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Reduced herbicide antagonism through novel spray application techniques

By

Luke H. Merritt

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Reduced herbicide antagonism through novel spray application techniques

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Field studies were conducted to test three application methods for applying antagonistic herbicide combinations: 1) tank mix (TMX), 2) mix-in-line (MIL), and 3) separate boom (SPB). Sethoxydim applied with bentazon, glyphosate applied with dicamba or 2,4-D, and clethodim applied with dicamba or 2,4-D had higher efficacy when applied using the SPB method. Antagonism of all the herbicide combinations above was observed when applied using the TMX and MIL methods. In some cases, antagonism was avoided when using the SPB method. Three application methods tested in greenhouse studies were 1) TMX, 2) synthetic auxin applied first (AAF), and 3) synthetic auxin applied second (AAS). The AAS application method resulted in higher weed control than the TMX and AAF methods. Analysis done through liquid chromatography mass spectrometry supported the greenhouse results with higher rates of glyphosate detected with the AAS method.

DEDICATION

To my family and friends who have always supported me and had my back. You know who you are.

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CHAPTER I

INTRODUCTION

Herbicide Antagonism

Tank mixing herbicides is a logistical way to apply multiple modes of action in one herbicide application. The following is a simplistic example: if herbicide A is expected to have 40 percent control and herbicide B is expected to have a 30 percent control. The two herbicides when tank mixed together should have 58 percent control, also known as an additive effect (Colby 1967). Synergy is when herbicides mixed together have greater control than what each of the herbicides achieve individually (Colby 1967). An example of synergy would be when herbicides A and B mentioned earlier have 85 percent control. Antagonism is when herbicides mixed together resulted in lower control than the herbicides individually applied separately (Colby 1967; WSSA 2019). Again with herbicides A and B, an example of antagonism would be to have only 55 percent control. Herbicide antagonism costs growers millions of dollars due to the herbicides having reduced activity. Having a higher application rate can make up for antagonism in some cases, but that still leads to higher crop production costs (Flint and Barrett 1989, 1989b).

Colby (1967) came up with a mathematical equation to determine if herbicide combinations produce antagonistic or synergistic responses. Colby’s equation for testing a two herbicide combination is the following:

$$E = (X+Y) - (XY)/100 \tag{1.1}$$

Where X is percent control of herbicide A, Y is percent control of herbicide B, and E is the expected amount of control. This formula was manipulated from percent inhibition values to percent of control (Colby 1967; Flint et al. 1988). Even today, the Colby method is still widely used to determine antagonistic and synergistic combinations. There are limitations to the formula. Using the formula is a mathematical way to predict herbicide responses. The Colby method is more accurate when there is a 50 percent dose response curve (Colby 1967).

Many factors affect herbicide antagonism. Herbicide rate can affect antagonism and increasing the rate can overcome the antagonism taking place (Mueller et al. 1989; Zhang et al. 1995). Asymmetric interaction is when one herbicide antagonizes the activity of another, but not conversely (Green 1989). Adjuvants, such as crop oil concentrate and methylated seed oil, enhance herbicide activity by allowing the herbicide to penetrate the waxy cuticle layer on the outer surface of the leaf. While adjuvants can be useful, they can also cause a tank mixture to thicken and can make droplet size less uniform (Green 1989). Buffering agents can adjust the spray solution pH and prevent antagonistic ions from interacting if the antagonism is due to chemical antagonism (McMullan 2000). Penner (1989) found that adjuvants can reduce antagonism by increasing the absorption of the herbicide and prevent derivative forms of weak acidic herbicides from being formed and absorbed. Time can also prevent an antagonistic effect. If a herbicide application occurs immediately after it is tank mixed, antagonism may be avoided. Other factors that affect herbicide antagonism include plant species, herbicide safeners, other pesticides, growth stage, application method, and the environment (Green 1989).

Herbicide antagonism is classified into four mechanisms by Green (1989): 1) Biochemical antagonism is when one chemical causes a reduced amount of herbicide to reach the site of action. 2) Competitive antagonism is when an antagonist prevents the binding of the herbicide by binding at the active site. 3) Physiological antagonism is when two herbicides counteract each other due to having opposite effects. The mode of action of herbicides is a significant factor in physiological antagonism. 4) Chemical antagonism is when a herbicide reacts chemically to another chemical. The chemistry of herbicides affects chemical antagonism.

Antagonism and synergism studies were reviewed by Zhang et al. (1995). Thirty-three percent of 267 studies had synergistic combinations and 67 percent had antagonistic combinations. Acetyl Co-A Carboxylase (ACCase) inhibiting herbicides (WSSA Group 1) tanked mixed with synthetic auxins (WSSA Group 4) was a common antagonistic herbicide mix. New trait technologies have allowed cotton and soybeans to have tolerance to dicamba and 2,4-D, making antagonism in tank mixes a renewed concern. Synthetic auxins provide excellent broadleaf weed control, but ACCase herbicides must be added to control grass weeds. Controlling both grass and broadleaf weeds will be more difficult with antagonism occurring.

Relevancy and Justification

In 2017, Mississippi planted 886,262 hectares of soybeans (*Glycine max*); 254,952 hectares of cotton (*Gossypium hirsutum*); 210,437 hectares of corn (*Zea mays*); 18,211 hectares of wheat (*Triticum aestivum*); and 17,806 hectares of peanuts (*Arachis hypogaea*) (USDA 2017). The use of herbicides is necessary to control weeds in these row crop systems. Given an increase in herbicide resistant weeds, it is important to incorporate multiple modes of actions in a herbicide program.

Weed control is a crucial component for row crop production. A weed is defined as a plant that causes economic losses or ecological damage, creates health problems for humans or animals, or is undesirable where it is growing (WSSA 2016). Not only can weeds cost a producer in the current growing season, but also it can affect multiple future seasons due to weed seed production and the weed seedbank. For conventional tillage and no-tillage operations, 60 percent of the weed seedbank is in the upper 5 cm of the soil (Clements et al. 1996). A weed seedbank in a field may include tens of millions seeds per acre (Ross and Lembi 2009). It is estimated that weeds cause an annual 12 percent loss of crop yields in the United States, a 32-billion-dollar loss in crop production (Ross and Lembi 2009). Weeds compete with crops for light, water, and nutrients. The greater weed density and size, the more competition they pose to the crop (Van Heemst 1985; Vangessel et al. 1995). When a crop undergoes stress from weed competition, yield potential decreases (Buchanan and Burns 1970).

Herbicides are used to combat weeds in the critical period of crop production. Normally, the critical control period is just prior to planting all the way through canopy closure (Halford et al. 2001; Hall et al. 1992; Van Acker et al. 1993). It is estimated that 85 percent of cropland receives herbicide applications (Ross and Lembi 2009). In 1996, glyphosate resistant soybean and canola were introduced followed by cotton in 1997, then corn in 1999 (Owen and Zelaya 2005). Glyphosate is a non-selective herbicide that inhibits certain amino acids from forming in a plant (Ross and Lembi 2009). Glyphosate moves through the plant symplastically (Duke and Powles 2008). It kills new growth first at the growing point (Feng et al. 2003, NC State 2015). With the introduction of glyphosate tolerant crops, producers could spray glyphosate and kill the weeds but not injure the crop. Given the widespread use of glyphosate, weeds underwent heavy selection pressure (Dill et al. 2008; Duke and Powles 2008). When only the weeds that survived glyphosate applications produced seed, the proportion of the weed population that could withstand glyphosate increased. Today, glyphosate is increasingly becoming less effective in agricultural production due to an increase in weeds becoming resistant (Dill 2005; Duke and Powles 2008).

The 2016 growing season was the first year that Bayer released Roundup Ready 2 Xtend® soybeans (Bayer, St. Louis, Mo 63167) (Bayer 2019). This is significant in the fact that dicamba could be used in postemergence weed control in soybean production. The dicamba trait allows for a herbicide application of Roundup PowerMAX® (glyphosate) with XtendiMax® (dicamba). Bayer also released dicamba tolerant cotton. Corteva Agriscience released crops with 2,4-D tolerance, known as Enlist™ soybeans, cotton, and corn (Corteva Agriscience, Indianapolis, IN 46268) (Corteva 2019). Between the two trait tolerant systems, 2,4-D and dicamba may be utilized for post emergence applications in soybeans and cotton.

Some weeds species developed resistance to multiple herbicide sites of action. Barnyardgrass (*Echinochloa crus-galli*) has resistance to nine reported herbicide sites of action, including ACCase, ALS, EPSP synthase, and photosystem II inhibitors (Heap 2018). Waterhemp (*Amaranthus tuberculatus* var. *rudis*) and Palmer Amaranth (*Amaranthus palmeri*) combined have resistance to seven different herbicide sites of action (Heap 2018; MSU 2017). When weeds have resistance to multiple sites of action, it will require an application of multiple herbicides with different modes of action to control the weed. If antagonism takes place when mixing herbicides, the mixed product may not fully kill the plant as expected and can increase selection pressure for weeds resulting in greater resistance to other sites of action. According to Heap (2018), Mississippi has 23 unique cases of herbicide resistant weeds, ranking it sixth among U.S. states with the most resistant weeds.

While there is proof of herbicide antagonism taking place, there is a gap in understanding of what specifically happens. It is not completely understood how the herbicide particles interact with each other. With weeds, such as waterhemp and Palmer amaranth, with resistance to multiple sites of actions, tank mixing herbicides will be more prevalent to better control weeds and prevent further resistance from developing. It is important to make sure herbicide applications work correctly in order to prevent reapplication. Reapplications of herbicides cost the producer more money and increase the environmental load. When weeds continue to grow after a failed application crop yield can decrease and increased weed seed can enter the soil seedbank, which can further build resistance to other herbicide sites of action. The goal of this project is to study when the reaction takes place, what chemical reaction happens, and to find new ways to apply herbicides together to best avoid antagonistic effect.

The objective of this research is to 1) identify spray application techniques to reduce herbicide interaction time, 2) discover new ways to overcome herbicide antagonism, 3) develop recommendations to best apply antagonistic herbicides together to achieve maximum weed control.

CHAPTER II

REDUCING SETHOXYDIM ANTAGONISM WITH BENTAZON THROUGH SPRAY APPLICATION TECHNIQUE

Abstract

Sethoxydim and bentazon have been documented as an antagonistic combination in past literature. This documented antagonistic herbicide combination was used to determine if different application methods can reduce antagonism. Two studies were conducted at the Black Belt Experiment Station and at the R.R. Foil Plant Science Research Center in fallow fields with broadleaf signalgrass (*Urochloa platyphylla*) and Italian ryegrass (*Lolium perenne* ssp. *multiflorum*). A modified tractor mounted sprayer with eight nozzles was used to spray bentazon and sethoxydim with three different application methods: tank mix, mix-in-line, and separate boom. Bentazon and sethoxydim were applied at a rate of 840 g ai ha^{-1} and 140 g ai ha^{-1} with COC at 1% v/v, respectively for all treatments. Visual estimations of weed control were taken 7, 14, 21, and 28 days after application. Antagonism of sethoxydim with bentazon was observed in Italian ryegrass and broadleaf signalgrass control when the two herbicides were tank mixed or applied through the mix-in-line method. Applying through separate booms resulted in higher weed control than the tank mix and the mix-in-line method in control of both Italian ryegrass and broadleaf signalgrass. In some cases, applying sethoxydim with bentazon through separate booms overcame the antagonism.

Introduction

Sethoxydim is an acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicide. This herbicide blocks the process of fatty acid synthesis. ACCase inhibitors provide grass control but do not have any activity on broadleaf weeds (Bridges et al. 1981; Parker and Thompson Jr. 1982; Sims et al. 1982). Therefore, sethoxydim is used for annual and perennial grass control in several broadleaf crops (Ahrens 1994). Sethoxydim moves through the phloem and xylem, making it a systemic chemical (Ahrens 1994). Bentazon is a photosystem II inhibitor. Bentazon works by binding the QB-binding niche of the D1 protein, therefore inhibiting photosynthesis. Bentazon provides control of annual broadleaf species in soybeans, peanuts, corn, sorghum, and rice production. Bentazon stops photosynthesis in the leaves of the plant acropetal to the application point (Potter 1977).

Antagonism of sethoxydim applied with bentazon is well documented in literature. Tank mixing sethoxydim with bentazon reduces sethoxydim activity on grass weed species in the field (Bridges et al. 1981; Hartzler and Foy 1983; Horng and Ilnicki 1982; Nalewaja et al. 1980; Rhodes Jr. and Coble 1984). Sequential applications of sethoxydim and bentazon have shown in some cases to prevent the antagonism from occurring (Rhodes Jr. and Coble 1984). Increasing the sethoxydim rate reduced the severity of the antagonism (Rhodes Jr. and Coble 1984). Rhodes Jr. and Coble (1984b) found that the application of bentazon with sethoxydim decreased foliar adsorption of ^{14}C applied as ^{14}C-sethoxydim by 50% in goosegrass (*Eleusine indica*). Carrier volume can influence efficacy of sethoxydim. Lassiter and Coble (1987) found that a carrier volume of 94 and 187 L ha^{-1} had better control large crabgrass than 374 L ha^{-1} for sethoxydim applied alone and sethoxydim and bentazon sequential application. There was no carrier volume effect for the sethoxydim and bentazon tank mix treatment (Lassiter and Coble 1987).

A tractor (John Deere 5400 series) mounted dual boom sprayer with eight nozzles was modified to spray using one of three application methods: 1) the two herbicides tanked mixed in a can and sprayed through a single boom (Figure 2.1), 2) two herbicides from separate cans mixed in the boom line and applied through a single boom (Figure 2.2), and 3) two herbicides from separate cans applied through both booms at the same time (Figure 2.3). Each herbicide combination was applied with the three different application methods.

Figure 2.1 Explanation of the tank mix application method.

A tractor (John Deere 5400 series) mounted three-point hitch dual boom sprayer was modified to make three different herbicide applications. The cans containing herbicides were pressurized through a mounted air compressor. Two sets of eight 430 series, 2-way manifold (TeeJet Technologies, Glendale Heights, Illinois 60139) were mounted to the sprayer with two separate control boxes in the cab of the tractor. Tank mix applications were mixed together in a can and applied through one, 430 series manifold.

Figure 2.2 Explanation of the mix-in-line application method.

Mix-in-line applications were made from two separate cans, connected to separate manifolds. The two lines coming out of the manifolds were connected to a “T” that would allow the herbicides to mix before coming out of the nozzles. This “T” was only used in the mix-in-line applications and was removed for the other application types.

Figure 2.3 Explanation of the separate boom application method.

Separate nozzle applications were made from two cans containing separate herbicides and applied through separate manifolds. One manifold controlled one boom while the second manifold controlled the other boom. One button on each control box in the tractor were pressed to activate both cans spraying at the same time through both booms.

Even though tank mixing herbicides is a simple way to apply multiple herbicides at one time, applying the herbicides separately may prevent antagonism from occurring. This study compared herbicide antagonistic responses based on the three herbicide application methods. It was hypothesized that applying the herbicides through the mix-in-line and dual boom method will overcome the antagonism normally seen in tank mixing.

Materials and Methods

Two field study replications were conducted at the Black Belt Experiment Station in Brooksville, MS and at the R.R. Foil Plant Science Research Center in Starkville, MS on March 29, 2019, and May 20, 2019, respectively. The study consisted of an untreated check plus five treatments, each with four replications in a randomized complete block design. An untreated check was used for weed control evaluations. All treatments with bentazon (Broadloom™, UPL, King of Prussia, PA 19406) and sethoxydim (Poast®, BASF, Research Triangle Park, NC 27709) were made at the rate of 840 g ai ha^{-1} and at 140 g ai ha^{-1} with crop oil concentrate (COC) (AGRI-DEX®, Helena, Collierville, TN 38017) at 1% v/v, respectively. The treatments were 1) bentazon, 2) sethoxydim, 3) bentazon tanked mixed with sethoxydim, 4) bentazon and sethoxydim applied through the mix-in-line method, and 5) bentazon and sethoxydim applied at the same time trough different booms. The modified tractor mounted sprayer described in the introduction was used to make all herbicide applications. Ammonia and water were run through the entire spray system in between each treatment change. Applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). Applications were made with a natural population of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) at an average height of 18 cm at the first study at the Black Belt Experiment Station. The second study at the R.R. Foil Plant Science Research Center had had Oregon Grown, Gulf Variety, Italian ryegrass drilled into the field at a rate of 112 kg ha^{-1} on March 20, 2019 along with a natural population of broadleaf signalgrass (*Urochloa platyphylla*). Applications were made with Italian ryegrass at an average height of 15 cm and broadleaf signalgrass at an average height of 9 cm. Visual estimation of injury (control) ratings were taken at 7, 14, 21, and 28 days after application (DAA). The Colby method was used to determine if herbicide combinations are antagonistic (Colby 1967). All results were run through SAS 9.4 through PROC GLIMMIX with Sidak’s adjustment and P value = 0.05.

Results and Discussion

Due to bentazon having no grass control, any visual estimations of weed control of sethoxydim with bentazon that was less than sethoxydim applied alone is antagonistic. Control ratings taken 7 DAA (Table 2.1) showed sethoxydim having less than 20% grass control of all grass species at both field locations. No differences of control of Italian ryegrass at the Black Belt Experiment Station and broadleaf signal grass at the R.R. Foil Plant Science Research Center were observed. The sethoxydim applied alone treatment and sethoxydim applied with bentazon through separate booms (SPB) had similar weed control of Italian ryegrass at the R.R. Foil Plant Science Research Center (Table 2.1). Applying sethoxydim with bentazon through tank mixing (TMX) and through the mix-in-line (MIL) method had lower control of Italian ryegrass than sethoxydim applied alone, but the three application methods were not statistically different. These results are inconclusive due to the ratings being taken only 7 DAA.

Table 2.1 Comparison of sethoxydim applied alone with all three applications methods of sethoxydim with bentazon 7 days after application at the Black Belt Experiment Station (Black Belt) and the R.R. Foil Plant Science Research Center (R.R. Foil).

