

8-12-2016

Auxin Herbicide Effects on Glyphosate Efficacy and Cotton (*Gossypium Hirsutum*) Yield

Chad Lee Smith

Follow this and additional works at: <https://scholarsjunction.msstate.edu/td>

Recommended Citation

Smith, Chad Lee, "Auxin Herbicide Effects on Glyphosate Efficacy and Cotton (*Gossypium Hirsutum*) Yield" (2016). *Theses and Dissertations*. 832.
<https://scholarsjunction.msstate.edu/td/832>

This Dissertation - Open Access is brought to you for free and open access by the Theses and Dissertations at Scholars Junction. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Scholars Junction. For more information, please contact scholcomm@msstate.libanswers.com.

Auxin herbicide effects on glyphosate efficacy and cotton (*Gossypium hirsutum*) yield.

By

Chad Lee Smith

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Weed Science
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

August 2016

Copyright by
Chad Lee Smith
2016

Auxin herbicide effects on glyphosate efficacy and cotton (*Gossypium hirsutum*) yield.

By

Chad Lee Smith

Approved:

Daniel B. Reynolds
(Major Professor)

David R. Shaw
(Committee Member)

Darrin M. Dodds
(Committee Member)

Michael S. Cox
(Committee Member & Graduate Coordinator)

J. Mike Phillips
(Committee Member & Department Head)

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Chad Lee Smith

Date of Degree: August 12, 2016

Institution: Mississippi State University

Major Field: Weed Science

Major Professor: Daniel B. Reynolds

Title of Study: Auxin herbicide effects on glyphosate efficacy and cotton (*Gossypium hirsutum*) yield.

Pages in Study 74

Candidate for Degree of: Doctor of Philosophy

Field, greenhouse and laboratory experiments were implemented to investigate the effects of auxin herbicides on growth and yield of cotton in glyphosate based systems. Field experiments evaluated the effect of rate and timing of dicamba or 2,4-D exposure when applied in glyphosate-resistant cotton. Increasing rates of either dicamba or 2,4-D resulted in increased injury and yield reductions. Initial injury symptomology was similar for cotton exposed at vegetative and reproductive stages. When cotton was exposed to auxin herbicides during vegetative growth, injury increased with time, while foliar injury during reproductive growth was stagnant and often decreased with time. Subsequently, the strongest correlations to yield loss and injury were from later evaluations of vegetative timings. Recovery from injury due to auxin herbicide exposure was dependent upon favorable environmental conditions; however, recovery was often superficial and masked significant yield loss.

Greenhouse studies evaluated the impact of the diglycolamine dicamba salt on the movement of ^{14}C radio-labeled potassium salt glyphosate in barnyardgrass and johnsongrass. Increasing glyphosate rate increased total absorption of glyphosate in both

species. Total absorption of glyphosate was not impacted by the presence of dicamba, for either johnsongrass or barnyardgrass. Dicamba did not consistently alter the translocation of glyphosate in johnsongrass; however, dicamba did reduce glyphosate translocation in barnyardgrass. Total amount of translocated glyphosate was 2.6 to 4.6% and 3.8 to 6.8% of applied in barnyardgrass and johnsongrass, respectively. Reduced translocation in barnyardgrass was a result of increased glyphosate accumulation in the distal portion of the treated leaf. Increasing the rate of glyphosate did overcome the dicamba induced antagonism; however, altered translocation of glyphosate has been documented to be a precursor to herbicide resistance.

DEDICATION

For my family...yes, all of you.

ACKNOWLEDGEMENTS

I would like to thank the entire faculty and staff in the department of Plant and Soil Sciences at Mississippi State University, most importantly my major professor Dr. Dan Reynolds, as well as my committee members Dr. Darrin Dodds, Dr. David Shaw, Dr. Michael Cox, and Dr. Mike Phillips. I have the utmost respect for all of you and no words can express my gratitude for the knowledge I have gained under your tutelage.

I would also like to thank all the people that have fostered my knowledge of agriculture in my life, most importantly my father Donald, and my brother Cale, as well as, Dr. Andy Kendig, Dr. Reid Smeda, Dr. Harry James, Dr. Trent Irby, Mr. Dwight Abbey and Mr. Jayson Shriver. Every one of you has contributed to my knowledge of agriculture through your leadership, expertise and encouragement and all of you have contributed to this degree, whether you know it or not.

Lastly but not least, I would also like to thank my mother Virginia, and my wife Whitney for their support and their belief that I could accomplish something as big as this in my life. I couldn't have made it without either of you. As for you Ivy, I'm hoping that only your mother will remember all those late nights that went into this.

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	viii
CHAPTER	
I. INTRODUCTION	1
Auxin-mimicking herbicides	3
Auxinic-resistant cotton.....	5
Hazards with auxinic-resistant cotton	7
Literature Cited.....	9
II. COTTON RESPONSE TO SUBLETHAL CONCENTRATIONS OF 2,4-D AND DICAMBA.....	15
Abstract.....	15
Introduction	16
Materials and Methods	19
Results and Discussion	22
Response to Dicamba	23
Response to 2,4-D	26
Conclusions	28
Literature Cited.....	39
III. EFFECTS OF DICAMBA ON GLYPHOSATE UPTAKE AND MOVEMENT IN BARNYARDGRASS (<i>ECHINOCHLOA</i> <i>CRUS-GALLI</i>) AND JOHNSONGRASS (<i>SORGHUM</i> <i>HALEPENSE</i>)	44

Abstract.....	44
Materials and Methods	50
Results and Discussion	53
Barnyardgrass uptake and translocation of ¹⁴ C glyphosate	53
Johnsongrass uptake and translocation of ¹⁴ C glyphosate.....	57
Literature Cited.....	68

LIST OF TABLES

2.1 Monthly and yearly accumulated precipitation in centimeters from Starkville, MS and from Brooksville, MS in 2009, 2010 and 2011.....31

2.2 Monthly and yearly mean high and low temperature from Starkville, MS and Brooksville, MS in 2009, 2010 and 2011.....32

2.3 Planting, application and harvest dates for dicamba and 2,4-D trials conducted at Starkville, MS and Brooksville, MS in 2009, 2010 and 2011.....33

2.4 Cotton injury and percent yield reduction^a following dicamba exposure at Brooksville, MS in 2009, 2010, and 2011 and at Starkville, MS in 2010, and 2011.....34

2.5 Cotton injury and percent yield reduction^a in yield following 2,4-D exposure at Brooksville, MS in 2009, 2010, and 2011 and at Starkville, MS in 2010, and 2011.35

2.6 Pearson correlation coefficients of injury at 1 and 3 WAT to reductions in cotton yield. Data are pooled over years and locations.36

3.1 Percent of the applied ¹⁴C-glyphosate recovered from the water and chloroform wash of the treated leaf and the total plant absorption and recovery of ¹⁴C in barnyardgrass after 24 hours.^a62

3.2 Partitioned and total amounts of ¹⁴C-glyphosate found in the treated barnyardgrass leaf after 24 hours.^a.....63

3.3 Partitioned amounts and sum of ¹⁴C-glyphosate translocated away from the treated leaf of barnyardgrass after 24 hours.^a64

3.4 Amount of ¹⁴C-glyphosate recovered from the water and chloroform wash of the treated leaf and the total plant absorption and recovery of ¹⁴C in johnsongrass after 24 hours.^a65

3.5	Partitioned and total amounts of ^{14}C -glyphosate found in the treated johnsongrass leaf after 24 hours. ^a	66
3.6	Partitioned amounts and sum of ^{14}C -glyphosate translocated away from the treated leaf of johnsongrass after 24 hours. ^a	67

LIST OF FIGURES

2.1	Percent cotton yield loss as a function of the rate of dicamba exposure at the vegetative growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011.	36
2.2	Percent cotton yield loss as a function of the rate of dicamba exposure at the reproductive growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011	37
2.3	Percent cotton yield loss as a function of the rate of 2,4-D exposure at the vegetative growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011	37
2.4	Percent cotton yield loss as a function of the rate of 2,4-D exposure at the reproductive growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011	38

CHAPTER I

INTRODUCTION

Weed control is an important component of producing a high-yielding, quality, crop of cotton (*Gossypium hirsutum*). Cotton weed control, up until 1995, involved an integrated combination of biological, cultural, mechanical and chemical tactics. The cost of weed control prior to 1995 was estimated to be approximately 10% of the total budget for a cotton crop (Vargas et al. 1996). By 2016, weed control costs, including herbicides and trait technology fees, were estimated to be 20% of the budget for a cotton crop in Mississippi (MSU 2016).

In 1995, bromoxynil-resistant cotton was released and became the first genetically modified variety of cotton, followed shortly by glyphosate-resistant (Roundup Ready®) cotton in 1997 (Carpenter and Gianessi 2001; Silvers et al. 2003). Glyphosate is a non-selective, broad-spectrum herbicide that provides excellent control of numerous weed species (Duke and Powles 2008; Ferrell and Witt 2002; Franz et al. 1997). In 2000, only three years after introduction, glyphosate-resistant varieties accounted for 54% of cotton planted in Mississippi (Carpenter and Gianessi 2001). As of 2015, 100% of the planted cotton in Mississippi was glyphosate-resistant (USDA 2015).

The first generation glyphosate-resistant gene inserted into cotton did not impart resistance at all growth stages and were only labeled for glyphosate postemergence (POST) applications up to the fourth-leaf vegetative stage (V-4). Applications of

glyphosate on cotton that was more mature than V-4 could cause both reduced pollination and boll retention, thus reducing final yields (Pline et al. 2002; Viator et al. 2003). The second generation, and current, glyphosate-resistant gene in cotton was marketed under the trade name Roundup Ready Flex® and has a trait that allows for POST applications of glyphosate throughout the growing season to cotton in any growth stage (May et al. 2004). The option to make glyphosate applications at multiple timings throughout the season allow for flexibility of application periods while still providing satisfactory weed control (Main et al. 2007).

Glyphosate applications in glyphosate-resistant cotton have been shown to be an economical and effective weed control program for cotton production systems (Askew and Wilcut 1999; Scott et al. 2002). Askew et al. (2002) found that late season weed control was excellent and net returns from sequential glyphosate treatments were as good if not better than weed control programs using herbicides other than glyphosate. However, as hectarage of glyphosate-resistant crops increased, the subsequent overreliance on glyphosate lead to concerns about shifting weed populations and future development of weed resistant to glyphosate (Culpepper 2006; Culpepper et al. 2006; Duke and Powles 2009; Kruger et al. 2009; Webster and Sosnoskie 2010)

When glyphosate-resistant cotton was released, glyphosate controlled 74 of the 76 world's worst weed species, including all the *Amaranthus* spp. (Franz et al. 1997; Holm et al. 1977). *Amaranthus* spp. have been shown to impact cotton yield in the southern United States (Keeley et al. 1987; Rowland et al. 1999). Palmer amaranth (*Amaranthus palmeri* S. Wats.), is an extremely competitive *Amaranthus* because it is the fastest growing amaranth (Horak and Loughin 2000) and it has the capability to produce a large

number of seeds when in very low densities (Bensch et al. 2003). Palmer amaranth densities between 1 to 10 plants per 9.1 m of row have been shown to reduce cotton canopy volume by 35 and 45% with 6 and 10 weeks of competition, respectively (Morgan et al. 2001). The same study noted that one Palmer amaranth plant per 9.1 m of row reduced yield by 13.4% and yield reductions increased linearly to 56.9% when the population was increased to 10 plants per 9.1 m of row. A single Palmer amaranth plant in 3 m of row has been shown to reduce yields by up to 28%, in addition to creating difficulties for the cotton harvester and increasing the time taken to harvest by 3.5 fold (Smith et al. 2000).

In recent years, numerous weeds have developed resistance to glyphosate in Mississippi. By 2016, critical weeds such as Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer), spiny amaranth (*Amaranthus spinosus* L.), Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), goosegrass (*Eleusine indica* (L.) Gaertn.), horseweed (*Conyza canadensis* (L.) Cronq.), common ragweed (*Ambrosia artemisiifolia* L.), johnsongrass (*Sorghum halepense* (L.) Pers.) and giant ragweed (*Ambrosia trifida* L.) had all developed resistance to the herbicide glyphosate (Heap 2016). The impact of competitive weeds, such as Palmer amaranth, only becomes more significant and control becomes more difficult when the weed is glyphosate-resistant.

Auxin-mimicking herbicides

Auxin-mimicking herbicides, also termed synthetic auxins, or growth-regulator herbicides, are a family of herbicides that affect the auxin hormone indol-3-yl-acetic acid (IAA) in plants (Cobb and Reade 2010; Fedtke 1982). Other than substituted quinolone carboxylic acids, all other classes of auxinic herbicides have herbicidal activity only on

dicot plants (Grossmann 1998). Other classes include benzoic acids like dicamba (3, 6-dichloro-2-methoxybenzoic acid) and phenoxy alcanoic acids like 2, 4-D (2, 4-dichloro phenoxyacetic acid). Widespread use of auxinic herbicides has been documented in cotton production; however, cotton is extremely susceptible to injury from this class of herbicides and thus applications are restricted to the early preplant (EPP) timing (Baker 1993; Everitt and Keeling 2007; Eubank et al. 2008; MSU-MAFES 2016; Keeling et al. 1989; York et al. 2004).

The first herbicidal effects of 2,4-D were documented in 1944 (Hamner and Tukey 1944). After the discovery, 2,4-D was rapidly produced and utilized in the US. This was due to cost effective and efficient synthesis on the manufacturing side, while producers were provided with the ability to use a foliar applied herbicide at low doses to selectively control dicot weeds in monocot crops (Cobb and Reade 2010). When 2,4-D was re-registered in June 2005, the Environmental Protection Agency (EPA) calculated the annual usage of 2,4-D to be 13.6 million kg, while home and garden use was estimated to be an additional 7.3 million kg annually (EPA 2005). After the discovery of the benzoic acid auxin family in 1958, which included dicamba, it also became a significant class of herbicides across multiple crops (Rao 2000).

Both 2,4-D and dicamba have chemical profiles that favor minimal soil and groundwater risk. Groundwater contamination risk is minimal due to little persistence in the environment. Dicamba has an average soil organic carbon coefficient (K_{oc}) value of 2 ml/g indicating weak soil adsorption and suggesting a leaching risk but actual potential for soil leaching is generally considered minimal due to rapid soil degradation (Shaner 2014). Movement of dicamba can occur if degradation is slowed in situations of lower

temperatures (Comfort et al. 1992) or when not enough time is allowed for degradation to occur prior to a hydrological event (Scifres et al. 1973), or if applied to extremely dry soil (Ochsner et al. 2006). Degradation of dicamba results in the non-herbicidal metabolite 3,6-dichlorosalicylic acid (DCSA), which can be readily bound to soils at very broad pH ranges (Murray and Hall 1989). While 2,4-D has a Koc range of 20 to 100 ml/g (Shaner 2014) and has a greater potential to be sorbed to soil, the leaching potential can increase in environments with low pH or temperature (Johnson et al. 1995). Grover (1977) directly compared the leaching potential of dicamba and 2,4-D in sandy loam, loam, and heavy clay soils, and determined that dicamba had greater soil mobility than 2,4-D in every soil type.

Both dicamba and 2,4-D also have minimal mammalian toxicity. The acute oral LD₅₀ of dicamba is 1700 mg/kg in rats and acute dermal LD₅₀ >2000 mg/kg in rabbits. While 2,4-D is 764 to 639 mg/kg in rats and a dermal LD₅₀ of >2000 mg/kg in rabbits. These levels are considered to be representative of a relatively low toxicity compared to other pesticides (Ware 2005; Shaner 2014). For both 2,4-D and dicamba, the aggregate risk levels for acute, short, and long term exposure are well below thresholds set by the EPA (EPA 2005; EPA 2006).

Auxinic-resistant cotton

In 2005, Monsanto Company® announced that they were in the development stage of dicamba-resistant cotton genetics obtained from a license with the University of Nebraska (Behrens et al. 2007; Monsanto 2005). The soil bacterium *Pseudomonas maltophilia* (strain DI-6), which is responsible for the soil metabolism of dicamba into 3,6-dichlorosalicylic acid (DCSA), was used to create the dicamba monooxygenase

(DMO) gene that imparts dicamba-resistance in plants. The enzyme in DMO responsible for conversion to DCSA is O-demethylase, which contains a ferredoxin similar to ferredoxin in plant chloroplasts. By using an agrobacterium gene transfer, plants can be inserted with the DMO gene that allows the breakdown of dicamba within the plant thus allowing the plant to convey herbicidal resistance to dicamba (Behrens et al. 2007).

Monsanto is currently marketing dicamba-resistant cotton under the brand name Bollgard II Xtendflex™, which also includes glyphosate and glufosinate-resistant trait technology (Monsanto 2016).

