MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 42-2014 (YEAR 3)
FINAL REPORT

TITLE: DETERMING THE EFFECT OF DICAMBA RATE AND APPLICATION TIMING ON SOYBEAN GROWTH AND YIELD

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EXECUTIVE SUMMARY

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With crops containing auxin-resistant traits being commercially sold and grown, producers are expected to gain production benefits. However, additional precautions will have to be taken by producers to ensure they do not injure susceptible crop and non-crop species. Spray drift, contaminated spray equipment, and volatility are some concerns that must be addressed to prevent injury to susceptible plant species due to dicamba exposure.

Experiments were conducted to evaluate the effect of application timing and rate on soybean injury from dicamba. The diglycoamine formulation of dicamba (Clarity 4L) was used in these experiments. Separate experiments for each objective were conducted over six site years in four different locations.

Dicamba was applied at 1X (0.56 kg ae/ha), 1/4X, 1/16X, 1/256X, 1/1024X, and 0X rates at the V3 and R1 growth stages. In other experiments, an application rate of 1/16X (0.00875 kg ae/ha) dicamba was applied to soybeans weekly until the soybeans reached physiological maturity. Soybean growth stage was carefully recorded at each application using the Fehr and Caviness (1977) stages of soybean development in order to determine the most sensitive application timing.

Visual injury estimates, plant heights, and yield data were collected for all experiments. Significant visual injury occurred from all dicamba treatments (26 to 98%). Soybean height and yield reductions did not exhibit an interaction; however, both rate of application and application timing had a significant effect. Dicamba applied at 1X, 1/4X, 1/16X, 1/64X, 1/256 X, and 1/1024X rate resulted in a 99, 86, 58, 30, 20, and 10% yield reduction, respectively, when averaged over application timings.

When averaged over all rates of application, the VE and R1 application timings resulted in 41 and 46% yield reductions, respectively.

The most sensitive growth stages of soybeans to dicamba at 28 days after treatment were V4, V5, and R1, with 42, 45, and 38% injury, respectively. No significant visual injury, height reductions, and yield reductions were measured after the R4 growth stage, which corresponded with the 8 week application timing. Yield reductions were greatest at weeks where applications were applied at the V4, V5, R1, and R2 growth stages, with 40, 51, 46, and 41% injury, respectively.

These results indicate that even low rates of dicamba applied to or in contact with susceptible soybean will cause visible injury and yield reductions if applied as late as the R4 developmental stage.

Overall, visual injury increased over time; i.e., visual injury ratings were greater at 28 than at 7 days after treatment.

Soybean was most sensitive to dicamba when it was in the late vegetative and early reproductive growth stages. Similar to what was observed from the 2,4-D timing experiment, no significant visual injury, height reductions, or yield reductions were measured after soybeans had reached the R4 stage.

Based on these data, soybean exposed to dicamba through R4 will suffer significant yield loss. Also, growth stage of the crop should be considered when assessing soybeans that have been exposed to dicamba, whether the exposure has come from accidental particle drift or a tank contamination situation.

In a situation of particle drift, tank contamination, or volatilization, dicamba can be detrimental to a soybean crop well into reproductive development. Soybeans are extremely sensitive to dicamba; a rate as low as 0.00055 kg ae/ha can result in a 10% yield loss.

Soybean exposure to dicamba in late vegetative through early reproductive growth stages is likely to result in the greatest yield losses.

Introduction

Dicamba (3, 6 dichloro-2-methoxybenzonic acid) is a synthetic auxin herbicide used for broadleaf weed control, and is also commonly referred to as a growth regulator herbicide (Senseman 2007). Dicamba is widely used at a relatively low cost to producers; it does not persist in the soil, and has proven to show little to no toxicity hazards (Behrens et al. 2007). Dicamba is a corn and wheat herbicide that has historically been used as a postemergence (POST) herbicide to control dicotyledon weeds (Senseman 2007).

