Dicamba + glyphosate	dicamba + glyphosate	7	18	c	34	c	803	b	5675	ab
Glufosinate	dicamba + glyphosate	7	24	bc	51	b	3305	b	3265	b
Glufosinate	glufosinate	7	21	bc	52	b	903	b	3387	b
Nontreated control	-	-	133	a	135	a	13569	a	10175	a

[A] Dicamba, glufosinate, and glyphosate were applied at 599, 594, and 1543 g ai ha^{-1}. All treatments included a water conditioning agent/nonionic surfactant blend at 1% v/v (Class Act® Ridion®, Winfield Solutions, LLC, St. Paul, MN 55164).

[B] Palmer amaranth height and above-ground fresh biomass recorded 35 DAIT.

[C] Means within a column followed by the same letter do not differ significantly at $P = 0.05$ based on Tukey's HSD.

[D] A year by treatment interaction was observed for Palmer amaranth height and biomass at $P = 0.05$; therefore, data were analyzed and presented separately for each year.

[E] Abbreviation: DAIT, days after initial treatment.

Table 3.6. Physiological measurements following herbicide applications^{ABCD}

	Measurement Timings (DAIT)^{E}													
Treatment	1		3		6		8		11		13		35	
Mid-day photosynthesis (μmol CO_2 m^{-2} s^{-1})														
Dicamba + glufosinate +glyphosate	1.84	c	8.92	c	9.18	c	20.23	a	27.38	ab	30.94	a	24.54	a
Dicamba + glyphosate fb glufosinate 7 DAIT	29.31	b	36.02	ab	25.33	ab	4.16	b	11.92	c	31.36	a	23.29	a
Glufosinate fb dicamba + glyphosate 7 DAIT	3.76	c	18.62	bc	13.21	bc	11.33	ab	21.59	bc	27.29	a	21.07	a
Nontreated control	37.70	a	44.58	a	33.57	a	26.09	a	36.48	a	27.14	a	23.39	a
Stomatal conductance (mmol H_2O m^{-2} s^{-1})														
Dicamba + glufosinate +glyphosate	0.16	bc	0.14	a	0.38	a	0.27	a	0.29	a	0.26	a	0.16	a
Dicamba + glyphosate fb glufosinate 7 DAIT	0.25	b	0.25	a	0.29	ab	0.15	a	0.21	a	0.27	a	0.16	a
Glufosinate fb dicamba + glyphosate 7 DAIT	0.05	c	0.19	a	0.16	ab	0.13	a	0.18	a	0.19	a	0.13	a
Nontreated control	0.47	a	0.29	a	0.26	b	0.21	a	0.30	a	0.18	a	0.17	a
Respiration (μmol CO_2 m^{-2} s^{-1})														
Dicamba + glufosinate +glyphosate	-2.66	c	-3.21	b	-3.68	b	-1.50	ab	-1.41	a	-1.49	b	-	-
Dicamba + glyphosate fb glufosinate 7 DAIT	-1.24	a	-1.70	a	-1.76	a	-1.68	ab	-1.34	a	-1.34	b	-	-

Glufosinate fb dicamba + glyphosate 7 DAIT	-2.34	bc	-2.30	ab	-1.91	a	-2.11	b	-1.67	a	-0.72	a	-	-
Nontreated control	-2.00	b	-1.99	a	-1.99	a	-1.23	a	-1.42	a	-0.98	ab	-	-
PSII Quantum Yield														
Dicamba + glufosinate +glyphosate	0.41	b	0.63	b	0.64	ab	0.63	ab	0.77	a	0.77	a	-	-
Dicamba + glyphosate fb glufosinate 7 DAIT	0.77	a	0.77	a	0.77	a	0.46	b	0.63	b	0.71	a	-	-
Glufosinate fb dicamba + glyphosate 7 DAIT	0.41	b	0.62	b	0.47	b	0.54	b	0.75	a	0.75	a	-	-
Nontreated control	0.77	a	0.76	a	0.79	a	0.79	a	0.77	a	0.77	a	-	-

[A] Abbreviation: DAIT, days after initial treatment, PSII, photosystem II.

[B] Photosynthesis and leaf stomatal conductance measurements were recorded at solar noon while respiration and PSII quantum yield measurements were recorded from dark-adapted plants.

[C] A treatment by measurement timing was observed for photosynthetic assimilation, leaf stomatal conductance, foliar dark respiration, and PSII quantum yield at P = 0.05; therefore, data were analyzed separately for each measurement timing.

[D] Dicamba, glufosinate, and glyphosate were applied at 186, 198, 514 g ai ha^{-1}.

[E] Means for each type of measurement within a column followed by the same letter do not differ significantly at P = 0.05 based on Tukey’s HSD

Table 3.7. Palmer amaranth leaf area index and biomass[ABCD]

Treatment	Leaf Area (cm^2)		Fresh Leaf Biomass (g) 14 DAIT		Fresh Leaf Biomass (g) 35 DAIT	
Dicamba + glufosinate +glyphosate	157.70	b	6.50	b	12.42	a
Dicamba + glyphosate fb glufosinate 7 DAIT	228.09	b	8.91	b	6.12	b
Glufosinate fb dicamba + glyphosate 7 DAIT	192.78	b	7.43	b	9.68	ab
Nontreated control	569.66	a	14.96	b	11.68	a

[A] Abbreviation: DAIT, days after initial treatment.

[B] Dicamba, glufosinate, and glyphosate were applied at 186, 198, 514 g ai ha^{-1}.

[C] Leaf area and biomass data collected 14 DAIT, then Palmer amaranth stems with no leaves were allowed to regrow until 35 DAIT when leaf biomass was collected for the second time.

[D] Means within a column followed by the same letter do not differ significantly at $P = 0.05$ based on Tukey's HSD.

Chapter 4

Dicamba Retention in Commercial Sprayers Following Triple Rinse Cleanout Procedures and Soybean Response to Contamination Concentrations[3]

4.1. Abstract

Background: The commercial launch of dicamba-tolerant (DT) crops has resulted in increased dicamba usage and a high number of dicamba off-target movement complaints on sensitive soybeans (*Glycine max* L.). Dicamba is a synthetic auxin and low dosages as 0.028 g ae ha^{-1} can induce injury on sensitive soybean. Tank contamination has been identified as one of the sources for unintended sensitive crop exposure. Labels of new dicamba formulations require a triple rinse cleanout procedure following applications. Cleanout efficacy may vary based on sprayer type and procedure followed. This study was performed to quantify dicamba retention in commercial sprayers and assess risk for crop injury from remaining contaminants.

Results: Results indicate triple rinse with water was comparable to cleanout procedures utilizing ammonium, commercial tank cleaners, and glyphosate in rinses. Dicamba contaminants in final rinsates resulted in <15% visual injury and no yield response when applied to sensitive soybeans at R1 stage. A survey of 25 agricultural sprayers demonstrated a cleanout efficacy of 99.996% by triple rinsing with water following applications of dicamba at 560 g ae ha^{-1}, with concentrations of less than 1 ug mL^{-1} detected rinsates from the fourth rinse. A dose response experiment predicted dosages causing 5% visual injury and yield loss were 0.1185 and 2.8525 g ae ha^{-1}.

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[3] Authors: Frances B Browne, Xiao Li, Katilyn J Price, Jianping Wang; Yi Wang; Greg R Kruger; Jeff Golus; Gabrielle de Castro Macedo; Bruno Vieira, Tyler Sandlin

However, symptomology was observed for all dosages tested including rate as low as 0.03 g ae ha^{-1}.

<u>Conclusion</u>: The results from this study suggest triple rinsing with sufficient amount of water (≥ 10% of tank volume) is adequate for the removal of dicamba residues from sprayers to avoid sensitive soybean damage. This study can provide producers with confidence in cleanout procedures following dicamba applications, and aid to minimize risk for off-target movement through tank contamination.

4.2. Introduction

Recent commercialization of DT cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* (L). Merril) has provided US producers with a new tool to control herbicide-resistant broadleaf weeds which is rampant in many cotton and soybean producing states. The rapid increase in dicamba usage in DT crops has led to unprecedented numbers of off-target movement complaints with an estimated 1.46 million hectares of sensitive soybean damaged in the first year following commercialization of the technology (Bradley 2017). Primary sources of dicamba off-target movement have been identified as spray particle drift, volatility, and sprayer contamination (Kniss 2018; Egan et al. 2014; Egan and Mortensen 2012; Boerboom 2004; Griffin et al. 2013; Mueller et al. 2013; Mueller et al. 2019; Soltani et al. 2016). Adverse weather conditions can strongly influence the amount of herbicide movement away from the target site via particle drift and volatility (Egan et al. 2014; Egan and Mortensen 2012; Boerboom 2004; Griffin et al. 2013; Mueller et al. 2013; Mueller et al. 2019). Tank contamination in commercial sprayers is likely the most preventable form of unintended exposure (Werle et al. 2018).

Effective cleanout procedures need to be identified to minimize dicamba retention and reduce risk for crop injury.

Dicamba is a synthetic auxin that can induce distinct injury on sensitive plants including leaf cupping, leaf crinkling, stem twisting, chlorosis on terminal leaves, abnormal leaf venation, swollen petiole bases, stunting, and necrosis, etc (Griffin et al. 2013; Soltani et al. 2016; Shaner 2014). Soybean response to dicamba has been widely studied and dosages as low as 0.028 g ae ha^{-1} (0.005% of 560 g ae ha^{-1} use rate) have been shown to cause symptomology (Kniss 2018; Egan et al. 2012; Soltani et al. 2016; Soloman and Bradley 2012; Robinson et al. 2013). The degree of damage can differ based on the crop stage at the time of exposure, with consistently higher yield loss potential for reproductive stages as opposed to vegetative stages by two to six-fold (Kniss 2018; Griffin et al. 2013; Soltani et al. 2016). For example, Griffin et al. (2013) observed 4 and 15% soybean yield losses following dicamba exposure during V2-V3 stages at 4.4 and 17.5 g ae ha^{-1}, respectively, and 10 and 36% reductions exposed during R1 stages using the same rates. In another study, the dicamba dosage predicted to cause 5% yield loss was 5.8 and 1.0 g ae ha^{-1} for V2-3 and R1 stages, respectively (Soltani et al. 2016). Similarly, a meta-analysis pooled data from multiple published studies found dosages predicted to cause 5% soybean yield loss ranged from 1.6 to 24, 1.2 to 47, and 0.15 to 14 g ae ha^{-1} for V1-3, V4-7, and R1-2 stages, respectively (Kniss 2018). Available literature indicates the potential for soybean recovery is greatest when exposed at vegetative stages and exposure during reproductive stages has the highest risk for yield reduction (Kniss 2018; Egan et al. 2014; Griffin et al. 2013; Soltani et al. 2016). However, soybean yield loss due to dicamba exposure can be inconsistent due to environmental conditions and plant vigor (Kniss 2018; Egan et al. 2014; Egan and Mortensen

2012; Griffin et al. 2013; Soltani et al. 2016; Andersen et al. 2004; Al-Khatib and Peterson 1999; Weidenhamer et al. 1989; Kelley et al. 2005).