The use of dicamba in dicamba-resistant cotton provides efficacy on numerous annual and perennial dicotyledonous weeds. Dicamba has been shown to control difficult to control herbicide-resistant weeds such as pigweed spp. (*Amaranthus* spp.), horseweed, common ragweed, giant ragweed, field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* (L.) Scop.) (MSU-MAFES 2016; Eubank et al. 2008; Everitt and Keeling 2007; Swanton et al. 2007). In addition, dicamba has been shown to broaden the broadleaf weed control spectrum of other POST herbicides in corn (Arnold et al. 2005; Nurse et al. 2007; Swanton et al. 2007) and the use of dicamba in cotton could provide similar results (Reynolds 2014). The use of dicamba in cotton would provide an alternative method for broadleaf weed control and more importantly, an alternative method for controlling glyphosate-resistant weeds (Duke 2015; Green 2014; Reynolds 2014).

Wright et al. (2010) documented the ability to create “robust crop resistance” to 2,4-D via bacterial aryloxyalkanoate dioxygenase (ADD) enzymes. Specifically, when the plant is transformed inserting the ADD-12 gene, the plant then has the ability to

metabolize 2,4-D into dichlorophenol thus conveying plant resistance to 2,4-D (Skelton et al. 2014). Currently 2,4-D resistant cotton is being developed by Dow Agrosciences® (DAS) under the trademark Enlist™. Traits that impart glyphosate, glufosinate and 2,4-D resistance are stacked together and will be a part of the Enlist™ cotton program that is currently planned for launch in the 2016 growing season (DAS 2016).

Hazards with auxinic-resistant cotton

Due to the additional weed control options needed to control glyphosate-resistant weeds, the speed of adoption and use of auxinic-resistant cropping systems is expected to be similar to that of glyphosate-resistant crops, providing the new herbicide resistant technology provides satisfactory weed control and is economically competitive (Dill et al. 2008; Riar et al. 2013; Shaner and Beckie 2014). Cotton, like all broadleaves that do not possess genes for auxinic herbicide resistance, is extremely sensitive to injury from dicamba and 2,4-D (Egan et al. 2014; Marple et al. 2007; Smith and Weise 1972). Currently, producers are not only concerned about controlling glyphosate resistant weeds but off-target movement as well. When surveyed in 2013, 68% of professional cotton consultants in the Mid-South listed off-target movement of auxinic herbicides as a concern with new herbicide-resistant cropping systems (Riar et al. 2013). Additional problems could arise from auxin injury to surrounding non-target areas via herbicide volatilization (Egan and Mortensen 2012; Behrens and Lueschen 1979; Riar et al. 2013).

Marple et al. (2007) simulated herbicide drift using fractional rates of auxin herbicides and found that six- to eight-node cotton was visibly injured from either dicamba or 2,4-D applications at 1/400X of the labeled rate, or 1.4 g ae ha⁻¹. Although all rates of dicamba caused visible injury on cotton, the injury was not as severe as from 2,4-

D applications. In addition, cotton yield reductions were not found at dicamba rates of 1/200th or less, but all applications rates of 2,4-D, including the lowest rate of 1/400th X, resulted in yield reductions. Prior research has also indicated that early season auxin injury can be a poor indicator of final effects on cotton yield (Everitt and Keeling 2009; Johnson et al. 2012). Minimal research exists evaluating the timing of the auxin exposure on cotton in conjunction with sublethal concentrations. Experiments simulating auxin exposure were conducted to evaluate cotton injury and yield reductions from multiple rates of dicamba and 2,4-D.

As both 2,4-D and dicamba-resistant cotton traits will be stacked with glyphosate-resistant traits, simultaneous applications of auxin herbicides with glyphosate will be commonplace. Dicamba and 2,4-D have both been shown to antagonize control of johnsongrass when tankmixed with glyphosate (Flint and Barrett 1989). Additionally, barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) control using glyphosate can be antagonized by non-auxinic herbicides (Norris et al. 2001; Starke and Oliver 1998).

Potential for reduced grass control could exist in auxin-resistant herbicide systems. This research examined the reduction in uptake and translocation of glyphosate in johnsongrass and barnyardgrass due to the presence of the diglycolamine salt formulation of dicamba.

Literature Cited

- Arnold RN, Smeal S, O'Neill MK (2005) Broadleaf weed control in field corn with postemergence herbicides. *J Crop Mgmt* doi:10.1094/CM-2005-0224-01-RS
- Askew SD, Wilcut JW (1999) Cost and weed management with herbicide programs in glyphosate-resistant cotton (*Gossypium hirsutum*). *Weed Technol* 13:308-313
- Askew SD, Bailey WA, Scott GH, Wilcut JW (2002) Economic assessment of weed management for transgenic and nontransgenic cotton in tilled and nontilled systems. *Weed Sci* 50:512-520
- Baker RS (1993) Response of cotton (*Gossypium hirsutum*) to preplant-applied hormone-type herbicides. *Weed Technol* 1:150-153
- Behrens M R, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. *Sci*. 316:1185-1188
- Behrens R, Lueschen WE (1979) Dicamba volatility. *Weed Sci* 27:486-493
- Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci* 51:37-43
- Carpenter J., Gianessi L (2001) Agricultural biotechnology: Updated benefit estimates. Washington, DC: National Center for Food and Agricultural Policy. <http://www.ncfap.org/documents/updatedbenefits.pdf>. Accessed April 4, 2016
- Cobb AH, Reade JRH (2010) *Herbicides and Plant Physiology*. 2nd edn. West Sussex, U.K. Wiley-Blackwell. Pp 133-155
- Comfort SD, Inskeep WP, Macur RE (1992) Degradation and transport of dicamba in a clay soil. *J Environ Qual* 21:653-658
- Culpepper AS (2006) Glyphosate-induced weed shifts. *Weed Technol* 20:277-281
- Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Sci* 54:620-626
- [DAS] Dow Agrosiences. (2016) Enlist™ weed control system. <http://www.enlist.com/en>. Accessed April 20, 2016
- Dill GM, Jacob CA, Padgett SR (2008) Glyphosate-resistant crops: adoption, use and future considerations. *Pest Manag Sci* 64: 326–331

- Duke SO (2015) Perspectives on transgenic, herbicide-resistant crops in the USA almost 20 years after introduction. *Pest Manag Sci* 71:652-657
- Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. *Pest Manag Sci* 64:319-325
- Duke SO, Powles SB (2009) Glyphosate-resistant crops and weeds: Now and in the future. *AgBioForum*. 12:346-357
- Egan JF, Barlow KM, Mortensen (2014) A meta-analysis on the effects of 2,4-D and dicamba drift on soybean and cotton. *Weed Sci* 62:193-206
- Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. *Environ Toxicol Chem* 31:1023-1031
- Eubank TW, Poston DH, Nandula VK, Koger CH, Shaw DR, Reynolds DB (2008) Glyphosate-resistant horseweed (*Conyza canadensis*) control using glyphosate-, paraquat-, and glufosinate-based herbicide programs. *Weed Technol* 22:16-21
- [EPA] Environmental Protection Agency (2005) Reregistration eligibility decision for 2,4-D. http://archive.epa.gov/pesticides/reregistration/web/pdf/24d_red.pdf. Accessed Nov 23 2015
- [EPA] Environmental Protection Agency (2006) Reregistration eligibility decision for dicamba. <http://www2.epa.gov/nscep>. Accessed Dec 02, 2015
- Everitt JD, Keeling JW (2007) Weed control and cotton (*Gossypium hirsutum*) response to preplant applications of dicamba, 2,4-D, and diflufenzopyr plus dicamba. *Weed Technol* 21:506-510
- Everitt JD, Keeling, JW (2009) Cotton growth and yield response to simulated 2,4-D and dicamba drift. *Weed Technol* 23:503-506
- Fedtke C (1982) *Biochemistry and Physiology of Herbicide Action*. Berlin. Springer-Verlag. Pg 158-176
- Ferrell JA, Witt WW (2002) Comparison of glyphosate with other herbicides for weed control in corn (*Zea mays*): efficacy and economics. *Weed Technol* 16:701-706
- Flint JL, Barrett M (1989) Antagonism of glyphosate toxicity to johnsongrass (*Sorghum halepense*) by 2,4-D and dicamba. *Weed Sci* 37:700-705
- Franz JE, Mao MK, Sikorski JA (1997) *Glyphosate: A unique global herbicide*. ACS monograph 189. American Chemical Society, Washington, D.C. 653 p

- Green JJ (2014) Current state of herbicides in herbicide-resistant crops. *Pest Manag Sci* 70:1351-1357
- Grossmann K (1998) Quinclorac belongs to a new class of highly selective auxin herbicides. *Weed Sci* 46:707-716
- Grover R (1977) Mobility of dicamba, picloram and 2,4-D in soil columns. *Weed Sci* 25:159-162
- Hamner CL, Tukey HB (1944) The herbicidal action of 2,4 dichlorophenoxyacetic and 2,4,5 trichlorophenoxyacetic acid on bindweed. *Science* 18:154-155
- Heap I (2016) The International Survey of Herbicide Resistant Weeds. <http://www.weedscience.com>. Accessed March 20, 2016
- Holm L, Doll J, Holm E, Pancho J, Herbenger J (1997) *World Weeds: Natural Histories and Distributions*. New York. J. Wiley and Sons, Inc. Pg 226-235
- Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. *Weed Sci* 48:347-355
- Johnson WG, Lavy TL, Gbur EE (1995) Sorption, mobility and degradation of triclopyr and 2,4-D on four soils. *Weed Technol* 43:678-684
- Johnson VA, Fisher LR, Jordan DL, Edmisten KE, Stewart AM, York AC (2012) Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2,4-D. *Weed Technol* 26:195-206
- Keeley PE, Thullen RJ, Carter CH (1987) Repeated annual applications of herbicides for control of rhizome johnsongrass. *Weed Sci* 35:75-80
- Keeling JW, Henniger CG, Abernathy JR (1989) Horseweed (*Conyza canadensis*) control in conservation tillage cotton (*Gossypium hirsutum*). *Weed Technol* 3:399-401
- Kruger GR, Johnson WG, Weller SC, Owen MDK, Shaw DR, Wilcut JW, Jordan DL, Wilson RG, Bernards ML, Young BG (2009) U.S. grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. *Weed Technol* 23:162-166
- Main CL, Jones MA, Murdock EC (2007) Weed response and tolerance of enhanced glyphosate-resistant cotton to glyphosate. *J Cotton Sci* 11:104-109
- Marple ME, Al-Khatib K, Shoup D, Peterson DE, Claassen M (2007) Cotton response to simulated drift of seven hormonal-type herbicides. *Weed Technol*. 21:987-992

- May OL, Culpepper AS, Cerny RE, Coots CB, Corkern CB, Cothren JT, Croon KA, Ferreira KL, Hart JL, Hayes RM, Huber SA, Martens AB, McCloskey WB, Oppenhuizen ME, Patterson MG, Reynolds DB, Shappley ZW, Subramani J, Witten TK, York AC, Mullinix, Jr BG (2004) Transgenic cotton with improved resistance to glyphosate herbicide. *Crop Sci* 44:234-240
- MSU (2016) Cotton 2016 planning budgets.
<http://www.agecon.msstate.edu/whatwedo/budgets/docs/MSUCOT16.pdf>.
Accessed March 20, 2016
- MSU-MAFES (2016) Weed control guidelines for Mississippi. Publication 1532.
- Monsanto (2016) Monsanto® research and development pipeline.
<http://www.monsanto.com/products/pages/research-development-pipeline.aspx>.
Accessed April 20, 2015
- Monsanto (2005) Monsanto® Press Release. <http://news.monsanto.com/press-release/monsanto-unl-sign-agreement-develop-dicamba-tolerant-crops>. Accessed March 22, 2016
- Morgan GD, Baumann PA, Chandler JM (2001) Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. *Weed Technol* 15:408-412
- Murray MR, Hall JK (1989) Sorption-desorption of dicamba and 3,6-dichlorosalicylic acid in soils. *J Environ Qual* 18:51-57
- Norris JL, Shaw DR, Snipes CE (2001) Weed control from herbicide combinations with three formulations of glyphosate. *Weed Technol* 15:552-558
- Nurse RE, Hamill AS, Swanton CJ, Tardif FJ, Sikkema PH (2007) Weed control and yield response to foramsulfuron in corn. *Weed Technol* 21:453-458
- Ochsner TE, Stephens BM, Koskinen WC, Kookana RS (2006) Sorption of a hydrophilic pesticide: effects of soil water content. *J Soil Sci Soc Am* 70:1991-1997
- Pline WA, Viator R, Wilcut JW, Edmisten KL, Thomas J, Wells R (2002) Reproductive abnormalities in glyphosate-resistant cotton caused by lower CP4-EPSPS levels in male reproductive tissues. *Weed Sci* 50:384-447
- Rao VS (2000) Principles of Weed Science. 2nd ed. Boca Raton, FL: CRC Press. Pp 75-76

- Reynolds DZ (2014) Management of glyphosate resistant Palmer amaranth in Bollgard II® XtendFlex™ cotton. M.S.thesis. Mississippi State Univ. 109 p
- Rowland MW, Murry DS, Verhalen LM (1999) Full-season Palmer amaranth (*Amaranthus palmeri*) interference with cotton (*Gossypium hirsutum*). *Weed Sci* 47:305-309
- Riar DS, Norsworthy JK, Steckel LE, Stevenson, IV DO, Bond JA (2013) Consultant perspectives on weed management needs in midsouthern United States cotton: a follow-up survey. *Weed Technol* 27:778-787
- Scott GH, Askew SD, Wilcut JW (2002) Glyphosate systems for weed control in glyphosate-tolerant cotton (*Gossypium hirsutum*). *Weed Technol* 16:191-198
- Scrifres CJ, Alien TJ, Leinweber CL, Pearson KH (1973). Dissipation and phytotoxicity of dicamba residues in water. *J Env Qual* 2:306-309
- Silvers CS, Gianessi LP, Carpenter JN, Sankula S (2003) Current and potential role of transgenic crops in U.S agriculture *In* A. Shrestha ed. *Cropping Systems: Trends and Advances*. Binghamton, NY: The Haworth Press. Pp 501-516
- Shaner DL (2014) *Herbicide Handbook*. 10th edn. Lawrence, KS: Weed Science Society of America, Pp 240-242
- Shaner DL, Beckie HJ (2014) The future for weed control and technology. *Pest Manag Sci* 70:1329-1339
- Smith DT, Baker RV, Steele GL (2000) Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). *Weed Technol* 14:122-126
- Smith DT, Wiese AF (1972) Cotton response to low rates of 2,4-D and other herbicides. *Tx Agri Exp St Bulletin*:B-1120. Pp 8
- Skelton JJ, Simpson DM, Lygin AV, Riechers DE (2014) Metabolic fate of 2,4-D in Enlist™ soybean . *Proc Weed Sci Soc Amer* Pp 356
- Starke RJ, Oliver LR (1998) Interaction of glyphosate with chlorimuron, fomesafen, imazethapyr, and sulfentrazone. *Weed Sci* 46:652-660
- Swanton CJ, Gulden RH, Chandler K (2007) A rationale for atrazine stewardship in corn. *Weed Sci* 55:75-81
- USDA (2015) Cotton varieties planted 2015 crop. <https://www.ams.usda.gov/mnreports/cnavar.pdf>. Accessed March 20, 2016

- Vargas RN, Fischer WB, Kempen HM, Wright SD (1996) Cotton Weed Management *In* Hake SJ, Kerbhy T, Hake K ed. Cotton Production Manual. Oakland, CA. University of California: Division of Agriculture and Natural Resources. Pp 187-202
- Viator RP, Jost PH, Senseman SA, Cothorn JT (2003) Effect of glyphosate application timings and methods on glyphosate-resistant cotton. *Weed Sci* 52:147-151
- Ware GW (2005) Complete Guide to Pest Control with and without Chemicals. 4th ed. Willoughby, OH: Meister Media Worldwide. Pp. 315-344
- Webster TM, Sosnoskie LN (2010) Loss of glyphosate efficacy: a changing weed spectrum in Georgia cotton. *Weed Sci* 58:73-79
- Wright TR, Shan RG, Walsh TA, Lira LM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. *Proc Natl Acad Sci USA* 107:20240-20245
- York AC, Culpepper AS, Stewart AM (2004) Response of strip-tilled cotton to preplant applications of dicamba and 2,4-D. *J Cotton Sci* 8:213–222

CHAPTER II
COTTON RESPONSE TO SUBLETHAL CONCENTRATIONS OF 2,4-D
AND DICAMBA

Abstract

Use of auxin-resistant cotton will increase exposure of non-auxin tolerant cotton to synthetic auxins, which can result in significant injury. Field experiments were conducted to determine the response of cotton to 2,4-D and dicamba. Cotton injury from herbicides and yield reductions were dependent upon the amount and timing of exposure. Increasing herbicide application rate resulted in greater injury. At one week after exposure, injury during early season, vegetative growth was similar to injury from exposure during late season, reproductive growth. By the third week after exposure, cotton injury was more pronounced from auxin herbicides applied at vegetative timings rather than reproductive timings. Yield loss was correlated to visual injury and strongest at later evaluations of vegetative timings. Exposure of reproductively growing cotton to 17.5 g ae/ha⁻¹ of dicamba or 8.8 g ae/ha⁻¹ of 2,4-D resulted in 15 and 12% injury at three weeks after application, and a yield loss of 28 and 34%, respectively.