Cotton and soybeans exposed to dicamba, even at ultra-low concentrations, will likely have injury (Egan et al. 2014). Symptomology observed from dicamba is typical of that of most auxin herbicides; e.g., epinastic twisting of the stems and petioles, cupping of the leaves, and swelling of the stems. All of these symptoms can be followed by chlorosis, inhibition of growth, wilting, and necrosis (Senseman 2007). The extent of symptomology from dicamba exposure can be highly dependent on the amount of dicamba to which the plant is exposed.

Dicamba has been used for weed control for over fifty years, and is one of the earliest-used herbicides. Throughout this time of use, little resistance to the herbicide has been recorded. With little weed resistance occurring, it is unlikely that an acceleration in dicamba-resistant weed species will occur like that from the overreliance on glyphosate (Johnson, W. et al.

2012). Monsanto Company is anticipating the release of a cropping system with crops that will be resistant to dicamba and glyphosate; this release is pending regulatory approval (Monsanto Company 2014). This seed technology will be utilized to better control resistant weed species not easily controlled with the current available technology.

This new technology works because of the insertion of a gene from the soil bacterium *Stenotrophomonas maltophilla* that allows plants to metabolize dicamba (Nandula 2010; Johnson, W. et al. 2012). With the use of dicamba in this tolerant cropping system, producers will receive advantages that include the use of multiple chemistries for broadleaf weed control that will enhance efforts to control glyphosate-resistant weeds. Thus, the risk of developing additional weed resistance will be reduced by using herbicides with multiple modes of action (Nandula 2012).

Dicamba is likely to cause damage to susceptible crops, and also has the potential to cause a yield loss to non-target plant species when applications are being made nearby (Egan et al. 2014). Applications of dicamba have the potential to not only physically drift to susceptible plant species, but also will volatilize to off-target application areas (Strachan et al. 2013). If proper application practices are not followed, there will likely be many incidents where injury to susceptible crops will occur due to tank contaminations (Johnson, V. et al. 2012).

Through previous research, we know that soybeans are far more sensitive to dicamba than to 2,4-D (Robinson et al. 2013; Egan et al. 2014; Sciumbato et al. 2004; Wax et al. 1969; Johnson, V. et al. 2012; Andersen et al. 2004; Kelley et al. 2005). Soybeans that have been exposed to dicamba will have yield losses (Johnson, V. et al. 2012; Wax et al. 1969; Kelley et al. 2005) that will depend on the rate of dicamba applied. Conversely, dicamba is far less injurious to cotton than 2,4-D (Wax et al. 1969; Marple et al. 2008; Everitt and Keeling 2009). As expected, when soybeans are exposed to dicamba at increased rates, a greater amount of injury is likely to occur (Robinson et al. 2013; Sciumbato et al. 2004; Weidenhamer et al. 1989). Previous research has indicated that at rates of 2.3 g ha^{-1} or greater, apical meristem death in soybeans will likely occur (Wax et al. 1969; Robinson et al. 2013).

It has been suggested that visual injury where soybeans have been exposed to dicamba could be an indicator of yield loss, meaning that where greater visual injury was observed, a greater yield loss was also recorded (Johnson,V. et al. 2012; Egan et al. 2014). Reduction in plant height has also been suggested as an indicator for yield loss where soybeans have been exposed to dicamba (Weidenhamer et al. 1989). However, visual injury as an indicator for yield loss can be a difficult tool to utilize because it is subjective and could vary greatly depending on individual evaluations. It has also been suggested that visual injury as an indicator could overestimate the predicted yield loss (Egan et al. 2014). This overestimation could easily occur due to the plant being able to grow out of injury from applications that have been made in early growth stages.

Growth stage of soybean at the time of dicamba exposure is important. In a study conducted by Griffin et al. (2013), dicamba applications were made at the V4 and R1 growth stages, and the greatest yield loss occurring from the R1 application. In another study where diacamba was applied at the V2, V5, and R2 growth stages, greatest yield losses occurred from the R2 application (Wax et al. 1969). Weidenhamer et al. (1989) applied dicamba at pre-bloom and mid-bloom growth stages and found minimal yield differences due to application timing; this minimal difference could have been due to later application at the pre-bloom timings. Thus, it is important to document growth stage when evaluating a misapplication or accidental exposure of soybeans to dicamba.