Treatment[1]	**Application method**	**Black Belt**		**R.R. Foil**			
		Italian ryegrass		**Italian ryegrass**		**Broadleaf signalgrass**	
		Observed	**Expected**[2]	**Observed**	**Expected**	**Observed**	**Expected**
		%					
Sethoxydim alone		0^{a}		16^{a}		0^{a}	
Bentazon alone		0^{a}		0^{c}		0^{c}	
Sethoxydim with bentazon	tank mix	0^{a}	0	4^{b}	16	0^{a}	0
Sethoxydim with bentazon	mix-in-line	0^{a}	0	3^{bc}	16	0^{a}	0
Sethoxydim with bentazon	separate boom	0^{a}	0	10^{ab}	16	0^{a}	0

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicate significance.

[1] All treatments with bentazon and sethoxydim were applied at 840 and 140 g ai ha^{-1} with COC at 1% v/v, respectively.

[2] Expected responses were calculated using Colby's equation E = (X + Y) – (XY)/100, any response of sethoxydim with bentazon that was significantly lower than sethoxydim alone is considered antagonistic.

The control ratings taken 21 DAA showed differences among the application methods. For Italian ryegrass control at the Black Belt Experiment Station, sethoxydim applied with bentazon through SPB was the only application method that resulted in similar weed control as sethoxydim applied by itself, with control being 60 and 51%, respectively (Table 2.2). Applying sethoxydim with bentazon by the TMX and MIL methods resulted in 30% control of Italian ryegrass, while sethoxydim by itself resulted in 60% control (Table 2.2). This shows that applying sethoxydim with bentazon with the TMX and MIL methods had lower weed control that sethoxydim applied with bentazon through SPB. Antagonism of sethoxydim being observed when tank mixed with bentazon coincides with findings from Minton et al. (1989) and Rhodes, Jr. and Coble (1984). Minton et al. (1989) observed an 18% reduction in barnyardgrass control when bentazon was tank mixed with sethoxydim, and Rhodes Jr. and Coble (1984) observed a 5-10% decrease in control of multiple grass species when sethoxydim was applied with bentazon. Reduced sethoxydim efficacy was observed when bentazon was added in applications to control Texas panicum *(Panicum texanum*) and southern crabgrass (*Digitaria ciliaris*) control in peanuts (Grichar 1991). Sethoxydim applied with bentazon was antagonistic when the two herbicides were tank mixed or applied through MIL applications (Table 2.2). Results of Italian ryegrass control at the R.R. Foil Plant Science Research Center were slightly different from the results at the Black Belt Experiment Station. Applying sethoxydim with bentazon through SPB and TMX were similar as sethoxydim alone despite the 27% difference in mean control of Italian ryegrass 21 DAA (Table 2.2) Control of broadleaf signal grass was highest with sethoxydim applied alone (50%, Table 2.2). All three application methods with sethoxydim applied with bentazon had significantly lower weed control than sethoxydim applied alone (Table 2.2).

Table 2.2 Comparison of sethoxydim applied alone with all three applications methods of sethoxydim with bentazon 21 days after application at the Black Belt Experiment Station (Black Belt) and the R.R. Foil Plant Science Research Center (R.R. Foil).

Treatment[1]	**Application method**	**Black Belt**		**R.R. Foil**			
		Italian ryegrass		**Italian ryegrass**		**Broadleaf signalgrass**	
		Observed	**Expected**[2]	**Observed**	**Expected**	**Observed**	**Expected**
				%			
Sethoxydim alone		60^{a}		77^{a}		50^{a}	
Bentazon alone		0^{c}		0^{c}		0^{b}	
Sethoxydim with bentazon	tank mix	30^{b}	60	43^{ab}	77	10^{b}	50
Sethoxydim with bentazon	mix-in-line	30^{b}	60	38^{b}	77	0^{b}	50
Sethoxydim with bentazon	separate boom	51^{a}	60	50^{ab}	77	10^{b}	50

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicate significance.

[1] All treatments with bentazon and sethoxydim was applied at 840 and 140 g ai ha^{-1} with COC at 1% v/v, respectively.

[2] Expected responses were calculated using Colby's equation $E = (X + Y) - (XY)/100$, any response of sethoxydim with bentazon that was significantly lower than sethoxydim alone is considered antagonistic.

Results of mean weed control taken 28 DAA are displayed in Table 2.3. Italian ryegrass control at the Black Belt and Experiment and at the R.R. Foil Plant Science Research Center were highest with sethoxydim applied alone (68 and 93%, respectively) and with sethoxydim applied with bentazon through SPB (61 and 50%, respectively; Table 2.3). At Black Belt, applying sethoxydim with bentazon with the MIL method and TMX resulted in 43 and 45% control, respectively (Table 2.3). At R.R. Foil, applying sethoxydim with bentazon with the MIL method and TMX both resulted in 43% control of Italian ryegrass (Table 2.3). This was significantly lower than the 61% control at Black Belt and the 50% control of Italian ryegrass at R.R. Foil with the sethoxydim applied with bentazon through SPB treatment (Table 2.3). Applying sethoxydim with bentazon through SPB resulted in similar weed control as sethoxydim applied alone in control of broadleaf signalgrass. Control of broadleaf signalgrass through tank mixing sethoxydim with bentazon was significantly lower than applying sethoxydim with bentazon through separate booms.

Table 2.3 Comparison of sethoxydim applied alone with all three applications methods of sethoxydim and bentazon 28 days after application at the Black Belt Experiment Station (Black Belt) and the R.R. Foil Plant Science Research Center (R.R. Foil).

Treatment[1]	**Application method**	**Black Belt**		**R.R. Foil**			
		Italian ryegrass		**Italian ryegrass**		**Broadleaf signalgrass**	
		Observed	**Expected**[2]	**Observed**	**Expected**	**Observed**	**Expected**
		%					
Sethoxydim alone		68^{a}		93^{a}		78^{a}	
Bentazon alone		0^{c}		0^{c}		0^{d}	
Sethoxydim with bentazon	tank mix	43^{b}	68	43^{b}	93	10^{c}	78
Sethoxydim with bentazon	mix-in-line	35^{b}	68	43^{b}	93	18^{bc}	78
Sethoxydim with bentazon	separate boom	61^{a}	68	83^{a}	93	50^{ab}	78

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicate significance.

[1] All treatments with bentazon and sethoxydim was applied at 840 and 140 g ai ha^{-1} with COC at 1% v/v, respectively.

[2] Expected responses were calculated using Colby's equation $E = (X + Y) – (XY)/100$, any response of sethoxydim with bentazon that was significantly lower than sethoxydim alone is considered antagonistic.

Conclusion

Antagonism of sethoxydim by bentazon was observed in Italian ryegrass control when the two herbicides were TMX or applied through the MIL methods. Applying sethoxydim with bentazon through SPB did overcome the antagonism in some cases. When mixed together, sethoxydim H^+ hydroxyl group and Na^+ ions from bentazon exchange to form a sodium salt of sethoxydim (Wanamarta et al. 1989). This sodium salt of sethoxydim is more polar and therefore absorption of sethoxydim is inhibited. Delaying the timing of sethoxydim and bentazon mixing until touching the plant leaf may have allowed enough time for enough sethoxydim to enter the plant before chemically reacting to bentazon.

CHAPTER III

COMPARING ANTAGONISTIC RESPONSES OF TANK MIXED AND SEQUENTIAL APPLICATIONS OF SETHOXYDIM WITH BENTAZON

Abstract

Previous studies have documented the antagonism resulting between sethoxydim and bentazon tank mixes. A greenhouse study was conducted to mimic field studies done to test different application techniques and their ability to reduce antagonism between sethoxydim and bentazon. Two studies were conducted in the greenhouse at the R.R. Foil Plant Science Research Center with broadleaf signalgrass (*Urochloa platyphylla*), giant foxtail (*Setaria faberi*), and barnyardgrass (*Echinochloa crus-galli*). A two-nozzle research track sprayer was used to spray bentazon and sethoxydim with three different application methods: tank mix, sequential application with bentazon applied first, and sequential application with bentazon applied second. Bentazon and sethoxydim applications were made at the rate of 840 g ai ha^{-1} and at 140 g ai ha^{-1} with COC at 1% v/v, respectively for all treatments. Visual estimations of injury ratings were taken 7, 14, 21, and 28 days after application. Biomass was also collected 28 days after application. Reduced efficacy of sethoxydim from adding bentazon is observed through 28 days after herbicide application, based on estimations of visual injury controls. Based on biomass weight results, no antagonism was observed with bentazon and sethoxydim applied together. Application methods showed no differences in herbicide efficacy.

Introduction

Tank mixing herbicides is a common practice in crop production. The ability to mix herbicides in one tank load and apply it to fields reduces time, labor, fuel, and other equipment cost compared to applying each herbicide in a separate load. When herbicides are tank mixed together, antagonism may occur. Herbicide Antagonism is the interaction of two or more herbicides such that the effect when combined is less than the predicted effect based on the activity of each chemical applied separately (WSSA 2019). If antagonism occurs when mixing herbicides, herbicide efficacy and weed control will be reduced.

Antagonism of sethoxydim applied with bentazon occurs when the two herbicides are tank mixed (Bridges et al. 1981; Hartzler and Foy 1983; Horng and Ilnicki 1982; Nalewaja et al. 1980; Rhodes Jr. and Coble 1984). Increasing the sethoxydim rate reduced the severity of the antagonism (Rhodes, Jr. and Coble 1984). Field experiments conducted by Rhodes, Jr. and Coble (1984) showed that sequential applications of sethoxydim and bentazon in broadleaf signalgrass (*Urochloa platyphylla*) prevented the antagonism from occurring. The sequential applications were made 5 minutes apart in those studies. Rhodes, Jr. and Coble (1984) found that order of sequential applications did not affect efficacy of sethoxydim. Carrier volume can influence efficacy of sethoxydim. In research conducted by Lassiter and Coble (1987), a carrier volume of 94 and 187 L ha^{-1} had better control of large crabgrass than 374 L ha^{-1} for sethoxydim applied alone and sethoxydim and bentazon sequential application. No carrier volume effect was observed for the sethoxydim and bentazon tank mix treatment (Lassiter and Coble 1987).

The objective of this study was to mimic the field study in Chapter II with application methods of sethoxydim with bentazon treatments. The objectives of the study were 1) to measure herbicide efficacy of sethoxydim with bentazon treatments through visual estimations of weed control and biomass weights and 2) determine if application method would reduce antagonism of sethoxydim applied with bentazon.

Materials and Methods

Two greenhouse studies were conducted at the R.R. Foil Plant Science Research Center in Starkville, MS. The two studies were planted on February 25, 2019 and March 23, 2019. The study was conducted using 0.95 L pots filled with Gro Metro-Mix potting soil (Sun Gro Horticulture, Agawam, MA 01001). Pots were seeded with the following grass species: barnyardgrass (*Echinochloa crus-galli*), broadleaf signalgrass (*Urochloa platyphylla*), and giant foxtail (*Setaria faberi*). The plants were placed into the greenhouse to germinate and grow. After emergence, the plants were thinned to one plant per pot. The study consisted of an untreated check plus five treatments, each with four replications in a randomized complete block design. Untreated checks were used to compare weed control. All treatments with bentazon (Broadloom™, UPL, King of Prussia, PA 19406) and sethoxydim (Poast®, BASF, Research Triangle Park, NC 27709) were applied at 840 g ai ha^{-1} and at 140 g ai ha^{-1} with crop oil concentrate (COC) (AGRI-DEX®, Helena, Collierville, TN 38017) at 1% v/v, respectively. The treatments were 1) bentazon, 2) sethoxydim, 3) bentazon tanked mixed with sethoxydim, 4) bentazon applied first followed by sethoxydim, and 5) sethoxydim applied first followed by bentazon. Sequential applications were made within 30 seconds of the first application. This was done to attempt to mimic the application of separate booms in the tractor field study in Chapter II. Ammonia and water were run through the sprayer system for cleanout between each treatment change. Treatments were applied with a two-nozzle research track sprayer (Generation III, DeVries Manufacturing Inc., Hollandale, MN). Treatments were applied using HYPRO Ultra Lo-Drift 120-02 (ULD) nozzles, at 140 L ha^{-1}, at 276 kPa, and at 6.7 km h^{-1}. Applications were made on March 22, 2019 and April 22, 2019, for the first and second study respectively. The grasses were sprayed at an average height of 10 cm for barnyardgrass, 10 cm for broadleaf signalgrass, and 13 cm for giant foxtail. Visual estimations of injury (control) ratings were taken 7, 14, 21, and 28 days after application (DAA) along with biomass after 28 days of application. The biomass samples were dried for 48 hours at 60°C and dry weights were recorded. All data collected was analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05. Due to no statistical differences found, all results were pooled across all three grass species. The Colby method was used to determine if herbicide tank mixes were antagonistic (Colby 1967).

Results and Discussion

Ratings 7 DAA showed no difference among application method, as shown in Table 3.1. The tank mix, sequential application with bentazon applied first, and sequential application with sethoxydim applied first had 11, 12, and 13% control, respectively (Table 3.1). Sethoxydim alone had 22% control, which was significantly higher than all three treatments with sethoxydim and bentazon. The three application methods did not overcome antagonism of grass control with sethoxydim applied with bentazon. When looking at the 28 DAA control ratings, the sequential application with bentazon applied first had 70% control, which was similar to the sequential application with sethoxydim applied first with 71% control (Table 3.1). Also, at 28 DAA, sethoxydim applied by itself had 78% control, which was similar to both sequential applications (Table 3.1). The tank mix treatment of sethoxydim with bentazon resulted in lower control than sethoxydim applied by itself (61 to 78%, respectively). When comparing dry biomass weights however, there was no statistical difference between sethoxydim by itself and the three sethoxydim with bentazon treatments with different application method (Table 3.1). Grichar

(1991) saw reduced sethoxydim efficacy when bentazon was added for Texas panicum *(Panicum texanum*) or southern crabgrass (*Digitaria ciliaris*) control in peanuts. This also coincides with findings from Rhodes, Jr and Coble (1984). In their research of looking at application variables affecting antagonism between bentazon and sethoxydim, tank mixing herbicides resulted in less weed control than sethoxydim alone. Sequential applications in their study prevented the antagonism, with the order of sequential application having no effect. Minton et al. (1989) observed antagonistic responses of barnyardgrass control when sethoxydim or quizalofop was tank mixed with broadleaf herbicides. When imazaquin, chlorimuron, or lactofen was applied 24 hours after sethoxydim or quizalofop, barnyardgrass control was not affected (Minton et al. 1989). When the order was reversed to where sethoxydim or quizalofop was applied 24 hours before imazaquin, chlorimuron, or lactofen, barnyardgrass control was antagonized (Minton et al. 1989).

Table 3.1 Comparison of dry biomass weights and visual estimations of weed control of barnyardgrass (*Echinochloa crus-galli*), broadleaf signalgrass (*Urochloa platphylla*), and giant foxtail (*Setaria faberi*)pooled together 7 and 28 days after application of sethoxydim applied alone with all three applications methods of sethoxydim and bentazon.

		7 DAA[2]		**28 DAA**		**Dry biomass weight**	
Treatment[1]	**Application Method**	**Observed**	**Expected[3]**	**Observed**	**Expected**	**Observed**	**Expected**
		%				g	
Sethoxydim alone		22^{a}		78^{a}		0.341^{a}	
Bentazon alone		0^{c}		0^{c}		2.285^{b}	
Sethoxydim and bentazon	tank mix	11^{b}	22	61^{b}	78	0.576^{a}	0.343
Sethoxydim and bentazon	sequential[4] application with bentazon applied first	12^{b}	22	70^{ab}	78	0.384^{a}	0.343
Sethoxydim and bentazon	sequential application with sethoxydim applied first	13^{b}	22	71^{ab}	78	0.424^{a}	0.343

Data were analyzed using Sidak’s comparison method. LS-means with different letters within the same column indicate significance.

[1] All treatments with bentazon and sethoxydim were applied at 840 and 140 g ai ha^{-1} with COC at 1% v/v, respectively.

[2] Days after application

[3] Expected responses were calculated using Colby’s equation $E = (X + Y) - (XY)/100$, any response of sethoxydim with bentazon significantly lower than sethoxydim alone is considered antagonistic.

[4] Sequential applications were made within 30 seconds after the first application.

As expected, bentazon had no grass control throughout all the ratings. This means that any treatments with lower weed control than sethoxydim applied by itself is antagonistic. When statistics were run for each control rating timing and biomass weights, the two trials and the three grass species were not statistically different. Therefore, all statistics were run with the two trial replications and with the three grass species pooled together.

Based on the visual estimations of weed control, antagonism of sethoxydim with bentazon was seen with the control ratings at 7 and 28 DAA. While the sequential applications were antagonistic 7 DAA, they had the same level of control as sethoxydim alone 28 DAA based on visual estimations of weed control. According to the dry biomass weights however, no antagonism of sethoxydim with bentazon was seen.

Conclusion

Reduced efficacy of sethoxydim from adding bentazon is observed through 28 days after herbicide application, based on estimations of visual weed controls. Application method did not reduce antagonism of sethoxydim with bentazon. Wanamarta et al. (1989) found that sethoxydim H^+ hydroxyl group and Na^+ ions from bentazon exchange to form a sodium salt of sethoxydim. This sodium salt of sethoxydim is more polar and therefore absorption of sethoxydim is inhibited. Applying sequential trials close together may not allow sethoxydim to be absorbed before mixing with bentazon on the leaf of the plant. More research needs to be done to analyze interaction of sethoxydim with bentazon on the leaf of a plant. When looking at dry biomass weights, no antagonism was observed when adding bentazon with sethoxydim applications and no difference observed between applications methods.

CHAPTER IV

REDUCING GLYPHOSATE ANTAGONISM WITH 2,4-D AND DICAMBA THROUGH SPRAY APPLICATON TECHNIQUE

Abstract

Dicamba and 2,4-D traits have been added to soybean and cotton, allowing for over the top applications of these herbicides. Methods to avoid antagonism of glyphosate by dicamba or 2,4-D should be utilized to achieve optimum weed control. This study was conducted at the Black Belt Experiment Station (Black Belt) and at the R.R. Foil Plant Science Research Center (R.R. Foil) in fallow fields with browntop millet (*Urochloa ramosa*), broadleaf signalgrass (*Urochloa platyphylla*) and Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) pressure. A tractor mounted dual boom sprayer was modified to spray three application methods: two herbicides tanked mixed, two herbicides in separate tanks mixed in the boom line, and two herbicides in separate tanks applied through separate booms simultaneously. Two salt formulations of dicamba and two salt formulations of 2,4-D were applied with glyphosate through the three application methods to determine difference in herbicide efficacy based on salt formulation and application method. Rates for the first trial at R.R. Foil were applied at 281 and 533 g ae ha^{-1} for dicamba and 2,4-D, respectively. Rates of dicamba, and 2,4-D increased to 562 and 1065 g ae ha^{-1}, respectively in the next two trials at Black Belt and R.R. Foil. Glyphosate was applied at 434 g ae ha^{-1} in all trials. Applying glyphosate with dicamba or 2,4-D resulted in the highest control when applied through separate booms. Antagonism of glyphosate was reduced by using the

separate boom application method. Antagonism of glyphosate from dicamba and 2,4-D was observed through the tank mix and mix-in-line application method.