Recovery from auxin injury was dependent upon environmental conditions during and following an exposure event. As a result, regression modeling of yield to herbicide rate indicated that dicamba and 2,4-D applied to cotton in the vegetative stage resulted in greater variation than applications to cotton in the reproductive growth stage.

Auxin injury on reproductively growing cotton was less affected by environmental conditions, but the reduced foliar symptomology often underestimated yield loss. The ability of cotton to visibly recover from auxin injury during vegetative growth and throughout the growing season could mask potential yield loss and this research suggests that patterning yield loss from early season injury events would be difficult at best, due to geographical variations in climate.

Nomenclature: Dicamba; 2,4-D; cotton, *Gossypium hirsutum* L..

Key words: Auxin, vegetative, reproductive, injury, yield loss.

Introduction

The successful production of a cotton crop requires the removal of most, if not all weeds in the field. Failure to control weeds often results in decreased lint yield and fiber quality, as well as increased production costs (Buchanan and Burns 1971; Morgan et al. 2001; Rowland et al. 1999; Vargas et al. 1996). The development of herbicide resistant weeds, specifically glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats), have created additional difficulties for cotton production (Culpepper et al. 2006; MacRae et al. 2013; Morgan et al. 2001; Sosnoskie and Culpepper 2014). Advancements in cotton traits could provide additional options for control of weeds, including those resistant to glyphosate. Currently dicamba and 2,4-D resistant cotton are commercially available by Monsanto and Dow Agrosiences, respectively. Cotton possessing these traits would be tolerant to auxin herbicides such as dicamba or 2,4-D (Behrens et al. 2007; DAS 2016; Monsanto 2015; Wright et al. 2010). Both dicamba and 2,4-D herbicides have been shown to be effective in controlling numerous broadleaf weeds, including those that are glyphosate-resistant (Chandi et al. 2013; Eubank et al. 2008; Johnson et al. 2010;

Merchant et al. 2014). Injury from applications of auxinic herbicides to non-target species, either via drift, misapplication or volatilization has been historically well documented (Johnson et al. 2012; Sciumbato et al. 2004; Weidenhamer et. al. 1989). Unique symptomology, and the extreme sensitivity to low rates, allow auxin herbicide injury to non-target plants to be easily identified and documented. (Colquhoun et al. 2014; Hatterman-Valenti and Mayland 2005; Hemphill and Montgomery 1981; Kruger et al. 2012; Lanini and Carrithers 2000; Marple et al. 2008)

Exposure of cotton to auxin herbicides can affect growth and reduce lint yield and fiber quality (Marple et al. 2007; Marple et al. 2008; Smith and Wiese 1972). The magnitude of auxinic injury on cotton can be affected by many factors. Staten (1946) documented 2,4-D injury to cotton, specifically differences in severity of injury due to herbicide rate and formulation, citing increased injury from ester formulations. This was confirmed by Smith and Wiese (1972) who further determined that dicamba was not as injurious to cotton, when compared to 2,4-D ester or amine formulations. Similar results were also reported by Marple et al. (2007), who went on to note that visual injury from 2,4-D amine applied to six- to eight-leaf cotton varied with weather conditions, and that increased uptake could occur in hot conditions. The same study also found that post-drift symptomology from dicamba did not accurately reflect yield effects, yet concluded 2,4-D symptomology was well correlated to yield reductions. Johnson et al. (2012) determined that 2,4-D applied to 20 to 30 cm tall cotton at a rate of 20 g ha⁻¹ could significantly reduce seed cotton yield. Early season injury evaluations, plant heights, node counts, and boll counts were poorly correlated to yield loss from sublethal concentrations of dicamba or 2,4-D.

Inconsistent conclusions of correlating injury effects and of yield loss could be a result of differences in application timing. Marple et al. (2008) evaluated applications of dicamba and 2,4-D at two rates (2.8 or 1.4 g ae ha⁻¹) on three- to four-leaf, eight-node, 14-node, or 18-node cotton and found that yield reductions from dicamba and 2,4-D can be a function of crop growth stage. While cotton was susceptible to injury at early growth stages, yield reductions only occurred when 2,4-D was applied at 2.8 g ae ha⁻¹ to three- to four-leaf cotton. The authors further noted that the observed injury could be a function of a short growing season at the trial location. A study conducted by Everitt and Keeling (2009) found that yield reduction was not significant when cotton received 2,4-D at 2.8 g ai ha⁻¹ applied at pinhead square. When the rate was increased to 28 g ai ha⁻¹, yields were reduced by 68%, but it was concluded that visual injury overestimated yield loss. Recent research from Byrd et al. (2016) found that 2,4-D had the greatest impact on yield when exposure occurred at first bloom.

Patterning yield loss has historically been calculated from either differences in seed cotton yield (Everitt and Keeling 2009; Johnson et al. 2012) or as a percent of the untreated (Marple et al. 2007; Marple et al. 2008), resulting in widespread inconsistencies in data (Egan et al. 2014). Yield response to herbicide rate has often utilized linear, exponential, and log-logistic rate-yield response curves (Johnson et al. 2012; Marple et al. 2007; Seefeldt et al. 1995; Snipes et al. 1992; Snipes et al. 1991). However, crops with low levels of auxin exposure can recover from injury and can even result in increased yields, or hormesis (Schabenberger et al. 1999; Solomon and Bradley 2014), which are often omitted or poorly accounted for, when utilizing linear, exponential, or log-logistic curves (Bohnenblurst et al. 2016; Egan et al. 2014). Research has suggested auxin

herbicide injury response data would be better fit if they were normalized by calculating yield as a percent of the untreated (Egan et al. 2014; Oliver 1988) and fit utilizing a logarithmic based linear model (Askew et al. 2002; Egan and Mortensen 2012; Gilreath et al. 2000; Hedges et al. 1999; Sirons et al. 1982).

Currently in the Mid-South, there is limited published data on the effects of 2,4-D (Byrd et al. 2016) on cotton growth and yield, and no published data exists for dicamba. In addition, data are varied for predicting yield loss from exposure and therefore draw different conclusions (Egan et al. 2014), which only highlights the need for evaluating the significance of auxin herbicides on cotton yield. A poll of cotton consultants in the mid-south found that 68% listed off-target movement of auxinic herbicides as a future concern (Riar et al. 2013). The objectives of this research were to utilize dicamba and 2,4-D, at logarithmically reduced rates, to simulate sublethal exposure at both vegetative and reproductive growth stages to evaluate cotton growth effects while patterning yield loss.

Materials and Methods

Two experiments, one with dicamba (Clarity, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) and one with 2,4-D (2,4-D Amine 4, Agri-Star Albaugh Inc., 1525 NE 36th St., Ankeny, Iowa, 50021), were conducted to determine the impact of logarithmically decreasing herbicide rates and the timing of their exposure on injury and yield of cotton. Studies were conducted in 2009, 2010, and 2011 at the Black Belt Branch Experiment Station in Brooksville, MS and in 2010 and 2011 at the W.B. Andrews Agricultural Systems Research Center in Starkville, MS. At all site-years, both experiments were conducted in the same field with a 15.2 m buffer planted between experiments. To reduce off target movement and increase yield sample size, treatments

were applied to plots eight rows wide, measuring 7.7 m wide and 13.7 m long with an additional four untreated border rows established between plots.

Cotton (in 2009, Stoneville “ST 4554B2RF”, Bayer CropScience, 2 T.W Alexander Drive, Research Triangle Park, NC 27709 and in 2010 and 2011, Deltapine “DP 0924 B2RF”, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167) was planted at 153140 seeds ha⁻¹ in rows spaced 0.97 m apart. The soil series at the Starkville location was Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts), while the Brooksville location was Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts). Trials were fertilized with 101 kg ha⁻¹ of nitrogen using 32% UAN sidedressed in the third week after planting. All applications for insects and harvest aids were made based on Mississippi State University Extension Service recommendations. Experiments were defoliated when 60% of the bolls were open in the untreated checks.

Trials were conducted under dryland conditions and were maintained weed-free throughout the duration of the studies, with a PRE application of fluometuron (Cotoran, Makhteshim Agan of North America, 3120 Highwoods Blvd, Suite 100, Raleigh, NC 27604) at 1.12 kg ai ha⁻¹ and POST applications of glyphosate (Roundup Powermax®, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.) at 0.87 kg ae ha⁻¹, pyriithiobac-sodium (Staple LX®, Dupont Crop Protection, Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898) at 34.0 g ai ha⁻¹, and hand weeding as needed.

Both the dicamba and 2,4-D trials were conducted using a factorial arrangement of treatments within a randomized complete block design with four replications. Application timing and herbicide rate served as the main factors. The two application timings for the studies were an early timing, applied during vegetative growth, or a late timing, applied during reproductive growth. Vegetative application timings were applied on five- to 10-node cotton and reproductive application timings were applied on 12- to 16-node cotton. The 1X rate of 2,4-D was 560 g ae ha⁻¹ and the 1X rate of dicamba was 1120 g ae ha⁻¹. The treatment factor of rate was derived from fractioning the 1X rate of each herbicide using a logarithmically decreasing distribution. The fractional rates were 1X, 1/4X, 1/16X, 1/64X, and 1/256X of the herbicide, thus applied rates were 560, 140, 35, 8.8, and 2.2 g ae ha⁻¹ for 2,4-D and 1120, 280, 70, 17.5, and 4.4 g ae ha⁻¹ for dicamba. Untreated checks (UTC) were included with both application timings. All treatments were applied in a sequentially increasing fashion, beginning with the lowest rate. Machinery and materials were cleaned with ammonium hydroxide and water between all timings and experiments.

Treatments were applied with a tractor-mounted sprayer traveling 5 kph and utilized compressed air at 221 kpa to deliver 140 L ha⁻¹ from nozzles with flat fan tip (Teejet XR8002, Spraying Systems Co., North Ave. & Schmale Rd, Wheaton, IL 60189-7900). The boom was 3.85 m wide and was shielded to reduced spray drift. Cotton injury was visually evaluated using a scale where 0 = no injury and 100 = total plant death (Frans et al. 1986).

Treatments were evaluated for injury at one and three weeks after treatment (WAT) and seed cotton yield was harvested from the second, third, sixth, and seventh

rows in each plot. Yield data were transformed to calculate percent change compared to the UTC utilizing the Abbott (1925) formula. For all site-years, reductions in plot yield were calculated using the UTC treatment mean from their corresponding application timing. Data were analyzed using the PROC GLIMMIX function in SAS software (SAS Institute Inc., Cary, NC, 27513) and mean separation was at confidence level of $\alpha=0.05$. Site-year was treated as a random effect and herbicide timing and rate were treated as main effects (Blouin et al. 2011). Pearson correlation coefficients and $P > F$ values were determined for injury at one and three WAT vs. yield reduction. A regression model of herbicide rate vs. yield reduction was fit utilizing the natural log (ln) to linearize data and account for negative values of yield reduction (Hedges et al. 1999).

Results and Discussion

The residual plots were examined for both the dicamba and 2,4-D experiments, and outliers were found in the yield data (data not presented). Outliers were found consistently in the yield data from the Brooksville 2009 site-year, in both the dicamba and 2,4-D studies. At the Brooksville 2009 site-year, the UTC mean yields were 1066 and 1844 kg ae ha⁻¹ in the dicamba and 2,4-D experiments, respectively. The UTC means from Brooksville in 2009 were comparatively lower for both experiments, than pooled means of other site-years. This was not unexpected for the site-year, as both studies were subject to environmental factors that resulted in poor yield potential (MSU-MAFES 2009). The summer drought began with no rainfall and a mean high temperature of 33.7 C in June, and continued through July and August, which had only 19 cm of rainfall combined (Table 2.1 and 2.2). The drought was followed by extremely long periods of heavy rain in September (25 cm) and October (29 cm) which contributed to

significant yield loss when harvest for both studies occurred by hand on December 10th (Table 2.3). All other site-years were able to be mechanically harvested. Subsequently, the yield data from the Brooksville 2009 site-year was concluded to be an outlier and removed from analysis.

Response to Dicamba

Crop injury from dicamba was consistent with auxin herbicide symptomology including leaf cupping, epinasty and red stems (Sciumbato et al. 2004). Injury was similar to that found by Everitt and Keeling (2009) and Johnson et al. (2012). At one WAT, the main factor of timing was not significant and there was no interaction between application timing and rate; therefore, data were pooled across timing (Table 2.4). The insignificance of application timing indicates that initial dicamba injury one WAT will appear similar when cotton is either in the vegetative or early reproductive stages. The sub factor of rate was significant as evident by the stepwise reduction in injury as rate decreased. Injury from dicamba was 46, 33, 26, 18, and 6% for the 1120, 280, 70, 17.5, and 4.4 g ae ha⁻¹ rates, respectively. The 6% injury from 4.4 g ae ha⁻¹ rate was greater than the UTC at one WAT.

At three WAT, the factor of timing was significant with respect to visual injury. Dicamba applied at rates of 1120, 280, 70, 17.5, and 4.4 g ae ha⁻¹ resulted in 66, 50, 34, 30, and 20% injury and 50, 39, 30, 15, and 1% injury from vegetative and reproductive timings, respectively. With the exception of the 70 g ae ha⁻¹ rate, comparable rates of dicamba resulted in significantly more injury from application at the vegetative timing compared to the reproductive timing. The change in factor significance from one WAT to three WAT was due to increased injury between evaluation timings of the vegetative

applications. From one WAT to three WAT, injury increased between 8 to 20% for all dicamba treatments at the vegetative timing, while the change in injury from treatments at the reproductive timing was not greater than 7% (Table 2.4). Increased injury from applications in the vegetative stage was not unexpected as visual injury from dicamba manifests itself in new, actively expanding foliage. As potential vegetative growth was reduced by the reproductive timing, the early application timing would contribute to more visual injury as the plants would be producing greater vegetative growth. Everitt and Keeling (2009) found similar results as dicamba applied to four to five leaf cotton were more injurious than applications at bloom. Injury effects were dependent upon rate with increasing rates resulting in greater injury. While the least injurious rate for both timings was the 4.4 g ae ha⁻¹ rate, the vegetative timing was more injurious (20%) than the reproductive timing (1%), which was not different from the UTC.

Mean seed cotton yield of the UTC was 2816 kg ae ha⁻¹ from the pooled site-years. Application timing and rate were both found to be significant with respect to seed cotton yield. Applications of dicamba at 1120, 280, 70, 17.5, and 4.4 g ae ha⁻¹ rate resulted in a yield reduction of 81, 57, 29, 23, and 18% from the vegetative and 90, 81, 53, 28, and 7% from the reproductive timings (Table 2.4). Seed cotton yield following applications of dicamba at 4.4 g ae ha⁻¹ was not different from the UTC when applied at the reproductive timing; however, applications made at the vegetative timing resulted in an 18% reduction in yield. Dicamba applied at 17.5 g ae ha⁻¹ resulted in at least a 23% yield reduction at either application timing.

Dicamba applied at the reproductive timing had a greater impact on yield than the vegetative timing when applied at 70 or 280 g ae ha⁻¹, while any rate above 17.5 g ae ha⁻¹

resulted in at least a 23% reduction in yield. Differences in yield due to application timing were found from dicamba applied at 1120 g ae ha⁻¹, which resulted in an 81 to 90% yield reduction. Neither application timing of dicamba at the 1120 g ae ha⁻¹ rate resulted in complete loss of yield.

Regression modeling of yield response to dicamba rate is presented by evaluation timing because timing was significant. Compared to reproductive timing, the vegetative timing of dicamba had more variation across site-years and resulted in the regression model accounting for less total variability as evidenced by the coefficient of determination of $R^2 = 0.4124$ (Figure 2.1). Yield response to rates of dicamba at the reproductive timing were more consistent and was also evidenced by increased model fit of $R^2 = 0.9203$ (Figure 2.2). Although yield response to dicamba was more consistent at the reproductive timing, the correlation of injury to reductions in yield was not as strong for reproductive timings compared to vegetative timings at three WAT (Table 2.4). Pearson correlation coefficients of injury to yield were 0.79 at one WAT and were 0.91 and 0.69 at three WAT for the vegetative and reproductive timings, respectively. The weaker linear relationship at one WAT vs. yield and three WAT vs. yield was likely a result of reduced vegetative symptomology in conjunction with the reproductive timing having a greater impact on yield loss (Table 2.4). Despite differences amongst timings, all coefficients indicated a strong relationship between injury and predicted yield loss. Johnson et al. (2012) found significant correlations between dicamba injury and seed cotton yield but subsequently ruled them inconclusive because the absolute value of the coefficient was not greater than 0.6.