Materials and Methods

During the 2012 and 2013 growing seasons, multiple experiments were conducted at four locations in the southeastern United States to determine the effect of dicamba application rate and timing on soybean growth and yield. All experiments were conducted on 3.9-m wide by 12.2-m long plots (equivalent to four rows on 38-in-wide rows). The two center rows were treated with the herbicide and the outside rows were used as a buffer to reduce the potential for herbicide contamination across other treatments. Each experiment had four replicates at each location. The diglycolamine salt of dicamba was used for all objectives.

Dicamba application rate and timing effect on soybean growth and yield

Experiments were conducted at the BlackBelt Experiment Station in Brooksville, MS (2012 and 2013), the R. R. Foil Plant Science Research Center in Starkville, MS (2012 and 2013), the Delta Research and Extension Center in Stoneville, MS (2013), and the Rohwer Research Station in Rohwer, AR (2013). Planting date, seeding rates, and seed variety varied among locations (Table 3.1).

Experiments were conducted as a randomized complete block design with a two-factor factorial arrangement of treatments. Factor A consisted of application timing at the V3 and R1 growth stages. Factor B consisted of the rate of dicamba based off of a 1X rate of dicamba that was equivalent to 0.56 kg ae/ha. This 1X rate was titrated and additional fractional rates of 1/4X, 1/16X, 1/64X, 1/256X, and 1/1024X which corresponded to 0.56, 0.14, 0.0035, 0.00875, 0.00219, and 0.00055 kg ae/ha were applied. The study also contained untreated check plots at all locations for comparison purposes.

All treatments were applied using a two-row (1.9m wide) shielded tractor-mounted spray boom calibrated to deliver a spray volume of 140 L/ha. TeeJet XR 8002 tips were used in 2012 and TTI 11002 spray tips were used in 2013. Plots were maintained weed-free throughout the growing seasons to prevent any weed interference. Herbicide and insecticide applications were applied throughout the growing season according to standard management practices.

Data collection consisted of visual evaluations at 7, 14, 21, and 28 days after treatment (DAT). Visual evaluations were recorded as a percentage of overall soybean injury that ranged from 0 (no injury) to 100 (plant death). Visual evaluations were collected at all locations with the exception of Rohwer. Height of six plants was measured at the end of the growing season at all locations except Rohwer. Yield data were collected from the treated area of each plot at all locations using a mechanical harvester. Data were combined over all locations, analyzing location and year as a random effect. Data were subjected to analysis using SAS 9.3 with

PROC GLIMMIX and means were separated by LSMEANS (α=0.05).

Dicamba application timing using a low dose application rate

Experiments were conducted the BlackBelt Experiment Station in Brooksville, MS (2012 and 2013), the R. R. Foil Plant Science Research Center in Starkville, MS (2012 and 2013), the Delta Research and Extension Center in Stoneville, MS (2013), and the Rohwer Research Station in Rohwer, AR (2013). Planting date and seeding rate varied among locations (Table 3.1).

Experiments were conducted as a randomized complete block design. A single low dose application rate of dicamba (0.00875 kg ae/ha) was applied at weekly intervals. Applications were made beginning one week after plant emergence, and each additional application was made at weekly intervals until plants began to naturally senesce. Growth stages of soybeans were carefully determined at each weekly application in order to evaluate the growth stage of soybeans most sensitive to exposure to dicamba. Soybean growth stages were determined based on the developmental scale developed by Fehr and Caviness (1977).

The experiments also contained untreated check plots at all locations for comparison purposes. All treatments were applied using a two-row (1.9m wide) hand held boom with a CO_2 backpack sprayer calibrated to deliver a spray volume of 140 L/ha. Teejet XR 8002 spray tips were used in 2012 and TTI 11002 spray tips were used in 2013. Plots were maintained weed-free throughout the growing seasons to prevent weed interference. Herbicide and insecticide applications throughout the growing season were applied according to standard management practices.

