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

TITLE: DETERMING THE EFFECT OF 2,4-D RATE AND APPLICATION TIMING ON SOYBEAN GROWTH AND YIELD

PI: DAN REYNOLDS

EXECUTIVE SUMMARY

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With the development of cropping systems containing new auxin-resistant traits, producers will have additional weed control options. These traits will offer many benefits to producers, but will also require additional precautions to ensure they do not injure susceptible crop and non-crop species. Susceptible plant species could be subjected to trace amounts of 2,4-D from spray drift, contaminated spray equipment, and volatility from applications applied to tolerant crops.

The dimethylamine (DMA) salt of 2,4-D was used to evaluate the effect of application timing (soybean growth stage) and rate on soybean growth and yield.

Applications of 2,4-D were made at a 1X (0.56 kg ae/ha), 1/4X, 1/16X, 1/256X, and 0X rate at various soybean growth stages. Soybean growth stage was recorded at each application in order to determine the stage most sensitive to 2,4-D application. Visual injury estimates, plant heights, and yield data were collected from all experiments.

2,4-D applied to soybean at the labeled rate (1X) and 1/4X labeled rate at the V3 and R1 growth stages resulted in significant visual injury and height reductions through 28 days after treatment (DAT). Application at V3 vs. R1 resulted in greater visual injury to treatment plants. Application of the 1X rate applied at the V3 stage resulted in more height reduction than did the same rate applied at R1.

When the 1X and 1/4X rates were applied to soybean at stages V3 and R1, yield reductions were significant. Application of both rates applied at V3 vs. R1 resulted in the greater yield reduction.

These results indicate that 2,4-D applied to susceptible soybean at normal and 1/4X label rates will result in significant visual injury, and height and yield reductions.

Yield reductions in soybean resulting from application of 2,4-D applied at the 1/4X rate were measured from VE through R4, indicating that susceptible soybean will be injured and sustain yield loss for most of its growth and reproductive period if contacted by even a reduced rate of 2,4-D. Based on these data, all soybean growth stages through R4 are susceptible to injury and yield loss from accidental drift of or tank contamination with 2,4-D. Thus, producers should take caution when making applications of 2,4-D to a site that has susceptible crops/plants nearby.

INTRODUCTION

The herbicide 2,4-D has been used for weed control in cropping systems since its initial discovery during the Second World War (Peterson, 1967). The dimethylamine salt of 2,4-D is a member of the phenoxy herbicide family and is typically applied as a postemergence (POST) application to control many broadleaf weeds (Senseman 2007).

Symptomology from 2,4-D application to most broadleaf plants is typical of most auxin herbicides; e.g, epinastic twisting of the stems and petioles, cupping and strapping of the leaves, and swelling of the stems. All of these symptoms are followed by chlorosis at the plant's growing point, growth inhibition, wilting, and necrosis (Senseman 2007).

Over all the years of 2,4-D usage, little resistance to the herbicide has been recorded. With little resistance occurring over the many decades it has been used for weed control, it is believed that it is unlikely that an acceleration in 2,4-D-resistant weed species will occur like we have witnessed with the overreliance on glyphosate (Johnson,W. et al. 2012).

Dow AgroSciences™ anticipates releasing crops for use in a cropping system that will be resistant to 2,4-D and other MOAs, pending regulatory approval (Randolph and Barr 2014). This seed technology is possible due to the insertion of a gene, AAD-1, that allows the plants to metabolize the 2,4-D herbicide (Nandula 2010; Johnson, W. et al. 2012). This resistant gene was derived from *Sphingobium herbicidovorans*, which is a soil bacterium capable of degrading many chemicals in the environment (Song 2014). This new technology will offer producers a way to control glyphosate-resistant weed species, as well as allow for additional modes of action to be utilized for overall improved weed control. By using a diverse selection of herbicides for optimal weed control, producers will be reducing the risk of developing additional weed resistance within their cropping system (Nandula 2010).

These new technologies offer many advantages, but with these advantages come many challenges that must be taken into consideration. Herbicides such as 2,4-D have the potential to greatly damage any susceptible crops and potentially result in a severe yield loss (Egan et al. 2014). Auxin herbicide applications have the potential to not only physically drift to susceptible plant species but also to volatilize to off-target areas (Strachan et al. 2013). Also, if proper application practices are not performed by producers, there will likely be many incidents where injury to susceptible crops will occur due to tank contaminations (Johnson, V. et al. 2012). Producers who choose to utilize these technologies will have to use great care to prevent damage to their own or neighboring susceptible crops.