Response to 2,4-D

Injury observed from 2,4-D was similar to previously documented literature, including leaf twisting, epinasty, and malformed growth (Sciumbato et al. 2004). The main factor of timing was not significant for injury one WAT and there was no interaction between timing and rate. Therefore, cotton injury at one WAT did not differ between vegetative or reproductive growth stages; therefore, data were pooled across application timing. Application rate was significant and increased injury was a function of increased rate. Injury from 2,4-D at one WAT was 47, 29, 14, 9, and 5% for the 560, 140, 35, 8.8, and 2.2 g ae ha⁻¹ rates respectively (Table 2.5). Cotton injury from different 2,4-D application rates was chained, and the 2.2 g ae ha⁻¹ application rate did not result in visual injury different from the untreated check.

At three WAT, application timing and rate were significant, with respect to visual cotton injury. Vegetative applications of 2,4-D at 560, 140, 35, 8.8, and 2.2 g ae ha⁻¹ rates resulted in 78, 49, 43, 37, and 26% injury while reproductive applications resulted in 53, 26, 20, 12, and 7% injury, respectively (Table 2.5). Increased injury from vegetative applications was due to presence of symptomology on expanding foliage. Like other auxinic herbicides, symptomology of 2,4-D in cotton is more prevalent during active foliar growth and is subsequently reduced when cotton progresses to reproductive growth stages (Marple et al. 2008). The production of new foliage can also cause increased injury with time, as the vegetative application timings had a greater change in foliar growth between one to three WAT, resulting in significance of the timing factor at three WAT. There were greater changes in injury from one to three WAT from vegetative timings of 2,4-D, than changes in injury from reproductive timings. Everitt and Keeling (2009)

documented a similar stagnation in injury response between one and two WAT when blooming cotton was treated with 2,4-D. All rates of 2,4-D applied during vegetative growth had greater injury than the corresponding rate applied at the reproductive stage. Differences in application timing were also noted by Marple et al. (2008) who documented that cotton injury from 2,4-D was more severe from applications at three to four leaf than 8-, 14-, or 18-nodes.

Mean seed cotton yield of the UTC was 2936 kg ha⁻¹ (Table 2.5). Analysis of yield reductions found that application timing was significant and that the magnitude of yield loss from 2,4-D was dependent upon the timing of exposure. Application rate was also significant and evident as greater rates resulted in greater yield loss. The 560, 140, 35, 8.8, and 2.2 g ae ha⁻¹ application rates of 2,4-D applied vegetatively reduced yield by 85, 65, 45, 26, and 8%, whereas reproductive applications reduced yield by 98, 90, 70, 34, and 11%, respectively (Table 2.5). Reduction in yield from 2,4-D applied at 2.2 g ae ha⁻¹ was not different from the UTC at either application timing. Any rate of 2,4-D at 8.8 g ae ha⁻¹ or greater, resulted in a yield reduction of at least 25%, regardless of application timing. Applications at a reproductive growth stage had a greater impact on yield for the majority of the rates. The 560, 140 and 35 g ae ha⁻¹ 2,4-D rates applied at the reproductive timing resulted in an additional 13 to 25% reduction in yield than comparable rates at the vegetative timing. Yield loss from a vegetative application of 2,4-D at 560 g ae ha⁻¹ was equivalent to a reproductive application at the 140 g ae ha⁻¹ rate.

Regression modeling of yield response to rate of 2,4-D is presented by application due to the significance of application timing. Regression of yield response to 2,4-D concentration indicated variation across all vegetatively applied rates and was reflected in

the coefficient of determination of $R^2=0.6745$ (Figure 2.3). Yield response from reproductive applications produced more consistent data across rates, as indicated in the coefficient of determination of 0.9154 (Figure 2.4).

All correlation tests of injury percentage to yield reduction percentage were significant (Table 2.6). Coefficients of yield to injury 1 WAT were 0.74 while coefficients to yield to injury 3 WAT were stronger from applications made at the vegetative (0.91) vs. the reproductive (0.76) timing. These data build on work by Johnson et al. (2012) who found significant, but weak, correlations between visual 2,4-D injury and cotton yield.

Conclusions

Injury data responded accordingly to rate for both dicamba and 2,4-D; however application timing was not significant one WAT, but was at three WAT (Table 2.4 and 2.5). Marple et al. (2008) found that vegetative applications resulted in increased exhibition of auxin symptomology that peaks at 4 WAT. Subsequently, both herbicides resulted in more symptomology and greater assessment of injury from later evaluations. In addition, yield responses were a function of application rate; however, yield loss was often greater from reproductive applications that were also often underestimated in visual injury evaluations (Table 2.4 and 2.5). Cotton yield loss from exposure at reproductive growth stages has been documented to be a result of increased hormone production resulting in abortion of reproductive structures (Guinn 1982; Fedtke and Duke 2005; Marple et al. 2007; Porter et al. 1959). In addition, the ability of cotton to continue vegetative growth following auxin exposure could also contribute to final yield potential (Marple et al. 2008; Smith and Wiese 1972; Snipes et al. 1991). Vegetative recovery

from a low dose of 2,4-D or dicamba on cotton would be dependent upon an extended period of favorable environmental conditions that contribute to growth (Snipes et al. 1991). As a result, yield response to rates of vegetatively applied dicamba and 2,4-D had more variation (Figures 2.1 and 2.3) that was likely due to rainfall differences observed in June, July and August of 2010 and 2011 (Table 2.1). Egan et al. (2014) noted that environmental variability could affect results from auxin injury trials. These data support that conclusion as vegetative applications often had site-years with increased variation of yield response to rate which were likely a result of environmental conditions surrounding the application event, as well as the subsequent period of recovery, regrowth and yield. Marple et al. (2008) stated that weather conditions contributed to full recovery of cotton injury from 2,4-D; however, it should be noted that the variety used for the research was documented to have enhanced tolerance to 2,4-D (Reiger et al. 1986). This research suggests that patterning yield loss from early season injury events would be difficult at best, due to geographical variations in climate. The variation in change of visual injury from one to three WAT, for both dicamba and 2,4-D, could also create difficulties in the prediction of the severity of an event, while the ability of cotton to visibly recover from auxin injury throughout the growing season (Hamilton and Arle 1979; Marple et al. 2008) could also mask potential yield loss.

Despite variation from environmental conditions, the results of both dicamba and 2,4-D studies were more consistent than other studies (Egan et al. 2014; Johnson et al. 2012; Smith and Wiese 1972). Improved modeling and data consistency led to better differentiation due to normalizing yield data by calculating loss as a function of the untreated, as suggested by others (Abbott 1925; Egan et al. 2014; Griffin et al. 2013;

Oliver 1988). The utilization of a natural log (ln) model of regression improved regression fit (Clewer and Scarisbrick 2001; Hedges et al. 1999; Egan et al. 2014). Improved yield consistency was achieved by increasing the harvested sample size to four rows and utilizing larger plots, while yield differentiation between rates was maximized by utilizing a broader range of herbicide rates in both trials. By invoking logarithmically decaying rates of herbicide, as outlined by Seefeldt et al. (1995), a more consistent dose-response of injury and yield were found compared to other existing data (Everitt and Keeling 2009; Johnson et al. 2012; Lanini and Carrithers 2000; Marple et al. 2007). These data also suggest that cotton exposure to dicamba in the vegetative stage can reduce yield. This contrasts Marple et al. (2008) and Johnson et al. (2012) who did not consistently document yield loss from early- or late- season applications of dicamba. The consistent impact of dicamba on yield at the reproductive stage also agrees with predictive modeling done by others (Egan et al. 2014; Everitt and Keeling 2009) who determined that cotton was most sensitive to dicamba at early flowering stage. Early season exposure may have more potential to recover but are also more susceptible to environmental factors. However, in instances where tank contamination of an auxinic herbicide occurs later in season, calculations based on an estimated rate of application could be useful in estimating yield loss.

Future research is needed to determine if irrigation could reduce variation and quantify the difference between treatment rates or timings. In addition, irrigation should be further investigated as a potential tool to improve recovery following auxin exposure.

Table 2.1 Monthly and yearly accumulated precipitation in centimeters from Starkville, MS and from Brooksville, MS in 2009, 2010 and 2011.

Month	Precipitation				
	2009	2010		2011	
	Brooksville	Brooksville	Starkville	Brooksville	Starkville
	cm				
January	19	13	15	16	14
February	9	9	7	7	7
March	7	15	9	15	16
April	8	15	11	23	31
May	17	20	16	7	5
June	0	13	6	5	13
July	13	6	8	12	11
August	6	10	4	2	5
September	25	1	6	26	18
October	18	9	3	3	3
November	4	24	17	9	9
December	10	4	3	12	16
Total	136	139	106	137	148

Table 2.2 Monthly and yearly mean high and low temperature from Starkville, MS and Brooksville, MS in 2009, 2010 and 2011.

	Mean Temperature									
	2009		2010				2011			
	Brooksville		Brooksville		Starkville		Brooksville		Starkville	
	High	Low	High	Low	High	Low	High	Low	High	Low
January	13.7	0.3	8.9	-2.6	9.3	-2.2	10.7	-1.2	10.9	0
February	16.6	0.9	14.6	-4.7	9.7	-1.7	16.2	2	15.7	2.8
March	20.5	7.5	20	4.7	16.4	4.9	21.2	5.4	20.4	7.1
April	24.6	9.2	27.6	7.6	25.8	11.1	25.4	10.8	25.8	12.7
May	29.4	18	29.4	16.5	29.2	18.4	26.8	14.6	27.8	15.5
June	33.7	20.8	33.5	22.6	33.8	22.9	33.8	20.6	34.5	21.4
July	32.8	17.8	33.7	22.7	34.7	23.7	32.6	22.3	34.4	22.2
August	31.4	20.1	34.9	22.8	35.9	23.5	34.4	21.1	34.9	21.2
September	28.8	19.2	33	16	33.4	17.5	28.2	15.5	28.8	14.9
October	22.2	10	27.2	8.5	27.5	9.2	23	6.9	23.5	6.8
November	18.4	5.1	19.6	4.4	20	5.8	20.4	4.5	19.4	5.5
December	10.8	0.3	11.3	-2.6	11.1	-1.3	13.6	1.6	13.7	1.7
Year	23.6	10.8	24.5	9.7	23.9	11	23.9	10.3	24.2	11

Table 2.3 Planting, application and harvest dates for dicamba and 2,4-D trials conducted at Starkville, MS and Brooksville, MS in 2009, 2010 and 2011.

Stage	Date				
	2009	2010		2011	
	Brooksville	Brooksville	Starkville	Brooksville	Starkville
	Planted				
Seed	May 28	May 13	May 12	May 26	May 11
	Application				
Vegetative	July 14	June 22	June 15	July 7	June 20
Reproductive	August 10	July 11	July 6	July 19	July 18
	Harvest				
Mature	December 10	October 18	October 8	October 17	October 3

Table 2.4 Cotton injury and percent yield reduction^a following dicamba exposure at Brooksville, MS in 2009, 2010, and 2011 and at Starkville, MS in 2010, and 2011.

Dicamba Rate		Timing	Cotton response ^b		
Fractional	Actual – g ae ha ⁻¹ –		Visual Injury		Yield Reduction
			1 WAT ^c	3 WAT	
			%		
1X	1120	Vegetative	46 a	66 a	81 a
		Reproductive		50 b	90 a
1/4X	280	Vegetative	33 b	50 b	57 b
		Reproductive		39 c	81 a
1/16X	70	Vegetative	26 c	34 dc	29 c
		Reproductive		30 d	53 b
1/64X	17.5	Vegetative	18 d	30 d	23 c
		Reproductive		15 e	28 c
1/256X	4.4	Vegetative	6 e	20 de	18 cd
		Reproductive		1 f	7 de
UTC ^d	n/a	Vegetative	0 f	0 f	0 e
		Reproductive			

^a Yield data from Brooksville in 2009 were omitted from analysis due to extreme rainfall prior to harvest. Mean yield for all site-site years was 2816 kg ha⁻¹ and percent yield reduction was calculated as function of the mean plot yield of the untreated control.

^b Means within a column followed by the same letter are not significantly different.

^c There was no interaction between timing and rate at 1 WAT and the main effect of timing was not significant therefore data were pooled across timing.

^d The UTC represents the untreated control for both application timings.

Table 2.5 Cotton injury and percent yield reduction^a in yield following 2,4-D exposure at Brooksville, MS in 2009, 2010, and 2011 and at Starkville, MS in 2010, and 2011.

Dicamba Rate		Timing	Cotton response ^b		
Fractional	Actual – g ae ha ⁻¹ –		Visual Injury		Yield
			1 WAT ^c	3 WAT	Reduction
			%		
1X	560	Vegetative	47 a	78 a	85 b
		Reproductive		53 b	98 a
1/4X	140	Vegetative	29 b	49 bc	65 c
		Reproductive		26 e	90 ab
1/16X	35	Vegetative	14 c	43 cd	45 d
		Reproductive		20 e	70 c
1/64X	8.8	Vegetative	9 d	37 d	26 e
		Reproductive		12 f	34 e
1/256X	2.2	Vegetative	5 de	26 e	8 f
		Reproductive		7 fg	11 f
UTC ^d	n/a	Vegetative	0 e	0 g	0 f
		Reproductive			

^a Yield data from Brooksville in 2009 were omitted from analysis to extreme rainfall prior to harvest. Mean yield for all site-site years was 2936 kg ha⁻¹ and percent yield reduction was calculated as function of the mean plot yield of the untreated control.

^b Means within a column followed by the same letter are not significantly different.

^c There was no interaction between timing and rate at 1 WAT and the main effect of timing was not significant therefore data were pooled across timing.

^d The UTC represents the untreated control for both application timings.

Table 2.6 Pearson correlation coefficients of injury at 1 and 3 WAT to reductions in cotton yield. Data are pooled over years and locations.

Herbicide	Evaluation Timing	Variable	P > F	Correlation coefficient
Dicamba	Pooled	Injury 1 WAT vs. yield	< 0.0001	0.79
	Vegetative Reproductive	Injury 3 WAT vs. yield	< 0.0001	0.91
		Injury 3 WAT vs. yield	0.0010	0.69
2, 4-D	Pooled	Injury 1 WAT vs. yield	< 0.0001	0.74
	Vegetative Reproductive	Injury 3 WAT vs. yield	< 0.0001	0.91
		Injury 3 WAT vs. yield	< 0.0001	0.76

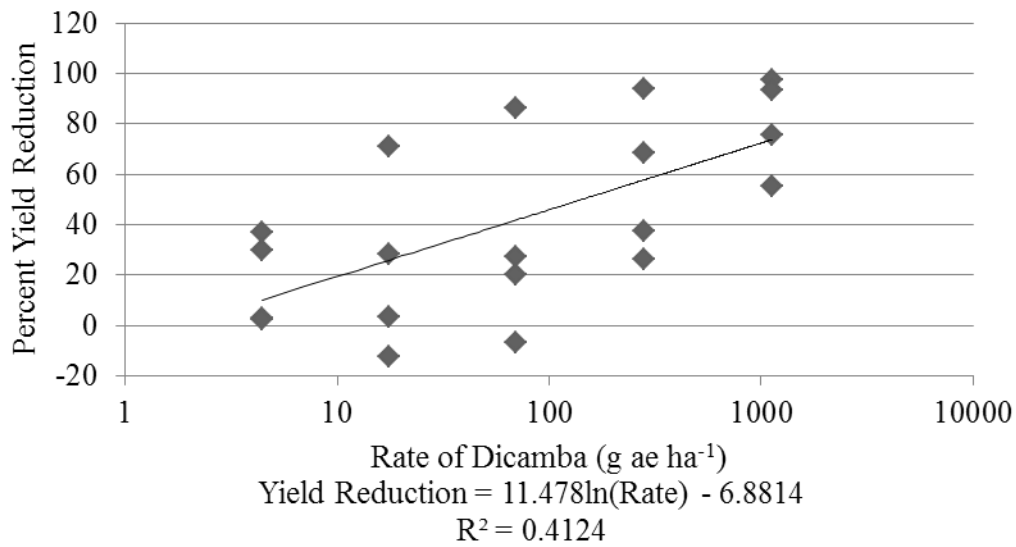


Figure 2.1 Percent cotton yield loss as a function of the rate of dicamba exposure at the vegetative growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011.