Row crop producers often use sprayers to apply multiple pesticides, as owning a sprayer dedicated to dicamba applications is expensive and unrealistic. Failure to remove dicamba residues from a sprayer could result in multiple exposures to sensitive crops throughout the season. Labels of new dicamba formulations require a triple rinse cleanout procedure following applications to minimize risk for unintended exposure to sensitive crops (Anonymous 2018a,b; Anonymous 2019a). Using a tank cleaning agent in the second rinse is recommended, but not required in these labels. Although dicamba is formulated as a water-soluble product, removal from spray equipment is difficult and residues can readily adhere to plastic and rubber surfaces (Boerboom 2004; Cundiff et al. 2017; Steckel et al. 2005; Osbourne et al. 2015). Modern self-propelled sprayers often have large tanks and multiple filters, screens, end caps, valves and nozzles that can trap dicamba deposits. Published data is very limited on dicamba clean out efficacy in agricultural sprayers. More information is needed to determine the effectiveness of cleanout procedures and soybean response to remaining contaminants. Therefore, the objective of this study was to evaluate triple rinse cleanout procedures for dicamba residue removal efficacy in agricultural sprayers following applications and assess sensitive soybean response to various concentrations of dicamba residue that may not be removed from sprayer equipment.

4.3. Materials and Methods

4.3.1. Cleanout procedure comparisons

Field and laboratory experiments were conducted in January 2017 to evaluate three commercial sprayers for dicamba residue retention following four triple-rinse cleanout procedures as shown in Table 1. Hagie Upfront STS 10 (Hagie Manufacturing Co., Clarion, IA 50525), John Deere 6700 (John Deere and Co., Moline, IL 61265) and SprayCoupe 4660 (AGCO, Duluth, GA 30096) sprayers with polyethylene tanks and capacities of 3570 L, 1590 L, and 1580 L, respectively, were used to apply diglycolamine salt of dicamba (Clarity®, BASF Corporation, Research Triangle Park, NC, 27709) at a rate of 1.12 kg ae ha^{-1} in a carrier volume of 93.5 L ha^{-1}. Herbicide solution was mixed for 378 L of water in each sprayer at initial application. Herbicide solution was agitated for 10 minutes before being sprayed on fallow fields until no droplets were emitted from of nozzles in order to empty solution in hose and pump. Following herbicide application, remaining herbicide solution in the tank was drained completely. For each rinse during cleanout procedures, 378 L of water and the assigned cleaning treatment were added to the tank. After agitating for 5 minutes, half of the cleaning solution was sprayed out before rinsate samples were collected from the left, middle, and right sections of the boom simultaneously and combined into a single sample. Sprayer tanks were not drained in between rinses during cleanout procedures. A fourth rinse using only water was included to demonstrate the cleaning efficacy of each triple-rinse procedure since label only mandates triple-rinse. All four cleaning procedures were repeated three times on each sprayer in field and experiment was repeated twice.

Rinsates were collected in 1 L glass jars, and 1/3 of each sample from the various boom sections were mixed in a single jar before being placed on ice and later frozen at -20°C for future analytical analysis. Samples were thawed before aliquoting 1.5 mL into 2 mL Eppendorf® tubes (Eppendorf North America, Hauppauge, NY, 11788), which were subsequently centrifuged for 2 minutes at 15,000 rpm to remove any debris. After centrifugation, 1 mL of the cleaned sample was pipetted into 2 mL glass chromatography vials. Prior to centrifuging, samples from the first and second rinses were diluted 1:100 and 1:10 respectively, as concentrations were too high for accurate chromatography analysis. All processed samples were analyzed through high performance liquid chromatography (HPLC) using an Agilent 1260 Infinity series apparatus (Agilent Technologies, Santa Clara, CA, 95051) equipped with a diode array detector (1260 Infinity Diode Array Detector VL, Agilent Technologies, Santa Clara, CA, 95051) and an Agilent Poreshell 120EC-C18 column (4 µm, 4.6 × 250 mm). The total runtime of the chromatography for each sample was 10 min (flow rate of 1 mL min^{-1}, injection volume of 100 µL) with a constant mobile phase of 50% 10 mM phosphoric acid and 50% acetonitrile. The analytical wavelength was set to 230 nm and peaks were integrated from the DAD spectrum. Detection limit of dicamba was 0.1 µg mL^{-1}. Analytical standards were developed using HPLC-grade water and formulated dicamba (Clarity®, BASF Corporation, Research Triangle Park, NC, 27709) to include ten concentrations ranging from 0.25 to 50 µg ml $^{-1}$. Known concentrations were regressed against their respective concentrations in Sigmaplot 13.0 (Systat Software, San Jose, CA 95131), and the resulting linear equation was used to determine dicamba concentrations in sprayer rinsate samples.

4.3.2. Rinsate application on sensitive soybean bioassays

Rinsates collected from the fourth rinses from all cleanout procedures were applied over the top of sensitive soybeans at early-bloom (R1) stage at EV Smith Research and Extension Center at Shorter, AL (32°29'45.6"N 85°53'25.2"W) on August 15, 2017 to demonstrate dicamba clean out efficacy by triple-rinse procedures. A Roundup-Ready Soybean variety ‘P76T54R2’ (Pioneer®, Corteva Agriscience, Johnston Iowa, 20131) was planted at 346,000 seeds ha^{-1} on 91 cm row spacing and managed with local recommendations. Plots were 7.6 m long by 3.7 m wide with four rows of soybean. A total of 12 treatments (3 sprayers by 4 cleanout procedures) were sprayed over the top of soybean in a randomized complete block design, plus a non-treated control. Each treatment contained all the replications from the spray cleaning study described above. Samples collected from left, middle, and right boom sections from the fourth rinse of each treatment replication were combined into a single sample and applied on the middle two rows in each plot. Rinsate samples were applied with a CO_2-pressurized backpack sprayer equipped with four Turbo TeeJet induction (TTI110025) nozzles (TeeJet Technologies, Wheaton, IL, 60187) at speed of 6.4 km ha^{-1}, 335 kpa pressure and a carrier volume of 187 L ha^{-1} to ensure good coverage. Visual injury was estimated on a scale of 0 (no injury) to 100 (complete mortality) at 7, 14, and 21 days after treatment (DAT). All plots were maintained weed free with appropriate herbicides and hand weeding. Soybean yield was collected on November 29, 2017, by machine harvesting the middle two rows.

All data collected were subjected to a mixed model analysis of variance using PROC GLIMMIX in SAS 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513). Cleanout procedure and sprayer were considered fixed effects, while replication was a random effect and all interactions were considered. Means comparisons were generated using Tukey's Honest Significant Difference (HSD) test with *P*=0.05.

4.3.3. Dicamba cleanout survey on commercial sprayers

A survey was conducted across 25 commercial sprayers from various row crop farms in AL, GA, and FL in October 2019 to further assess cleanout efficacy of triple rinsing with water following dicamba applications. Sprayer models, manufacturers, and locations can be found in Table 2. All sprayers have polyethylene tanks except for sprayer ID # 4 which has a stainlesssteel tank. Sprayers were thoroughly cleaned before mixing dicamba by rinsing tank and flush spray line with clean water at 15% tank capacity. Dicamba (Xtendimax® with VaporGrip® Technology, Monsanto Co., St. Louis, MO, 63167) was applied at 560 g ae ha^{-1} in tank mixture with a drift reduction agent (Intact, Precision Laboratories, LLC, Waukegan, IL, 60085) at 0.5% v v^{-1}. All sprayers were loaded at 15% tank capacity and agitated for 10 minutes before application at 140 L ha^{-1}. A standard procedure of four rinses with water were conducted on all sprayers in this survey at 15% tank capacity during each rinse. Water was agitated for 5 minutes in the tank before collection process was initiated. At each rinse, half of the water added to tank was sprayed out before sample collection from the left, middle, and right sections of the boom simultaneously. Sprayer tanks were drained after initial application and in between all rinses. Samples were immediately placed in a cooler on ice, then frozen at -20°C until further analysis. Rinsate samples were processed in laboratory and analyzed by HPLC with the procedure as previously described. Rinsate samples collected from left, middle, and right sections of the boom were analyzed separately to reveal potential boom section effect. Dicamba concentrations in rinsates were analyzed in a mixed model analysis of variance using PROC GLIMMIX in SAS 9.4, with fixed effects of boom section and number of rinses and random effect of sprayer ID number.

4.3.4. Soybean response to dicamba concentrations

In order to evaluate soybean response to different dicamba concentrations, field studies were conducted during summers of 2017, 2018, and 2019 at the E.V. Smith Research Center in Macon County, AL (32°29'45.6"N 85°53'25.2"W) and the West Central Research and Extension Center in Lincoln County, NE (41° 05’ 15.98” N 100° 46’ 39.42”W) in 2019. Air temperatures at the time of applications ranged from 33 to 35° C with humidity of 48 to 72% across all sites. Dates for soybean planting, dicamba application, and harvest as well as temperature and humidity at the time of application can be found in Table 3. Plots were 7.6 m long by 1.2 m wide and consisted of four rows of ‘P76T54R2’ (Pioneer®, Corteva Agriscience, Johnston Iowa, 20131) and ‘NK S24-K2’ (NK®, Syngenta Crop Protection, Greensboro, NC, 2749) soybean varieties in the AL and NE field studies, respectively. Soybeans were planted at 346,000 seeds ha^{-1} on 91 and 76 cm row spacing in AL and NE, respectively. Randomized complete block design with four replications was used at each location. Broadcast applications of dicamba at 0.03, 0.14, 0.70, 1.40, 3.51, 14.04, 35.07, and 140.28 g ae ha^{-1} were used to simulate tank contamination at low concentrations. These dosages represent 0.25, 1, 5, 10, 25, 100, 250, and 1000 µg mL^{-1}, respectively. These concentrations were applied to soybeans at first to mid-bloom stage with a CO_2-pressurized backpack sprayer equipped with four Turbo TeeJet Induction (TTI110025) nozzles (TeeJet Technologies, Wheaton, IL, 60187) delivering 187 L ha^{-1} at 6.4 km hr^{-1} and 335 kpa pressure.