Introduction

Antagonism can take place when glyphosate is mixed with dicamba or 2,4-D. (Flint and Barrett 1989). Translocation of glyphosate decreased when 2,4-D or dicamba was added, and less glyphosate was taken in through the treated leaf (Flint and Barrett 1989; Ou et al. 2018). Increasing the amount of glyphosate overcame the reduced translocation when mixed with dicamba or 2,4-D (Flint and Barrett 1989). Blackshaw et al. (2006) found evidence of antagonism with clethodim and 2,4-D when looking at volunteer wheat control. With consumers showing concern for health and environmental effects from herbicide use, increasing use rates of herbicides to make up for antagonism may bring more concerns from consumers to the agriculture industry (Byrne et al. 1991; Dunlap and Beus 1992; Myers et al. 2016).

Flint and Barrett (1989) looked at antagonism of glyphosate with dicamba and 2,4-D. Normally, dicamba and 2,4-D will have negligible effect on grasses. When Flint and Barrett (1989) compared treatments, he considered any herbicide mixtures that produced more fresh weight compared to the glyphosate alone as antagonistic. Flint and Barrett (1989) observed antagonism in the shoot weights of johnsongrass (*Sorghum halepense*) with glyphosate mixed with 2,4-D. Flint and Barrett (1989) also observed root and shoot growth suppression was reduced with glyphosate mixed with dicamba compared to glyphosate alone. Flint and Barrett (1989) found herbicide rate and salt formulation influenced antagonism.

The higher the rate of 2,4-D or dicamba used, the less activity glyphosate elicited. Flint and Barrett (1989) found that when 2,4-D or dicamba was added to glyphosate, less absorption of glyphosate took place in the leaves. Glyphosate was less effective in controlling johnsongrass when reduce translocation occurred. When dicamba was mixed with glyphosate, more of the absorbed glyphosate stayed in the leaves compared to glyphosate alone. Flint and Barrett (1989) went on to state that more glyphosate was retained in the treated section of the plant when it was 2,4-D or dicamba was present. In association with this, less glyphosate was found in the remaining parts of the plant, including the roots. Since the glyphosate stayed in the treated section, it did not move throughout the plant as it normally would. Flint and Barrett (1989) also noted that there was a general decrease of the mass of glyphosate lost from the roots when mixed with 2,4-D or dicamba. The cause of the decrease of glyphosate lost through the roots is due to a reduction in the amount of glyphosate going through the plant when mixed with 2,4-D. Flint and Barrett (1989) concluded that the toxicity of glyphosate to johnsongrass decreases when mixed with 2,4-D or dicamba. Flint and Barrett (1989) went on to say that increasing the herbicide rate of glyphosate could overcome the antagonism, which is supported by Green (1989).

Ou et al. (2018) looked at the combination of glyphosate and dicamba to control kochia (*Kochia scoparia*). In a greenhouse experiment, Ou et al. (2018) applied two different rates of dicamba with glyphosate. The treatments were 560 g ae ha^{-1} (treatment (Trt) 1), a normal field rate, and 1400 g ae ha^{-1} (Trt 2), 2.5 times a normal field rate. The dicamba susceptible population had 47 percent control when combined with glyphosate at 350 g ae ha^{-1}, half the recommended field rate. When 420 g ae ha^{-1} of glyphosate was applied with dicamba, there was only 50 percent control. Ou et al. (2018) went on to state that glyphosate alone had best control of the dicamba susceptible kochia when compared to dicamba combinations with the same dose of glyphosate. When glyphosate was applied at 840 g ae ha^{-1}, it had 95 percent control of dicamba susceptible kochia (Ou et al. 2018). When dicamba was applied with glyphosate at 70 g ae ha^{-1}, 140 g ae ha^{-}

^{1}, 280 g ae ha^{-1}, and 560 g ae ha^{-1} there was 80, 82, 91, and 87 percent control respectively (Ou et al. 2018). This shows glyphosate being antagonized by dicamba.

The dicamba susceptible kochia absorbed more glyphosate when glyphosate was mixed with dicamba than glyphosate applied alone 24 hours after treatment (Ou et al. 2018). Ou et al. (2018) then reported no differences in glyphosate absorption 72 and 168 hours after treatment. When glyphosate was mixed with dicamba less glyphosate translocated in the plant. Dicamba is an auxin herbicide; it can cause metabolic and physical reactions to the plant within hours of application. This means growth of the plant can be inhibited. Glyphosate can transport down the phloem of plants (Bromilow et al. 1993). Dicamba can weaken the phloem, meaning glyphosate may be restricted in its movement throughout the plant (Ou et al. 2018). If glyphosate is restricted in its ability to be absorbed and translocated within the plant, then optimum control of the plant may not be reached. Glyphosate could also cause lower translocation of dicamba. Ou et al. (2018) observed in later time points that dicamba shown reduced translocation. Glyphosate inhibits the EPSPS enzyme. This effects amino acid production in plants. When glyphosate kills the plant, the phloem ceases to operate in the plant (Amrhein et al. 1980; De Maria et al. 2006). Like glyphosate, dicamba also needs the phloem to move throughout the plant. If the phloem stops working, then dicamba is left idle.

It is important to note observed antagonism of glyphosate with dicamba and 2,4-D because of recently released dicamba and 2,4-D tolerant crops. Over the top applications of dicamba can now be applied across soybeans and cotton with the dicamba tolerant traits. Soybeans and cotton with 2,4-D tolerance has also been released to allow over the top applications of 2,4-D in these crops. Roundup Ready 2 Xtend® soybeans (Bayer, St. Louis, Mo 63167) (Bayer 2019) contain both dicamba and glyphosate tolerance. Corteva Agriscience released crops with 2,4-D tolerance, known as Enlist™ soybeans, cotton, and corn (Corteva Agriscience, Indianapolis, IN 46268) (Corteva 2019). Glyphosate and 2,4-D can be applied over the top in Enlist™ crops.

This study looked at antagonism of glyphosate applied with dicamba or 2,4-D, and how application method can reduce antagonism of glyphosate. Each herbicide combination was applied using three application methods: 1) the two herbicides tanked mixed together, 2) each of the two herbicides is in a separate can that is mixed in the boom line and applied through a single boom, and 3) each of the two herbicides in separate cans and applied in a separate boom at the same time. One salt formulation of glyphosate was tested for antagonism with different salt formulations of dicamba and 2,4-D. The objective of this study is to evaluate efficacy of glyphosate in grass weed species and how it is affected when mixed with dicamba or 2,4-D with each different herbicide application method.

Materials and Methods

This trial was conducted in the fall of 2018 at the R.R. Foil Plant Science Research Center in Starkville, MS on October 5, 2018, Black Belt Experiment Station in Brooksville, MS on March 28, 2019, and again at the R.R. Foil Plant Science Research Center on May 20, 2019. This trial consisted of 17 treatments each with four replications, in a randomized complete block design. An untreated check was used for weed control evaluations. A tractor mounted sprayer described in the introduction of Chapter II was used to make herbicide applications using the three application methods: 1) the two herbicides tanked mixed in a can and sprayed through a single boom, 2) each of the two herbicides is in a separate can and then mixed in the boom line and applied through a single boom, and 3) each of the two herbicides is in a separate can and applied at the same time through a dual boom. Each herbicide combination was applied with the three different application methods. For the first trial at the R.R. Foil Plant Science Research Center, all treatments with dicamba and 2,4-D were made applied at 281 and 533 g ae ha^{-1}, respectively. For the second trial replication at the Black Belt Experiment Station and the third trial replication at the R.R. Foil Plant Science Research Center, the dicamba and 2,4-D applications were increased to 562 and 1065 g ae ha^{-1}, respectively. Salt formulations tested in all three trial replications were bis-aminopropyl methylamine (BAPMA) salt of dicamba (Engenia®, BASF, Research Triangle Park, NC 27709), diglycolamine (DGA) salt of dicamba with vapor grip (FeXapan®, Corteva Agriscience, Indianapolis, IN 46268), choline salt of 2,4-D (Enlist One®, Corteva Agriscience), and dimethylamine (DMA) salt of 2,4-D Amine (Weed Rhap® A-4D, Helena Chemical, Collierville, TN 38017). All treatments with glyphosate were made at a rate of 434 g ae ha^{-1} using Roundup PowerMAX® (Bayer, St. Louis, MO 63167) for all three trial replications. Table 4.1 displays all the treatment combinations used in all three trial replications.

Table 4.1 Complete treatment combination list for the glyphosate antagonism field study.

Treatment	**Application Method**	**Herbicide Active Ingredient(s)**
1	single application	BAPMA salt of dicamba
2	single application	DGA salt of dicamba with vapor grip
3	single application	choline salt of 2,4-D
4	single application	DMA salt of 2,4-D Amine
5	single application	glyphosate
6	tank mix	BAPMA salt of dicamba and glyphosate
7	mix-in-line	BAPMA salt of dicamba and glyphosate
8	separate boom	BAPMA salt of dicamba and glyphosate
9	tank mix	DGA salt of dicamba with vapor grip and glyphosate
10	mix-in-line	DGA salt of dicamba with vapor grip and glyphosate
11	separate boom	DGA salt of dicamba with vapor grip and glyphosate
12	tank mix	choline salt of 2,4-D and glyphosate
13	mix-in-line	choline salt of 2,4-D and glyphosate
14	separate boom	choline salt of 2,4-D and glyphosate
15	tank mix	DMA salt of 2,4-D Amine and glyphosate
16	mix-in-line	DMA salt of 2,4-D Amine and glyphosate
17	separate boom	DMA salt of 2,4-D Amine and glyphosate

All applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). Visual estimation of injury (control) ratings were taken 7, 14, 21, and 28 days after application (DAA). The first trial replication at the R.R. Foil Plant Science Research Center had a natural stand of browntop millet (*Urochloa ramosa*) that was sprayed at an average height of 15 cm and 9 cm, respectively. The second trial replication at the Black Belt Experiment Station had a natural thick stand of Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*), and applications were made with the Italian ryegrass at an average height of 18 cm. The third study at the R.R. Foil Plant Science Research Center had had Oregon Grown, Gulf Variety, Italian ryegrass drilled into the field at a rate of 112 kg ha^{-1} on March 20, 2019 along with a natural population of broadleaf signalgrass (*Urochloa platyphylla*). Applications were made with Italian ryegrass at an average height of 15 cm and broadleaf signalgrass at an average height of 9 cm. All data collected was analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05. The Colby method was used to determine if herbicide combinations are antagonistic (Colby 1967).

Results and Discussion

When analyzing results taken 7 DAA, data for Italian ryegrass control from the Black Belt Experiment Station and R.R. Foil Plant Science Research Center were pooled together due to no significant difference being found between the two locations. The herbicide combinations applied also showed no significant difference therefore data were also pooled across all herbicide combination for each weed specie for data taken at 7 DAA and 28 DAA. Due to dicamba and 2,4-D having no control of grass species in all three trial replications, any weed control with glyphosate applied with dicamba or 2,4-D treatments less than glyphosate applied alone is considered antagonistic.

The results of browntop millet control 7 DAA was 100, 98, 40, and 99% for the glyphosate alone, tank mix (TMX), mix-in-line (MIL), and separate boom (SPB) methods, respectively (Figure 4.1) Due to the TMX and SPB methods not being significantly lower than glyphosate alone, no antagonism was observed. The MIL application method resulted in lower browntop millet control, but it was due to improper mixing in the line. In all the treatments with the MIL application, a block of dead browntop millet would be followed by a block of browntop millet that looked unaffected. The auxins in this trial had zero browntop millet control. It is believed that the control unit did not mix the herbicides properly. It appeared that glyphosate came out the boom, then the auxin, then back to glyphosate. The MIL method at the fall application at the R.R. Foil Plant Science Research Center was applied by using one manifold instead of two manifolds with a "T" in the line. It was because of the browntop millet control failure that the MIL method was modified to using two manifolds with a "T" in the line for the applications made at the Black Belt Experiment Station in the spring of 2019 and again at the R.R. Foil Plant Science Research Center in the summer of 2019.

For Italian ryegrass control 7 DAA, applying glyphosate with dicamba or 2,4-D through SPB was the only application method that resulted in weed control similar to glyphosate applied by itself (Figure 4.1). The SPB application method and glyphosate applied alone had 40 and 49% control, respectively (Figure 4.1). As show in Figure 4.1, applying glyphosate with dicamba or 2,4-D through TMX or the MIL method resulted in weed control lower than the SPB application method. The TMX and MIL methods resulted in 26 and 14% control respectively, which was significantly lower than the 49% control from glyphosate applied alone. Broadleaf signalgrass control was higher at 35% control when glyphosate was applied alone. Among the three application methods, applying glyphosate with dicamba or 2,4-D was higher at 19% control with the SPB application. The TMX method and the MIL method resulted in 10 and 3% control, respectively, which resulted in lower weed control than the SPB application method (Figure 4.1). All three application methods with glyphosate applied with dicamba or 2,4-D were still antagonistic due to all three methods having less control that glyphosate applied by itself, as shown in Figure 4.1. The results of the TMX application method being antagonistic in this trial is also supported by Flint and Barrett (1989), where glyphosate activity on johnsongrass was reduced when dicamba or 2,4-D was applied with glyphosate.

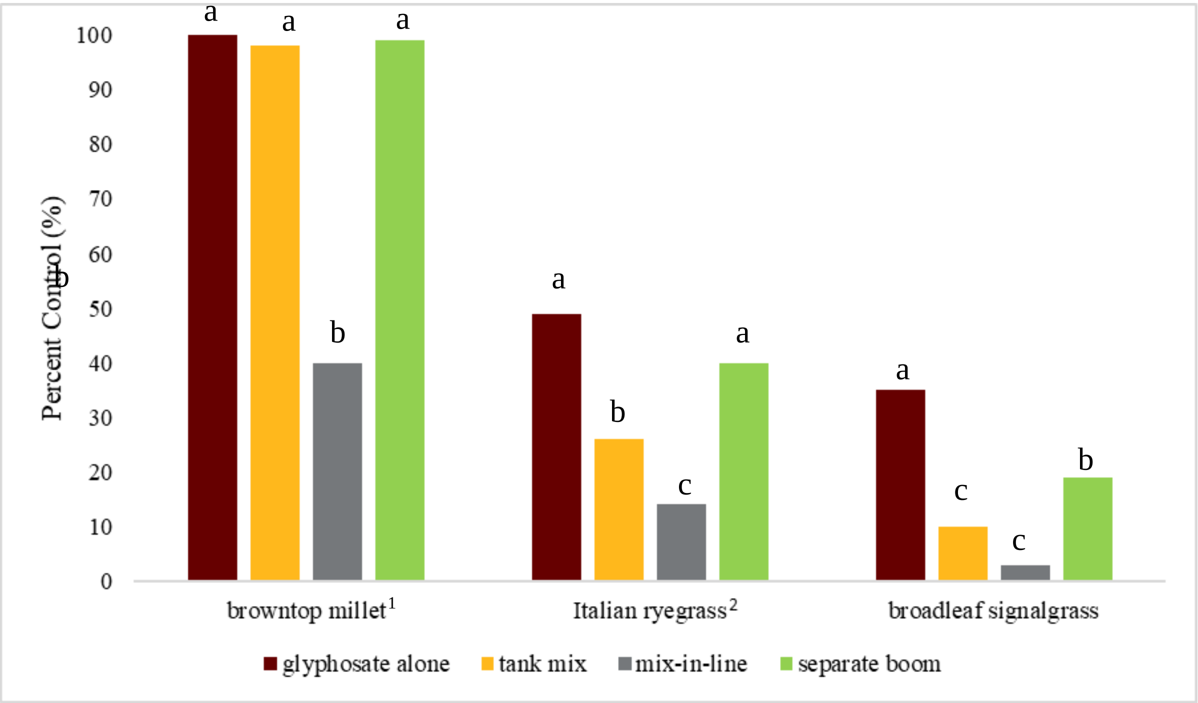

Figure 4.1 Comparison of application methods in control of browntop millet, Italian ryegrass, and broadleaf signalgrass 7 days after application.

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same grass specie column indicate significance.

[1] Dicamba and 2,4-D applications for browntop millet control were made applied at 281 and 533 g ae ha^{-1}, respectively. Glyphosate was applied at a rate of 434 g ae ha^{-1}.

[2] Dicamba and 2,4-D applications for Italian ryegrass and broadleaf signalgrass control were increased to 562 and 1065 g ae ha^{-1}, respectively. Glyphosate was applied at a rate of 434 g ae ha^{-1}.

For the first trial at the R.R. Foil Plant Science Research Center, all treatments with dicamba and 2,4-D were made applied at 281 and 533 g ae ha^{-1}, respectively. For the application at the Black Belt Experiment Station and the third trial replication at the R.R. Foil Plant Science Research Center, the dicamba and 2,4-D applications were doubled to 562 and 1065 g ae ha^{-1}, respectively. Both dicamba and 2,4-D were applied at a half rate for the first trial, then increased to a full rate for the last two trials at the Black Belt Experiment Station and the summer trial at the R.R. Foil Plant Science Research Center. Increased antagonism was seen due to increasing the rate of dicamba and 2,4-D. Increasing the rate of dicamba and 2,4-D also showed more antagonism with tank mix applications 7 DAA.