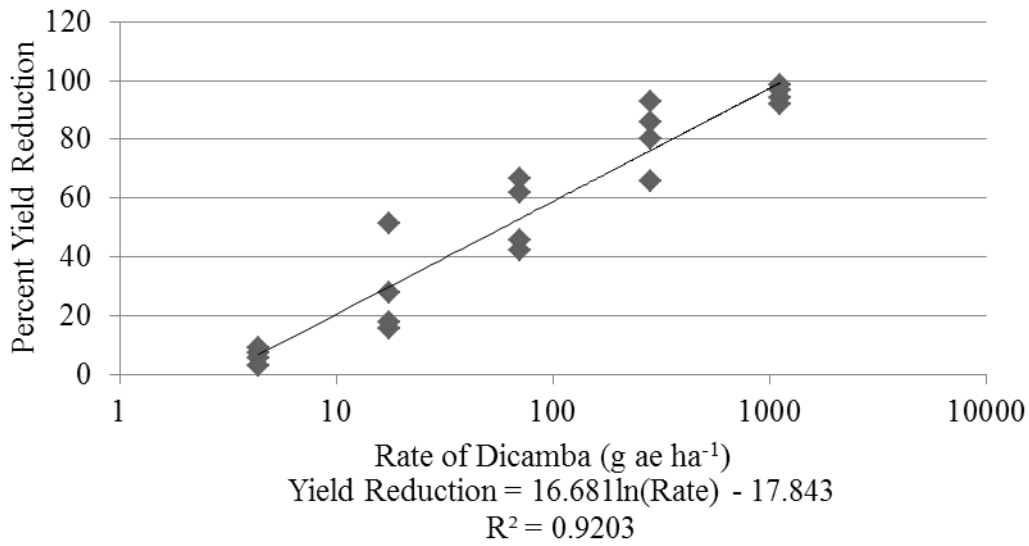


Figure 2.2 Percent cotton yield loss as a function of the rate of dicamba exposure at the reproductive growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011

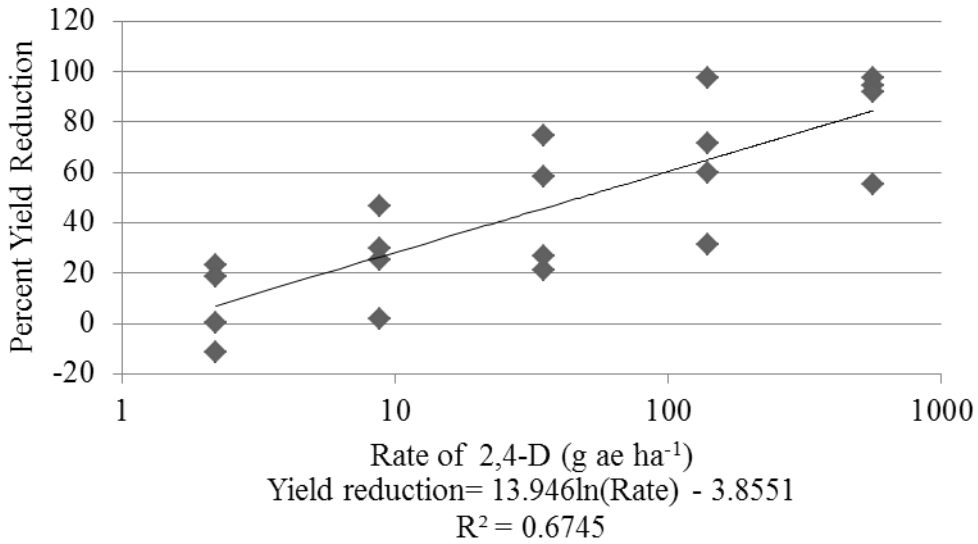


Figure 2.3 Percent cotton yield loss as a function of the rate of 2,4-D exposure at the vegetative growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011

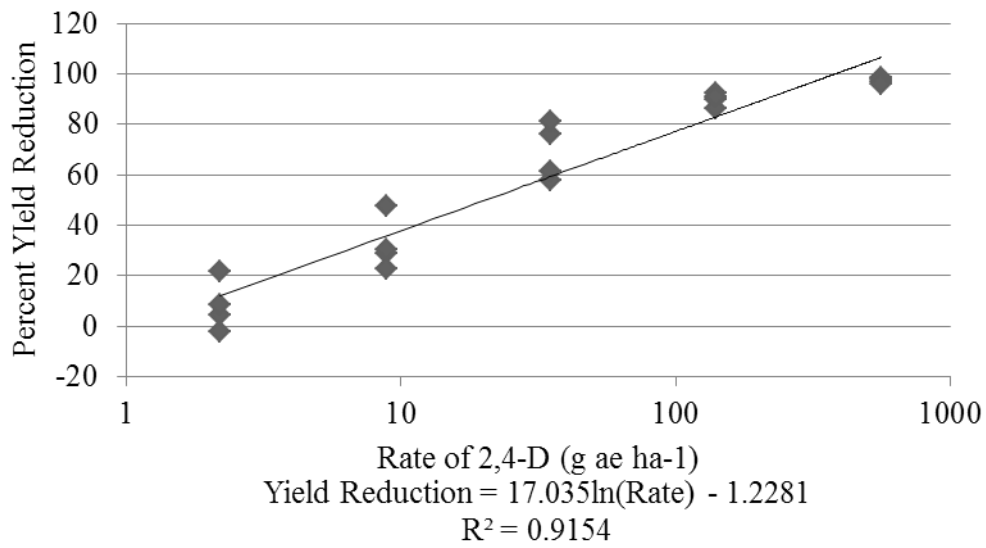


Figure 2.4 Percent cotton yield loss as a function of the rate of 2,4-D exposure at the reproductive growth stage from Brooksville, MS and Starkville, MS in 2010 and 2011

Literature Cited

- Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265-267
- Askew SD, Wilcut JW, Cranmer JR (2002) Cotton (*Gossypium hirsutum*) and weed response to flumioxazin applied preplant and postemergence directed. *Weed Technol* 16:184-190
- Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. *Science* 316:1185-1188
- Bohnenblust EW, Vaudo AD, Egan JF, Mortensen, Tooker JF, (2016) Effects of the herbicide dicamba on nontarget plants and pollinator visitation. *Environ Toxicol Chem* 35:144-151
- Blouin DC, Webster EP, Bond JA (2011) On analysis of combined experiments. *Weed Tech* 25:165-169
- Buchanan GA, Burns ER (1971) Weed competition in cotton. II. cocklebur and redroot pigweed. *Weed Sci* 19:580-582
- Byrd SA, Collins GD, Culpepper AS, Dodds DM, Edmisten KL, Wright DL, Morgan GD, Baumann PA, Dotray PA, Manuchehri MR, Jones AS, Grey TL, Webster TM, Davis JW, Whitaker JR, Roberts PM, Snider JL, Porter WM (2016) Cotton stage of growth determines sensitivity to 2,4-D. *Weed Technol in press*
- Chandi A, Jordan DL, York AC, Milla-Lewis SR, Burton JD, Culpepper AS, Whitaker JR (2013) Interference and control of glyphosate-resistant and -susceptible Palmer amaranth (*Amaranthus palmeri*) populations under greenhouse conditions. *Weed Sci* 61:259-266
- Clewer AG, Scarisbrick DH (2001) Curve fitting *in* Practical Statistics and Experimental Design for Plant and Crop Science. New York: John Wiley and Sons. Pp 87-101
- Colquhoun, J. B., D. J. Heider, and R. A. Rittmeyer. 2014. Relationship between visual injury from synthetic auxin and glyphosate herbicides and snap bean and potato yield. *Weed Technol* 28:671-667
- Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Sci* 54:620-626
- [DAS] Dow Agrosiences (2016) Enlist™ weed control system. <http://www.enlist.com/en>. Accessed April 18, 2016

- Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. *Environ Toxicol Chem* 31:1023-1031
- Egan JF, Barlow KM, Mortensen DA (2014) A meta-analysis on the effects of 2,4-D and dicamba drift on soybean and cotton. *Weed Sci* 62:193-206
- Eubank TW, Poston DH, Nandula VK, Koger CH, Shaw DR, Reynolds DB (2008) Glyphosate-resistant horseweed (*Conyza canadensis*) control using glyphosate-, paraquat-, and glufosinate-based herbicide programs. *Weed Technol* 22:16-21
- Everitt JD, Keeling JW (2009) Cotton growth and yield response to simulated 2,4-D and dicamba drift. *Weed Technol* 23:503-506
- Fedtko C, Duke SO (2005) Herbicides. Pages 247-330 *in* Hock B, Elstner EF eds. *Plant Toxicology*, 4th ed. New York: Marcell Decker
- Frans RE, Talbert R, Marx D, Crowley H (1986) Experimental design and techniques for measuring and analyzing plant responses to weed control practices *in* N. D. Camper, ed. *Research Methods in Weed Science*. 3rd ed. Champaign, IL: Southern Weed Science Society. Pp. 37-38
- Griffin JL, Bauerle MJ, Stephenson III DO, Miller DK, Boudreaux JM (2013) Soybean response to dicamba applied at vegetative and reproductive growth stages. *Weed Technol* 27:696-703
- Gilreath JP, Chase CC, Locascio SJ (2000) Crop injury from sublethal rates of herbicide. III. Pepper. *Hort Sci* 36:677-681
- Guinn G (1982) Cause of boll shedding in cotton. *USDA tech bulletin*:1672. 21 p
- Hamilton KC, Arle HF (1979) Response of cotton (*Gossypium hirsutum*) to dicamba. *Weed Sci* 27:604-607
- Hatterman-Valenti H, Mayland P (2005) Annual flower response to simulated 2,4-D and dicamba spray drift. *Hort Sci* 40:680-684
- Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* 80:1150-1156
- Hemphill DD, Montgomery ML (1981) Response of vegetable crops to sublethal application of 2,4-D. *Weed Sci* 29:632-635
- Johnson VA, Fisher LF, Jordan DL, Edmisten KE, Stewart AM, York AC (2012) Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2,4-D. *Weed Technol* 26:195-206

- Johnson WG, Young B, Matthews J, Marquardt P, Slack C, K. Bradley K, York A, Culpepper S, Hager A, Al-Khatib K, Steckel L, Moechnig M, Loux M, Bernards M, Smeda R (2010) Weed control in dicamba-resistant soybeans. *Crop Manage* doi: 10.1094/CM-2010-0920-01-RS
- Kruger GR, Johnson WG, Doohan DJ, Weller SC (2012) Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. *Weed Technol* 26:256-260
- Lanini WT, Carrithers V (2000) Simulated drift of herbicides on grapes, tomatoes, cotton, and sunflower. *Proc Calif Weed Conf* 52:107-110
- Merchant RM, Culpepper AS, Eure PM, Richburg JS, Braxton LB (2014) Salvage Palmer amaranth programs can be effective in cotton resistant to glyphosate, 2,4-D, and glufosinate. *Weed Technol* 28:316-322
- Monsanto (2015) Monsanto® research and development pipeline. <http://www.monsanto.com/products/pages/research-development-pipeline.aspx>. December 18, 2015
- MacRae AW, Webster TM, Sonoskie LM, Culpepper AS, Kichler JM (2013) Cotton yield loss potential in response to length of Palmer amaranth (*Amaranthus palmeri*) interference. *Jrn Cotton Sci* 17:227-232
- Marple ME, Al-Khatib K, Shoup D, Peterson DE, Claassen M (2007) Cotton response to simulated drift of seven hormonal-type herbicides. *Weed Technol* 21:987-992
- Marple ME, Al-Khatib K, Chandler JM (2008) Cotton injury and yield as affected by simulated drift of 2,4-D and dicamba. *Weed Technol* 22:609-614
- Morgan GD, Baumann PA, Chandler JM (2001) Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. *Weed Technol* 15:408-412
- MSU-MAFES (2009) Mississippi cotton variety trials. *Information Bulletin* 456.
- Oliver LR (1988) Principles of weed threshold research. *Weed Technol* 2:398-403
- Porter Jr. WK, Thomas CH, Baker JB (1959) A three-year study of the effect of some phenoxy herbicides on cotton. *Weeds* 7:341-348
- Reiger C, Dilbeck RE, Undersander DJ, Quisenberry JE (1986) Cotton resistance to 2,4-dichlorophenoxy acetic acid. *Crop Sci* 26:376-377

- Riar DS, Norsworthy JK, Steckel LE, Stephenson IV DO, Bond JA (2013) Consultant perspectives on weed management needs in Midsouthern United States cotton: a follow-up survey. *Weed Technol* 27:778-787
- Rowland MW, Murray DS, Verhalen LM (1999) Full-season Palmer amaranth (*Amaranthus palmeri*) interference with cotton (*Gossypium hirsutum*). *Weed Sci* 47:305-309
- Schabenberger O, Tharp BE, Kells JJ, Penner D (1999) Statistical tests for hormesis and effective dosages in herbicide dose response. *Agron J* 91:713-721
- Sciumbato AS, Chandler JM, Senseman SA, Bovey RW, Smith KL (2004) Determining exposure to auxin-like herbicides. I. quantifying injury to cotton and soybean. *Weed Technol* 18:1125-1134
- Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships *Weed Technol* 9:218-227
- Sirons GJ, Anderson GW, Frank R, Ripley BD (1982) Persistence of hormone-type herbicide residue in tissue of susceptible crop plants. *Weed Sci* 30:572-578
- Smith DT, Wiese AF (1972) Cotton response to low rates of 2,4-D and other herbicides. *Tx Agri Exp St Bulletin*: B-1120. 8 p
- Snipes CE, Street JE, Mueller TC (1991) Cotton (*Gossypium hirsutum*) response to simulated triclopyr drift. *Weed Technol* 5:493-498
- Snipes CE, Street JE, Mueller TC (1992) Cotton (*Gossypium hirsutum*) injury from simulated quinclorac drift. *Weed Sci* 40:106-109
- Solomon CB Bradley KW (2014) Influence of application timings and sublethal rates of synthetic auxin herbicides on soybean. *Weed Technol* 28:454-464
- Sosnoskie LM, Culpepper AS (2014) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. *Weed Sci* 62:393-402
- Staten G (1946) Contamination of cotton fields by 2,4-D or hormone-type weed sprays. *J Am Soc Agron* 38:536-544
- Vargas RN, Fischer WB, Kempen HM, Wright SD (1996) Cotton Weed Control. Pages 187-202 in Johnson Hake S, Kerby T, Hake K, eds. *Cotton Production Manual*. Oakland: University of California Division of Agriculture and Natural Resources, Publication 3352
- Weidenhamer JD, Triplett Jr. GB, Sobotka FE (1989) Dicamba injury to soybeans. *Agron J* 81:637-643

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. *Proc Natl Acad Sci USA* 107:20240–20245

CHAPTER III
EFFECTS OF DICAMBA ON GLYPHOSATE UPTAKE AND MOVEMENT IN
BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*) AND JOHNSONGRASS
(*SORGHUM HALEPENSE*)

Abstract

The adoption of auxin-resistant cotton will increase the use of dicamba, which has been shown to antagonize glyphosate when applied simultaneously. Greenhouse experiments were conducted to evaluate the effects of dicamba on absorption, uptake and translocation of glyphosate in barnyardgrass and johnsongrass. Radiolabeled ^{14}C -potassium salt glyphosate was applied with the diglycolamine salt formulation of dicamba. Plants were harvested 24 h after application and recovery of radiolabelled material was 82 to 89% for barnyardgrass and 77 to 85% for johnsongrass. The majority of applied ^{14}C for both species was found in the water wash of the treated area at harvest. Increasing glyphosate rate did increase total absorption in both species. Total amount of translocated glyphosate was 2.6 to 4.6% and 3.8 to 6.8% of applied in barnyardgrass and johnsongrass, respectively. No reductions in total absorbed glyphosate were observed in barnyardgrass when glyphosate was tankmixed with dicamba; however, glyphosate translocation was reduced when dicamba was applied with the 0.56 and 0.84 kg ae ha⁻¹ rates of glyphosate. Increasing glyphosate application rate to 1.12 kg ae ha⁻¹ did improve translocation of glyphosate to levels observed from glyphosate applied at 0.84 kg ae ha⁻¹

without dicamba. Reduced translocation was a result of increased glyphosate concentration in the distal portion of the treated leaf. Reduced translocation could not be confirmed in johnsongrass but increased glyphosate accumulation in the distal portion of the treated leaf did occur when dicamba was present. Although lethal concentrations of glyphosate were achieved after 24 h in the presence of dicamba, altered translocation of glyphosate has been characterized as a precursor to, and identifier of, glyphosate resistance. Subsequently, consideration should be taken to maximize the efficacy of glyphosate when applications are made to either barnyardgrass or johnsongrass.