Data collection consisted of visual evaluations at 7, 14, 21, and 28 DAT. Visual evaluations were recorded as a percentage of overall soybean injury that ranged from 0 (no injury) to 100 (plant death). Visual evaluations were collected at all locations with the exception of Rohwer. Height of six plants was measured at the end of the growing season at all locations except Rohwer. Yield data were collected from the treated area of each plot at all locations using a mechanical harvester. Data were combined over all locations, analyzing location and year as a random effect. Data were subjected to analysis using SAS 9.3 with PROC GLIMMIX and means were separated by LSMEANS (α=0.05).

Results and Discussion

Dicamba application rate and timing effect on soybean growth and yield

A significant rate by timing interaction was present for all rating intervals except 21 DAT. Therefore, data are shown for the rate and application timing combination in Table 3.2.

Visual injury at 7 DAT was significant for all application rates at both application timings. The lowest application rate resulted in visual injury ratings of 12 and 16% at the V3 and R1 application timings, respectively, at 7 DAT. **Therefore, even the lowest rate used in this study resulted in significant visual injury.**

Visual injury at 14 DAT was significant at all application rates and timings, and ranged from 99 to 23%, depending on the rate and application timing.

At 21 DAT visual injury ranged from 94 to 29%.

Visual injury at 28 DAT ranged from 98 to 27%, depending on application rate and timing.

Overall, visual injury increased over time; i.e., visual injury ratings were greater at 28 DAT than at 7 DAT.

Application timing and application rate were significant factors for plant height and height reductions, and no interactions were measured (Table 3.3). Plant height data were pooled over all site years and application timings.

Plant heights ranged from 17 cm at the 1X rate to 88 cm at the 1/1024X rate of dicamba, which was well below the 99 cm for the untreated.

Height reductions were calculated as a percentage based on plant heights collected from the untreated check plots. Significant height reductions were measured at all application rates, and ranged from 92% reduction from the 1X rate to 11% reduction from the 1/1024X rate.

Plant height data were analyzed and pooled over all site years and application rates (Table 3.4). Plants in the V3 application timing treatment were 66 cm tall, and those that received dicamba at the R1 application timing were 61 cm tall. Greater height reductions were measured in the R1 application timings, which resulted in a 41% reduction compared to 34% reduction in the V3 growth stage application. However, dicamba applied at both soybean growth stages resulted in shorter plants.

Yield and yield reductions have been pooled over site years and application timings (Table 3.3) and site years and application rates (Table 3.4).

Yields pooled over location and application timings were significantly affected by all application rates with the exception of the lowest rate of 1/1024X (Table 3.3).

Yields from the 1X, 1/4X, 1/16X, 1/64X, 1/256X, and 1/1024X treatment rates were 0, 387, 1376, 2526, 2961, and 3481 kg/ha, respectively. The lowest application rate yielded 3481 kg/ha, which was not significantly different from yield of the untreated check.

Yield reductions as a percent of the untreated check were significant for all application rates when data were pooled over locations and application timings. Yield reductions of 99, 86, 58, 30, 20, and 10% were measured for the 1X, 1/4X, 1/16X, 1/64X, 1/256X, and 1/1024X rates, respectively.

Yield and yield reductions were pooled over location and application rates, and are shown in data Table 3.4. Yield of soybeans that received applications at the V3 growth stage were 2141 kg/ha compared to 1990 kg/ha for the R1 growth stage treatment. Yield reductions when pooled over location and application rate were 46% for R1 growth stage treatment compared to 41% for the V3 growth stage treatment.

These data show that soybean that is contacted by even low levels of dicamba will be injured and suffer yield loss. Also, these results show that injury from dicamba can increase over time. Also, greater rates of dicamba result in greater visual injury, height reductions, and yield reductions. Where greater injury was observed, greater yield reductions also occurred. Greater height reductions corresponded with greater yield reductions as well. Soybeans exposed to dicamba, no matter the application rate, are more sensitive to dicamba at the R1 growth stage than at the V3 growth stage.

Dicamba application timing using a low dose application rate

Visual injury ratings are shown in Table 3.5, and plant height and yield are shown in Table 3.6.

At 7 DAT, visual injury was significant for all application intervals through R4. The greatest visual injury of 37% at 7 DAT was at the V1 growth stage.