Glyphosate applied alone had 100% control, which was the same control of browntop millet as glyphosate applied with dicamba or 2,4-D with the TMX and MIL application methods 28 DAA (Table 4.2). The MIL method had 84% control, which was the only method that resulted in control less than 100% of browntop millet 28 DAA (Table 4.2). Therefore, antagonism of browntop millet control was seen only with glyphosate applied with dicamba or 2,4-D with the MIL method. For control of Italian ryegrass at the Black Belt Experiment Station, the TMX application and the SPB application had 54 and 69% control, both statistically the same weed control at 28 DAA (Table 4.2). The MIL method had 50% control of Italian ryegrass, which was lower than the SPB application method but not the TMX method, as shown in Table 4.2. Italian ryegrass control 28 DAA at the R.R. Foil Plant Science Research Center was highest with glyphosate applied alone at 96% and with glyphosate applied with dicamba or 2,4-D with the SPB application method at 92% (Table 4.2). Tank mixing glyphosate with dicamba or 2,4-D resulted 78% control, which was lower control of Italian ryegrass than applying the same herbicide combinations through separate booms, as shown in Table 4.2. The MIL application method had 50% less control of Italian ryegrass, which was lower than the TMX application method (Table 4.2).

The results of broadleaf signal grass control 28 DAA were similar to control of Italian ryegrass at the R.R. Foil Plant Science Research Center. Applying glyphosate with dicamba or 2,4-D through separate booms had statistically the same weed control as glyphosate applied alone (65-65%, respectively, Table 4.2). Applications with the MIL and TMX methods had similar weed control of 18 and 39%, respectively, of broadleaf signal grass but both resulted in less control of 65% with the separate boom application method (Table 4.2).

Table 4.2 Comparison of application methods in control of browntop millet, Italian ryegrass, and broadleaf signalgrass at the Black Belt Experiment Station (Black Belt) and the R.R. Foil Plant Science Research Center (R.R. Foil) 28 days after application.

Application method[1]	**R.R. Foil (Fall 2018)**		**Black Belt (Spring 2019)**		**R.R. Foil (Summer 2019)**			
	Browntop millet		**Italian ryegrass**		**Italian ryegrass**		**Broadleaf signalgrass**	
	Observed	**Expected**[2]	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**
	%							
Glyphosate alone	100^{a}		80^{a}		96^{a}		65^{a}	
Dicamba or 2,4-D	0^{c}		0^{c}		0^{d}		0^{c}	
Tank mix	100^{a}	100	54^{ab}	80	78^{b}	96	39^{b}	65
Mix-in-line	84^{b}	100	50^{b}	80	48^{c}	96	18^{b}	65
Separate boom	100^{a}	100	69^{a}	80	92^{a}	96	65^{a}	65

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicate significance.

[1] Dicamba and 2,4-D applications for browntop millet control were made applied at 281 and 533 g ae ha^{-1}, respectively. Dicamba and 2,4-D applications for Italian ryegrass and broadleaf signalgrass control were increased to 562 and 1065 g ae ha^{-1}, respectively. Glyphosate was applied at a rate of 434 g ae ha^{-1} for control of all species.

[2] Expected responses were calculated using Colby's equation $E = (X + Y) - (XY)/100$, any response of glyphosate with dicamba or 2,4-D significantly less than glyphosate alone is considered antagonistic.

Conclusion

Overall, applying glyphosate with dicamba or 2,4-D was highest when they were applied separately through separate booms. In most cases, making applications through separate booms did not show antagonism while antagonism was seen with the TMX and MIL application methods. Keeping glyphosate apart from dicamba or 2,4-D by using the SPB method to apply them may keep the two herbicides from interacting chemically, resulting in improved grass control. Delaying the herbicide interactions until they land on the plant’s leaf may allow enough time for glyphosate to enter and kill the grass weed opposed to tank mixing them. More research needs to be done to investigate what chemical reactions are taking place when glyphosate is mixed with dicamba or 2,4-D. Applications with separate booms also increases the carrier volume from 140 to 280 L ha^{-1}. Instead of only one 02 size nozzle being used for TMX and MIL application methods, a dual boom would have two sets of 02 size nozzles making applications thus increasing the carrier volume.

The rate of the herbicides did affect antagonistic responses. The fall 2018 trial at the R.R. Foil Plant Science Research Center had dicamba and 2,4-D applied at a half rate while the trial at the Black Belt Experiment Station and the summer trial at R.R. Foil Plant Science Research Center applied dicamba and 2,4-D at a full rate. When glyphosate was applied with dicamba and 2,4-D at a half rate, the TMX application method was not antagonistic according to the visual estimations of weed control 7 and 28 DAA.

CHAPTER V

REDUCING CLETHODIM ANTAGONISM WITH 2,4-D AND DICAMBA THROUGH SPRAY APPLICATION TECHNIQUE

Abstract

Dicamba and 2,4-D traits have been added to soybean and cotton, allowing for over the top applications of these herbicides. This trial was developed to test application techniques to reduce antagonism of clethodim by dicamba or 2,4-D and achieve optimum weed control. This study was conducted at the Black Belt Experiment Station (Black Belt) and at the R.R. Foil Plant Science Research Center (R.R. Foil) in fallow fields with volunteer corn (*Zea mays*), browntop millet (*Urochloa ramosa*), broadleaf signalgrass (*Urochloa platyphylla*) and Italian ryegrass (*Lolium perenne* ssp. *multiflorum*). A tractor mounted dual boom sprayer with eight nozzles was modified to spray three application methods: two herbicides tanked mixed, two herbicides in separate tanks mixed in the boom line, and two herbicides in separate tanks applied through separate booms simultaneously. Two salt formulations of dicamba and two salt formulations of 2,4-D were applied with glyphosate through the three application methods to determine difference in herbicide efficacy based on salt formulation and application method. For the first trial at the R.R. Foil, dicamba and 2,4-D was applied at 281 and 533 g ae ha^{-1}, respectively. Rates of dicamba, and 2,4-D increased to 562 and 1065 g ae ha^{-1}, respectively for the next two trials at Black Belt and R.R. Foil. Clethodim was applied at 68 g ai ha^{-1} with NIS at a rate of 0.25% v/v in all trials. Among the three application methods, the separate boom application method resulted

in the highest control when applying clethodim with dicamba or 2,4-D, and in some cases reduced antagonism of clethodim. Antagonism of clethodim from dicamba and 2,4-D was observed through the tank mix and mix-in-line application method.

Introduction

Zollinger (2017) reported antagonism with dicamba and clethodim in specifically grass control. In a study looking at control of southern crabgrass (*Digitaria ciliaris*), clethodim applied 24 hours before a broadleaf herbicide showed little to no antagonism while the tank mixes were antagonistic (Grichar et al. 2002). Even though tank mixing herbicides is a simple way to apply multiple herbicides at one time, applying the herbicides separately may prevent antagonism form occurring.

Grichar et al. (2002) looking at control of southern crabgrass (*Digitaria ciliaris*), when clethodim applied 24 hours before a broadleaf herbicide showed little to no antagonism while the tank mixes were antagonistic (Grichar et al. 2002). When clethodim alone was applied, Grichar et al. observed 95 percent control in 1996 and less than 80 percent control of southern crabgrass in 1997 and 1999. When clethodim was followed by acifluorfen plus bentazon or 2,4-D, the control of southern crabgrass dropped to below 50 percent. The extra 24 hours allowed the herbicide to start working within the plant before a second herbicide could interfere with it. Broadleaf signalgrass (*Urochloa platyphylla*) control was also observed in this study. Clethodim had lower control of broadleaf signal grass when it was tanked mixed with each of the broadleaf herbicides in the study. Only three out of the six broadleaf herbicides tested showed antagonism when clethodim was applied 24 hours prior. Grichar et al. (2002) concluded that generally less antagonism occurred when clethodim was applied 24 hours before or after a broadleaf herbicide application.

Blackshaw et al. (2006) found antagonism with clethodim and 2,4-D amine. Blackshaw et al. (2006) conducted experiments looking at controlling volunteer wheat (*Triticum aestivum*). The experiments were conducted in fields planted to wheat in the early spring to replicate volunteer wheat. Herbicide combinations that were used in this study were clethodim and quizalofop-P alone, mixtures with 2,4-D, mixtures with bromoxynil, mixtures with bromoxynil plus MCPA, and mixtures of thifensulfuron plus tribenuron. As mentioned earlier, antagonism was observed with clethodim mixed with 2,4-D amine and quizalofop-P mixed with 2,4-D amine. To overcome the antagonism, he raised the rate of both herbicides. Even at the highest rate, antagonism was still observed. 2,4-D ester, however, did not show antagonistic responses. This shows that salt formulation can have an impact on antagonism.

Amine formulations are less efficient in moving into the leaf compared to ester formulations (Nice 2004). Amine formulations are more water soluble, meaning it takes longer for it to penetrate through the waxy cuticle layer of the leaf. Clethodim may be interacting with the 2,4-D amine sitting on the leaf. Potentially the 2,4-D amine could be coating the leaf and not allowing the clethodim to go through. Hard water can antagonize 2,4-D, by cations, such as Ca or Mg, bonding to the negative charged 2,4-D (Schortgen 2017). If cations bond to the 2,4-D, a large molecule may be sprayed on the plant. The large molecule is less efficient in penetrating through the leaf cuticle.

With weeds, such as waterhemp (*Amaranthus tuberculatus* var. *rudis*) and Palmer amaranth (*Amaranthus palmeri*), developing resistance to multiple sites of actions, tank mixing herbicides will be more prevalent to better control weeds and prevent further resistance from developing. It is important to make sure herbicide applications work effectively in order to prevent reapplication. Reapplications of herbicides cost the producer more money and increase the environmental load. Letting weeds to continue to grow after a failed application can potentially decrease crop yield, allow more weed seed to enter the soil seedbank, and build resistance to other herbicide sites of action. The goal of this review was to look at specific chemical reactions between 2,4-D, dicamba, glyphosate, and clethodim takes place and what reactions occur within the plants.

Applying herbicide combinations different ways may reduce antagonistic effects. In a study looking at control of southern crabgrass (*Digitaria ciliaris*), clethodim applied 24 hours before a broadleaf herbicide showed little to no antagonism while the tank mixes were antagonistic (Grichar et al. 2002). Even though tank mixing herbicides is a simple way to apply multiple herbicides at one time, applying the herbicides separately may prevent antagonism form occurring.

This study looked at antagonism of clethodim applied with dicamba or 2,4-D, and how application method can reduce antagonism of clethodim. This study applied herbicides using three methods: 1) the two herbicides tanked mixed together, 2) each of the two herbicides is in a separate can that is mixed in the boom line and applied through a single boom, and 3) each of the two herbicides in separate cans and applied in a separate boom at the same time. Clethodim was tested for antagonism with different salt formulations of dicamba and 2,4-D. The objective of this study is to evaluate efficacy of clethodim in grass weed species and how it is affected when mixed with dicamba or 2,4-D with each different herbicide application method.

Materials and Methods

This trial was conducted in the fall of 2018 at the R.R. Foil Plant Science Research Center (R.R. Foil) in Starkville, MS on October 6, 2018, the Black Belt Experiment Station (Black Belt) in Brooksville, MS on March 29, 2019, and again at the R.R. Foil Plant Science

Research Center on May 20, 2019. This trial consisted of 17 treatments each with four replications, in a randomized complete block design. An untreated check was used for weed control evaluations. A tractor mounted sprayer described in the introduction of Chapter II was used to make herbicide applications using the three application methods: 1) the two herbicides tanked mixed in a can and sprayed through a single boom, 2) each of the two herbicides is in a separate can and then mixed in the boom line and applied through a single boom, and 3) each of the two herbicides is in a separate can and applied at the same time through a dual boom. Each herbicide combination was applied with the three different application methods. For the first trial at the R.R. Foil Plant Science Research Center, all treatments with dicamba and 2,4-D were applied at 281 and 533 g ae ha^{-1}, respectively. For the second trial replication at the Black Belt Experiment Station and the third trial replication at the R.R. Foil Plant Science Research Center, the dicamba and 2,4-D applications were increased to 562 and 1065 g ae ha^{-1}, respectively. Salt formulations tested in all three trial replications were bis-aminopropyl methylamine (BAPMA) salt of dicamba (Engenia®, BASF, Research Triangle Park, NC 27709), diglycolamine (DGA) salt of dicamba with vapor grip (FeXapan®, Corteva Agriscience, Indianapolis, IN 46268), choline salt of 2,4-D (Enlist One®, Corteva Agriscience), and dimethylamine (DMA) salt of 2,4-D Amine (Weed Rhap® A-4D, Helena Chemical, Collierville, TN 38017). All treatments with clethodim (Select Max®, Valent, Walnut Creek, CA 94569) were made at a rate of 68 g ai ha^{-1} with a nonionic surfactant (NIS) (Activate Plus™, Winfield United, River Falls, WI 54022) at 0.25% v/v for all three trial replications. Table 5.1 displays all the treatment combinations used in the three trial replications.

Table 5.1 Complete treatment combination list for the clethodim antagonism field study.

Treatment	**Application Method**	**Herbicide Active Ingredients(s)**
1	single application	BAPMA salt of dicamba
2	single application	DGA salt of dicamba with vapor grip
3	single application	choline salt of 2,4-D
4	single application	DMA salt of 2,4-D Amine
5	single application	clethodim with NIS
6	tank mix	BAPMA salt of dicamba and clethodim with NIS
7	mix-in-line	BAPMA salt of dicamba and clethodim with NIS
8	separate boom	BAPMA salt of dicamba and clethodim with NIS
9	tank mix	DGA salt of dicamba with vapor grip and clethodim with NIS
10	mix-in-line	DGA salt of dicamba with vapor grip and clethodim with NIS
11	separate boom	DGA salt of dicamba with vapor grip and clethodim with NIS
12	tank mix	choline salt of 2,4-D and clethodim with NIS
13	mix-in-line	choline salt of 2,4-D and clethodim with NIS
14	separate boom	choline salt of 2,4-D and clethodim with NIS
15	tank mix	DMA salt of 2,4-D Amine and clethodim with NIS
16	mix-in-line	DMA salt of 2,4-D Amine and clethodim with NIS
17	separate boom	DMA salt of 2,4-D Amine and clethodim with NIS

All applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. In the fall, 2018 study, the applications of the separate booms were made at 70 L ha^{-1} at 13.4 km h^{-1} at 276 kPA. This was done to achieve the same GPA application volume of 140 L ha^{-1} because two sets of nozzles were being used. This method was then reverted to an application volume of 140 L ha^{-1} with a speed of 6.7 km h^{-1} due to carrier volume having effects on efficacy (Sperry 2019). The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). The first trial replication at the R.R. Foil Plant Science Research Center had a stand of volunteer corn (*Zea mays*) and natural population of browntop millet (*Urochloa ramosa*) that were sprayed at an average height of 15 cm and 9 cm, respectively. The second trial replication at the Black Belt Experiment Station had a natural uniform population of Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*), and applications were made with the Italian ryegrass at an average height of 18 cm. The third study at the R.R. Foil Plant Science Research Center had Oregon Grown, Gulf Variety, Italian ryegrass drilled into the field at a rate of 112 kg ha^{-1} on March 20, 2019 along with a natural population of broadleaf signalgrass (*Urochloa platyphylla*). Applications were made with Italian ryegrass at an average height of 15 cm and broadleaf signalgrass at an average height of 9 cm. Visual estimation of injury control ratings were taken 7, 14, 21, and 28 days after application (DAA). All data collected was analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05. The Colby method was used to determine if herbicide combinations are antagonistic (Colby 1967).

Results and Discussion

No grass control was observed from any dicamba or 2,4-D application in all three trial replications. Any weed control lower than clethodim applied alone is considered antagonistic. Data from the visual estimation of weed control ratings at 7, 21, and 28 DAA were pooled across herbicide combination within each grass species due to no difference found in herbicide combination.

Control ratings taken 7 DAA showed little activity from clethodim due to the timing being close from application (Table 5.2). No significant difference was found among application methods for both volunteer corn and browntop millet at the R.R. Foil Plant Science Research Center in the fall of 2018, as shown in Table 5.2. At 7 DAA, volunteer corn control ranged from 21-25% and browntop millet control was 0% for all herbicide combinations. Applying clethodim with dicamba or 2,4-D through the tank mix (TMX) or the separate boom (SPB) methods resulted in 2 and 0.25% control, respectively, which was similar to the 1% control from applying clethodim alone in Italian ryegrass at the Black Belt Experiment Station (Table 5.2). At R.R. Foil 7 DAA, the SPB application had 9% control of Italian ryegrass, and it was the only application method when applying clethodim with dicamba or 2,4-D that resulted in similar control of Italian ryegrass as clethodim alone, which resulted in 15% control (Table 5.2). The TMX, mix-in-line (MIL), and SPB applications resulted in 4, 3, and 9% control of Italian ryegrass 7 DAA (Table 5.2) The only difference among application methods in Italian ryegrass control 7 DAA was the SPB application was higher at 9% than the 3% from the MIL method. No difference of Italian ryegrass control was found among the three application methods at the R.R. Foil 7 DAA. No differences of any treatment were found for control of broadleaf signal grass at the R.R. Foil 7 DAA (Table 5.2). The variability of control among each grass species is due to clethodim having little activity 7 DAA.

Table 5.2 Comparison of application method of control of multiple grass species 7 days after application at the R.R. Foil Plant Science Research Center (R.R. Foil) and at the Black Belt Experiment Station (Black Belt).

Application method[1]	R.R. Foil (Fall 2018)				Black Belt (Spring 2019)		R.R. Foil (Summer 2019)			
	Volunteer corn		Browntop millet		Italian ryegrass		Italian ryegrass		Broadleaf signalgrass	
	Observed	Expected[2]	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
	%									
Clethodim alone	25^{a}		0^{a}		1^{ab}		15^{a}		3^{a}	
Dicamba or 2,4-D	0^{b}		0^{a}		0^{b}		0^{c}		0^{a}	
Tank mix	23^{a}	25	0^{a}	0	2^{a}	1	4^{bc}	15	0^{a}	3
Mix-in-line	21^{a}	25	0^{a}	0	0^{b}	1	3^{c}	15	0^{a}	3
Separate boom	24^{a}	25	0^{a}	0	0.25^{ab}	1	9^{ab}	15	1^{a}	3

Data were analyzed using Sidak’s comparison method. LS-means with different letters within the same column indicates significance.