Soybean visual injury was estimated on a scale of 0 (no injury) to 100 (complete mortality) 14 and 21 DAT. Soybean yield was collected at each location by a plot combine from the center two rows. Data collected were subjected to a mixed model analysis of variance using PROC GLIMMIX in SAS 9.4. Dicamba concentration and location were considered fixed effects, while replication was a random variable, and all interactions were examined. If an interaction was significant, data was analyzed and presented by the fixed effects at each level. Means comparisons were generated using Tukey’s HSD test with *P* = 0.05. A non-linear regression model was fitted to the dry weight and visual estimations of injury data using the *DRC* package in R software (R Foundation for Statistical Computing, Wienna, Austria) (Knezevic et al. 2007). The effective-dose to reduce 5% and 10% of plant biomass and cause 5% and 10% visual estimations of injury (ED_5 and ED_{10}) were estimated using a four-parameter log logistic equation:

$$y = c + \{d - c/1 + exp[b(log\ x - log\ e)]\}$$

in which y corresponds to the biomass reduction and visual estimations of injury (%), x represents dicamba dosage, b is the slope at the inflection point, c is the lower limit of the model, d is the upper limit, and e is the inflection point which represent the dosage that caused 50% injury or 50% yield loss. Visual injury and yield data were pooled over years and locations for non-linear regression to provide maximum prediction power to the model.

4.4. Results and Discussion

4.4.1. Comparison of four cleanout procedures for dicamba residue retention

No sprayer by cleanout procedure interactions were observed for dicamba residue retention at any rinse. Therefore, data were combined to show cleanout procedure and sprayer effects individually (Table 4). Dicamba concentrations in the first rinse were similar for all cleanout procedures. Residues detected in the Hagie Upfront STS 10 exceeded those in the John Deere 6700 by 272 mg L^{-1}, possibly due to smaller tank size of John Deere 6700. Dicamba concentrations in the rainsate from the second rinse was similar for all cleanout procedures and sprayers. Triple rinsing with water resulted in higher dicamba concentrations in the third rinse as compared to ammonium fb glyphosate fb water, with 3.56 and 0.72 µg mL^{-1} detected in rinsates, respectively. The Hagie Upfront STS 10 retained more dicamba than John Deere 6700 at the third rinse with 1.8 µg mL^{-1} higher in concentrations. Dicamba concentrations in the fourth rinse did not differ significantly for any sprayer or cleanout procedure and did not exceed 1.25 µg mL^{-}

^{1}, which is equivalent to 0.18 g ae ha^{-1} in a sprayer delivering 140 L ha^{-1}. This concentration surpassed the lowest dicamba dosage expected to induce soybean symptomology and slightly exceeded the dosage predicted to cause 5% yield loss which are 0.03 and 0.15 g ae ha^{-1}, respectively (Kniss 2018; Griffin et al. 2013; Soltani et al. 2016; Robinson et al. 2013).

4.4.2. Application of fourth rinsates on sensitive soybean bioassays

When the fourth rinsates were applied directly to sensitive soybeans at mid-bloom, typical dicamba symptomology of leaf cupping, stem twisting, and chlorosis was observed. However, visual injury estimations did not exceed 15% for any treatment and yield was not significantly reduced by remaining dicamba residues from any clean out procedure and any sprayer (data not shown). These data indicate sensitive soybeans could tolerate dicamba contaminants remaining in sprayers following triple rinse cleanout procedures without risk for yield loss. However, environmental conditions can largely influence soybean response to dicamba and injury could be more severe during times of drought stress and higher temperatures (Kniss 2018; Mueller et al. 2013; Mueller et al. 2019; Al-Khatib and Peterson 1999). These low concentrations of dicamba residue may still pose a concern for sensitive soybean.

Inman et al. (2014) reported triple rinse with water was comparable to ammonia and commercial tank cleaners for dicamba residue removal following applications of 560 g ae ha^{-1}, with 0.006% of original concentrations remaining in final rinses. Dicamba residue retention was higher in this study with up to 0.015% (1.25 µg mL^{-1}) of the original application concentrations detected, which was probably due to higher initial use rate of dicamba at 1.12 kg ae ha^{-1}. Cundiff et al. (2017) found that hose material had a greater influence on dicamba retention in sprayer equipment than triple rinse cleanout procedures of water versus ammonia, likely due to material porosity of hose interior where residues can settle. Certain hose material deteriorate quicker than others after repeated pressurization during application which led to more dicamba retention. Osborne et al. (2015) conducted an anonymous sprayer survey and reported 98 to 100% cleanout efficiency, and average dicamba concentrations of 245 µg mL^{-1} reduced to 0.41 µg mL^{-1} after triple rinses with water. Results of this study combined with previous literature demonstrate addition of a cleaning agent may not provide additional benefit for dicamba removal than triple rinse with water, hose type and the age of sprayer equipment is likely a concern (Cundiff et al. 2017).

4.4.3. Survey of commercial sprayers for dicamba residue retention following triple rinse with water

Concentrations collected from the left, middle, and right sections of the boom for individual rinses performed on each sprayer was not significant as a fixed effect in the model; therefore, concentrations were combined over sections. Dicamba concentration in the sprayer tank was calculated at 4000 µg mL^{-1} when mixed at 560 g ae ha^{-1} rate and applied at 140 L ha^{-1}. The average dicamba concentrations detected in the first, second, third, and fourth rinses were 100.75, 6.78, 0.79, and 0.17 µg mL^{-1}, respectively, indicating cleanout efficacy improved with each additional rinse (Table 5). Dicamba concentrations detected in the first rinse were not uniform across sprayers and detection ranged from 0.10 to 664.82 µg mL^{-1} which is likely a result of vastly different sprayer systems and equipment ages.[20,24] In general, sprayers that retained higher concentrations in initial rinses also retained higher amounts of dicamba throughout the rinses, such as sprayer ID no. 3, 18 and 25.

The three sprayers with highest dicamba concentrations in the first rinse were two John Deere R4030s and a tractor mounted sprayer (ID no. 3, 18, and 25) with concentrations ranging from 287.17 to 664.82 µg mL^{-1} (Table 5). The greatest dicamba retention at the second rinse was observed on sprayer ID no. 25, 18, and 12, with concentrations of 84.29, 18.61, and 10.90 µg mL^{-1}, respectively. The three highest dicamba concentrations in rinsates collected at the third rinse were detected from sprayers previously identified (ID No. 3, 18, 25) for retaining the greatest amounts of dicamba in the first rinse, ranging from 2.18 to 6.89 µg mL^{-1}. All sprayers retained 0.68 µg mL^{-1} or less of dicamba at the fourth rinse, with the majority (18 sprayers) below the instrument detection limit of 0.1 µg mL^{-1}, indicating near complete cleanout efficacy on these sprayers. Based on initial tank concentration (4000 µg mL^{-1}) and average concentrations generated from the 4th rinse (0.17 µg mL^{-1}), triple rinse with water procedure cleaned out 99.996% of dicamba in sprayers surveyed. Final dicamba concentrations in this survey, where the dicamba in-season use rate (560 g ae ha^{-1}) was applied initially, did not surpass the lowest dosage expected to cause yield loss (0.15 g ae ha^{-1} or 1.07 µg mL^{-1}). Three sprayers (ID No. 3, 24, 25) retained concentrations with potential to induce symptomology based on available literature (Kniss 2018; Soltani et al. 2016; Robinson et al. 2013; Soloman and Bradley 2014). Three sprayers (ID No. 4, 16, and 22) showed abnormally low dicamba concentrations in the initial rinsate samples, ranging from 0.10 to 2.70 µg mL^{-1}. The explanation for this observation is unknown but certain sprayer design may allow more complete tank drainage following dicamba application, thus led to low concentrations detected in the first rinse. Further investigation is needed to study how sprayer design affects chemical retention in these sprayers.

4.4.4. Soybean dose-response to dicamba concentrations

A dicamba concentration by site-year interaction was observed for soybean visual injury at 14 and 21 DAT. Data were analyzed and presented separately for each site-year (Table 6). Soybean visual injury was observed for all concentrations tested and increased from 1 to 100% as dicamba dosage increased from 0.25 µg mL^{-1} to 1000 µg mL^{-1} across all site-years. The two highest dosages tested in this study resulted in consistently higher injury of 49% or greater across all rating dates and site-years. Dicamba concentrations of 0.25 and 1 µg mL^{-1} did not induce visual injury greater than 18% and soybean responses were variable across site-years.

Soybean yield was also variable across site-years and ranged from 852 to 4934 kg ha^{-1} in the nontreated control (Table 7). The AL site experienced extended periods of drought in the summer of 2019 which likely caused the lower yields across all treatments and a lack of differences. Across all site-years, soybean yield was not significantly reduced for dicamba dosages of 5 µg mL^{-1} or less as compared to the nontreated control. A dicamba dosage of 10 µg mL^{-1} resulted yields 725 kg ha^{-1} lower than the nontreated control in AL during 2017. No other site-year observed a loss from this dosage, and applications of 25 µg mL^{-1} did not result in yield reductions for any site. Yield loss resulted from 100 µg mL^{-1} was observed at the 2017 AL and 2019 NE sites with reductions of 819 and 2026 kg ha^{-1}, respectively. Three out of four sites observed yield loss greater than 50% following dicamba applications at 250 µg mL^{-1}. The most severe yield loss was caused by applications of 1000 µg mL^{-1} (1/4 of labeled use rate at 560 g ae ha^{-1}), and resulted in lower yield for all sites, ranging from 0 to 442 kg ha^{-1}. Non-linear regression using indicated significant relationships between dicamba dosages to soybean visual injury and yield loss (Table 8, Figure 1 and 2). The ED_5 and ED_{10} are estimated as 0.1185 and 0.4143 g ae ha^{-1} for visual injury and 2.8525 and 4.9602 g ae ha^{-1} for yield loss, respectively. These ED_5 and ED_{10} of this study are within the range of dosages causing 5% injury and yield loss in the published studies (Kniss 2018; Soltani et al. 2016; Griffin et al. 2013; Robinson et al. 2013; Soloman and Bradley 2014).