Visual injury ratings at 14 DAT were significant for applications made at the VE through R4 growth stages, with the greatest visual injury occurring at the VE, V1, V2, V3, V4, and V5 growth stages (38, 38, 39, 39, 37 and 38%, respectively).

Visual injury at 21 DAT was significant at the VE through R4 growth stages. Visual injury ratings were greatest from applications made at the V1 through R1 growth stages (37, 40, 41, 38, 44, 34, and 37%, respectively).

Visual injury at 28 DAT was significant at the V1 through R4 growth stages, with the greatest occurring at the V4, V5, V6, and R1 growth stages (42, 45, 38, and 40%, respectively).

Plant height and percent height reductions, calculated based on the untreated check plots, were both collected for this experiment (Table 3.6). Plants were significantly shorter following applications made at the VE, V1, V2, V3, V4, V5, R2, R3, and R4 growth stages (84, 90, 90, 83, 70, 66, 70, 83, and 93 cm, respectively) compared to 101 cm for the untreated check.

Significant plant height reductions were measured following applications made at the VE through R4 growth stages, and were 16, 11, 10, 20, 30, 34, 43, 30, 18, and 8%, respectively. The greatest height reductions of 34 and 43% were measured following applications made at the V5 and R1 growth stages, which corresponded to applications made at 5 and 6 weeks after emergence.

Yield was significantly lower following applications made at the VE through R4 growth stages. Yields ranged from 3828 to 1906 kg/ha, depending on application timing.

Percent yield reductions were significant where applications were made at the V2 though R4 growth stages. Greatest yield reductions occurred in treatments receiving applications at the

V4, V6, and R1 growth stages, resulting in 40, 51 and 46% yield losses, respectively. These particular application timings correspond with the late vegetative and early reproductive growth stages.

These data indicate that, like the previous experiment, dicamba injury increases over time. Plant height reduction was a good indicator of yield reduction; i.e., the greatest height reduction occurred where the greatest yield losses occurred.

Soybeans were most sensitive to dicamba when it was in the late vegetative and early reproductive growth stages. Similar to what was observed from the 2,4-D timing experiment, no significant visual injury, height reductions, or yield reductions were measured after soybeans had reached the R4 stage.

Based on these data it can be determined that soybean exposed to dicamba through R4 will suffer significant yield loss. Also, growth stage of the crop should be considered when assessing soybeans that have been exposed to dicamba, whether the exposure has come from accidental particle drift or a tank contamination situation.

In summary, these data indicate that in a situation of particle drift, tank contamination, or volatilization, dicamba can be detrimental to a soybean crop well into reproductive development. Soybeans are extremely sensitive to dicamba; a rate as low as 0.00055 kg ae/ha can result in a 10% yield loss.

Soybean exposure to dicamba in late vegetative or early reproductive growth stages is likely to result in the greatest yield losses.

Table 3.1. Planting year, location, date, seeding rate, and seed variety information for dicamba application rate and timing experiments.

Year	Location	Planting Date	Variety	Seeding rate
2012	Starkville	May 15	AG 4932	140,000 seeds/ac
2012	Brooksville	May 1	AG 4932	140,000 seeds/ac
2013[a]	Starkville	May 30	PKP 95Y61	138,000 seeds/ac
2013[a]	Brooksville	May 22	PKP 95731	140,000 seeds/ac
2013	Stoneville	May 16	PKP 94Y82	140,000 seeds/ac
2013	Rohwer	June 25	HBK 4950	130,000 seeds/ac

[a] Determinate varieties, all other locations were planted with indeterminate varieties.

Table 3.2. Visual injury ratings at 7, 14, 21, and 28 DAT for the effect of dicamba application rate and timing on soybean growth and yield[a]

	Days After Treatment							
	7		14		21		28	
	Growth Stage		Growth Stage		Growth Stage		Growth Stage	
Rate[b]	V3	R1	V3	R1	V3	R1	V3	R1
	--------%-------		--------%--------		--------%--------		--------%--------	
1X	85a	70b	99a	91b	94a	92a	98a	93a
1/4X	68b	58c	83c	73d	85b	75c	86b	75c
1/16X	35d	31d	45e	38f	54d	45e	52d	44e
1/64X	18e	20e	33fg	31g	35fg	37f	39f	38f
1/256X	16e	17e	31g	27gh	38f	33fg	35fg	31gh
1/1024X	12e	16e	23h	27gh	29g	30g	26h	27h
0X[c]	0f	0i	0i	0i	0h	0h	0i	0i