[1] For the tank mix, mix-in-line, and separate boom method, dicamba and 2,4-D applications for volunteer corn and browntop millet control were made applied at 281 and 533 g ae ha^{-1}, respectively. Dicamba and 2,4-D applications for Italian ryegrass and broadleaf signalgrass control were increased to 562 and 1065 g ae ha^{-1}, respectively. Clethodim was applied at a rate of 68 g ai ha^{-1} with NIS at 0.25% v/v for control of all species in all treatments.

[2] Expected responses were calculated using Colby’s equation $E = (X + Y) – (XY)/100$, any response of clethodim with dicamba or 2,4-D significantly less than clethodim alone is considered antagonistic.

Applying clethodim with dicamba or 2,4-D through TMX or SPB resulted in no difference in control of volunteer corn and browntop millet versus clethodim applied alone 21 DAA at the 2018 fall trial at the R.R. Foil (Table 5.3). The MIL method resulted in 54 and 12% control of volunteer corn and browntop millet, respectively, 21 DAA at the R.R. Foil. This resulted in lower weed control than the TMX and SPB application methods for volunteer corn and browntop millet control 21 DAA, as shown in Table 5.3. For control of Italian ryegrass at the Black Belt 21 DAA, the TMX, MIL, and SPB application methods resulted in 39, 30, and 48% control (Table 5.3). The SPB method had a higher control but was not different than the TMX method. The MIL method, however, had lower weed control than the SPB method. As shown in Table 5.3, the clethodim alone treatment, which resulted in 43% control, was similar across as all three application methods for Italian ryegrass control at Black Belt 21 DAA. When applying clethodim with dicamba or 2,4-D, applying the herbicides through separate booms resulted in 70% control of Italian ryegrass 21 DAA, which was the same as clethodim alone at the R.R. Foil in the summer of 2019 (Table 5.3). Applying clethodim with dicamba or 2,4-D through TMX and MIL methods resulted in 54 and 41% control of Italian ryegrass at the R.R. Foil 21 DAA (Table 5.3). The TMX method had lower control of Italian ryegrass compared to the SPB method, and the MIL method had lower control of Italian ryegrass than the TMX method (Table 5.3). Clethodim alone resulted in 33% control of broadleaf signalgrass 21 DAA (Table 5.3). The TMX and SPB application methods resulted in 13 and 34% control, respectively of Italian ryegrass. Compared to the clethodim alone treatment, both the TMX and SPB methods did not reduce control of broadleaf signal grass 21 DAA, but the TMX method resulted in lower control than the SPB application method (Table 5.3).

Table 5.3 Comparison of application method of control of multiple grass species 21 days after application at the R.R. Foil Plant Science Research Center (R.R. Foil) and at the Black Belt Experiment Station (Black Belt).

Application method[1]	R.R. Foil Plant Science (Fall 2018)				Black Belt (Spring 2019)		R.R. Foil Plant Science (Summer 2019)			
	Volunteer corn		Browntop millet		Italian ryegrass		Italian ryegrass		Broadleaf signalgrass	
	Observed	Expected[2]	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
	%									
Clethodim alone	89^{a}		23^{a}		43^{ab}		70^{a}		33^{ab}	
Dicamba or 2,4-D	0^{c}		0^{c}		0^{c}		0^{d}		0^{d}	
Tank mix	79^{a}	89	18^{a}	23	39^{ab}	43	54^{b}	70	13^{bc}	33
Mix-in-line	54^{b}	89	12^{b}	23	30^{b}	43	41^{c}	70	5^{c}	33
Separate boom	83^{a}	89	20^{a}	23	48^{a}	43	68^{a}	70	34^{a}	33

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicate significance.

[1] For the tank mix, mix-in-line, and separate boom method, dicamba and 2,4-D applications for volunteer corn and browntop millet control were made applied at 281 and 533 g ae ha^{-1}, respectively. Dicamba and 2,4-D applications for Italian ryegrass and broadleaf signalgrass control were increased to 562 and 1065 g ae ha^{-1}, respectively. Clethodim was applied at a rate of 68 g ai ha^{-1} with NIS at 0.25% v/v for control of all species in all treatments.

[2] Expected responses were calculated using Colby's equation $E = (X + Y) - (XY)/100$, any response of clethodim with dicamba or 2,4-D significantly less than clethodim alone is considered antagonistic.

All treatments with dicamba and 2,4-D were made applied at 281 and 533 g ae ha^{-1}, respectively at the first trial in the fall of 2018 at the R.R. Foil Plant Science Research Center. For the application at the Black Belt Experiment Station and the third trial replication at the R.R. Foil Plant Science Research Center, the dicamba and 2,4-D applications were doubled to 562 and 1065 g ae ha^{-1}, respectively. Both dicamba and 2,4-D were applied at a half rate for the first trial, then increased to a full rate for the last two trials at the Black Belt Experiment Station and the summer trial at the R.R. Foil Plant Science Research Center. Increased antagonism was observed due to increasing the rate of dicamba and 2,4-D in the last two trial replications.

Applying dicamba or 2,4-D with clethodim with the TMX and SPB methods resulted in 88 and 92% control of volunteer corn, which was similar to the 96% control from the clethodim alone treatment 28 DAA (Table 5.4). The MIL method resulted in 60% control of volunteer corn 28 DAA, which was lower control than the clethodim applied alone treatment (Table 5.4). The SPB and the TMX application methods resulted in 34 and 27% control of browntop millet 28 DAA, respectively, which was similar in control (Table 5.4). The SPB application method resulted in 62% control of Italian ryegrass 28 DAA at Black Belt (Table 5.4). This was the only application method that resulted in Italian ryegrass control similar to the 63% control from applying clethodim alone. Both, the TMX and MIL methods had 48 and 37% control of Italian ryegrass 28 DAA at Black Belt, respectively. Both methods had lower control of Italian ryegrass at Black Belt than the SPB and clethodim alone treatments (Table 5.4). Italian ryegrass control 28 DAA at R.R. Foil was highest with clethodim alone and the SPB application method, which was 89 and 93% control respectively (Table 5.4). The TMX and MIL methods resulted in 75 and 54% control of Italian ryegrass, respectively, 28 DAA at R.R. Foil. Like the Italian ryegrass control results from Black Belt, the TMX and MIL methods resulted in lower control. Applying clethodim with dicamba or 2,4-D through separate booms was the only application method that resulted in control of Italian ryegrass at the Black Belt Experiment Station and at the R.R. Foil Plant Science Research Center that was similar to applying clethodim alone (Table 5.4). Applying clethodim with dicamba or 2,4-D through TMX or the MIL application methods resulted in lower control of Italian ryegrass compared to the SPB application method, therefore showing an antagonistic reaction.

Table 5.4 Comparison of application method of control of multiple grass species 28 days after application at the R.R. Foil Plant Science Research Center (R.R. Foil) and at the Black Belt Experiment Station (Black Belt).

Application method[1]	**R.R. Foil (Fall 2018)**				**Black Belt (Spring 2019)**		**R.R. Foil (Summer 2019)**			
	Volunteer corn		**Browntop millet**		**Italian ryegrass**		**Italian ryegrass**		**Broadleaf signalgrass**	
	Observed	**Expected[2]**	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**
	%									
Clethodim alone	96^{a}		49^{a}		63^{a}		93^{a}		50^{a}	
Dicamba or 2,4-D	0^{c}		0^{c}		0^{c}		0^{d}		0^{c}	
Tank mix	88^{a}	96	27^{ab}	49	48^{b}	63	75^{b}	93	29^{ab}	50
Mix-in-line	60^{b}	96	19^{b}	49	37^{b}	63	54^{c}	93	14^{b}	50
Separate boom	92^{a}	96	34^{a}	49	62^{a}	63	89^{a}	93	42^{a}	50

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column indicates significance.

[1] For the tank mix, mix-in-line, and separate boom method, dicamba and 2,4-D applications for browntop millet control were made applied at 281 and 533 g ae ha^{-1}, respectively. Dicamba and 2,4-D applications for Italian ryegrass and broadleaf signalgrass control were increased to 562 and 1065 g ae ha^{-1}, respectively. Clethodim was applied at a rate of 68 g ai ha^{-1} with NIS at 0.25% v/v for control of all species in all treatments.

[2] Expected responses were calculated using Colby's equation $E = (X + Y) - (XY)/100$, any response of clethodim with dicamba or 2,4-D significantly less than clethodim alone is considered antagonistic.

Blackshaw et al. (2006) found antagonistic responses of goosegrass four weeks after application when clethodim was applied with 2,4-D amine. Underwood et al. (2016) also observed antagonistic response of volunteer corn control when clethodim was mixed with dicamba. Clethodim alone, TMX, and SPB methods had 50, 29, and 42% control of broadleaf signalgrass 28 DAA, respectively (Table 5.4). Those three methods had highest control of broadleaf signalgrass, while the MIL method was the only application method that had lower control of broadleaf signalgrass 28 DAA compared to clethodim alone (Table 5.4). This shows that TMX method was not antagonistic in control of broadleaf signalgrass. The natural population of broadleaf signalgrass varied greatly across the field. This could have affected control ratings of broadleaf signalgrass, making results more variable.

Conclusion

Overall, applying clethodim with dicamba or 2,4-D through separate booms resulted in increased herbicide efficacy than the TMX and MIL application methods. Making applications through separate booms reduced antagonism while antagonism was observed with the TMX and MIL application methods. Keeping clethodim separated from dicamba or 2,4-D by using separate booms to apply them may keep the two herbicides from chemically interacting. Delaying the herbicide interactions until they land on the plant’s leaf may allow enough time for glyphosate to enter and kill the grass weed opposed to tank mixing them. More research needs to be done to investigate what chemical reactions, if any, are taking place when glyphosate is mixed with dicamba or 2,4-D. Applications with separate booms doubles the carrier volume from 140 to 280 L ha^{-1}. Instead of only one 02 flow-rate nozzle being used for the tank mix and mix-in-line application methods, a dual boom would have two sets of 02 flow-rate nozzles making applications, thus increasing the carrier volume.

The rate of the herbicides did affect antagonistic responses. The fall 2018 trial at the R.R. Foil Plant Science Research Center had dicamba and 2,4-D applied at a half-rate while the trial at the Black Belt Experiment Station and the summer trial at R.R. Foil Plant Science Research Center applied dicamba and 2,4-D at a full rate. When clethodim was applied with dicamba and 2,4-D at a half rate, the TMX application method was not antagonistic according to the visual estimations of weed control 7, 21 and 28 DAA.

CHAPTER VI

COMPARING ANTAGONISM OF GLYPHOSATE TANKED MIXED WITH DICAMBA OR 2,4-D VERSUS FACTORY PREMIXES

Abstract

This study was conducted at the Black Belt Experiment Station (Black Belt) and at the R.R. Foil Plant Science Research Center (R.R. Foil) in fallow fields with volunteer corn (*Zea mays*), browntop millet (*Urochloa ramosa*), broadleaf signalgrass (*Urochloa platyphylla*) and Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) pressure. This trial was developed to compare tank mixes and factory premixes of the same herbicide active ingredients at the same ae rates. The study consisted of seven treatments with four replications, in a randomized complete block design. Dicamba, 2,4-D, and glyphosate rates for all treatments were 318 g ae ha^{-1}, 640 g ae ha^{-1}, and 630 g ae ha^{-1}, respectively. The seven treatments were 1) choline salt of 2,4-D (Enlist One®, Corteva Agriscience, Indianapolis, IN 46268), 2) glyphosate (Roundup Powermax®, Bayer, St. Louis, MO 63167), 3) premix of 2,4-D and glyphosate (Enlist Duo®, Corteva Agriscience), and 4) choline salt of 2,4-D (Enlist One®) tank mixed with glyphosate (Roundup Powermax®). The applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). No differences in herbicide efficacy were observed among the tank mixes and the premixes of the same herbicide active ingredients.

Introduction

Green (1989) stated that formulations may affect mixture performance, therefore may potentially cause antagonism of herbicides. Different formulations of herbicides when mixed may be incompatible, leading to poor performance (Johanson and Kaldon 1972). Incompatible interactions may form agglomerates, crystals, phase separation, or a failed spray uniformity caused by thickening (Green 1989). This eight-treatment study was developed to compare factory premixes and tank mixes of glyphosate with dicamba or 2,4-D. The objective was to evaluate herbicide efficacy based on comparable tank mixes and factory premixes of the same active ingredients.

Materials and Methods

This study was replicated three times at the R.R. Foil Plant Science Research Center (R.R. Foil) in Starkville, MS on October 6, 2018, Black Belt Experiment Station (Black Belt) in Brooksville, MS on March 29, 2019, and again at the R.R. Foil Plant Science Research Center on May 20, 2019. The study consisted of four treatments with four replications, in a randomized complete block design. 2,4-D and glyphosate rates for all treatments were 640 and 630 g ae ha^{-1}, respectively. The treatments were as follows: 1) choline salt of 2,4-D (Enlist One®, Corteva Agriscience, Indianapolis, IN 46268), 2) glyphosate (Roundup Powermax®, Bayer, St. Louis, MO 63167), 3) premix of 2,4-D and glyphosate (Enlist Duo®, Corteva Agriscience), 4) choline salt of 2,4-D (Enlist One®) tank mixed with glyphosate (Roundup Powermax®). The applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). An untreated check was used for weed control evaluations. A John Deere 5400 series tractor with four nozzles was used to spray the study.

The first trial replication at the R.R. Foil Plant Science Research Center had a natural stand of volunteer corn (*Zea mays*) and browntop millet (*Urochloa ramosa*) that were sprayed at an average height of 15 cm and 9 cm, respectively. The second trial replication at the Black Belt Experiment Station had a natural population of Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*), and applications were made with the Italian ryegrass at an average height of 18 cm. The third study at the R.R. Foil Plant Science Research Center had had Oregon Grown, Gulf Variety, Italian ryegrass drilled into the field at a rate of 112 kg ha^{-1} on March 20, 2019 along with a natural population of broadleaf signalgrass (*Urochloa platyphylla*). Applications were made with Italian ryegrass at an average height of 15 cm and broadleaf signalgrass at an average height of 9 cm. Visual estimations of injury (control) ratings were taken 7, 14, 21, and 28 days after application (DAA). All data collected was analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05. The Colby method was used to determine if herbicide combinations were antagonistic (Colby 1967).

Results and Discussion

With 2,4-D showing no grass control in all three trial replications, any herbicide combination resulting in lower grass control than glyphosate applied alone is considered antagonistic. Results for this study were similar at all three locations. Observed control ratings of browntop millet 7 DAA applications ranged from 90-99% for all treatments with glyphosate in it (Table 6.1) No differences were observed in the premix and tank mix treatments. Italian ryegrass control at the Black Belt 7 DAA was 38% with glyphosate alone. All treatment combinations with glyphosate had 36-41% control of Italian ryegrass at Black Belt (Table 6.1). Glyphosate alone resulted in 80% control of Italian ryegrass 7 DAA at the R.R. Foil. 68-78% control of Italian ryegrass at the R.R. Foil was achieved with all glyphosate combinations with 2,4-D

(Table 6.1). No differences among the premix and tank mix treatments were observed in Italian ryegrass control at both locations. No difference in tank mix and premix treatments was observed in control of broadleaf signalgrass 7 DAA (Table 6.1).

Table 6.1 Visual Estimation of control of multiple grass species 7 days after application at the R.R. Foil Plant Science Research Center (R.R. Foil) and at the Black Belt Experiment Station (Black Belt).

Treatment[1]	Mixture type	R.R. Foil (Fall 2019)		Black Belt (Spring 2019)		R.R. Foil (Summer 2019)			
		Browntop millet		Italian ryegrass		Italian ryegrass		Broadleaf signalgrass	
		Observed	Expected[2]	Observed	Expected	Observed	Expected	Observed	Expected
		%							
Glyphosate alone		90^a		38^a		80^a		45^a	
2,4-D alone		0^b		0^b		0^b		0^b	
Glyphosate with 2,4-D	tank mix	99^a	90	41^a	38	78^a	80	30^a	45
Glyphosate with 2,4-D	premix	93^a	90	36^a	38	68^a	80	38^a	45

Data were analyzed using Sidak’s comparison method. LS-means with different letters within the same column indicates significance.

[1] 2,4-D, and glyphosate applications were applied at 640 and 630 g ae ha^{-1}, respectively.

[2] Expected responses were calculated using Colby’s equation E = (X + Y) – (XY)/100, any response of glyphosate with dicamba or 2,4-D significantly less than glyphosate alone is considered antagonistic.

The 28 DAA rating showed no differences in premixes and tank mixes in control of browntop millet, Italian ryegrass, and broadleaf signalgrass, as observed in Table 6.2. This could be due to the rate of the herbicides used. No consistent numerical difference was observed across the pre-mixes versus the tank mixes. The pre-mix of glyphosate and dicamba only had numerically higher weed control than the tank mix of glyphosate and dicamba with Italian ryegrass at the Black Belt Experiment Station. The pre-mix of glyphosate and 2,4-D was only numerically higher in weed control of broadleaf signalgrass than the tank mix of glyphosate and 2,4-D. In all other cases, the tank mix was numerically higher than the pre-mix or the weed control results were similar.

Table 6.2 Visual Estimation of control of multiple grass species 28 days after application at the R.R. Foil Plant Science Research Center (R.R. Foil) and at the Black Belt Experiment Station (Black Belt).

Treatment[1]	**Mixture type**	**R.R. Foil (Fall 2019)**		**Black Belt (Spring 2019)**		**R.R. Foil Plant Science (Summer 2019)**			
		Browntop millet		**Italian ryegrass**		**Italian ryegrass**		**Broadleaf signalgrass**	
		Observed	**Expected**[2]	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**
		%							
Glyphosate alone		100^{a}		47^{a}		98^{a}		85^{a}	
2,4-D alone		0^{b}		0^{b}		0^{b}		0^{b}	
Glyphosate with 2,4-D	tank mix	99^{a}	100	69^{a}	47	94^{a}	98	73^{a}	85
Glyphosate with 2,4-D	premix	100^{a}	100	68^{a}	47	93^{a}	98	79^{a}	85

Data were analyzed using Sidak’s comparison method. LS-means with different letters within the same column indicates significance.

[1] 2,4-D and glyphosate applications were applied at 640 and 630 g ae ha^{-1}, respectively.