Visual injury was observed for all dosages tested in this study, which correlates to previous studies where at least 5% visual injury was predicted with dicamba dosages as low as 0.028 g ae ha^{-1} (Kniss 2018; Soltani et al. 2016; Robinson et al. 2013). A study by Griffin et al. (2013) predicted soybean injury of 19% is likely to occur from exposure to dicamba at 1.1 g ae ha^{-1} during reproductive stages 7 to 14 DAT. A similar dosage in this study of 1.4 g ae ha^{-1} (10 µg mL^{-1}) resulted in 15 to 40% injury across all sites. Although previous literature predicts 5% soybean yield loss with dosages as low as 0.15 g ae ha^{-1}, soybeans in this study were able to tolerate dosages up to 0.7 g ae ha^{-1} (5 µg mL^{-1}) with no yield loss (Kniss 2018; Griffin et al. 2013; Soltani et al. 2016). Research by Kelley et al. (2005) observed 25 and 41% visual injury resulted from dicamba applications of 0.56 and 5.6 g ae ha^{-1} at R2 stage with no yield loss and 7% yield loss, respectively. Foster et al. (2018) reported 9% and 30% yield loss for dosages of 2.2 and 8.8 g ae ha^{-1} applied at reproductive stages. Injury up to 37% observed in the current study from 0.7 g ae ha^{-1} (5 µg mL^{-1}) with no impact on yield, indicating a substantial amount of injury can occur without affecting yield under certain conditions. In a nutshell, results from this study demonstrate concentrations greater than 1.25 µg mL^{-1} (0.18 g ae ha^{-1}) has not been found on any sprayer tested if triple rinse cleanout procedures were followed. There is reasonable confidence to state that soybean yield loss from dicamba residue remained in sprayer after triple rinse procedure using sufficient amount of water is unlikely to occur.

4.5. Discussion

Dicamba concentrations from the final rinse in the sprayer survey may cause visual symptomology on sensitive soybean but were not higher than the lowest dosage expected to cause soybean yield loss. Dicamba concentrations in the final rinse were not detectable in the majority of the sprayers surveyed. Higher amounts of dicamba may be retained when higher rates are applied. Several sprayers in both studies did retain enough dicamba residues in final rinsates to cause observable symptomology based on dose responses in previous studies (Kniss 2018; Soltani et al. 2016; Robinson et al. 2013; Soloman and Bradley 2014). Bioassay where actual rinsates were applied to soybeans at first bloom in 2017, which is the most sensitive growth stage for yield loss, resulted in <15% symptomology and no yield loss (Kniss 2018; Egan et al. 2014). These observations are consistent with the findings from the dose-response portion of this study where concentrations of 1 µg mL^{-1} generated 18% or less visual injury and a lack of yield response. Published literature and results of this study suggest the safety margin for sensitive soybeans not to be damaged by dicamba residues in sprayers does exist, but it is fairly narrow (0.68 µg mL^{-1} dicamba found in 4th rinse and 1.07 µg mL^{-1} may cause soybean yield loss) so growers must be cautious when conducting triple rinse procedure (Kniss 2018; Griffin et al. 2013; Soltani et al. 2016; Cundiff et al. 2017).

A few precautionary measures for cleaning sprayer equipment include draining the tank thoroughly after dicamba application and between each rinse, using sufficient volumes of water (at least 10% tank capacity), washing the top of the tank, flushing hoses, cleaning end caps, soaking nozzles, and washing the exterior of the sprayer (Steckel et al. 2005; Johnson et al. 2012). Failure to drain sprayer tanks in between rinses can impede proper dilution and result in higher herbicide retention (Johnson et al. 2012). Additionally, periodic maintenance of sprayer equipment and replacement of damaged parts such as hoses and nozzles is often recommended to limit spaces where residues can become lodged (Cundiff et al. 2017). Commercial tank cleaning agents have been shown to increase removal efficacy on flumioxazin and other herbicides with low water solubility which tend to precipitate to the bottom of the tank and spray hose (Shaner et al. 2014; Johnson et al. 2012; Anonymous 2019b). However, addition of tank cleaning agent in the second rinse did not significantly improve dicamba clean out efficacy in this study due to its high-water solubility.

Dicamba off-target movement concerns are not likely to be completely resolved in coming years and proper technology stewardship will be necessary to minimize impacts on sensitive crops and environment. Data generated from dicamba retention in commercial sprayers and soybean dose response experiments in this study can provide guidelines to ensure proper stewardship of new technology and avoid potential self-inflicted damage on sensitive crops. More research is needed on understanding how different sprayer designs, tank materials, boom length, hoses, and tank plumbing affecting dicamba retention.

4.6. Acknowledgements

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Table 4.1. Cleanout procedures, agents, and, amounts.

Rinse	Cleaning Agent[A]	Amount added to tank
Procedure 1		
1	Water	378.00 L
2	Water	378.00 L
3	Water	378.00 L
4	Water	378.00 L
Procedure 2		
1	Ammonium	11.34 L
2	Glyphosate	6.24 kg ai
3	Water	378.00 L
4	Water	378.00L
Procedure 3		
1	Ammonium	11.34 L
2	Fimco™ detergent	0.90 kg
3	Water	378.00 L
4	Water	378.00L
Procedure 4		
1	Ammonium	11.34 L
2	Protank® detergent	0.95 L
3	Water	378.00 L
4	Water	378.00 L

[A] Manufacturers for cleaning agents: ammonium (10% ammonium hydroxide), Great Value™ clear ammonium all-purpose cleaner, KIK International LLC, 33 Macintosh Blvd Concord, Ontario, Canada L4K 4L5; glyphosate, Roundup Powermax®, Bayer CropScience, St. Louis, MO 63167; Fimco™ spray tank neutralizer and cleaner, Fimco Industries, North Sioux City, SD 57049; Protank® liquid cleaner, Winfield Solutions, LLC, St. Paul, MN 55164.

Table 4.2. Sprayer ID, model, boom width, and tank capacity for sprayer survey.

ID No.	Location	Model[ABCD]	Boom Width (m)	Tank Capacity (L)
1	Baldwin Co., AL	JD 4630	24	2271
2	Coffee Co., AL	JD 4730	33	3028
3	Coffee Co., AL	JD R4030	33	3028
4	Dallas Co., AL	JD R4030	33	3785
5	Dallas Co., AL	JD R4030	27	3028
6	Geneva Co., AL	JD 4730	30	3028
7	Henry Co., AL	Tractor mounted	7	568
8	Henry Co., AL	Tractor mounted	11	568
9	Henry Co., AL	Tractor mounted	7	568
10	Henry Co., AL	JD 6700	11	1136
11	Henry Co., AL	Tractor mounted	11	1136
12	Henry Co., AL	JD 6700	11	1136
13	Limestone Co, AL	MudMaster™	9	416
14	Limestone Co, AL	JD 6700	11	1590
15	Macon Co., AL	Tractor mounted	7	454
16	Jackson Co., FL	JD 4730	27	3028
17	Santo Rosa Co., FL	Tractor mounted	7	454
18	Berrien Co., GA	Tractor mounted	11	1136
19	Berrien Co., GA	JD 4730	33	3028
20	Irwin Co., GA	Tractor mounted	16	1136
21	Irwin Co., GA	JD 4730	33	3028
22	Tift Co., GA	Tractor mounted	11	1893
23	Tift Co., GA	JD R4030	33	3028
24	Tift Co., GA	JD R4030	33	3028
25	Worth Co., GA	JD R4030	33	3028

[A] Abbreviations: JD, John Deere

[B] John Deere sprayers manufactured at John Deere, Moline, IL, 61265.

[C] Mudmaster™ manufactured at Bowman Manufacturing Co., Inc., Newport, AR 2112.

[D] Manufacturing information not available for tractor mounted sprayers.

Table 4.3. Dates of soybean planting, dicamba application, harvest, and temperature and humidity at time of application.

Year	Location	Planting date	Application date	Harvest date	Temperature at application °C	Relative humidity at application %
2017	Macon Co., AL	6/15/2017	8/15/2017	11/29/2017	33	72
2018	Macon Co., AL	6/21/2018	8/08/2018	11/06/2018	34	48
2019	Macon Co., AL	5/29/2019	7/10/2019	11/17/2019	35	55
2019	Lincoln Co., NE	6/03/2019	7/15/2019	10/14/2019	33	54

Table 4.4. Dicamba concentrations in rinsates following cleanout procedures in different sprayers[ABCD].

	Rinse							
	1		2		3		4	
Cleaning Procedure	Concentration (μg mL^{-1})							
Triple rinse with water	298.32	a	21.12	a	3.56	a	1.25	a
Ammonium fb glyphosate fb water	521.98	a	16.67	a	0.72	b	0.67	a
Ammonium fb Fimco™ fb water	373.13	a	29.75	a	1.21	ab	0.90	a
Ammonium fb Protank™ fb water	472.65	a	29.96	a	1.28	ab	0.50	a
Sprayer								
Hagie Upfront STS 10	543.05	A	31.75	A	2.55	A	1.09	A
John Deere 6700	270.34	B	16.94	A	0.75	B	0.91	A
SprayCoup 4660	436.16	AB	24.44	A	1.78	AB	0.48	A

[A] Abbreviation: fb, followed by.

[B] Dicamba (Clarity®, BASF Corporation, Research Triangle Park, NC 27709) initially applied at 1.12 kg ae ha^{-1}.

[C] No sprayer by treatment interactions were observed at *P* = 0.05; therefore, data were combined to show sprayer and treatment effects individually.

[D] Means followed by the same uppercase letter within a sprayer column or lowercase letter within a cleaning procedure column are not significantly different based on Tukey's HSD at *P* = 0.05.

Table 4.5. Dicamba concentration in surveyed sprayers rinsates from each rinse[AB].

	Rinse			
	1	2	3	4
ID No.	Dicamba Concentration (µg mL^{-1})[C]			
1	27.76	0.86	0.10	< 0.10
2	44.13	0.10	0.74	0.17
3	294.96	9.10	2.18	0.68
4	0.54	0.20	0.17	< 0.10
5	24.08	3.55	0.00	0.00
6	41.83	0.62	0.14	< 0.10
7	57.87	0.10	0.10	< 0.10
8	55.20	0.10	0.25	< 0.10
9	82.31	1.51	0.07	< 0.10
10	154.28	7.13	0.12	< 0.10
11	31.28	0.10	0.10	0.12
12	146.78	10.90	0.15	0.07
13	60.41	0.12	0.17	< 0.10
14	70.22	3.18	0.23	< 0.10
15	17.25	0.10	0.10	< 0.10
16	2.70	0.10	0.10	< 0.10
17	73.77	0.10	0.10	< 0.10
18	287.17	18.61	6.89	< 0.10
19	74.91	5.75	0.08	0.16
20	145.09	8.41	0.10	< 0.10
21	14.97	0.43	0.21	< 0.10
22	0.10	0.77	0.10	< 0.10
23	96.79	4.97	1.02	< 0.10
24	49.59	7.70	1.18	1.00
25	664.82	84.69	5.35	0.47
Average	100.75	6.78	0.79	0.17

[A] Dicamba (Xtendimax® with Vaporgrip Technology®, Monsanto Co., St. Louis, MO 63167) was applied at 560 g ae ha^{-1} and tank mixed with a drift reduction agent (Intact) at 1% v v^{-1}.