Nomenclature: glyphosate; dicamba; barnyardgrass, *Echinochloa crus-gall* (L.) Beauv.

ECHCG; johnsongrass, *Sorghum halepense* (L.) Pers. SORHA

Key words: Translocation, antagonism, tank mixture, interaction

Introduction

Glyphosate (N-(phosphonomethyl glycine) is a broad spectrum herbicide with activity on both broadleaf and grass weeds (Dill et al. 2010; Shaner 2014a). Originally discovered to have herbicidal activity in 1970 by J.E. Franz (Franz et al. 1997), early usage patterns were limited to fallow fields, rights-of-way, and industrial areas due to its non-selective nature (Dill et al. 2010). Glyphosate usage increased after 1996 when Monsanto released glyphosate-resistant soybeans, which included a genetically modified trait in the crop that imparted herbicide tolerance. By 2014, maize (*Zea mays* L.), cotton(*Gossypium hirsutum* L.), canola (*Brassica napus* L.), sugarbeet(*Beta vulgaris* L.), alfalfa(*Medicago sativa* L.), as well as soybean(*Glycine max* (L.) Merr.), were all available with the glyphosate resistant trait (Duke 2015; Duke and Powles 2009). Today,

herbicide resistant crops often contain more than one herbicide-resistant trait and it is expected that in the future, the glyphosate-resistant trait will be combined with traits conferring resistance to acetolactate synthase (ALS), 4-hydroxyphenylpyruvate dioxygenase (HPPD) and acetyl CoA carboxylase (ACCase) inhibiting herbicides, as well as auxin mimicking herbicides such as dicamba and 2,4-D (Duke 2015; Green et al. 2008; Stewart et al. 2010).

Recently developed by Monsanto, Roundup Ready 2 Xtend™ is the trade name for soybeans that contain genes that confer herbicide tolerance to both dicamba and glyphosate, while Bollgard II® XtendFlex™ is the trade name given to cotton containing genes conferring tolerance to glyphosate, glufosinate and dicamba (Monsanto 2015a.) In addition, Monsanto has plans to market a diglycolamine (DGA) formulation of dicamba named XtendiMax™ to be utilized as a component of a tankmix for their dicamba resistant crops (Monsanto 2015b). The development of crops stacked with multiple herbicide resistant traits are seen as a tool to help manage naturally developing herbicide resistant weeds (Duke 2015; Duke and Powles 2009; Shaner 2014b; Stewart et al. 2010). However, the utilization of multiple herbicides in a single application will occur, as it is a common practice termed tankmixing. The herbicide mixture improves the spectrum and/or duration of weed control and reduces application costs, while also mitigating the development of herbicide resistant weeds (Bruff and Shaw 1992; Edwards et al. 2014; Knezevic et al. 2009; Shaw and Arnold 2002; Vangessel et al. 2000).

Although the benefits of mixing multiple herbicides are well stated, tankmixing herbicides can often result in changes of herbicidal efficacy. Synergism is when two separate herbicides, when compared to each herbicide individually, result in greater

efficacy on a weed of a single species. Antagonism is when the combination of two separate herbicides, when compared to each individually, results in reduced efficacy on a single species (Colby 1967; Rummens 1975).

The susceptibility of glyphosate to antagonism has been well documented. Although it is dependent upon the weed species, antagonism of glyphosate activity occurs predominantly in monocots (Baylis 2000; Merchant et al. 2013; Selleck and Baird 1981). Appleby and Somabhi (1978) demonstrated that antagonism of glyphosate can occur when it is mixed with a triazine herbicide, such as atrazine or simazine. They speculated that when glyphosate was tankmixed in a spray solution, glyphosate was physically bound to inert materials in the other herbicide. Stahlman and Phillips (1979) confirmed Appleby and Somabhi's binding theory and concluded that clay particles in triazine formulations were responsible for binding glyphosate in the spray solution. Antagonism of glyphosate activity from triazine or ALS-inhibiting herbicides has been noted to be minor and quantifiable by the Colby method (Colby 1967; Shaw and Arnold 2002). Further research has shown that triazine or ALS-inhibiting herbicides can remain effective tankmix partners with glyphosate when both compounds are used at full rates (Appleby and Somabhi 1978; Hydrick and Shaw 1994; Starke and Oliver 1998; Wilson and Worsham 1988).

The identification and documentation of herbicides that can cause glyphosate antagonism has become more prevalent as glyphosate usage has increased over time. Antagonism of glyphosate has now been documented with tankmixes of many common cotton and soybean herbicides including dicamba and 2,4-D (Selleck and Baird 1981; Flint and Barrett 1989a), glufosinate (Bethke et al. 2013), metribuzin (Hydrick and Shaw

1994), chlorimuron, imazaquin, cloransulam-methyl, pyrithiobac (Shaw and Arnold 2002), fomesafen (Starke and Oliver 1998), flumiclorac (Nandula et al. 2012), monosodium methanearsonate (MSMA) (Burke et al. 2007; Koger et al. 2007), and primisulfuron (Damalas and Eleftherohorinos 2001). In 2008, Monsanto recommended that to sustainably use glyphosate, growers should avoid tankmixing any herbicide that is known to antagonize glyphosate (Gustafson 2008). Currently the two most prevalent herbicide resistant traits, glyphosate and glufosinate-resistance, are often stacked in the same crop despite antagonism from a tankmixed application (Bethke et al. 2013).

Similar to glyphosate, the adoption of auxin-resistant cropping systems will likely result in increased amounts of auxin herbicides applied. As the usage of auxin herbicides increase, documentation of herbicidal antagonism is needed as new combinations of herbicides are applied across a greater range of species. Auxin herbicides have already been documented to reduce herbicidal activity in glyphosate (Flint and Barrett 1989a; O'Sullivan and O'Donovan 1980), ALS-inhibiting (Damalas and Eleftherohorinos 2001) and certain ACCase-inhibiting herbicides (Blackshaw et al. 2006; Mueller et al. 1989). Damalas and Eleftherohorinos (2001) observed that the addition of dicamba to primisulfuron reduced johnsongrass control by 43% compared to primisulfuron alone. Low rates of dicamba were shown to reduce the phytotoxicity of reduced rates of glyphosate in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and wild oats (*Avena fatua* L.), however similar to antagonism from ALS and triazine herbicides, the antagonism was overcome by glyphosate application rate (O'Sullivan and O'Donovan 1980).

Herbicide efficacy is a function of uptake and translocation and can be dependent upon the formulation (Belles et al. 2006; Feng et al. 1998; Li et al. 2005). Antagonism can also be dependent upon the formulation of either herbicide involved (Flint and Barrett 1989a; Green 1989; Hart and Wax 1996). O'Donovan and O'Sullivan (1982) found that the amine and ester formulations of 2,4-D varied in the level to which they antagonized paraquat activity. Hart and Wax (1996) determined that different formulations of dicamba resulted in different levels of antagonism of johnsongrass control with imazethapyr. Koger et al. (2005) suggested that the use of a potassium (K) salt formulation of glyphosate instead of an isopropylamine (IPA) salt, varied uptake and translocation of glyphosate.

More research about how the upcoming auxin-resistant traits and subsequent new combinations of herbicides will impact weed control is needed (Merchant et al. 2013). Flint and Barrett (1989a) documented reduced control of johnsongrass with the IPA salt formulation of glyphosate when mixed with the dimethylamine (DMA) salt formulation of dicamba. However, no research is available on interactions between the more recently developed K glyphosate salt and DGA salt of dicamba. Recently, the translocation of DGA dicamba salt in barnyardgrass was reduced when applied with IPA salt glyphosate (Huff 2010). The objective of this research was to evaluate any potential antagonistic effects that the DGA salt formulation of dicamba may have on the uptake and translocation of the K salt formulation of glyphosate when applied to johnsongrass and barnyardgrass.

Materials and Methods

Separate greenhouse studies were conducted on johnsongrass and barnyardgrass to assess the effect of the DGA salt of dicamba on uptake and translocation of the K salt of glyphosate. Both studies were identically designed as a randomized complete block with four replications and were repeated twice for each species. Barnyardgrass and johnsongrass seeds were planted in cone shaped containers¹ measuring 3.8 cm in diameter and 21 cm tall. Seeds were planted 1 cm deep in a commercial growth media² and containers were sub-irrigated for the duration of the studies. Seedlings were thinned to 1 plant per container following emergence. Studies were conducted in a greenhouse with a photoperiod of 14 h via supplemental lighting³ and a day/night temperature cycle of 32/25 C, respectively.

Herbicide treatments were applied to plants at the four \pm one leaf stage for all studies. Prior to application, plants of similar size and vigor were grouped by rep to improve homogeneity. Both the radiolabeled and non-radiolabeled portions of the treatments utilized distilled water to prevent any cation interaction in the spray solution (Chahal et al. 2013; Roskamp et al. 2013). A potassium salt formulation of glyphosate (N-(phosphonomethyl glycine)⁴ and a DGA salt formulation of dicamba (3,6-dichloro-2-methoxybenzoic acid)⁵ were utilized for the nonradiolabeled portion. Glyphosate was applied at 0.28, 0.56, and 0.84 kg ae ha⁻¹. Dicamba at 0.56 kg ai ha⁻¹ was tankmixed with

¹ Ray Leach SC10 Super Cone-Tainer, Stuewe and Sons, Inc., 31933 Rolland Drive, Tangent, Oregon 97389 USA.

² Metro-Mix 350, Hummert Inc., 4500 Earth City Expressway, Earth City, MO 63045 USA.

³ General Electric Sodium Vapor Lamps, GE 85379 Lucalox LU400, GE Lighting, Nela Park, 1975 Noble Road, Cleveland, Ohio 44112 USA.

⁴ Roundup Powermax, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

⁵ Clarity, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709.

glyphosate at 0.28, 0.56, 0.84, and 1.12 kg ae ha⁻¹. An untreated check was also included for comparison.

The rates of glyphosate in the tankmix treatments were chosen as other research has found that increasing the rate of glyphosate overcomes antagonism (Flint and Barrett 1989a; Koger et al. 2007). Treatments were applied in a spray chamber calibrated to travel 4.8 kph and deliver 140 L ha⁻¹ at 221 kpa through a XR110015E flat fan spray tip⁶. The nozzle tip was 48 cm above the top collar of the plants.

Radiolabeled ¹⁴C-glyphosate [¹⁴C- phosphonomethyl with a specific activity of 1.85 Gbq mmol⁻¹, and 99.5% radio-chemical purity]⁷ was dissolved into an aliquot from each of the previously mixed non-radiolabeled solutions to create a final radiolabeled solution of 1.45 Kbp per 10 µl. Prior to application, a 2.5 cm long piece of adhesive backed paper⁸ was placed on the fourth leaf midway between the tip and the collar on the adaxial surface. After the application of the non-radiolabeled portion, the covered treatment area was pressed onto a horizontal section of vinyl board⁹ that was preadjusted to the height of the treated leaf. To maintain a horizontal position, a removable glue dot¹⁰ was placed between the board and the abaxial leaf surface of the treated area. The adaxial paper was removed so that the radiolabeled portion of the treatments could be applied. Each plant was dosed using micropipette with a 10 µl aliquot of the radiolabeled treatment solution, evenly dispersed across the treatment area in 20-25 spots.

⁶ Teejet, Spraying Systems Co., 200 W. North Ave, Glendale Heights, IL 60139.

⁷ American Radiolabeled Chemicals, 101 ARC Drive. Saint Louis, MO 63146.

⁸ Post-It Office Note, 3M Corporate Headquarters, 3M Center, St. Paul, MN 55144.

⁹ Barrette Vinyl Fence Rail, Barrette Outdoor Living, 7830 Freeway Circle, Middleburg Hts., OH 44130.

¹⁰ Removable Glue Dots, Glue Dots Intl., N117,W18711 Fulton Drive, Germantown, WI 53022.

Plants were harvested and partitioned 24 hours after treatment (HAT) to maximize recovery of ^{14}C -glyphosate and translocation differences (Feng et al. 1998; Feng et al. 2000). Removal of the treated leaf from the board was aided with warm air from a handheld hair dryer¹¹. The treated area was excised and washed in 10 ml of distilled water for 10 s to remove any ^{14}C -glyphosate remaining on the leaf surface followed by a 10 ml chloroform wash for 10 s to remove ^{14}C -glyphosate contained in epicuticular wax. The treated area was then placed in a 20 ml glass scintillation vial¹² while all other plant tissue was placed in coin envelopes. The remaining foliage around the treated area was partitioned into distal and proximal leaf tissue. The distal leaf portion was from the treated area to the leaf tip and the proximal leaf portion was from the treated area to the collar, including the leaf sheath. The remainder of the plant was separated at the node where the treated leaf joined the stem. From the node of the treated leaf to the base of the plant was considered to be the lower plant portion while all tissue above the node was considered to be the upper plant portion. Water was used to remove growing medium from the roots.

All plant parts were lyophilized¹³ and oxidized¹⁴ to convert all ^{14}C to $^{14}\text{CO}_2$. A liquid scintillation cocktail mixture was then used to capture¹⁵ evolved $^{14}\text{CO}_2$ and fluoresce¹⁶ the captured ^{14}C . A 1 ml aliquot was pulled from the water and chloroform leaf washes of the treated area and then added to a liquid scintillation cocktail¹⁷ to induce

¹¹ Conair 1875 Dryer, Conair Corporation, 1 Cummings Point Road, Stamford, CT 06902.

¹² Foil-lined High Performance Glass Vial. Perkin-Elmer, 940 Winter St. Waltham, Massachusetts 02451.

¹³ FreeZone 18L, LabConCo, 8811 Prospect Ave., Kansas City, MO 64132-2696.

¹⁴ Model A307 Sample Oxidizer, Perkin Elmer, 940 Winter St. Waltham, Massachusetts 02451.

¹⁵ Carbo-Sorb E, Perkin Elmer, 940 Winter St. Waltham, Massachusetts 02451.

¹⁶ Permafluor E+, Perkin Elmer, 940 Winter St. Waltham, Massachusetts 02451.

¹⁷ Ultima Gold, Perkin Elmer, 940 Winter St. Waltham, Massachusetts 02451.

fluorescence for quantification. Quantification of disintegrations per minute (DPM) for each sample was conducted on a liquid scintillation counter¹⁸. The scintillation counter utilized an internal quench curve and samples were counted for 5 minutes. Counts from the aliquots of the leaf washes were back calculated to compensate for the total volume. Total recovery of radioactivity was calculated as a sum of the leaf washes, the plant foliage and the roots. Total plant fraction was calculated as a sum of all portions of plant foliage and roots. Treated leaf totals were calculated as the sum of the radioactivity of the proximal, distal, and treated leaf tissue. The total for the plant outside of the treated area was calculated as the sum of the tissue above and below the treated node, as well as the roots. Data were analyzed in SAS software¹⁹ utilizing PROC GLIMMIX. Repetitions of studies were combined for analysis and pairwise mean comparisons were calculated with an alpha=0.05.

Results and Discussion

Barnyardgrass uptake and translocation of ¹⁴C glyphosate

Total recovery of ¹⁴C ranged from 82 to 89% and was similar to recoveries from other studies conducted with the IPA salt glyphosate (Feng et al. 1998. Kirkwood et al. 2000). Recovery levels from treatments of glyphosate alone generally decreased with the rate of glyphosate, and a significant reduction was found between the 0.84 and 0.28 kg ae ha⁻¹ rates (Table 3.1). As there is little in-vivo metabolism of glyphosate, translocation is primarily via the phloem where glyphosate is shifted to the roots where it is exuded and degraded by soil bacterium (Coupland and Caseley 1979; Cobb and Reade 2010; Fedtke

¹⁸ Tri-Carb 2810TR, Perkin Elmer, 940 Winter St. Waltham, Massachusetts 02451.

¹⁹ SAS Institute Inc., 100 SAS Campus Drive., Cary, NC 27513-2414.

and Duke 2005; Rodrigues et al. 1982). Geiger and Bestman (1990) concluded that there can be a self-limiting response from higher rates of glyphosate, where rapid phytotoxic action can result in a decreased translocation. Feng et al. (1998) suggested that reduced glyphosate recovery could be a function of translocation speed, and as critical glyphosate concentrations were slowed or reduced within a plant, translocation of glyphosate to capillary roots could increase. The difference between recovery of ^{14}C -glyphosate at the 0.84 to 0.28 kg ae ha⁻¹ rates could be attributed to the speed at which glyphosate is moved to, and exuded from, the capillary roots, which may have been lost in harvest.

Conversely, total recovery from tankmix treatments were unchanged across all glyphosate rates and could be a function of slower movement and loss through the capillary roots (Flint and Barrett 1989b). Soil media was tested, however no treatments were found to have ^{14}C levels above background (data not presented).