[2] Expected responses were calculated using Colby’s equation E = (X + Y) – (XY)/100, any response of glyphosate with dicamba or 2,4-D significantly less than glyphosate alone is considered antagonistic.

Conclusion

No differences were observed across the factory premix and the tank mix herbicide treamtents of the same active ingredient. None of the herbicide mixes with glyphosate showed antagonistic responses when comparing them to glyphosate applied alone. If the glyphosate rate was decreased to a half rate for all treatments, differences in treatments may be observed. Trials in the greenhouse of in field conditions need to be done to see if differences would be seen if glyphosate was decreased.

CHAPTER VII

REDUCING ANTAGONISTIC EFFECTS OF GLYPHOSATE AND CLETHODIM WITH 2,4-D AND DICAMBA THROUGH THE USE OF SEQUENTIAL APPLICATIONS

Abstract

Two greenhouse studies were planted at the R.R. Foil Plant Science Research Center in Starkville, MS. Pots were seeded with barnyardgrass (*Echinochloa crus-galli*), broadleaf signalgrass (*Urochloa platyphylla*), and giant foxtail (*Setaria faberi*). The study consisted of 16 treatments with four replications per treatment in a randomized complete block design. The treatments included four herbicide combinations: 1) clethodim with dicamba, 2) clethodim with 2,4-D, 3) glyphosate with dicamba, and 4) glyphosate with 2,4-D. Clethodim, glyphosate, dicamba, and 2,4-D were applied singularly, resulting in four treatments. The four-herbicide combinations were applied with three different application methods, resulting in the final 12 treatments. Treatments were applied with a two-nozzle research spray chamber with three application methods: 1) tank mix, 2) sequential applications where the synthetic auxin was applied first followed by glyphosate or 2,4-D (auxin applied first), and 3) sequential applications where glyphosate or clethodim was applied first followed by the synthetic auxin herbicide (auxin applied second). Dicamba, 2,4-D, and glyphosate applications were applied at 562, 1065, and 434 g ae ha^{-1}, respectively for all treatments. All treatments with clethodim were applied at a rate of 63 g ai ha^{-1} with NIS at 0.25% v/v. Visual estimation of injury control ratings were taken 7, 14, 21, and 28 days after application (DAA). Biomass samples were taken after the 28 DAA

rating then dried for 48 hours at 60°C and dry weights were recorded. Visual estimations of control ratings showed the auxin applied second had higher control than the tank mix and auxin applied first methods for all four herbicide combinations. The auxin applied second method also resulted in lower biomass weights than the tank mix and auxin applied first application methods. Antagonism of clethodim and glyphosate was observed when they were tank mixed with dicamba and 2,4-D.

Introduction

Clethodim is used in postemergence herbicide applications to control many annual and perennial grass species, but an adjuvant needs to be applied with clethodim to get maximum efficacy on grass weeds (Ahrens 1994). Clethodim has no activity on broadleaf weeds (Ahrens 1994). Clethodim is in the cyclohexanedione chemical family, which inhibits acetyl-CoA carboxylase (ACCase), an enzyme involved in fatty acid synthesis (Ahrens 1994; Burton et al. 1987). Glyphosate is a nonselective herbicide that controls most annual and perennial weeds, but performs really well on annual grasses (Ahrens 1994). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which leads to depletion of aromatic amino acids needed for protein synthesis, and glyphosate is primarily translocated in the symplast (Ahrens 1994).

Dicamba and 2,4-D are both synthetic auxins. Dicamba is used to control annual broadleaf weed species and can suppress some perennial broadleaf weeds (Ahrens 1994). 2,4-D also controls many broadleaf weeds but has very little activity on grass (Ahrens 1994). Dicamba and 2,4-D can be moved throughout the plant through the phloem, but dicamba can also move throughout the xylem (Ahrens 1994). Dicamba and 2,4-D acidify the cell wall through stimulating the membrane-bound ATPase proton pump (Ahrens 1994). The primary action of dicamba and 2,4-D is that it increases RNA, DNA, and protein synthesis, which leads to uncontrolled cell division and growth (Ahrens 1994).

This greenhouse study was developed to mimic the field studies in Chapters IV and V with glyphosate and clethodim antagonism with dicamba and 2,4-D. Herbicide combinations were applied in three different ways 1) tank mix, 2) sequential applications with the auxin applied first, and 3) sequential applications with the auxin applied second. The objective of this study was to evaluate glyphosate and clethodim efficacy, and how applying them with dicamba or 2,4-D using each application method affects it.

Materials and Methods

Two greenhouse studies were planted at the R.R. Foil Plant Science Research Center in Starkville, MS on February 1, 2019, and February 25, 2019. The study was conducted using 0.95 L pots filled with Gro Metro-Mix potting soil (Sun Gro Horticulture, Agawam, MA 01001). Pots were seeded with the following grass species: barnyardgrass (*Echinochloa crus-galli*), broadleaf signalgrass (*Urochloa platyphylla*), and giant foxtail (*Setaria faberi*). The plants were placed into the greenhouse to germinate and grow. After emergence, the plants were thinned to one plant per pot. The study consisted of 16 treatments with four replications per treatment in a randomized complete block design. Treatment combinations are listed in Table 7.1. Dicamba (FeXapan®, Corteva Agriscience, Indianapolis, IN 46268), 2,4-D (Enlist One®, Corteva Agriscience), and glyphosate (Roundup PowerMAX®, Bayer, St. Louis, MO 63167) applications were applied at 562, 1065, and 434 g ae ha^{-1}, respectively for all treatments. All treatments with clethodim (Select Max®, Valent, Walnut Creek, CA 94569) were applied at a rate of 63 g ai ha^{-1} with nonionic surfactant (NIS) (Activate Plus™, Winfield United, River Falls, WI 54022) at 0.25%

v/v. Untreated checks were used to compare weed control. Each herbicide combination was applied three separate ways, 1) a tank mix 2) sequential applications where the synthetic auxin was applied first followed by glyphosate or 2,4-D (auxin applied first), and 3) sequential applications where glyphosate or clethodim was applied first followed by the synthetic auxin herbicide (auxin applied second). In the sequential application treatments, the second herbicide was applied around 30 seconds after the first herbicide. This was done to best mimic the field tractor studies using the same herbicide rates and combinations. Ammonia and water were sprayed through the sprayer system for cleanout between each herbicide change.

Table 7.1 Complete treatment combination list.

Treatment	Application Method	Herbicide(s)
1	single application	dicamba
2	single application	clethodim with NIS
3	single application	glyphosate
4	single application	2,4-D
5	tank mix	dicamba and glyphosate
6	sequential application with auxin applied first	dicamba followed by glyphosate
7	sequential application with auxin applied second	glyphosate followed by dicamba
8	tank mix	2,4-D and glyphosate
9	sequential application with auxin applied first	2,4-D followed by glyphosate
10	sequential application with auxin applied second	glyphosate followed by 2,4-D
11	tank mix	dicamba and clethodim with NIS
12	sequential application with auxin applied first	dicamba followed by clethodim with NIS
13	sequential application with auxin applied second	clethodim with NIS followed by dicamba
14	tank mix	2,4-D and clethodim with NIS
15	sequential application with auxin applied first	2,4-D followed by clethodim with NIS
16	sequential application with auxin applied second	clethodim with NIS followed by 2,4-D

The grasses were sprayed at an average height of 10 cm for barnyardgrass, 10 cm for broadleaf signalgrass, and 13 cm for giant foxtail. Treatments were applied on February 25, 2019 and March 22, 2019, for the first and second trials, respectively with a two-nozzle research spray chamber generation III (Generation III, DeVries Manufacturing Inc., Hollandale, MN). The applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzles used were HYPRO Ultra Lo-Drift 120-02 (ULD). Visual estimation of injury (control) ratings were taken 7, 14, 21, and 28 days after application (DAA). Biomass samples were taken after the 28 DAA rating then dried for 48 hours at 60°C and dry weights were recorded. All data collected was analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05. Antagonism was determined by using the Colby Method (Colby 1967).

Results and Discussion

When analyzing the 7 DAA control results, species were different across herbicide response. Table 7.2 lists all control results of broadleaf signalgrass, giant foxtail, and barnyardgrass control at 7 DAA. Results of broadleaf signalgrass control for the tank mix (TMX), synthetic auxin applied first (AAF), and synthetic auxin applied second (AAS) were 17, 8, and 32%, respectively, when clethodim was applied with dicamba. The AAS method had higher broadleaf signalgrass control than the AAF method, as shown in Table 7.2. When clethodim was applied with 2,4-D, the TMX, AAF, and the AAS method resulted in 15, 8, and 33% control, respectively. Again, the AAS method had higher control compared to the reverse order of the AAF (Table 7.2). When clethodim was applied with dicamba or 2,4-D, only the AAS method had similar control of broadleaf signalgrass as clethodim by itself (Table 7.2). Broadleaf signalgrass showed no difference among herbicide application methods for glyphosate applied with dicamba and glyphosate applied with 2,4-D combinations. When glyphosate was applied with dicamba, the TMX, AAF, and the AAS methods resulted in 11, 3, and 18% control, respectively (Table 7.2) For combinations of clethodim with dicamba and clethodim with 2,4-D, the only difference observed was the AAS method had higher control of broadleaf signalgrass than the AAF method. For all four herbicide combinations, the AAS method resulted in numerically higher control than by making applications with the AAF or TMX methods. When comparing clethodim applied alone to clethodim applied with any of the two auxins, the only application method that was similar was the AAS method (Table 7.2). Applying clethodim before dicamba or applying clethodim before 2,4-D resulted in broadleaf signalgrass control similar to clethodim applied alone. When comparing glyphosate applied alone to glyphosate applied with dicamba, the only application method that resulted in lower control of broadleaf signalgrass was when dicamba was applied first. Tank mixing 2,4-D with glyphosate also resulted in lower broadleaf signalgrass control than applying glyphosate by itself.

Giant foxtail showed similar results as broadleaf signalgrass. The glyphosate applied with dicamba through the TMX, AAF, and AAS methods resulted in 9, 3, and 14% control of giant foxtail, respectively. Glyphosate applied with 2,4-D through the TMX, AAF, and AAS methods resulted in 5, 8, and 19% control of giant foxtail, respectively. No difference among herbicide application methods for glyphosate applied with dicamba or 2,4-D was observed at 7 DAA (Table 7.2). The different application methods with clethodim applied with 2,4-D also showed no differences. Applying clethodim with dicamba resulted in 18, 14, and 35% control of giant foxtail when using the TMX, AAF, and AAS methods, respectively. The two sequential application methods again resulted in different levels of control. Applying dicamba second improved control compared to applying dicamba first (Table 7.2). When comparing glyphosate applied with dicamba or 2,4-D to glyphosate applied alone with all three application methods, all treatments had lower control of giant foxtail than glyphosate applied alone (Table 7.2). This is evidence of antagonism with glyphosate applied with dicamba or 2,4-D. For the herbicide combinations of clethodim applied with dicamba and clethodim applied with 2,4-D, applying 2,4-D second resulted in similar control of giant foxtail as applying clethodim alone in both cases (Table 7.2).

When clethodim was applied with dicamba, control of barnyardgrass resulted in 35, 27, and 33% control using the TMX, AAF, and AAS methods, respectively. Applying clethodim with 2,4-D resulted in 29, 32, and 43% control of barnyardgrass when using the TMX, AAF, and AAS methods, respectively. Ratings of barnyardgrass showed no antagonism when clethodim was applied with dicamba or 2,4-D (Table 7.2). Applying glyphosate with dicamba resulted in 17, 6, and 19% control of barnyardgrass with the TMX, AAF, and AAS application methods, respectively. Among all three application methods of glyphosate applied with dicamba, no differences of barnyardgrass control were observed. Also, it showed no antagonism of barnyardgrass control for all three application methods when compared to glyphosate applied alone (Table 7.2). The TMX, AAF, and AAS application methods resulted in 3, 2, and 14% control, respectively, of barnyardgrass with the glyphosate and 2,4-D combination (Table 7.2) The only application method that showed no antagonism of barnyardgrass control when glyphosate was applied with 2,4-D was the sequential application where 2,4-D was applied second. For all three grass species, the results indicate that antagonism is observed when glyphosate and clethodim were applied with dicamba and 2,4-D. Results may be variable because these ratings were taken only seven days after the applications were made. The full effects of each herbicide may not have taken place.

Table 7.2 Visual estimations of control of all grass species 7 days after application.

Treatment[1]	Broadleaf signalgrass				Giant foxtail				Barnyardgrass			
	single[2]	tank mix	auxin applied first[3]	auxin applied second[4]	single	tank mix	auxin applied first	auxin applied second	single	tank mix	auxin applied first	auxin applied second
	%											
Clethodim alone	47^{a}				43^{a}				57^{a}			
Glyphosate alone	26^{bc}				38^{a}				43^{ab}			
Dicamba alone	0^{d}				0^{e}				0^{c}			
2,4-D alone	0^{d}				0^{e}				0^{c}			
	expected control[5]				**expected control**				**expected control**			
Clethodim with dicamba	47	17^{bcd}	8^{cd}	32^{ab}	43	18^{cde}	14^{de}	35^{abc}	57	35^{abc}	27^{abc}	33^{abc}
Clethodim with 2,4-D	47	15^{bcd}	8^{cd}	33^{ab}	43	15^{de}	14^{de}	28^{abcd}	57	29^{abc}	32^{abc}	43^{ab}
Glyphosate with dicamba	26	11^{cd}	3^{d}	18^{bcd}	38	9^{e}	3^{e}	14^{de}	43	17^{bc}	6^{bc}	19^{bc}
Glyphosate with 2,4-D	26	3^{d}	11^{cd}	18^{bcd}	38	5^{e}	8^{e}	19^{bcde}	43	3^{c}	2^{c}	14^{bc}

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column of grass specie indicate significance.

[1] All applications with dicamba and 2,4-D were applied at 562 and 1065 g ae ha^{-1}, respectively. All treatments with glyphosate were applied at a rate of 434 g ae ha^{-1}. All treatments containing clethodim were applied at 68 g ai ha^{-1} with NIS at 0.25% v/v.

[2] Single application

[3] Sequential application where dicamba or 2,4-D was applied first followed by clethodim or glyphosate. Sequential applications were made 30 seconds after the first application.

[4] Sequential application where clethodim or glyphosate was applied first followed by dicamba or 2,4-D. Sequential applications were made 30 seconds after the first application.

[5] Expected responses were calculated using Colby's equation $E = (X + Y) – (XY)/100$. Any response of glyphosate with dicamba or 2,4-D significantly less than glyphosate alone or clethodim with dicamba or 2,4-D significantly less than clethodim alone is considered antagonistic.

Control ratings 14 DAA showed no difference among all three grass species, therefore data were pooled across grass species. The TMX, AAF, and AAS methods resulted in 32, 29, and 76% control, respectively, 14 DAA for the clethodim with dicamba herbicide combination (Table 7.3). When clethodim was applied with 2,4-D, the TMX, AAF, and AAS application methods resulted in 34, 36, and 69% control, respectively, 14 DAA. The TMX, AAF, and AAS application methods with glyphosate applied with dicamba resulted in an average control of 22, 28, and 69% control, respectively, for all grass species 14 DAA. Applying glyphosate with 2,4-D resulted in 24, 26, and 67% average control 14 DAA when using the TMX, AAF, and AAS application methods, respectively. For all clethodim and glyphosate combinations with dicamba or 2,4-D, the only the application method that was similar to applying glyphosate alone (76% control) or clethodim alone (83% control) 14 DAA was from the sequential application where the synthetic auxin was applied second (Table 7.3). The same was also true for control ratings taken 28 DAA, also shown in Table 7.3. As shown in Table 7.3, the AAS method resulted in around 89% control of grass species 28 DAA for all the herbicide combinations that were applied. This is similar to control obtained from applying clethodim and glyphosate by themselves, which resulted in 95 and 83% control, respectively (Table 7.3). TMX and the AAF methods had about half the control as the AAS application method and were antagonistic for all glyphosate and clethodim combinations with dicamba and 2,4-D (Table 7.3). Minton et al. (1989) observed antagonistic responses of barnyardgrass control when sethoxydim or quizalofop was tank mixed with broadleaf herbicides. When imazaquin, chlorimuron, or lactofen was applied 24 hours after sethoxydim or quizalofop, barnyardgrass control was not affected (Minton et al. 1989). When the order was reversed to where sethoxydim or quizalofop was applied 24 hours before imazaquin, chlorimuron, or lactofen, antagonism was observed with control of barnyardgrass (Minton et al. 1989).

Table 7.3 Visual estimations of control of all grass species pooled together 14 and 28 days after application.

Treatment[1]	14 DAA[2]					28 DAA				
	single application	expected control[3]	tank mix	auxin applied first[4]	auxin applied second[5]	single application	expected control	tank mix	auxin applied first	auxin applied second
	%									
Clethodim alone	83^{a}					95^{a}				
Glyphosate alone	76^{a}					83^{a}				
Dicamba alone	0^{c}					0^{c}				
2,4-D alone	0^{c}					0^{c}				
Clethodim with dicamba		83	32^{b}	29^{b}	76^{a}		95	48^{b}	42^{b}	90^{a}
Clethodim with 2,4-D		83	34^{b}	36^{b}	69^{a}		95	44^{b}	45^{b}	89^{a}
Glyphosate with dicamba		76	22^{b}	28^{b}	69^{a}		83	29^{b}	34^{b}	86^{a}
Glyphosate with 2,4-D		76	24^{b}	26^{b}	67^{a}		83	27^{b}	39^{b}	86^{a}

Data were analyzed using Sidak's comparison method. LS-means with different letters within the same column of DAA indicate significance.

[1] All applications with dicamba and 2,4-D were applied at 562 and 1065 g ae ha^{-1}, respectively. All treatments with glyphosate were applied at a rate of 434 g ae ha^{-1}. All treatments containing clethodim were applied at 68 g ai ha^{-1} with NIS at 0.25% v/v.

[2] Days after application.

[3] Expected responses were calculated using Colby's equation $E = (X + Y) - (XY)/100$. Any response of glyphosate with dicamba or 2,4-D significantly less than glyphosate alone or clethodim with dicamba or 2,4-D significantly less than clethodim alone is considered antagonistic.

[4] Sequential application where dicamba or 2,4-D was applied first followed by clethodim or glyphosate. Sequential applications were made 30 seconds after the first application.