[B] All sprayers received water at 15% tank capacity for all rinses

[C] No boom section by rinse interactions were observed at $P = 0.05$; therefore, data were combined across sections for each rinse to show sprayer average

Table 4.6. Soybean visual injury to various concentrations of dicamba under field conditions 14 and 21 DAT[ABCDE].

Concentration ($\mu g\ mL^{-1}$)	14 DAT				21 DAT			
	AL 2017	AL 2018	AL 2019	NE 2019	AL 2017	AL 2018	AL 2019	NE 2019
0.25	1 d	5 d	4 e	10 d	2.5 c	10 de	5 e	7 e
1.00	8 cd	1 d	16 de	17 d	4 c	3 e	18 de	17 e
5.00	10 cd	20 cd	31 d	33 c	13 c	15 de	31 d	37 d
10.00	15 c	-	31 d	37 c	14 c	-	30 d	40 cd
25.00	16 c	30 bc	26 d	-	13 c	29 cd	26 d	-
100.00	20 c	44 bc	55 c	47 b	15 c	44 bc	59 c	48 bc
250.00	49 b	53 b	76 b	48 b	50 b	56 b	78 b	55 b
1000.00	80 a	84 a	100 a	89 a	91 a	83 a	100 a	88 a

[A] Dicamba concentrations of 0.25, 1, 5, 10, 25, 100, 250, and 1000 represent dosages of 0.03, 0.14, 0.70, 1.40, 3.51, 14.04, 35.07, and 140.28 g ae ha^{-1}, respectively, at 140 L ha^{-1} application rate.

[B] Abbreviation: DAT, days after treatment

[C] A concentration by site-year interaction was observed for visual injury 14 and 21 DAT at $P = 0.05$; therefore, data were analyzed and presented separately by each site-year.

[D] Means within a column followed by the same letter do not differ significantly at $P = 0.05$ based on Tukey's HSD.

[E] – indicates where dosages were not tested in a specific site-year.

Table 4.7. Soybean yield response to various dicamba concentrations under field conditions [A].

Concentration (µg mL^{-1})	Yield (kg ha^{-1})[BC]							
	AL 2017		AL 2018		AL 2019		NE 2019	
0.00	3652	a	1150	abc	852	ab	4934	abc
0.25	3524	a	1337	a	921	ab	5019	ab
1.00	3371	a	1280	ab	931	ab	5117	a
5.00	3289	ab	1268	ab	999	a	4307	bc
10.00	2927	bc	-		921	ab	4112	c
25.00	3219	abc	832	bcd	885	ab	-	
100.00	2833	c	712	cd	842	ab	2908	d
250.00	1319	d	405	d	648	b	1539	e
1000.00	376	e	442	d	0	c	124	f

[A] A concentration by site-year interaction was observed for yield at *P* = 0.05; therefore, data were analyzed and presented separately by each site-year. Dicamba concentrations of 0.25, 1, 5, 10, 25, 100, 250, and 1000 represent dosages of 0.03, 0.14, 0.70, 1.40, 3.51, 14.04, 35.07, and 140.28 g ae ha^{-1} respectively at 140 L ha^{-1} application rate.

[B] Means within a column followed by the same letter do not differ significantly at *P* = 0.05 based on Tukey's HSD.

[C] – indicates where dosages were not tested in a specific site-years.

Table 4.8. Parameter estimates for non-linear regression[AB]

Data type	c ± SE	P value	b ± SE	P value	e ± SEM[C]	P value	ED_5 (g ae ha^{-1})	ED_{10} (g ae ha^{-1})
Visual injury	0	-	-0.5971 ±0.0530	<0.0001	16.4181 ±2.0377	<0.0001	0.1185 ±0.0535	0.4143 ±0.1434
Yield loss	-1.5831 ±2.2259	0.4782	-1.1190 ±0.1677	<0.0001	30.9877 ±3.8327	<0.0001	2.8525 ±1.1123	4.9602 ±1.5494

[A] Four parameter log logistic model is used, where c is the minimum value of response variable, d is the maximum value of response variable, e is the point of inflection, b is the slope of the curve at inflection point.

[B] Parameter c was set as 0% in non-linear regression for visual injury. Parameter d was set as 100% for both visual injury and yield loss in the non-linear regression.

[C] Also known as ED_{50}, effective dosage to cause 50% visual injury or reduce 50% of the yield.

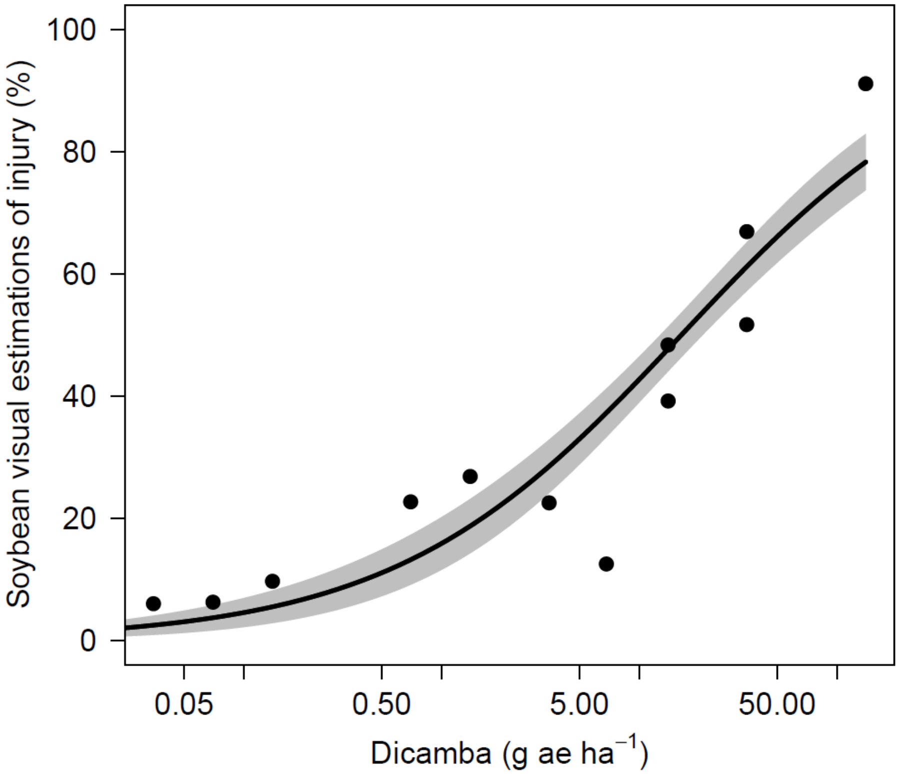

Figure 4.1. Soybean visual injury as affected by dicamba dosage.

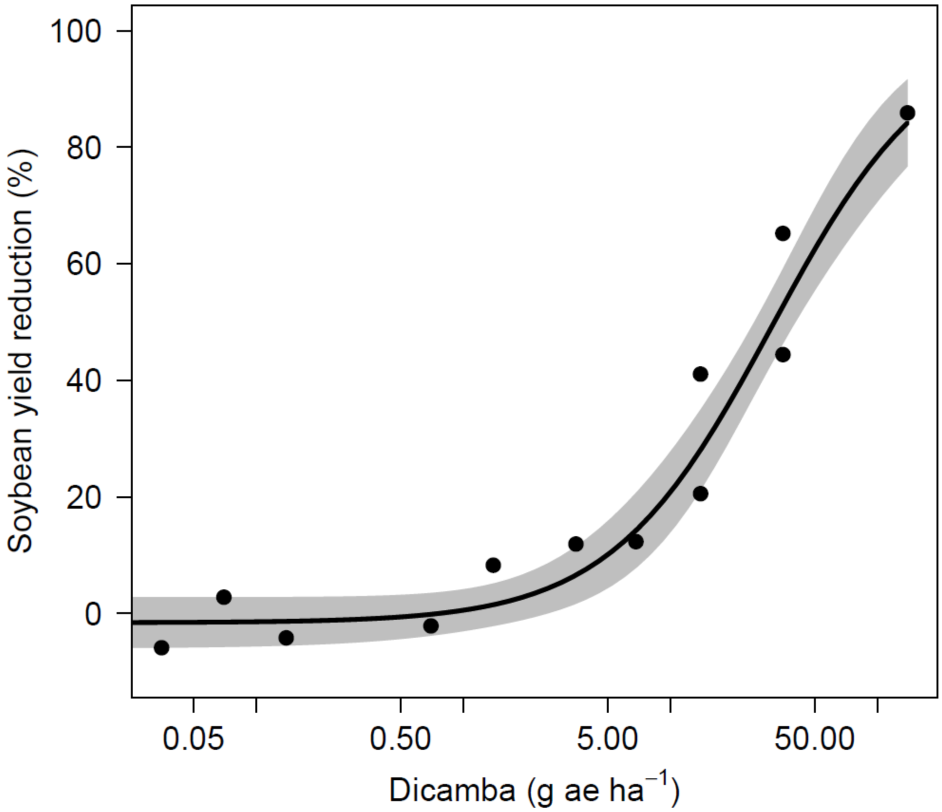

Figure 4.2. Soybean yield as affected by dicamba dosage.

Chapter 5

Lack of Soybean Yield Response to Dicamba Vapor Exposure[4]

5.1. Abstract

The commercial launch of dicamba-tolerant (DT) crops has led to an increased use of this herbicide, and large numbers of off-target movement complaints on sensitive soybeans with volatility identified as a potential source. To investigate the response of sensitive soybean to dicamba vapor in a field setting, experiments were conducted in Macon County, AL in 2018 and 2019 and Lincoln County, NE in 2019. The experiment was conducted by placing low tunnels sealed with plastic tarp and placed over two rows of sensitive soybean at R1 stage for 48 hrs to concentrate vapor emitted from two soil pans treated with dicamba at 0.56, 5.59, 56.42, 559.17, 5591.75, and 11183.51 g ae ha^{-1}. Air samples were collected with low volume air samplers from inside the low tunnels that received 56.42, 559.17, 5591.75 g ae ha^{-1}. Visual injury was recorded at 14 and 21 days after treatment (DAT), and yield was collected at harvest. Dicamba air concentration in low tunnels increased as dosages increased. Soybean injury did not exceed 45% at any rating date regardless of dosage and location. Two highest dosages caused more injury than lower dosages. Soybean height 21 DAT and crop yield was unaffected by dicamba dosage. The results of this study suggest visual injury caused by dicamba vapor is not a reliable indicator of yield response and soybean yield was not reduced by single exposure event to dicamba vapor.