The majority of applied ^{14}C was found in the water washes of the treated area and ranged from 64 to 74% (Table 3.1). Chloroform washes contained <0.3% of ^{14}C from all treatments and indicated minimal adsorption of K salt glyphosate in the epicuticular wax and was similar to results documented on the IPA salt formulation (Norsworthy et al. 2001; Sherrick et al. 1986). Recovery of ^{14}C in the total plant fraction ranged from 10 to 23% and agrees with others in that plant uptake is the largest hindrance to an application of glyphosate (Leaper and Holloway 2002). This was also evidenced by the significant reductions in recovered ^{14}C that corresponded to a stepwise reduction in glyphosate rate. Previous research has found that lower rates of glyphosate will decrease the total uptake and concentration within the plant (Cranmer and Linscott 1991; Feng et al. 1999; Feng et al. 2000; Norsworthy et al. 2001; Singh et al. 2011). Total absorption of K salt glyphosate

was similar to levels reported by others (Koger et al. 2005) and despite the response to rate, there were no reductions in total absorbed glyphosate found when the 0.28, 0.56 or 0.84 kg ae ha⁻¹ rates of glyphosate were combined with dicamba when compared to glyphosate alone (Table 3.1)

The greatest amount of ¹⁴C glyphosate within the plant was found in the treated leaf (Table 3.2). The addition of 0.56 kg ae ha⁻¹ of dicamba to glyphosate at 0.56 or 0.84 kg ae ha⁻¹ significantly reduced ¹⁴C glyphosate found in the treated area compared to where glyphosate was applied alone at the same rates. Differences observed in the proximal or distal leaf tissue, as well as leaf totals, were generally a function of glyphosate rate. However, the distal leaf tissue generally had greater amounts of ¹⁴C glyphosate when dicamba was included, which can often be an indicator of acropetal translocation and/or glyphosate resistance (Bostamam et al. 2012; Feng et al. 2004; Lorraine-Collwill et al. 2002)

Glyphosate content in the upper and lower plant tissue was also a function of glyphosate application rate as opposed to dicamba presence (Table 3.3). Total reductions in ¹⁴C root accumulation occurred only when 0.84 kg ae ha⁻¹ of glyphosate was combined with dicamba. However, compared to glyphosate alone, the accumulated glyphosate total outside the treated leaf was reduced when the 0.56 and 0.84 kg ae ha⁻¹ rates were applied with dicamba. Herbicide translocation is defined as the difference between the amount found in the treated leaf and the amount of herbicide found in locations outside of the treated leaf, and any decrease of that amount is considered to be a reduction in translocation (Dewey and Appleby 1983; Feng et al. 1998; Koger et al. 2005).

Burke et al. (2007) documented that the severe antagonism of glyphosate from MSMA often resulted in excellent control and altered translocation may not be of biological significance. Therefore, it could be contested that as the quantity of in vivo glyphosate likely reached a lethal level after 24 hours because levels are also likely to increase over time (Burke et al. 2007; Feng et al. 2000; Koger et al. 2005) and that differences in translocation would be negligible at the field level. However, good translocation is considered to be a strong indicator of plant death (Feng et al. 1998; Shaner 2009), but more importantly, altered glyphosate translocation has been characterized as a precursor to, and identifier of, glyphosate resistance (Burke et al. 2007; Nandula et al. 2015; Powles and Preston 2006; Shaner 2009; Vila-Aiub et al. 2012). Recently, glyphosate-resistant johnsongrass was characterized in Arkansas and translocation of glyphosate out of the treated leaf was found to be almost 20% less than susceptible biotypes (Riar et al. 2011b). Numerous other studies have identified glyphosate resistant weeds with altered translocation, especially acropetal movement, and translocation patterns were independent upon glyphosate formulation (Koger and Reddy 2005; Nandula et al. 2015; Norsworthy et al. 2001; Riar et al. 2011b; Ribeiro et al. 2015; Starke and Oliver 1998). Tankmixing herbicides with glyphosate can also result in antagonism that can shift herbicide uptake and translocation resulting in reduced control (Hydrick and Shaw 1994; Steele et al. 2008). Changes in glyphosate translocation can also naturally occur, as documented in some weeds with a natural tolerance to glyphosate (Koger et al. 2004; Reddy 2000; Norsworthy et al. 2001), or in instances of extreme environmental conditions, including temperature and humidity (Ahmadi et al. 1980 ; Masiunas and Weller 1988; Wicks and Hanson 1995).

Recent weed resistance modeling by Bagavathiannan et al. (2013) concluded that multiple applications of glyphosate in continuously cropped glyphosate-resistant cotton systems would likely produce glyphosate-resistant barnyardgrass by the ninth year. Botha et al. (2014) suggested that all glyphosate tankmix combinations that could result in reduced doses should be fully evaluated, across multiple environments, for performance and robustness in order to prevent glyphosate resistance. Therefore, utilizing weed size to determine the proper application rate and dosage of glyphosate is important to minimize risk of herbicide resistant weeds. These data suggest that the size of barnyardgrass in this study (<22 cm) and the current corresponding labeled rate of K-salt glyphosate²⁰ at 0.84 kg ae ha⁻¹ (Monsanto 2016), may not result in an effective dose of glyphosate when tankmixed with dicamba. However, an application of glyphosate at 1.12 kg ae ha⁻¹ with dicamba had glyphosate translocated to plant tissue outside of the treated leaf to levels found at the 0.84 kg ae ha⁻¹ rate of glyphosate alone (Table 3.3). This data is in agreement with others that increased glyphosate application rates could be utilized to offset any changes in translocation (Kirkwood et al. 2000; Norsworthy et al. 2001) and contrasting Sikkema et al. (2005), who suggested that lower production costs and adequate control of barnyardgrass could be achieved with an application of glyphosate at a rate of 0.45 kg ae ha⁻¹.

Johnsongrass uptake and translocation of ¹⁴C glyphosate

Recovery of ¹⁴C-glyphosate ranged from 77 to 85% (Table 3.4). Chloroform washes of the treated section resulted in < 0.7% of ¹⁴C captured glyphosate with no

²⁰ Roundup Powermax, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

significant differences among treatments. Water washes from the treated area contained the majority of the ^{14}C and ranged from 55 to 72% of the applied amount. Prior research has found that differences in the water wash were a function of rate as higher applied rates resulted in less ^{14}C found in the wash, while lower rates resulted in less ^{14}C absorbed (Feng et al. 2000). Norsworthy et al. (2001) noted the same response in barnyardgrass and hemp sesbania (*Sesbania herbacea* (Mill.) McVaugh) while attributing the relationship to lower rates of glyphosate having reduced concentration gradients when applied, which result in less absorption and greater residual on the leaf surface. Greater concentration gradients have been shown to be critical to increasing the speed and uptake of glyphosate (Cranmer and Linscott 1991; Kirkwood et al. 2000). Plants in this study were also harvested at 24 HAT and others have suggested that absorption would increase with additional time (Kirkwood et al. 2000; Feng et al. 2004; Norsworthy et al. 2001).

Increases in total ^{14}C glyphosate absorbed in each treatment were also a function of glyphosate rate and ranged from 13 to 24% of the applied. The addition of dicamba to any rate of glyphosate did not significantly affect the amount recovered from the treated area or the proximal portion of the treated leaf. However, when dicamba was added to the 0.84 kg ae ha⁻¹ rate of glyphosate, the amount of ^{14}C glyphosate found in the distal leaf portion doubled compared to the treatment without dicamba (Table 3.5). The tankmix of glyphosate at 1.12 kg ae ha⁻¹ and dicamba were found to be at a level similar to the 0.84 kg ae ha⁻¹ rate of glyphosate alone, possibly indicating outward flow of glyphosate as described by others (Dewey and Appleby 1983; Lorraine-Colwill 2002). In addition, total concentrations of glyphosate in the treated leaf increased when dicamba was applied with glyphosate at the 0.84 kg ae ha⁻¹ rate.

The amount of glyphosate translocated outside of the treated leaf ranged from 3.8 to 6.8% of applied (Table 3.6) and was similar to levels previously documented in johnsongrass using an IPA salt glyphosate (Camacho and Moshier 1991). Accumulation of glyphosate in the upper plant tissue and roots were generally greater when applied without dicamba. However, comparison of treatments by glyphosate rate and dicamba presence indicated that dicamba only reduced glyphosate accumulation in the upper plant tissue and in the roots at the 0.56 kg ae ha⁻¹ and 0.84 kg ae ha⁻¹ rates of glyphosate, respectively. In addition, the total amount of glyphosate translocated was reduced when dicamba was added to the 0.56 kg ae ha⁻¹ rate of glyphosate.

Flint and Barrett (1989a) documented reduced glyphosate translocation in johnsongrass when an IPA salt formulation was applied at the 0.28 and 0.84 kg ae ha⁻¹ and mixed with the DMA salt form of dicamba at 0.56 kg ae ha⁻¹. The total translocated ¹⁴C glyphosate from this research cannot confirm the same antagonism when using K salt glyphosate and DGA dicamba applied at those rates. This is likely due to differences in the timing of plant harvest. Flint and Barrett (1989a) harvested plants at 72 HAT and as a result, the majority of the dosed glyphosate was found in the roots and no consistent differences were found in the quantity of glyphosate in the shoots. Koger and Reddy (2005) documented pitted morningglory (*Ipomoea lacunosa* L.) to have a large increase translocated glyphosate root accumulations between 24 and 96 HAT. Feng et al. (1998) described damage to meristems at 72 HAT and was likely due to the self-limiting factor of glyphosate as described by Geiger and Bestman (1990) where translocation of glyphosate can initially progress rapidly but is then limited by the plant damage. Extending the duration of our studies would likely result in greater total accumulations of

glyphosate, especially in the roots, but would result in poorer estimations of translocation and would have reductions in ^{14}C recovery as described in other research (Feng et al. 1998; Feng et al. 2000). This research did find significant differences in total and partitioned glyphosate amounts in the treated leaf and whole plant. It confirms recent research (Feng et al. 1998; Feng et al. 2000) suggesting that harvesting 24 HAT maximizes translocation differences. This research did confirm a reduction of total translocated glyphosate as a result of a tankmix of the K salt formulation of glyphosate at $0.56 \text{ kg ae ha}^{-1}$ with the DGA salt formulation of dicamba at $0.56 \text{ kg ae ha}^{-1}$. In addition, the data from the distal portion of the treated leaf suggest that applications of glyphosate with dicamba can increase acropetal translocation on the treated leaf, while simultaneously reducing the level found in the upper portion of the plant (Dewey and Appleby 1983).

Although the addition of dicamba did not affect the total amount of glyphosate absorbed in the plant for any of the applied rates, altered translocation patterns were present and consideration should be taken when utilizing tankmixes of dicamba and glyphosate applied to control johnsongrass, as decreased translocation of glyphosate can lead to glyphosate-resistant weed populations (Nandula et al. 2015; Powles and Preston 2006; Vila-Aiub et al. 2012). Due to glyphosate-resistant weeds, utilization of dicamba-resistant cropping systems are expected to be rapidly adopted by producers upon release. With proper management, they will likely provide results similar to glyphosate- or glufosinate-resistant systems in providing equitable and efficacious control of weeds (Cahoon et al. 2015; Riar et al. 2011a). Their use, in conjunction with rotation of crops and herbicidal mode of action, should slow the development of herbicide resistant weeds

(Inman et al. 2016; Neve et al. 2011). However, the use of stacked trait technologies should not result in use of a single mode of action and weed management strategies should focus on preservation of all herbicide-resistant traits as well as any interactions that might occur between them.

Table 3.1 Percent of the applied ^{14}C -glyphosate recovered from the water and chloroform wash of the treated leaf and the total plant absorption and recovery of ^{14}C in barnyardgrass after 24 hours.^a

Herbicide Rate		^{14}C Recovered			
Glyphosate	Dicamba	Leaf Wash		Plant Fraction	Combined
–kg ae ha ⁻¹ –	–kg ai ha ⁻¹ –	H ₂ O	Chloroform	Total	Total
				%	
0.28	0	64 BC	0.3 A	11 E	82 C
0.56	0	70 ABC	0.2 AB	16 DC	87 AB
0.84	0	67 ABC	0.3 A	22 AB	90 A
0.28	0.56	72 AB	0.2 AB	12 E	84 BC
0.56	0.56	74 A	0.2 AB	15 DE	89 A
0.84	0.56	67 ABC	0.2 B	18 BC	86 ABC
1.12	0.56	64 C	0.3 A	24 A	88 AB

^a Means within a column followed by the same letter are not significantly different.

Table 3.2 Partitioned and total amounts of ¹⁴C-glyhosate found in the treated barnyardgrass leaf after 24 hours.^a

Herbicide Rate		¹⁴ C in Treated Leaf				
Glyphosate	Dicamba	Partitioned			Combined	
–kg ae ha ⁻¹ –	–kg ai ha ⁻¹ –	Treated area	Distal	Proximal	Total	
			%			
0.28	0	4.0 CD	1.0 DE	3.2 BC	8 E	
0.56	0	5.8 AB	2.5 CD	3.7 BC	12 CD	
0.84	0	6.9 A	4.5 BC	5.3 A	17 AB	
0.28	0.56	3.6 D	3.0 CD	2.5 C	9 DE	
0.56	0.56	4.3 CD	4.1 BC	3.7 BC	12 CD	
0.84	0.56	4.1 CD	6.3 B	4.8 AB	15 BC	
1.12	0.56	5.1 BC	9.7 A	4.2 AB	19 A	

^a Means within a column followed by the same letter are not significantly different.

Table 3.3 Partitioned amounts and sum of ^{14}C -glyphosate translocated away from the treated leaf of barnyardgrass after 24 hours.^a

Herbicide Rate		^{14}C Translocated to Plant			
Glyphosate	Dicamba	Partitioned		Combined	
–kg ae ha ⁻¹ –	–kg ai ha ⁻¹ –	Upper	Lower	Roots	Total
		%			
0.28	0	1.1 AB	1.1 B	0.4 C	2.6 C
0.56	0	1.4 AB	2.1 AB	0.9 AB	4.5 AB
0.84	0	1.8 A	2.7 A	1.0 A	5.5 A
0.28	0.56	1.1 AB	1.4 AB	0.4 C	2.9 C
0.56	0.56	0.8 B	1.2 B	0.8 ABC	2.7 C
0.84	0.56	1.1 AB	1.8 AB	0.5 BC	3.3 BC
1.12	0.56	1.5 AB	2.4 AB	0.7 ABC	4.6 AB

^a Means within a column followed by the same letter are not significantly different.

Table 3.4 Amount of ^{14}C -glyphosate recovered from the water and chloroform wash of the treated leaf and the total plant absorption and recovery of ^{14}C in johnsongrass after 24 hours.^a

Herbicide Rate		^{14}C Recovered			
Glyphosate –kg ae ha ⁻¹ –	Dicamba – kg ai ha ⁻¹ –	Leaf Wash		Plant Fraction	Combined
		H ₂ O	Chloroform	Total	Total
				%	
0.28	0	68 AB	0.6 A	13 C	79 AB
0.56	0	63 BCD	0.4 AB	14 C	77 B
0.84	0	58 D	0.6 A	17 BC	76 B
0.28	0.56	65 ABC	0.4 A	15 C	81 AB
0.56	0.56	72 A	0.4 AB	13 C	85 A
0.84	0.56	60 DC	0.4 A	22 AB	83 AB
1.12	0.56	55 D	0.5 A	24 A	79 AB

^a Means within a column followed by the same letter are not significantly different.

Table 3.5 Partitioned and total amounts of ¹⁴C-glyphosate found in the treated johnsongrass leaf after 24 hours.^a

Glyphosate –kg ae ha ⁻¹ –	Dicamba – kg ai ha ⁻¹ –	¹⁴ C in Treated Leaf			
		Treated area	Partitioned		Combined Total
			Distal %	Proximal	
0.28	0	3.9 B	1.9 BC	1.0 C	6.7 C
0.56	0	3.6 B	2.7 BC	1.3 BC	7.5 BC
0.84	0	4.7 AB	3.9 B	2.0 AB	10.6 B
0.28	0.56	4.4 AB	3.7 B	1.6 ABC	9.7 BC
0.56	0.56	3.6 B	3.5 B	1.3 ABC	8.8 BC
0.84	0.56	5.8 A	8.4 A	1.9 AB	16.1 A
1.12	0.56	5.7 A	9.4 A	2.2 A	17.4 A

^a Means within a column followed by the same letter are not significantly different.