[5] Sequential application where clethodim or glyphosate was applied first followed by dicamba or 2,4-D. Sequential applications were made 30 seconds after the first application.

Figure 7.1 displays mean biomass weights for all combinations of glyphosate or clethodim with dicamba and 2,4-D. For the herbicide combination of glyphosate with dicamba, the sequential application of glyphosate applied before dicamba resulted in 0.302 grams of dry weight, which was similar to the biomass as glyphosate applied alone. Applying dicamba sequentially first with glyphosate and tank mixing the two herbicides together resulted in 1.175 and 1.347 g of dry weight, respectively. Both resulted in higher biomass weight than glyphosate alone, therefore was antagonistic. When applying glyphosate with 2,4-D, only the sequential application were glyphosate was applied before 2,4-D had similar biomass as glyphosate applied alone (Figure 7.1). Applying glyphosate before 2,4-D resulted in dry biomass weight of 0.44 g. Applying glyphosate with 2,4-D through the TMX and AAF methods resulted in 1.244 and 0.955 g of biomass weight, respectively. The AAS method resulted in lower biomass than the AAF method. When comparing biomass weights of the three application methods for 2,4-D with glyphosate, only the TMX method was lower than the sequential application sequence of applying glyphosate before 2,4-D (Figure 7.1). Observing antagonism of glyphosate with dicamba or 2,4-D agrees with findings from Flint and Barret (1989) when reduced control of johnsongrass (*Sorghum halepense*) was observed when glyphosate was tank mixed with dicamba or 2,4-D. In addition, Damalas and Eleftherohorinos (2001) observed reduced antagonism of grass herbicides when sequential applications were made with the broadleaf herbicides applied five days before grass herbicides.

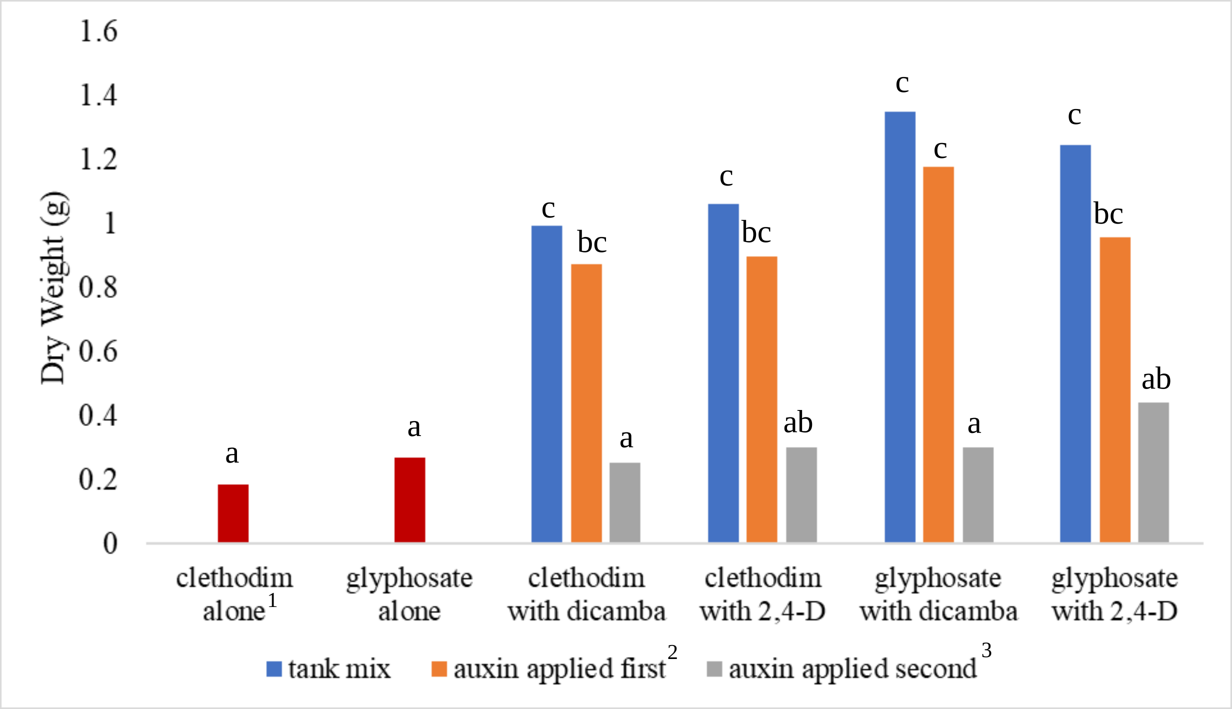

Figure 7.1 Comparison of dry biomass weights with dicamba, 2,4-D, and glyphosate combinations with different application methods.

Data were analyzed using Sidak's comparison method. LS-means with different letters indicate significance.

[1] All applications with dicamba and 2,4-D were applied at 562 and 1065 g ae ha^{-1}, respectively. All treatments with glyphosate were applied at a rate of 434 g ae ha^{-1}. All treatments containing clethodim were applied at 68 g ai ha^{-1} with NIS at 0.25% v/v.

[2] Sequential application where dicamba or 2,4-D was applied first followed by clethodim or glyphosate. Sequential applications were made 30 seconds after the first application.

[3] Sequential application where clethodim or glyphosate was applied first followed by dicamba or 2,4-D. Sequential applications were made 30 seconds after the first application.

The glyphosate with 2,4-D herbicide combination had similar biomass results as the clethodim with 2,4-D herbicide combination. The sequential application of applying clethodim before 2,4-D resulted in a biomass weight of 0.302 g, which was similar to 0.184 g from applying clethodim alone. When comparing the three application methods with 2,4-D and clethodim, only tank mixing the two herbicides together was lower than the sequential application of clethodim applied first (Figure 7.1). For the clethodim with dicamba herbicide combination, clethodim applied sequentially first before dicamba (0.254 g) had similar biomass as clethodim applied alone (Figure 7.1). Both, the sequential application of applying dicamba before clethodim (0.874 g) and tank mixing (0.992 g) the two herbicides had higher biomass weight than the sequential application where clethodim was applied before dicamba. For all four herbicide combinations, the AAS application method resulted in the lowest amount of biomass weights compared to the TMX and AAF application methods (Figure 7.1). Also seen in all four herbicide combinations, only the AAS method resulted in biomass weights similar to glyphosate and clethodim applied by themselves. Clethodim is known to show antagonism with 2,4-D when the two herbicides were tank mixed together (Blackshaw et al. 2006; Grichar et al. 2002). Grichar et al. (2002) made sequential applications of clethodim and broadleaf herbicides, both broadleaf herbicides applied first and broadleaf herbicides applied second, with 24 hours between each sequential application. In some cases, Grichar et al. (2002) found antagonism when clethodim followed the broadleaf herbicide in control of southern crabgrass (*Digitaria ciliaris*).

Conclusion

Based on the 14 DAA, 28 DAA, and biomass results, sequentially applications where the synthetic auxin was applied second reduced antagonism and, in some cases, showed no antagonism. Antagonism of glyphosate and clethodim with dicamba and 2,4-D was observed when the herbicides were tank mixed together as well as when the synthetic auxin was applied first in sequential applications. Due to the sequential application of the synthetic auxin applied second resulting in increased efficacy than the sequential application of applying the synthetic auxin first, antagonism may be due to the synthetic auxins keeping glyphosate or clethodim from entering the cuticle. Another reason could be that a chemical reaction may occur on the leaf of the plant, and the little time glyphosate has to enter the plant before the synthetic auxin is applied allows enough glyphosate to enter the plant. More research needs to be done to see if a reaction is occurring on the leaf and how much of each herbicide is entering the plant.

CHAPTER VIII

PLANT TISSUE ANALYSIS OF ANTGONISTIC HERBICIDE COMBINATIONS

Abstract

Barnyardgrass (*Echinochloa crus-galli*) was planted at the R.R. Foil Plant Science Research Center in Starkville, MS on January 17, 2019, February 25, 2019, March 23, 2019, and May 9, 2019. Each planting date was a replication of the 16 treatments. The treatments included four herbicide combinations: 1) clethodim with dicamba, 2) clethodim with 2,4-D, 3) glyphosate with dicamba, and 4) glyphosate with 2,4-D. Clethodim, glyphosate, dicamba, and 2,4-D were applied singularly, resulting in four treatments. The four-herbicide combinations were applied with three different application methods, resulting in the final 12 treatments. The three application methods were 1) a tank mix 2) sequential applications where the synthetic auxin was applied first followed by glyphosate or 2,4-D (auxin applied first), and 3) sequential applications where glyphosate or clethodim was applied first followed by the synthetic auxin herbicide (auxin applied second). Dicamba, 2,4-D, and glyphosate applications were applied at 562, 1065, and 434 g ae ha^{-1}, respectively for all treatments. All treatments with clethodim were applied at a rate of 63 g ai ha^{-1} with NIS at 0.25% v/v. Treatments were applied with a two-nozzle research spray chamber where the applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. Plants were clipped at the top leaf collar and sent to the Mississippi State Chemical Laboratory 24 hours after spray application. Detection of glyphosate, clethodim, dicamba, and 2,4-D was done using liquid chromatography mass spectrometry. The auxin applied second

method had more glyphosate and less dicamba and 2,4-D detected within each herbicide combination. When clethodim was applied with dicamba, clethodim sulfoxide detection with the auxin applied second application method was highest among the three application methods. 2,4-D detection was much higher in both tank mix applications with glyphosate and clethodim.

Introduction

Antagonistic responses from glyphosate and clethodim applied with dicamba and 2,4-D have been found in the previous chapters of this thesis as well as in other literature (Flint and Barrett 1989; Grichar et al. 2002; Ou et al. 2018; Underwood et al. 2016). Flint and Barrett (1989) measured shoot and root rates of johnsongrass (*Sorghum halepense*) when studying glyphosate is applied with dicamba or 2,4-D. Shoot growth suppression and reduced root growth in johnsongrass was seen when glyphosate was applied with dicamba or 2,4-D (Flint and Barrett (1989). Glyphosate absorption decreased when applied with dicamba or 2,4-D and led to less glyphosate translocation in johnsongrass (Flint and Barrett 1989). Results of antagonism between glyphosate and dicamba do vary some. Smith (2016) found that in barnyardgrass (*Echinochloa crus-galli*), only the translocation of glyphosate decreased when it was applied with dicamba, while absorption did not change. Flint and Barrett (1989) found when dicamba was applied with glyphosate resulted in more glyphosate staying in the treated leaf, but no chemical alteration from the herbicides mixing was found. Many different effects from within a plant sprayed with herbicides may lead to antagonism being seen. When sethoxydim and bentazon interact, the Na^+ ions from the sodium salt of bentazon exchange with the H^+ hydroxyl group of sethoxydim and formed the sodium salt of sethoxydim (Wanamarta et al. 1989). Absorption of glyphosate in barnyardgrass (*Echinochloa crus-galli*) decreased 10% when mixed with fomesafen and translocation out of the treated leaf decreased (Starke and Oliver 1998). Antagonism with clethodim applied with imazapic or CGA 362622 (trifloxysulfuron-sodium) is believed to be the result from the photosynthesis and/or growth rate being altered by imazapic or trifloxysulfuron-sodium (Burke and Wilcut 2003; 3003b).

While herbicide antagonism of glyphosate and clethodim with dicamba and 2,4-D is known to exist, there is a gap in understanding in what truly happens for the antagonism to occur. Plant tissues from the same treatments used in the greenhouse study in Chapter VII were analyzed for metabolite detection in plant tissue to study the amount of each herbicide that moves throughout the plant, and how herbicide combinations applied with three different application methods affect that movement.

Materials and Methods

Barnyardgrass (*Echinochloa crus-galli*) was planted in 0.95 L pots on January 17, 2019, February 25, 2019, March 23, 2019, and May 9, 2019, and were allowed to germinate and grow in a greenhouse at the R.R. Foil Plant Science Research Center. Barnyardgrass was thinned to one plant per pot after germination. The study consisted of 16 treatments plus an untreated check for comparison. Treatment combinations are listed in Table 8.1. Dicamba (FeXapan®, Corteva Agriscience, Indianapolis, IN 46268), 2,4-D (Enlist One®, Corteva Agriscience), and glyphosate (Roundup PowerMAX®, Bayer, St. Louis, MO 63167) applications were applied at 562, 1065, and 434 g ae ha^{-1}, respectively for all treatments. All treatments with clethodim (Select Max®, Valent, Walnut Creek, CA 94569) were applied at a rate of 63 g ai ha^{-1} with nonionic surfactant (NIS) (Activate Plus™, Winfield United, River Falls, WI 54022) at 0.25% v/v. Each herbicide combination was applied three separate ways, 1) a tank mix 2) sequential applications where the synthetic auxin was applied first followed by glyphosate or 2,4-D (auxin applied first), and 3) sequential applications where glyphosate or clethodim was applied first followed by the synthetic auxin herbicide (auxin applied second).The plants were grown to an average height of 26 cm and sprayed. Eight plants were sprayed per treatment. Applications for the four replications were made individually on February 22, 2019, April 4, 2019, May 2, 2019, and June 7, 2019. The applications were made at 276 kPa, with an application volume of 140 L ha^{-1}, at 6.7 km h^{-1}. The nozzle used was HYPRO Ultra Lo-Drift 120-02 (ULD). The plants were harvested 24 hours after the herbicides were applied. After 24 hours of application, leaf samples were collected. The leaf samples were composed of the leaves above the top collar. After the plants were cut at the top color, they were dipped in 60 mL of distilled water for 20 seconds and dipped in 20 mL chloroform for 20 seconds immediately after. The eight plants per treatment were combined into one sample. The samples were then sent to the Mississippi State Chemical Laboratory (MSCL) in Mississippi State, MS for plant tissue analysis. The four sample replications were sent to the lab on February 23, 2019, April 5, 2019, May 3, 2019, and June 8, 2019.

Table 8.1 Complete treatment combination list.

Treatment	Application Method	Herbicide(s)
1	single application	dicamba
2	single application	clethodim with NIS
3	single application	glyphosate
4	single application	2,4-D
5	tank mix	dicamba and glyphosate
6	sequential application with auxin applied first	dicamba followed by glyphosate
7	sequential application with auxin applied second	glyphosate followed by dicamba
8	tank mix	2,4-D and glyphosate
9	sequential application with auxin applied first	2,4-D followed by glyphosate
10	sequential application with auxin applied second	glyphosate followed by 2,4-D
11	tank mix	dicamba and clethodim with NIS
12	sequential application with auxin applied first	dicamba followed by clethodim with NIS
13	sequential application with auxin applied second	clethodim with NIS followed by dicamba
14	tank mix	2,4-D and clethodim with NIS
15	sequential application with auxin applied first	2,4-D followed by clethodim with NIS
16	sequential application with auxin applied second	clethodim with NIS followed by 2,4-D

At the MSCL, samples were analyzed using Liquid Chromatography with tandem mass spectrometry (LC/MS-MS) using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method (Lehotay et al. 2010). For testing of clethodim, each plant tissue sample was scissor chopped into fine pieces. Vegetation was weighed to 0.3 g and placed in a 50 mL polypropylene QuEChERS tube. Five g of quality control (QC) vegetation was placed into a QuEChERS tube for a BLANK and SPIKE. Fifteen mL of homogenizing bead was then added to each tube followed by two mL of high-performance liquid chromatography (HPLC) (Water, Optima™ LC/MS Grade, Fisher Scientific International, Pittsburgh, PA 15275) grade water. Samples were then placed in a plant tissue homogenizer (GenoGrind) (Geno/Grinder®, SPEX® SamplePrep, Metuchen, NJ 08840) for five minutes (Figure 8.1). Ten mL of acidified acetonitrile was added to each sample and then was GenoGrind for 5 minutes (Figure 8.1). QuEChERS salt was then added to the samples and placed in the GenoGrind for an additional five minutes. Samples were placed in the centrifuge for ten minutes at 4000 rpm and then poured into a new 15 mL polypropylene tube (Figure 8.1).

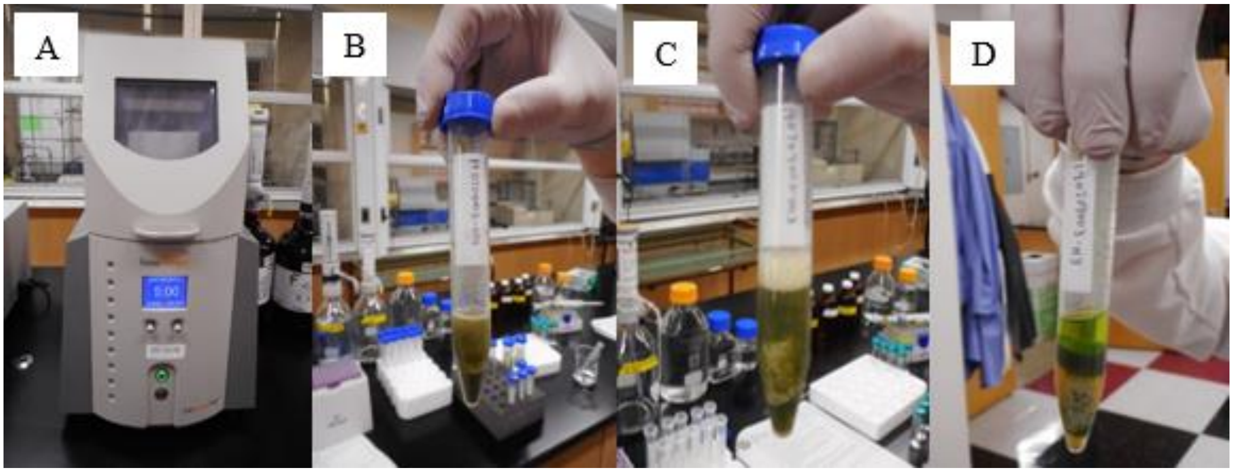

Figure 8.1 Clethodim sample through the preparation process for LC/MS-MS.

Photo A: pictured is the Geno/Grinder®. Photo B: the sample vile after HPLC grade water was added and then GenoGrind for five minutes. Photo C: sample vile after acidified acetonitrile was added then GenoGrind. Photo D: the clethodim sample after being centrifuge for two minutes (note the separation of materials in the clethodim samples).