5.2. Introduction

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[4] Authors: Frances B Browne, Xiao Li, Katilyn J Price, Ryan Langemeier, Greg R Kruger; Jeff Golus; Gabrielle de Castro Macedo, Bruno C. Vieira, Tyler Sandlin

The commercial launch of cotton (*Gossypium hirsutum* L.) and soybeans (*Glycine max* L.) crops tolerant to dicamba in 2016 provides a new tool to control herbicide resistant and problematic weeds. Unfortunately, dicamba off-target movement and damage to sensitive crops especially soybeans frequently raise concern in recent years due to increased usage throughout the growing season. Controversy exists among the scientific community regarding the source of exposure and the degree of soybean yield loss resulting from low doses of dicamba (Steckel et al. 2017; Werle et al. 2018). Tank contamination, particle drift, and volatility have been identified as major sources of off-target movement, with the latter two largely impacted by weather conditions during and after application (Soltani et al. 2026; Griffin et al. 2013; Mueller et al. 2019; Mueller et al. 2013). Dicamba volatility is perhaps the most challenging aspect to mitigate as certain environmental conditions associated with this route of herbicide loss, such as temperature inversions, can be difficult to identify and predict (Werle et al. 2018; Bush et al. 2019).

Dicamba is a synthetic auxin in the benzoic acid family of herbicides. Low doses are known to cause leaf cupping, stem twisting, chlorosis, and necrosis among other symptoms on sensitive plants (Soltani et al. 2016; Griffin et al. 2013; Shaner 2014). In the parent acid form, dicamba is highly volatile with a vapor pressure of 4.5×10^{-3} Pa at 25°C (Shaner 2014). New dicamba products registered for use in dicamba tolerant crops include a diglycolamine salt formulation (DGA) which includes a pH modifier of potassium acetate (Vaporgrip®), and an N,N-Bis-(3-aminopropyl) methylamine salt formulation (BAPMA) (Anonymous 2018a,b; Anonymous 2019). These formulations have been shown to possess lower potential for off-target movement caused by volatility (Anonymous 2018a, b; Anonymous 2019). Previous dicamba formulations such as dimethylamine (DMA) salt have been frequently associated with increased volatility as compared to DGA or BAPMA formulations (Egan and Mortensen 2012; Jones et al.

2019; Mueller et al. 2013). Multiple studies have reported volatility for all commercially available formulations (Mueller et al. 2013; Bish et al. 2019; Egan and Mortensen 2012; Jones et al. 2019; Mueller et al. 2019; Bauerle et al. 2015). Egan and Mortensen (2012) observed up to 94% reduction of volatility with DGA as opposed to DMA formulations; however, injury on sensitive soybeans was noted up to 23 m from the treated area. Recent studies further confirm off-target movement of new formulations due to volatility through both analytical and bioassay studies (Bish et al. 2019; Jones et al. 2019). Bish et al. (2019) reported dicamba concentrations of 22.6 and 25.8 ng m^{-3} 0.5 to 8 hours after 560 g ae ha^{-1} was applied for DGA and BAMPA salt formulations, respectively. Jones et al. (2019) conducted a volatility study and observed 5% visual injury at 30 and 24 m from sites where 560 g ae ha^{-1} of the DGA and BAPMA dicamba were applied, respectively. Dicamba labels elaborate many drift management practices to reduce dicamba volatility such as do not make applications during temperature inversions, avoid tank mixes that create low tank pH, ban DMA and DGA formulations or mixing ammonium sulfate with dicamba in applications over dicamba tolerant crops, and warn against use during periods of high temperatures or temperature inversions (Anonymous a,b; Anonymous 2019).

Although research indicates new dicamba formulations will decrease risks associated with volatility, some degree of off-target movement is likely to occur due to vapor pressures, high temperatures and low dosages capable of inducing injury on sensitive plants (Soltani et al. 2016; Griffin et al. 2013; Shaner 2014; Egan and Mortensen 2012). Available literature suggests dicamba dosages as low as 0.03 and 0.15 g ae ha^{-1} are capable of inducing 5% soybean visual injury and yield loss, respectively (Soltani et al. 2016; Griffin et al. 2013; Kniss 2018; Robinson et al. 2013). Unprecedented numbers of dicamba injury complaints on sensitive soybeans have followed commercialization of dicamba tolerant crops, estimated up to 3.6 million affected acres in 2017 (Bradley 2017). Furthermore, the extent of dicamba injury on sensitive crops due to volatility is likely to vary among years due to weather variability as temperature and humidity have been identified as major driving forces (Steckel et al. 2017; Mueller et al. 2019; Egan and Mortensen 2012; Mueller et al. 2013; Behrens and Lueschen 1979). Soybean response to dicamba particle drift has been widely studied in field and greenhouse experiments (Soltani et al. 2016; Griffin et al. 2013; Kniss 2018; Egan et al. 2014). However, majority of dicamba volatility studies in the literature were conducted in greenhouse or humidome settings with bioassay plants and very little research is available to provide information regarding soybean injury and yield response to dicamba vapor exposure under field conditions (Mueller et al. 2019; Mueller et al. 2013; Behrens and Lueschen 1979). In light of the heightened concerns associated with extensive dicamba usage and frequent off-target movement reports throughout mid-western and southern US, the potential for soybeans to be damaged by vapor exposure needs to be evaluated further (Steckel et al. 2017; Werle et al. 2018; Egan and Mortensen 2012; Behrens and Lueschen 1979; Egan et al. 2014). Therefore, the objective of this study was to evaluate sensitive soybean response to various concentrations of dicamba vapor under different field conditions without particle drift influence, and examine the relationship between dicamba vapor, soybean injury and yield response.

5.3. Materials and Methods

5.3.1. Experimental Location, Design and Crop Management

Field studies over three site-years were conducted at the EV Smith Research Center in Macon County, AL (32°29'45.6"N 85°53'25.2"W) in 2018 and 2019 and at the West Central Research and Extension Center in Lincoln County, NE (41° 05’ 15.98” N 100° 46’ 39.42”W) in 2019. Soybean varieties ‘P76T54’ (Pioneer®, Corteva Agriscience, Johnston Iowa, 20131) and

‘NK S24-K2’ (NK®, Syngenta Crop Protection, Greensboro, NC, 2749) were planted at 346,000 seeds ha^{-1} in the AL and NE field studies, respectively. Rows were spaced 91 and 76 cm for the AL and NE field studies, respectively. Planting dates were June 6, 2018 and May 29, 2019 in AL and June 3, 2019 in NE. Plots were 7.6 m long and encompass four rows of soybeans. The center two rows were used to receive dicamba vapor treatments and collect data from. Experiment used randomized complete block design, replicated three times in NE and four times in AL. Soybeans were maintained weed free through herbicides and handweeding. Fertilizer, pesticide and irrigation were applied as needed following local production recommendations.

5.3.2. Dicamba application to soil pans

Kalmia sandy loam soil characterized as fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Typic Hapludults was collected from Macon County, AL and used for experiments at the AL site. Cozad silt loam soil characterized as Coarse-silty, mixed, superactive, mesic Typic Haplustolls collected from Lincoln County, NE was used for the experiment at NE location. Greenhouse plastic trays 60 cm long by 30 cm wide were filled with mesh-screened soil. Soil was thoroughly watered to above saturation point and drained overnight before herbicide application the next morning. Dicamba (Xtendimax® with Vaporgrip® Technology, Bayer CropScience, St. Louis, MO 63167) was applied to soil pans at an off-site location at 0.56, 5.59, 56.42, 559.17, 5591.75, and 11183.51 g ae ha^{-1} then immediately placed under low tunnels to emit vapor. All dicamba applications in Alabama were made with a CO_2-pressurized backpack sprayer equipped with two Turbo TeeJet induction (TTI) 110025 nozzles (TeeJet Technologies, Wheaton, IL 60187) at 6.4 km ha^{-1} delivering 140 L ha^{-1}. Applications were made 0.5-1 km downwind to avoid spray drift damage to sensitive soybean. In Nebraska, dicamba applications were made using a three-nozzle track sprayer in the laboratory and transported on the back of a truck to the field. Application in NE were made using Teejet TTI 11002 nozzles delivering 140 L ha^{-1}. Treatments were applied August 8, 2018 and July 10 in 2019 in Alabama and July 15, 2019 in Nebraska.

5.3.3. Low tunnel installation and soybean field incubation

Polyvinyl chloride (PVC) pipes with 2.54 cm diameters were used to construct low tunnel skeletons. Four arches of 2.5 m long by 1.5 m wide were cut and glued to form a tunnel with final dimensions of 6.6 m long, 1.5 m wide, and 0.9 m tall. Low tunnel skeletons were transported to the field and placed over two middle rows of soybeans. Then, plastic sheeting (Husky®, 3.6 m wide × 9 m long clear 2-mil plastic sheeting, Husky Corporation, Pacific, MO 36069) was installed over the PVC low tunnel skeletons and sealed tightly on three ends by clamps and sand. Two pans of field soil were treated with dicamba as described below and immediately placed between the two center rows of soybean. Extensive care was taken to ensure the pans did not touch soybean foliage This methodology was adapted from a similar study conducted by Sosnoski et al. (2015) where 2,4-D volatility was assessed. After soil pans were placed in each plot, the last side of low tunnel was sealed to allow dicamba vapor to be trapped inside. Two rows of soybean were incubated for 48 hours inside sealed low tunnels before plastic sheeting and PVC skeletons were removed. Soybeans were in the R1 growth stage on date of treatment. Weather data was collected with a Davis Vantage Pro 2 weather station (Davis Instruments, Hayward, CA 94545) at soybean canopy height and weather observations during the first and second 24 hours were recorded which can be found in Tables 1 and 2. Low tunnels were sealed on all four edges which eliminated the potential for accidental dicamba particle drift. These field trials were conducted on university experimental stations where no large scale dicamba application occurred that can cause contamination to sensitive soybean plots during summer months; therefore, visual injury observed on soybeans was a response to dicamba vapor emitted from treated soil pans.