[a] means separated within date of injury ratings
[b]1X application rate equivalent to 0.56 kg ae/ha
[c] untreated check treatments

Table 3.3. Plant height, height reduction, yield, and yield reduction from dicamba application timing and rate effect on soybean growth and yield[ab]

Rate[c]	Height	Height Reduction	Yield	Yield Reduction
	------cm------	-----------%---------	-----kg/ha-----	---------%--------
1X	17g	92a	0f	99a
1/4X	41f	64b	387e	86b
1/16X	54e	45c	1376d	58c
1/64X	69d	30d	2526c	30d
1/256X	77c	22e	2961b	20e
1/1024X	88b	11f	3481a	10f
0X[d]	99a	0g	3745a	0g

[a] means separated within columns
[b] data pooled over all application timings
[c] 1X application rate equivalent to 0.56 kg ae/ha
[d] untreated check treatments

Table 3.4. Plant height, height reduction, yield, and yield reduction from dicamba application timing and rate effect on soybean growth and yield[ab]

Timing	Height	Height Reduction	Yield	Yield Reduction
	------cm------	-----------%---------	-----kg/ha----	----------%----------
V3	66a	34b	2141a	41b
R1	61b	41a	1990a	46a

[a] means separated within columns
[b] pooled over all application rates

Table 3.5. Visual injury ratings at 7, 14, 21, and 28 days after treatment for dicamba weekly application experiment[a]

	Days After Treatment			
Growth Stage[b]	7	14	21	28
	------------------------------------%	------------------------------------		
VE	19cd	38ab	29bc	34bc
V1	37a	38ab	37ab	32c
V2	22bcd	39a	40a	32c
V3	27b	39a	41a	34bc
V4	24bc	37ab	38a	42a
V5	23bcd	38ab	44a	45a
V6	16d	27cd	34ab	38abc
R1	23cd	33bc	37ab	40ab
R2	20cd	26d	29bc	32c
R3	17d	20d	22c	23d
R4	7e	10e	14d	12e
R5	3ef	3ef	4e	3f
R5.5	6ef	1fg	0e	8ef
R6	2ef	0.5fg	0e	1f
R6.5	2ef	2fg	1e	-
R7	3ef	2efg	2e	5ef
Untreated[c]	0f	0g	0e	0f

[a] means separated within each column
[b] all application timings received 0.00875 kg ae/ha of dicamba
[c] untreated check treatment

Table 3.6. Plant heights, height reductions, yield, and yield reductions for dicamba weekly application experiment[a]

	Height		Yield	
Growth Stage[c]	Height	Reduction[b]	Yield	Reduction[b]
	------cm-----	-------%-------	-------kg/ha------	--------%-------
VE	84cde	16cd	3104bcd	16cd
V1	90bcd	11cde	3308abc	9de
V2	90bcd	10cde	3217bcd	15cd
V3	83de	20c	2783cd	26c
V4	70e	30b	-	40ab
V5	66ef	34ab	2943bcd	15cde
V6	-	-	1906ef	51a
R1	-	43a	-	46a
R2	70e	30b	2119ef	41ab
R3	83de	18c	2464de	30bc
R4	93bcd	8de	2831cd	24c
R5	100ab	3ef	3694ab	6de
R5.5	102ab	3ef	3437abc	5de
R6	102a	2ef	3828a	2e
R6.5	97abc	5def	3227abcd	5de
R7	97abcd	3def	3582abc	3de
Untreated[c]	101ab	0f	3780a	0e

[a] means separated within columns
[b] percent calculated from comparison of untreated check
[c] all application timings received 0.00875 kg ae/ha of dicamba

Details of this study and its results are presented in a Master of Science thesis entitled "Determining the effect of auxin herbicide concentration and application timing on soybean growth and yield" by Alanna Blaine Scholtes. Click here to access the thesis.

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