Table 3.6 Partitioned amounts and sum of ¹⁴C-glyphosate translocated away from the treated leaf of johnsongrass after 24 hours.^a

Glyphosate –kg ae ha ⁻¹ –	Dicamba – kg ai ha ⁻¹ –	¹⁴ C Translocated to Plant			
		Partitioned		Roots	Combined Total
		Upper	Lower		
		%			
0.28	0	1.9 AB	2.8 AB	1.2 ABC	5.6 AB
0.56	0	2.2 A	2.8 AB	1.7 ABC	6.4 A
0.84	0	1.3 ABC	3.8 AB	1.8 A	6.8 A
0.28	0.56	1.2 ABC	3.6 AB	0.9 BC	5.2 AB
0.56	0.56	0.4 DC	2.6 B	0.9 C	3.8 B
0.84	0.56	0.8 BCD	4.6 A	1.0 BC	6.2 AB
1.12	0.56	0.7 BCD	3.8 AB	1.8 AB	6.2 AB

^a Means within a column followed by the same letter are not significantly different.

Literature Cited

- Ahmadi MS, Haderlie LC, Wicks GA (1980) Effect of growth stage and water stress on barnyardgrass (*Echinochloa crus-galli*) control and on glyphosate absorption and translocation. *Weed Sci* 28:277-282
- Appleby AP, Somabhi M (1978) Antagonistic effect of atrazine and simazine on glyphosate activity. *Weed Sci* 26:135-139
- Bagavathiannan, MV, Norsworthy JK, Smith KL, Neve P (2013) Modeling the evolution of glyphosate resistance in barnyardgrass (*Echinochloa crus-galli*) in cotton-based production systems of the Midsouthern United States. *Weed Technol* 27:475-487
- Baylis AD (2000) Why glyphosate is a global herbicide: strengths, weaknesses and prospects. *Pest Manag Sci* 56:299-308 (2000)
- Belles D, Shaner D, Westra P, Brunk G (2006) Comparison of efficacy, absorption and translocation of three glyphosate formulations on velvetleaf (*Abutilon theophrasti*). *Pest Manag Sci* 62:1177-1181
- Bethke RK, Molin WT, Sprague C, Penner D (2013) Evaluation of the interaction between glyphosate and glufosinate. *Weed Sci* 61:41-47
- Blackshaw RE, Harker KN, Clayton GW, O'Donovan JT (2006) Broadleaf herbicide effects on clethodim and quizalofop-p efficacy on volunteer wheat (*Triticum aestivum*). *Weed Technol* 20:221-226
- Bostamam Y, Malone JM, Dolman FC, Boutsalis P, Preston C (2012) Rigid ryegrass (*Lolium rigidum*) populations containing a target site mutation in EPSPS and reduced glyphosate translocation are more resistant to glyphosate. *Weed Sci* 60:474-479
- Botha GM, Burgos NR, Gbur EE, Allan EA, Salas RA, Scott RC (2014) Interaction of glufosinate with 2,4-D, dicamba, and tembotrione on glyphosate-resistant. *Am J Exp Agric* 4:427-442
- Bruff SA, Shaw DR (1992) Tankmix combinations for weed control in stale seedbed soybean (*Glycine max*). *Weed Technol* 6:45-51
- Burke IC, Koger CH, Reddy KN, Wilcut JW (2007) Reduced translocation is the cause of antagonism of glyphosate by MSMA in browntop millet (*Brachiaria ramosa*) and palmer amaranth (*Amaranthus palmerii*). *Weed Technol* 21:166-170
- Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. *Weed Technol* 29:758-770

- Camacho RF, Moshier LJ (1991) Absorption, translocation, and activity of CGA-136872, DPX-V9360, and glyphosate in rhizome johnsongrass (*Sorghum halepense*). *Weed Sci* 39:354-357
- Chahal GS, Jordan DL, Burton JD, Danehower D, York AC, Eure PM, Clewis B (2013) Influence of water quality and coapplied agrochemicals on efficacy of glyphosate. *Weed Technol* 26:167-176
- Cobb AH, Reade JPH (2010) *Herbicides and Plant Physiology*. 2nd edn. West Sussex, U.K. Wiley-Blackwell. Pp 133-155
- Colby SR (1967) Synergistic and antagonistic responses of herbicide combinations. *Weeds* 15:20-22
- Coupland D, Caseley JC (1979) Presence of ^{14}C activity in root exudates and guttation fluid from *Agropyron repens* treated with ^{14}C -labelled glyphosate. *New Phytol* 83:17-23
- Cranmer JR, Linscott DL(1991) Effects of droplet composition on glyphosate absorption and translocation in velvetleaf (*Abutilon theophrasti*). *Weed Sci* 39:251-254
- Damalas CA, Eleftherohorinos IG (2001) Dicamba and atrazine antagonism on sulfonylurea herbicides used for johnsongrass (*Sorghum halepense*) control in corn (*Zea mays*). *Weed Technol* 15:62-67
- Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A, Bleeke M, Honegger JJ, Farmer D, Wright D, Hauptfear EA (2010) Glyphosate: development, applications, and properties. Pages 1-34 in V. Nandula ed. *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*. Hoboken, NJ: John Wiley and Sons, Inc.
- Dewey SA, Appleby AP (1983) A comparison between glyphosate and assimilate translocation patterns in tall morningglory (*Ipomoea purpurea*). *Weed Sci* 31:308-314
- Duke SO (2015) Perspectives on transgenic, herbicide-resistant crops in the USA almost 20 years after introduction. *Pest Manag Sci* 71:652-657
- Duke SO, Powles SB (2009) Glyphosate-resistant crops and weeds: now and in the future. *AgBioForum* 12:346-357
- Edwards CB, Jordan DL, Owen MDK, Dixon PM, Young BG, Wilson RG, Weller SC, Shaw DR (2014) Benchmark study on glyphosate-resistant crop systems in the United States. Economics of herbicide resistance management practices in a 5 year field-scale study. *Pest Manag Sci* 70:1924-1929

- Fedtke C, Duke SO (2005) Herbicides. Pages 247-330 in Hock B, Elstner EF, eds. Plant Toxicology, 4th ed. New York: Marcell Decker
- Feng PCC, Ryerse JS, Sammons RD (1998) Correlation of leaf damage with uptake and translocation of glyphosate in velvetleaf (*Abutilon theophrasti*). Weed Technol 12:300-307
- Feng PCC, Pratley JE, Bohn JA (1999) Resistance to glyphosate in *Lolium rigidum*. II. Uptake, translocation, and metabolism. Weed Sci 47:412-415
- Feng PCC, Sandbrink JJ, Sammons RD (2000) Retention, uptake, and translocation of ¹⁴C-glyphosate from track-spray applications and correlation to rainfastness in velvetleaf (*Abutilon theophrasti*). Weed Technol 14:127-132
- Feng PCC, Minhtien T, Chiu T, Sammons RD, Heck GR, CaJacob CA (2004) Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation, and metabolism. Weed Sci 52:498-505
- Flint JL, Barrett M (1989a) Antagonism of glyphosate toxicity to johnsongrass (*Sorghum halepense*) by 2,4-d and dicamba. Weed Sci 37:700-705
- Flint JL, Barrett M (1989b) Interactions of glyphosate with 2,4-D or dicamba on field bindweed (*Convolvulus arvensis*). Weed Sci 37:12-18
- Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: A unique global herbicide. ACS monograph 189. American Chemical Society, Washington, D.C. 653 p
- Geiger DR, Bestman HD (1990) Self-limitation of herbicide mobility by phytotoxic action. Weed Sci 38:324-329
- Green JM, Hazel CB, Forney DR, Pugh LM (2008) New multiple-herbicide crop resistance and formulation technology to augment the utility of glyphosate. Pest Manag Sci 64:332-339
- Green JM (1989) Herbicide antagonism at the whole plant level. Weed Technol 3:217-226
- Gustafson DI (2008) Sustainable use of glyphosate in North American cropping systems. Pest Manag Sci 64:409-416
- Hart LM, Wax SE (1996) Dicamba antagonizes grass weed control with imazethapyr by reducing foliar absorption. Weed Technol 10:828-834
- Huff JA (2010) Interactions of glyphosate and dicamba in controlling key weed species. Ph.D dissertation. Mississippi State Univ. 66 p

- Hydrick DE, Shaw DR (1994) Effects of tankmix combinations of non-selective soil-applied herbicides on three weed species. *Weed Technol* 8:129-133
- Inman MD, Jordan DL, York AC, Jennings KM, Monks DW, Everman WJ, Bollman SL, Fowler JT, Cole RM, Soteris JK (2016)) Long-term management of Palmer amaranth (*Amaranthus palmeri*) in dicamba-tolerant cotton. *Weed Sci* 64:161-169
- Kirkwood RC, Hetherington R, Reynolds TL, Marshall G (2000) Absorption, localisation, translocation and activity of glyphosate in barnyardgrass (*Echinochloa crus-galli* (L) Beauv): Influence of herbicide and surfactant concentration. *Pest Manag Sci* 56:239-367
- Knezevic SZ, Datta AD, Scott J, Klein RN, Golus J (2009) Problem weed control in glyphosate-resistant soybean with glyphosate tank mixes and soil-applied herbicides. *Weed Technol* 23:507-512
- Koger CH, Burke IC, Miller DK, Kendig JA, Reddy KN, Wilcut JW (2007) MSMA antagonizes glyphosate and glufosinate efficacy on broadleaf and grass weeds. *Weed Technol* 21:159-165
- Koger CH, Reddy KN (2005) Glyphosate efficacy, absorption, and translocation in pitted morningglory (*Ipomoea lacunosa*). *Weed Technol* 53:277-283
- Koger CH, Poston DH, Reddy KN (2004) Effect of glyphosate spray coverage on control of pitted morningglory (*Ipomoea lacunosa*). *Weed Tech* 18:124-130
- Leaper CL, Holloway PJ (2002) Adjuvants and glyphosate activity. *Pest Manag Sci* 56:313-319
- Li J, Smeda RJ, Sellers BA, Johnson WG (2005) Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Sci* 53:153-159
- Lorraine-Colwill DF, Powles SB, Hawkes TR, Hollinshead PH, Warner SAJ, Preston C (2002) Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. *Pest Biochem Physiol* 74:62-72
- Masiunas JB, Weller SC (1988) Glyphosate activity in potato (*Solanum tuberosum*) under different temperature regimes and light levels. *Weed Sci* 36:137-140
- Merchant RM, Sosnoskie LM, Culpepper AC, Steckel LF, York AC, Braxton LB, Ford JC (2013) Weed response to 2,4-D, 2,4-DB, and dicamba applied alone or with glufosinate. *Jrnl of Cot Sci* 17:212-218
- Monsanto (2015a) Monsanto® Roundup Ready Xtend Crop System. <http://www.monsanto.com/products/pages/roundup-ready-xtend-crop-system.aspx> Accessed October 10, 2015

- Monsanto (2015b) Herbicide Application and Drift Management Vital to Weed Management in the Corn Belt. https://www.roundupreadyplus.com/Content/assets/media-room/RRXtendCS_IA_IL.pdf. Accessed November 10, 2015
- Monsanto (2016) Roundup Powermax label. <http://www.cdms.net/ldat/ld8CC010.pdf>. Accessed January 14, 2016
- Mueller TC, Witt WW, Barrett M (1989) Antagonism of johnsongrass (*Sorghum halepense*) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. *Weed Technol* 3:86-89
- Nandula VJ, Reddy KN, Koger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribero DN (2012) Multiple resistance to glyphosate and pyriithiobac in palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. *Weed Sci* 60:179-188
- Nandula VJ, Wright AA, VanHorn CR, Molin WT, Westra P, Reddy KN (2015) Glyphosate resistance in giant ragweed (*Ambrosia trifida* L.) from Mississippi is partly due to reduced translocation. *Am J Plant Sci* 6:2104-2113
- Neve P, Norsworthy JK, Smith KL, Zelaya IA (2011) Modeling glyphosate resistance management strategies for palmer amaranth (*Amaranthus palmeri*) in cotton. *Weed Technol* 25:335-343
- Norsworthy JK, Burgos NR, Oliver LR (2001) Differences in weed tolerance to glyphosate involve different mechanisms. *Weed Technol* 15:725-731
- O'Donovan JT, O'Sullivan PA (1982) Amine salts of growth regulator herbicides antagonize paraquat. *Weed Sci* 30:605-608
- O'Sullivan PA, O'Donovan JT (1980) Interaction between glyphosate and various herbicides for broadleaved weed control. *Weed Res* 20:255-260
- Powles SB, Preston C (2006) Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. *Weed Technol* 20:282-289
- Reddy K (2000) Factors affecting toxicity, absorption, and translocation of glyphosate in redvine (*Brunnichia ovata*). *Weed Technol* 14:457-462
- Riar DS, Norsworthy JK, Griffith GM (2011a) Herbicide programs for enhanced glyphosate-resistant and glufosinate-resistant cotton (*Gossypium hirsutum*). *Weed Technol* 25:526-534
- Riar DS, Norsworthy JK, Johnson DB, Scott RC, Bagavathiannan M (2011b) Glyphosate resistance in a johnsongrass ("*Sorghum halepense*") biotype from Arkansas. *Weed Sci* 59:299-304

- Ribeiro DN, Nandua VJ, Dayan FE, Rimando AM, Duke SO, Reddy KN, Shaw DR (2015) Possible glyphosate tolerance mechanism in pitted morningglory (*Ipomoea lacunosa* L.). *J Agric Food Chem* 63:1689-1697
- Rodrigues JJV, Worsham, AD, Corbin FT (1982) Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mays*) and soybeans (*Glycine max*). *Weed Sci* 30:316-320
- Roskamp JM, Chahal GS, Johnson WG (2013) The effect of cations and ammonium sulfate on the efficacy of dicamba and 2,4-D. *Weed Technol* 27:72-77
- Rummens FHA (1975) An improved definition of synergistic and antagonistic effects. *Weed Sci* 23:4-6
- Selleck GW, Baird DD (1981) Antagonism with glyphosate and residual herbicide combinations. *Weed Sci* 29:185-190
- Shaner DL (2009) Role of translocation as a mechanism of resistance to glyphosate.. *Weed Sci* 57:118-123
- Shaner DL, ed (2014a) *Herbicide Handbook*. 10th edn. Lawrence, KS: Weed Science Society of America. Pp 240-242
- Shaner DL (2014b) Lessons learned from the history of herbicide resistance. *Weed Sci* 62:427-431
- Shaw DR, Arnold JC (2002). Weed control from herbicide combinations with glyphosate. *Weed Sci* 16:1-6
- Sherrick SL, Holt HA, Hess FD (1986) Absorption and translocation of MON 0818 adjuvant in field bindweed (*Convolvulus arvensis*). *Weed Sci* 34:817-823
- Sikkema PH, Shropshire C, Hamill AS, Weaver SE, Cavers PB (2005) Response of barnyardgrass (*Echinochloa crus-galli*) to glyphosate application timing and rate in glyphosate-resistant corn (*Zea mays*). *Weed Technol* 19:830-837
- Singh M, Sharma SD, Ramirez AHM, Jhala AJ (2011) Glyphosate efficacy, absorption, and translocation in selected four weed species common to Florida citrus. *HorTechnology* 21:599-605
- Stahlman PW, Phillips WM (1979) Inhibition of glyphosate phytotoxicity. *Weed Sci* 27:575-577
- Starke RJ, Oliver LR (1998) Interaction of glyphosate with chlorimuron, fomesafen, imazethapyr, and sulfentrazone. *Weed Sci* 6:652-660

- Steele GL, Senseman SA, Sciumbato AS, Chandler JM (2008) Diuron reduces absorption and translocation of glyphosate in sharppod morningglory (*Ipomoea cordatotriloba*). *Weed Technol* 22:414-419
- Stewart Jr. CN, Peng, Y, Abercrombie LG, Halfhill M, Rao MR, Ranjan P, Hu J, Sammons D, Heck GR, Tranel PJ, Yuan JS (2010) Genomics of glyphosate resistance. Pages 149-164 in V. Nandula ed. *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*. Hoboken, NJ: John Wiley and Sons, Inc.
- Vangessel MJ, Ayeni AO, Majek BA (2000) Optimum glyphosate timing with or without residual herbicides in glyphosate resistant soybean (*Glycine max*) under full-season conventional tillage. *Weed Technol* 14:140-149
- Vila-Aiub MM, Balbi MC, Destefano AJ, Fernandez L, Hopp E, Yu Q, Powles SB (2012) Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. *Pest Manag Sci* 68:430-436
- Wicks JS, Hanson GE (1995) Effect of rainfall on glyphosate plus 2,4-D performance on *Echinochloa crus-galli*. *Weed Sci* 43:666-670
- Wilson JS, Worsham AD (1988) Combinations of nonselective herbicides for difficult to control weeds in no-till corn, *Zea mays*, and soybeans, *Glycine max*. *Weed Sci* 36:648-652