5.3.4. Data collection

Visual injury was estimated on a scale of 0 (no injury) to 100 (complete mortality) at 14 and 21 DAT. Soybean height was measured at 21 DAT in each plot on 10 random selected plants. Soybeans were maintained weed free through maturity and the middle two rows of each plot were mechanically harvested November 6, 2018 and November 11, 2019 in AL and October 14, 2019 in NE. Air samples were taken by low volume air pumps (SKC Aircheck 52 pumps. Eighty Four, PA 15330) and polyurethane foam (PUF) traps (Sorbent Tube, 22 × 100-mm size, 76 mm sorbent, Air Sampling Solutions and Expertise, Eighty Four, PA 15330) at 3 L min^{-1} in low tunnels that received 55.90, 559.17 and 5591.75 g ae ha^{-1} dosages during the second 24 hr of incubation. Air sampling tubing was placed directly above the soil pans in the middle of each low tunnel. PUF traps were analyzed by Mississippi State Chemical laboratory at Mississippi State University in Starkville, MS with a detection limit of 0.3 ng/PUF.

5.3.5. Statistical analysis

Data were subjected to a mixed model analysis of variance using PROC GLIMMIX in SAS 9.4 (Statistical Analysis Systems®, version 9.4; SAS Institute Inc., Cary, NC 27513). Dicamba dosage and location were considered fixed effects, while replication was a random variable. All interactions were examined. If an interaction was significant, data was presented at each level for both fixed effects. Means comparisons were generated using Tukey’s Honest Significant Difference (HSD) test with $P = 0.05$. Yield data was converted to percent of nontreated control (% NTC) to normalize location variances. A non-linear regression model was fitted to the yield reduction and visual injury data using the *DRC* package in R software (R Foundation for Statistical Computing, Wienna, Austria) (Knezevic et al. 2007). The effective-dose to reduce 5% and 10% visual estimations of injury (ED_5 and ED_{10}) or yield loss were estimated using a four-parameter log logistic equation:

$$y = c + \{d - c/1 + exp[b(log\ x - log\ e)]\}$$

in which y corresponds to the % of yield reduction and visual injury (%), x represents dicamba dosage in g ae ha^{-1}, b is the slope at the inflection point, c is the lower limit of the model, d is the upper limit, and e is the inflection point which represent the dosage that caused 50% injury or 50% yield reduction. Visual injury and yield data were pooled over years and locations for non-linear regression to provide maximum prediction power to the model.

5.4. Results and Discussion

Exterior air temperatures 24 hours after soil pans were placed under low tunnels were similar for AL and NE during 2018 and 2019 with averages of 27 and 29°C, respectively (Table 1). AL and NE air temperature averages during the second 24 hours were 26 and 28°C, respectively. Exterior temperature range during the whole incubation period was 22-34°C, 23-35°C and 17-33°C for AL 2018, AL 2019 and NE 2019 experiment, respectively. A weather station failure occurred in AL during 2019 so conditions inside of low tunnels were not recorded. At the NE site, temperatures inside of low tunnels varied greatly, ranging from 17 to 59 °C with a humidity of 59 to 100%. Environmental factors such as temperature and relative humidity have been identified as key drivers of dicamba volatility (Mueller et al. 2019; Mueller et al. 2013; Behrens and Lueschen 1979). Experiments conducted in the southeastern and midwestern US provide an opportunity to evaluate soybean response to dicamba vapor under different environmental conditions.

A site-year by dicamba dosage interaction was observed for visual injury 14 and 21 DAT; therefore, data were analyzed and presented separately for each site-year (Table 2). Visual injury ranged from 0 to 45% across all site-years with observations of typical dicamba symptomology such as leaf cupping, stem twisting, chlorosis, and damage to terminal buds. Visual injury increased 13 and 14% at the NE site from dosages of 5591.75 and 11183.51 g ae ha^{-1} at 21 DAT respectively, compared to 14 DAT ratings. When dosages of 55.90 g ae ha^{-1} or lower were applied to soil pans, less than 14% was observed across both ratings among all locations, meanwhile, 559.17 g ae ha^{-1} and higher dosages generated 7-45% injury on soybean bioassays. Visual injury did not exceed 45% regardless of dicamba dosage and location. Soybean visual injury resulted from dosages of 5,591.75 and 11,183.17 g ae ha^{-1} were similar or higher than lower dicamba dosages in each site-year regardless of rating timings, which indicate greater dicamba vapor emission and concentration inside sealed low tunnels resulted from higher dosages. Air samples collected with low volume air samplers suggested dicamba vapor concentrations inside sealed low tunnels were 1.72, 6.3 and 32.64 ng m^{-3} for dosages of 55.90, 559.17 and 5591.75 g ae ha^{-1}. Sosnoskie et al. (2015) observed 5 to 76% cotton injury from exposure to 2,4-D when volatiles were emitted under open-ended low tunnels for 48 hours. These data demonstrate low tunnel is a viable methodology in future studies to assess plant response to herbicide vapor.

Soybean height was not affected by dicamba dosage at any site-year; therefore, plant height data was not shown. There was no location × dicamba dosage interaction so soybean yield was pooled over 3 site-years for analysis. Soybean yield was not reduced by any dicamba vapor dosage in any site-year and ranged from 2,075 to 2,316 kg ha^{-1}. Non-linear regression analyses of soybean yield or visual injury against dosage indicated a significant relationship between dosage and visual injury but lack of correlation between the yield reduction and dosage (Figure 1 and Table 3). ED_5 and ED_{10} were predicted as 2.4495 and 27.3252 g ae ha^{-1}, respectively. No significant correlation was found between soybean yield to visual injury (Figure 2) as the models used failed to fit data properly. Contrary to the findings in this study, previous literature indicates soybean yield response to dicamba particle drift is highly correlated and reasonably predictable when exposure occurs during reproductive stages than vegetative stages (Kniss 2018; Egan et al. 2014). Literature also suggests soybean yield loss predictions from visual injury are not accurate, overestimation is likely due to subsequent growth and compensation during vegetative stages (Soltani et al. 2016; Kniss 2018; Egan et al. 2014; Foster et al. 2019).

Soybean injury can also be confounded by herbicide tank mix and spray adjuvants. Jones et al. (2018) found that two rates of DGA salt of dicamba and glyphosate tank mixtures at 8.75 + 13.44 and 2.19 + 3.36 g ae ha^{-1}, respectively, applied to sensitive soybeans at R1 stage resulted in 6% more visual injury as compared to dicamba alone at the same rates. Injury increases of 5 to 10% have been observed when dicamba at 0.056 g ae ha^{-1} was tank mixed with crop oil at 1% v v^{-1} (Andersen et al. 2004). Data from this study combined with previous studies indicate soybean response to dicamba via different exposure methods (particle drift vs vapor) can differ greatly. Other factors such as herbicide tank mixture, tank contamination, surfactant, crop stage at exposure, environmental conditions, drought stress, single vs multiple drift events, and duration of exposure can complicate diagnosis following drift events in field (Werle et al. 2018; Soltani et al. 2016; Griffin et al. 2013; Kniss 2018; Egan et al. 2014; Sall et al. 2020; Kelley et al. 2005; Kelley et al. 2005; aJones et al. 2018; Andersen et al 2004).

Soybeans are most vulnerable to yield loss resulted from dicamba exposure during reproductive stages as opposed to vegetative stages when plants have greater potential to recover from injury (Soltani et al. 2016; Griffin et al. 2013; Knis 2018; Egan et al. 2014). A meta-analysis conducted by Kniss (2018) found the lowest dicamba particle drift dosages known to result in a 5% yield reduction ranged from 1.2 to 47 and 0.15 to 14 g ae ha^{-1} for vegetative and reproductive stages, respectively. Experiments in this study were conducted on soybeans at first bloom to evaluate a worst-case scenario, assuming it is most sensitive to dicamba vapor at this growth stage. On the other hand, visual injury from vapor exposure in this study has not exceeded 45%, which created drastic contrast to visual injury caused by dicamba particle drift that often leads to over 50% or even total death with high dosages (Robinson et al. 2013; Soltani et al. 2016; Griffin et al. 2013). The fact that visual injury caused by dicamba vapor remained below 45% combined with lack of yield response indicate further study in which higher vapor concentration during incubation and more frequent exposure may be required. However, it is reasonable to speculate different entry mechanisms for these two exposure methods (particle drift vs vapor) may attribute to differences in injury and yield response of sensitive soybean.

Although soybean yield was not affected by dicamba vapor after a single exposure event, exposure to vapor and particle drift could occur at several growth stages throughout growing season that may lead to yield loss, which warrants further investigation. Meanwhile, as spray particle drift and vapor drift can occur simultaneously, current air sampling technique cannot differentiate dicamba vapor from particle drift and other forms of dicamba sampled in PUF traps which may confound observations from injured plants. Additional research effort will be needed to improve our understanding of how multiple drift events and length of exposure affect sensitive plants and create new techniques to better identify effects of different exposure method on sensitive plants following dicamba off-target movement in field.

5.5. Acknowledgments

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Table 5.1. Hourly air temperature on exterior and interior of low tunnels during first and second 24 hours

	Exterior Temperature (C°)						Interior Temperature (C°)		Interior Humidity (%)	
	AL 2018		AL 2019		NE 2019		NE 2019			
Hour	First 24 hours	Second 24 hours	First 24 hours	Second 24 hours	First 24 hours	Second 24 hours	First 24 hours	Second 24 hours	First 24 hours	Second 24 hours
1	31	30	30	31	29	27	46	41	74	74
2	32	31	31	33	30	28	51	41	70	82
3	33	32	31	33	31	29	55	46	71	82
4	33	33	32	33	32	30	58	50	72	81
5	34	34	35	34	32	31	59	52	72	80
6	34	30	34	33	32	31	58	52	70	79
7	32	25	33	30	33	32	56	51	71	78
8	29	24	33	30	29	31	53	48	74	77
9	29	24	31	29	28	30	45	45	70	76
10	26	24	28	28	27	28	41	41	77	79
11	24	23	28	26	25	26	34	31	82	89
12	24	23	27	26	24	26	25	27	95	98
13	23	23	27	26	23	26	24	26	100	100
14	24	23	26	25	23	25	23	26	100	100
15	23	23	26	25	23	25	23	24	100	100
16	23	22	25	24	21	24	23	24	100	100
17	23	22	25	24	20	23	22	23	100	100
18	23	22	25	24	19	22	21	22	100	100
19	22	22	24	23	18	21	19	21	100	100
20	23	22	25	24	17	21	18	21	100	100
21	24	23	25	25	18	22	17	21	100	100
22	26	24	27	28	21	23	20	24	100	100
23	28	25	28	29	24	25	30	26	92	61
24	30	27	30	31	25	27	38	28	80	59
Average	27	26	29	28	25	27	36	34	87	87

Table 5.2. Soybean visual injury resulted from dicamba vapor exposure

Dicamba Dosage[a]	14 DAT[b]			21 DAT		
	AL[c] 2018	AL 2019	NE 2019	AL 2018	AL 2019	NE 2019
g ae ha^{-1}		—— % ——			—— % ——	
0.56	0[d] b[e]	8 b	1 b	5 b	6 c	1 c
5.59	9 b	9 b	1 b	5 b	8 c	1 c
55.90	11 b	10 b	5 b	14 b	10 c	1 c
559.17	41 a	25 a	7 b	40 a	28 b	6 c
5591.75	43 a	33 a	13 b	45 a	35 ab	26 b
11183.51	40 a	39 a	29 a	36 a	44 a	43 a

[a] Dicamba was applied as the diglycolamine salt formulation (XtendiMax® with Vaporgrip® Technology, Bayer CropScience, St. Louis, MO 63167) to soil pans and place under low tunnels with two rows of soybeans.