The extraction liquid was filtered into an autosampler vial with a 0.45 micron polytetrafluoroethylene (PTFE) filter and was then analyzed using LC/MS-MS. The LC conditions, solvent gradient, MS conditions, and MS transitions are listed in Table 8.2.

Table 8.2 LC conditions, solvent gradient, MS conditions, and MS transitions for clethodim samples.

LC conditions	
Column	Agilent ZORBAX Eclipse Plus C18, RR HT, 2.1 x 50 mm, 1.8 µm (Cat. No. 959741-902) or equivalent
Injection Volume	5.00 µL
Column Temperature	40°C
Flow Rate	0.300 mL/min
Solvent A	Water + 1% Formic Acid
Solvent B	Acetonitrile + 1% Formic Acid
Stop Time	7.00 minutes
Post Time	4.00 minutes

Solvent gradient		
Time	**A (%)**	**B (%)**
1.00	90.00	10.00
3.00	10.00	90.00

MS conditions	
Gas Temp	300 °C
Gas Flow	5 L/min
Nebulizer	310 kPa
Sheath Gas Temp	250 °C
Sheath Gas Flow	11 L/min
Capillary	3500 V

MS transitions					
Analyte	**Precursor Ion**	**Product Ion**	**Fragmentor**	**Collison Energy**	**Polarity (+ or -)**
clethodim sulphone	392.1	300.1	109	13	+
clethodim sulphone	392.1	164	109	29	+
clethodim sulfoxide	376.1	206.1	109	13	+
clethodim sulfoxide	376.1	164	109	29	+
clethodim	360.1	166.1	104	25	+
clethodim	360.1	164.1	104	7	+

Dicamba and 2,4-D samples were saponified with sodium hydroxide, then further extracted with a QuEChERS based method. Plant tissue was scissor chopped and homogenized. Again, 0.3 g of tissue was weighed and placed into a polypropylene centrifuge tube. A matrix blank and matrix spike QC sample was prepared and added to a tube. One ceramic homogenizing bead was added to each sample tube followed by 20 mL of water. A total of 0.05 mL of NaOH solution was added to each sample and then samples were placed in the GenoGrind for five minutes. The samples then sat for 30 minutes with intermittent shaking. Five hundredths (0.05) mL of HCl solution was added to the samples, then again placed in the GenoGrind for five minutes. The samples were placed in a heated bath at a temperature of 65ºC for 30 minutes and then cooled to room temperature. Ten mL of acidified acetonitrile was added to each sample solution then placed in the GenoGrind for five minutes. Citric salts were then added to each sample followed by another five minute session in the GenoGrind. Samples were then centrifuged for ten minutes at 4000 rpm, and the extract liquid was placed into a new 15 mL polypropylene tube afterwards. Approximately one mL of each filtered sample solution was placed into the autosampler vial and was analyzed using LC/MS-MS. The LC conditions, solvent gradient, MS conditions, and MS transitions are described in Table 8.3.

Table 8.3 LC conditions, solvent gradient, MS conditions, and MS transitions for dicamba and 2,4-D samples.

LC conditions	
Column	Agilent ZORBAX Eclipse Plus C18, RR HT, 2.1 x 50 mm, 1.8 µm (Cat. No. 959741-902) or equivalent
Injection Volume	5.00 µL
Column Temperature	40°C
Flow Rate	0.300 mL/min
Solvent A	100% Water
Solvent B	100% Acetonitrile
Stop Time	6.00 minutes
Post Time	3.00 minutes

Solvent gradient		
Time	**A (%)**	**B (%)**
1.00	90.00	10.00
3.00	10.00	90.00

MS conditions	
Gas Temp	200 °C
Gas Flow	10 L/min
Nebulizer	310 kPa
Sheath Gas Temp	350 °C
Sheath Gas Flow	11 L/min
Capillary	4000 V

MS transitions					
Analyte	**Precursor Ion**	**Product Ion**	**Fragmentor**	**Collison Energy**	**Polarity (+ or -)**
5-hydroxy dicamba	235	190.9	74	1	-
5-hydroxy dicamba	235	155	74	9	-
DCSA	204.9	160.9	79	5	-
DCSA	204.9	125	79	21	-
Dicamba	219	175	65	1	-
Dicamba	219	145	60	5	-
2,4-D	219	125	60	13	-
2,4-D	219	125	60	29	-

Glyphosate samples were scissor chopped and 0.3 g of plant tissue was placed into a polypropylene QuEChERS tube. Five g of clean QC vegetation was also placed into a QuEChERS tube for a BLANK and SPIKE sample. Fifteen mL of homogenizing bead was added to each sample tube followed by ten mL of water and ten mL of methylene chloride. The tubes were then shaken and vented. The samples were then placed in the GenoGrind for 20 minutes and then centrifuged for ten minutes. An autosampler vial had 0.5 mL of sample, 0.25 mL of borate buffer, and 0.25 mL fluorenylmethoxycarbonyl (FMOC) vortex. The borate buffer was made from 0.5 g of Sodium Tetraborate in 10 mL of water. The FMOC-Cl was composed of 0.28 g of FMOC-Cl in ten mL of acetonitrile (ACN) (Figure 8.2).

A 1000 ng/mL curve point was then made from ten mL standard, 400 µL of water, 250 µL of borate buffer, and 250 µL of FMOC. The samples and curve points were then left to sit at room temperature for two hours. Four mL of pH 3 water was added to 15 mL polypropylene tubes, then samples were added to the tubes and rinsed with one mL of pH 3 water. The pH 3 water was made from combining five g of potassium phosphate monobasic to 500 mL of water, then pH was adjusted to 3.0 using 6 N HCl. The samples were placed into the centrifuge for five minutes (Figure 8.2). An Oasis HLB Solid Phase Extraction (SPE) cartridge was filled with five mL of MeOH and five mL of pH 3 water. The column in the SPE machine was not allowed to dry. Samples and calibration standards were then transferred onto individual columns. The columns were washed with five mL of pH 3 water and were pull vacuumed to dry for 5-10 minutes (Figure 8.2). Then, five mL of MeOH was used to elute each sample. Samples and standards were N-evap to less than one mL and reconstitute to one mL with MeOH (Figure 8.2).

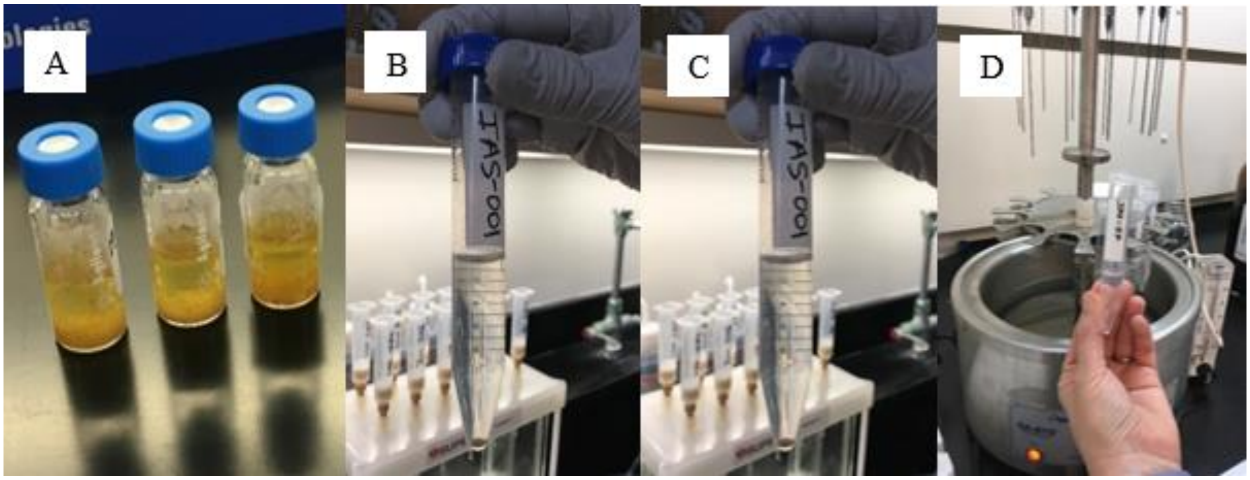

Figure 8.2 Preparation process of glyphosate samples for LC/MS-MS.

Photo A: image of the FMOC-CL mixture in a sample container. Photo B: glyphosate sample after being centrifuge for 5 minutes. Photo C: glyphosate samples in the SPE machine wash column being pull vacuumed to dry. Photo D: glyphosate samples being placed in the machine to be N-evap.

The vials were vortexed and transferred to autosampler vials to be analyzed. The LC conditions, solvent gradient, and MS conditions for all the glyphosate samples are described in Tables 8.8, 8.9, and 8.10, respectively. All data collected were analyzed through SAS 9.4 with PROC GLIMMIX with Sidak's adjustment and with P value set to 0.05.

Table 8.4 LC conditions, solvent gradient, MS conditions, and MS transitions for glyphosate samples.

LC conditions					
Column	SB C18				
Injection Volume	20.00 µL				
Column Temperature	40°C				
Flow Rate	0.300 mL/min				
Solvent A	100% Water + 5 mM Ammonium Acetate				
Solvent B	100% Acetonitrile				
Stop Time	6.00 minutes				
Post Time	3.00 minutes				
Solvent gradient					
Time		**A (%)**		**B (%)**	
1.00		95.00		5.00	
6.00		5.00		95.00	
MS conditions					
Gas Temp	350 °C				
Gas Flow	11 L/min				
Nebulizer	310 kPA				
Sheath Gas Temp	400 °C				
Sheath Gas Flow	11 L/min				
Capillary	3500 V				
MS transitions					
Analyte	**Precursor Ion**	**Product Ion**	**Fragmentor**	**Collison Energy**	**Polarity (+ or -)**
Glyphosate	392	179	100	24	+
Glyphosate	392	88	100	16	+
AMPA	334	179	100	11	+
AMPA	334	112	100	10	+

Results and Discussion

As mentioned earlier in the materials and methods section, all plant biomass material came from the top part of the plant, and then rinsed with water and chloroform to remove the cuticle layer of the leaves. All results should represent how much of each herbicide made it inside the plant and made its way to the growing point on top of the plant. When glyphosate was applied alone, an average of 5,363 parts per billion (ppb) of glyphosate was detected inside the barnyardgrass plants (Table 8.5). This was higher than 2,230 and 2,460 ppb of glyphosate detected from the tank mix applications of glyphosate with dicamba and glyphosate with 2,4-D, respectively. This evidence seems to support that when glyphosate is tank mixed with dicamba or 2,4-D, the amount of glyphosate at the growing point of the plant is reduced. This could lead to less glyphosate activity on a grass plant. The auxin applied second sequential applications were dicamba or 2,4-D was applied before glyphosate had 4,293 and 3,368 ppb of glyphosate detected for glyphosate applied with dicamba and glyphosate applied with 2,4-D, respectively (Table 8.5). Both sequential applications were the auxin herbicides were applied after glyphosate resulted in glyphosate detection similar as the glyphosate alone treatment.

Aminomethylphosphonic acid (AMPA) is a weak organic acid that is a degradation product of glyphosate when broken down in plants, soil, or water (Kawai et al. 1991; Manas et al. 2009). AMPA detection was variable in all treatments While the glyphosate alone treatment had the highest amount of AMPA detected at 51 ppb, no differences were seen among most treatments (Table 8.5). All treatments were similar to glyphosate alone except for the tank mix treatment of glyphosate with 2,4-D had lower AMPA detected (Table 8.5). Surprisingly, the dicamba alone treatment had the least amount of dicamba detected at 2,035 ppb. All three application methods of glyphosate with dicamba were similar, but the tank mix treatment had the highest amount of dicamba detected at 5,398 ppb (Table 8.5). The tank mix treatment of glyphosate with 2,4-D had the highest amount of 2,4-D detected at 10,861 ppb (Table 8.5). The auxin applied first and auxin applied second treatment with glyphosate and 2,4-D resulted in 4,495 and 4,985 ppb of 2,4-D, respectively.

Table 8.5 Plant tissue analysis results of glyphosate with dicamba and 2,4-D.

Treatment[1]	**Application method**	**Metabolite analyzed**		
		Glyphosate	**AMPA**[2]	**Synthetic Auxins**[3]
		parts per billion (ppb)		
Glyphosate	single application	5363^{a}	51^{a}	
Dicamba	single application			2035^{c}
2,4-D	single application			6391^{b}
Glyphosate with dicamba	tank mix	2230^{b}	16^{ab}	5398^{bc}
Glyphosate with dicamba	auxin applied first[4]	3412^{ab}	19^{ab}	3746^{bc}
Glyphosate with dicamba	auxin applied second[5]	4293^{ab}	16^{ab}	2468^{c}
Glyphosate with 2,4-D	tank mix	2460^{b}	1^{b}	10861^{a}
Glyphosate with 2,4-D	auxin applied first	2539^{b}	12^{ab}	4495^{bc}
Glyphosate with 2,4-D	auxin applied second	3368^{ab}	11^{ab}	4985^{bc}

Data were analyzed using Sidak's comparison. LS-means with different letters within the same column of herbicide analyzed indicate significance.

[1] All applications with dicamba and 2,4-D were applied at 562 and 1065 g ae ha^{-1}, respectively. All treatments with glyphosate were applied at a rate of 434 g ae ha^{-1}.

[2] Aminomethylphosphonic acid

[3] Synthetic auxins consist of dicamba or 2,4-D

[4] Sequential application where dicamba or 2,4-D was applied first followed by glyphosate. Sequential applications were made 30 seconds after the first application.

[5] Sequential application where glyphosate was applied first followed by dicamba or 2,4-D. Sequential applications were made 30 seconds after the first application.

Clethodim can dissipate so rapidly that detection in plant tissues may be difficult (You et al. 2014). Clethodim is mainly oxidized to clethodim sulfoxide or clethodim sulphone in field conditions (Ishimitsu et al. 2001). Clethodim and clethodim sulfoxide can be oxidized to clethodim sulphone by *m*-chloroperoxybenzoic acid (Ishimitsu et al. 2001). Clethodim was not detected in any of the samples from the four replications. In the fourth replication, the metabolites clethodim sulphone and clethodim sulfoxide were tested. The results listed in Table 8.6 show results from only the fourth replication of samples for the clethodim metabolites. No statistical analysis can be run on the clethodim metabolites due to only one replication worth of results. Based off the results from the fourth replication, clethodim sulfone detection was similar across all application methods for the clethodim applied with dicamba herbicide combination. Clethodim sulphone detection of the clethodim applied with dicamba combination resulted in 18, 17, and 17 ppb for the tank mix, auxin applied first, and the auxin applied second application methods, respectively (Table 8.6). Clethodim sulfoxide detection with the clethodim applied dicamba herbicide combination resulted in 100, 97, and 117 ppb for the tank mix, auxin applied first, and the auxin applied second application methods, respectively (Table 8.6). It appears that the auxin applied second method had a slight bump in clethodim sulfoxide compared to the other two application methods.

For the clethodim applied with 2,4-D herbicide combination, again clethodim sulphone did not show difference across the three application methods (Table 8.6). Surprisingly, clethodim sulfoxide detection was highest when applied with 2,4-D at 200 ppb with the tank mix application method. The auxin applied first and auxin applied second methods resulted in 139 and 159 ppb, respectively. Clethodim applied alone had 334 ppb of clethodim sulfoxide detected. All the clethodim applications with dicamba or 2,4-D were over 100 ppb lower than clethodim alone. Among the synthetic auxins, detection was highest at 16,404 ppb with clethodim applied with 2,4-D through the tank mix method (Table 8.6). This was also true with glyphosate applied with 2,4-D (Table 8.5). Among the clethodim applied with 2,4-D treatments, the auxin applied second method application method resulted in 11,814 ppb of 2,4-D detected, which is lower than the 2,4-D detected than the tank mix method (Table 8.6). The auxin applied first method had the lowest amount of 2,4-D detected among the three application methods at 7,507 ppb of 2,4-D detected. When clethodim was applied with dicamba, the tank mix application had higher dicamba detected at 10,750 ppb than the auxin applied first and auxin applied second application methods (Table 8.6). The auxin applied second and auxin applied first application methods of the herbicide combination of clethodim with dicamba had similar detection of dicamba at 6,050 and 5,316 ppb, respectively.

Table 8.6 Plant tissue analysis results of clethodim with dicamba and 2,4-D.

Treatment[1]	**Application method**	**Metabolite analyzed**		
		Clethodim sulphone	**Clethodim sulfoxide**	**Synthetic Auxins**[2]
		parts per billion (ppb)		
Clethodim	single application	34	334	
Dicamba	single application			4335^{e}
2,4-D	single application			8690^{bcd}
Clethodim with dicamba	tank mix	18	100	10750^{bc}
Clethodim with dicamba	auxin applied first[3]	17	97	5316^{de}
Clethodim with dicamba	auxin applied second[4]	17	117	6050^{de}
Clethodim with 2,4-D	tank mix	37	200	16404^{a}
Clethodim with 2,4-D	auxin applied first	23	139	7507^{cde}
Clethodim with 2,4-D	auxin applied second	30	159	11814^{b}

Data were analyzed using Sidak’s comparison. LS-means with different letters within the same column of herbicide analyzed indicate significance.

[1] All applications with dicamba and 2,4-D were applied at 562 and 1065 g ae ha^{-1}, respectively. All treatments containing clethodim were applied at 68 g ai ha^{-1} with NIS at 0.25% v/v.

[2] Synthetic auxins consist of dicamba and 2,4-D

[3] Sequential application where dicamba or 2,4-D was applied first followed by clethodim. Sequential applications were made 30 seconds after the first application.

[4] Sequential application where clethodim was applied first followed by dicamba or 2,4-D. Sequential applications were made 30 seconds after the first application.

Conclusion

Overall, the auxin applied second method had more glyphosate detected within each herbicide combination. It also had a lower amount of dicamba and 2,4-D detected in samples. This could explain the differences in grass control seen in Chapter VII when the same treatments at the same rates were used. Clethodim sulfoxide detection with the auxin applied second application method was highest with the clethodim and dicamba herbicide combination, but not with the clethodim and 2,4-D herbicide combination. 2,4-D detection was much higher in both tank mix applications with glyphosate and clethodim. More results from the clethodim samples need to be analyzed in order to draw assessments that are more accurate on the differences of application methods with the clethodim treatments.

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