[b] Abbreviation; DAT, days after treatment.

[c] A site-year by dosage interaction was observed at $P = 0.05$; therefore, data were analyzed be each site year separately.

[d] Visual injury was estimated on a scale of 0 (no injury) to 100 (complete mortality).

[e] Means followed by the same letter in a column are not significantly different based on Tukey's HSD at $P = 0.05$.

Table 5.3. Parameter estimates for non-linear regression[ab]

Data type	c ± SE	P value	b ± SE	P value	e ± SE	P value	ED_5 (g ae ha^{-1})[c]	ED_{10} (g ae ha^{-1})
Visual injury	0	-	-0.3098 ±0.0334	<0.0001	32867 ±11281	0.0047	2.4495 ±1.9219	27.3252 ±14.7299
Yield loss[d]	-	-	-	-	-	-	-	-

[a] Four parameter log logistic model is used, where c is the minimum value of response variable, d is the maximum value of response variable, e is the point of inflection, b is the slope of the curve at inflection point, x is dicamba dosage.

[b] Parameter c was set as 0% in non-linear regression for visual injury. Parameter d was set as 100% for both visual injury and yield loss in the non-linear regression.

[c] Also known as ED_{50}, effective dosage to cause 50% visual injury or reduce 50% of the yield.

[d] Model did not converge due to lack of trend in dataset.

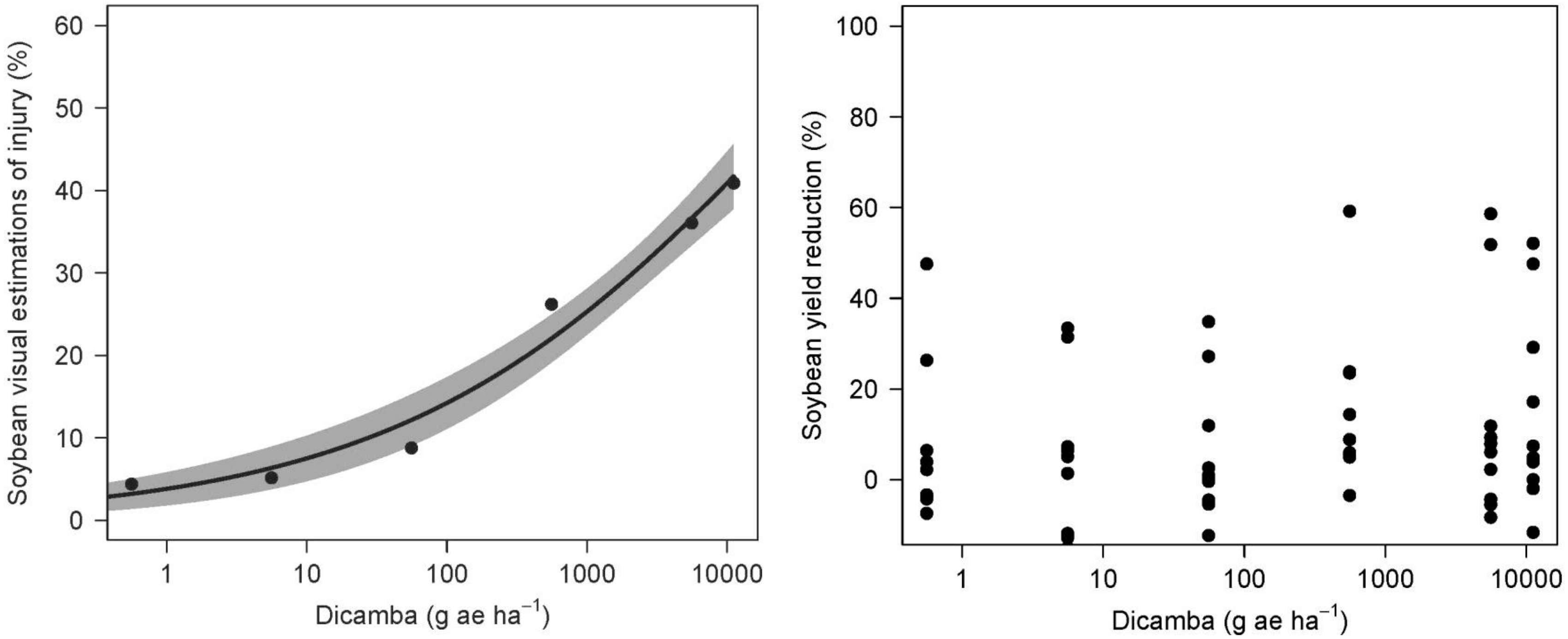

Figure 5.1. Soybean visual injury and yield reduction as affected by dicamba dosage sprayed on soil pans.

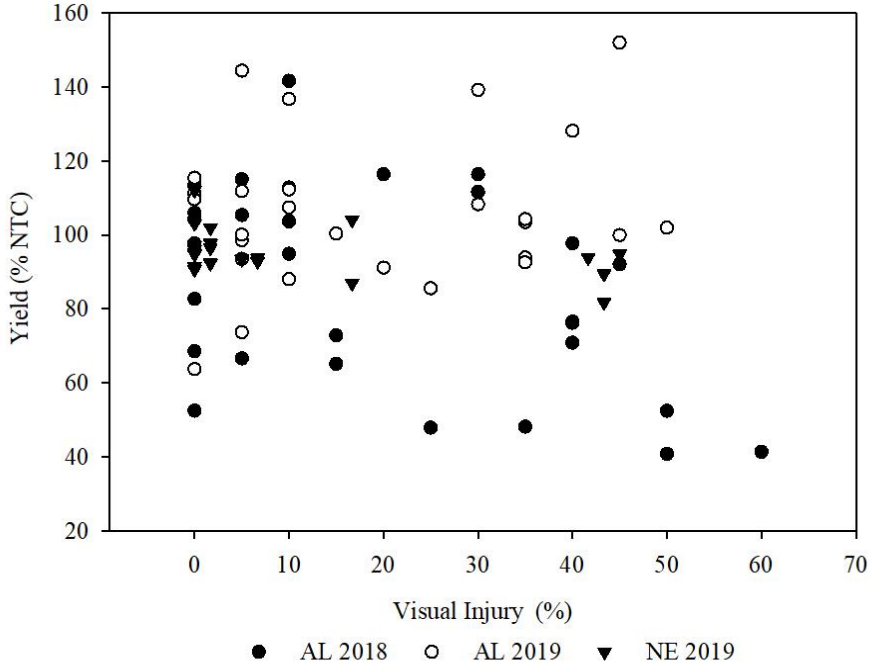

Figure 5.2. Soybean yield plotted versus visual injury.

Chapter 6

Conclusion

Palmer amaranth (*Amaranthus palmeri*) herbicide resistance to existing chemistries is a rapidly evolving problem and a major threat to crop production across the US. Commercial launch of cotton (*Gossypium hirsutum* L.) and soybeans (*Glycine* L.) with resistance to 2,4-D and dicamba has provided additional weed management strategies; however, a large number of off-target movement complaints have followed. Stewardship of synthetic auxins includes more than mitigation of off-target movement and producers are often encouraged to incorporate multiple modes of action into herbicide programs to maintain efficacy of 2,4-D and dicamba. The research in this dissertation addresses major agronomic concerns associated with Palmer amaranth infestations such as adequate activation and cotton tolerance of preemergence (PRE) herbicides, utility of rescue programs to control large escapes, and assess risk for off-target movement of dicamba.

Solubility was a major factor implicated in PRE herbicide activation and water volumes of 1.91, 0, 0.64, and 1.27 cm resulted in the greatest reductions in Palmer amaranth germination for acetochlor, fomesafen, fluridone, and pendimethalin, respectively. Results indicate label suggestions of 1.27 cm water for herbicide activation are sufficient; however, irrigation levels could be manipulated to reduce inputs and maximize Palmer amaranth control. Field studies suggest cotton safety up to two times the highest labeled rates for fomesafen combinations with acetochlor, diuron, fluridone, and prometryn. These results will provide producers with guidelines on choosing PRE herbicides for weed management programs which will reduce selection pressure for POST herbicides to control Palmer amaranth populations.

Results from field studies suggest sequential applications of synthetic auxins followed by glufosinate have the greatest potential to control large Palmer amaranth escapes as compared to the reverse sequence or tank mixture. However, control was not consistent among years and timely applications remain the most effect approach to control escapes. Glufosinate severely impaired Palmer amaranth photosynthesis as compared to dicamba. The only treatment to reduce Palmer amaranth regrowth compared to the nontreated control following leaf removal was dicamba applied 7 days before glufosinate. Multiple applications of POST herbicides with different modes of action increased control of large Palmer amaranth. However, rescue practice is strongly discouraged as environmental conditions can influence herbicide performance and control of large Palmer amaranth is not guaranteed.

Dicamba off-target movement via sprayer contamination and volatility was confirmed. Triple rinsing with water was comparable to glyphosate and commercial cleaning agents for dicamba removal following applications. Dicamba contaminants remaining in sprayers after completion of triple rinse procedures were not higher than the lowest dosages predicted to cause soybean yield loss. Some sprayers did retain enough dicamba in final rinses to cause observable symptomology in soybean and producers should remain cautious when cleaning equipment as the margin of safety is narrow. Soybean visual injury up to 45% resulted from dicamba vapor was observed; however, soybean yield was unaffected at all locations. These data suggest dicamba vapor alone may not result in major yield losses. However, dicamba vapor drift and particle drift could occur simultaneously and result in more severe injury. Proper stewardship of synthetic auxins such as incorporating additional modes of action into weed management programs and mitigation of off-target movement will be required to ensure long-lived viability in crop production systems.