Previous research where soybeans were exposed to 2,4-D indicate that soybean response resulted in immediate twisting of the stems and petioles (epinastic response), and slight cupping and strapping of the leaves became noticeable over time (Wax et al. 1969; Johnson, V. et al. 2012; Robinson et al. 2013; Kelley et al. 2005).

Plant stunting, leaf burning, and necrosis occurred when soybeans were exposed to extremely high rates of 2,4-D (Kelley et al. 2005; Johnson, V. et al. 2012). Numerous studies have indicated that 2,4-D is not as injurious to soybeans as dicamba is (Andersen et al. 2004; Sciumbato et al. 2004); however, 2,4-D is more injurious to cotton when compared to dicamba

(Wax et al. 1969; Marple et al. 2008; Everitt and Keeling 2009).

Higher rates of 2,4-D have not necessarily always resulted in plant death, but higher application rates of 2,4-D have resulted in plant height reduction (Kelley et al.,2005; Andersen et al. 2004; Robinson et al. 2013). An experiment conducted by Kelley et al. (2005) resulted in an 18 to 25% final plant height reduction where 2,4-D had been applied. In the same study, yield reductions were greatest where 180 g ae/ha of 2,4-D were applied at the R2 growth stage (Kelley et al. 2005)

Soybeans that have been exposed to 2,4-D at earlier growth stages show less visual injury than those that have been exposed to 2,4-D after bloom (Wax et al. 1969). However, in this same experiment conducted by Wax et al. (1969), it was observed that application timing, no matter the growth stage at application, did not greatly reduce seed yield in comparison to other auxinic herbicides.

MATERIALS AND METHODS

During the growing seasons of 2012 and 2013, six experiments were conducted at four locations in the southeastern United States. Experiments were conducted to determine the effect of 2,4-D rate and application 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-inch-wide row spacing). 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 among treatments. Each treatment had four replications at each location. The dimethylamine formulation of 2,4-D was used for all experiments.

2,4-D application rate and timing effect of soybean growth and yield

Experiments were conducted during the growing seasons of 2012 and 2013 to evaluate the effect of 2,4-D application timing and rate on soybean growth and yield. Experiments were conducted at BlackBelt Experiment Station in Brooksville, MS (2012 and 2013), R. R. Foil Plant Science Research Center in Starkville, MS (2012 and 2013), Delta Research and Extension Center in Stoneville, MS (2013), and Rohwer Research Station, Rohwer, AR (2013). Planting date, seeding rate, and seed variety varied among locations (Table 2.1).

Experiments were conducted as a randomized complete block design with a two-factor factorial arrangement of treatments. Factor A consisted of two application timings, one at the V3 growth stage and the other at the R1 growth stage (Fehr and Caviness 1977).

Factor B consisted of 2,4-D rate applied, and rates were based off a 1X rate of 2,4-D equivalent to 0.56 kg ae/ha. This 1X rate was titrated and fractional rates were applied as the experimental treatments. The 1X, 1/4X, 1/16X, 1/64X, and 1/256X titrations corresponded to 0.56, 0.14, 0.0035, 0.00875, and 0.00219 kg ae/ha. The study also contained untreated check plots at all locations for comparison purposes.

All treatments were applied using a two-row (1.9-m-wide) shielded tractor-mounted spray boom 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 any weed interference. Herbicide and insecticide applications were applied throughout the growing season according to standard management practices.

Data collection consisted of visual injury evaluations at 7, 14, 21, and 28 DAT. Visual injury was recorded as a percentage ranging from 0 (no injury) to 100 (plant death). Visual evaluations were collected at all locations except Rohwer.

Height of six plants in each plot 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 random effects. Data were subjected to analysis using SAS 9.3 with PROC GLIMMIX and means were separated by LSMEANS (α=0.05).

2,4-D application timing experiment using a single low-dose application rate

Experiments were conducted during the 2013 growing season to evaluate the effect of 2,4-D application timing on soybean growth and yield. Experimental tests were conducted at BlackBelt Experiment Station in Brooksville, MS (2013), R. R. Foil Plant Science Research Center in Starkville, MS (2013), Delta Research and Extension Center, Stoneville, MS (2013), and Rohwer Research Station in Rohwer, AR (2013). Planting date, seeding rates, and seed variety varied among locations (Table 2.1).

Experiments were conducted as a randomized complete block design. A single low-dose rate of 2,4-D equivalent to the 1/4X rate (0.14 kg ae/ha) from the previous experiment was applied at weekly intervals. Applications were made beginning one week after plant emergence, with each additional application made at weekly intervals until the plants began to naturally senesce. Soybean growth stage was carefully determined at each weekly application in order to evaluate at which growth stage soybeans are most sensitive to exposure to 2,4-D. Soybean growth stages were determined based on the developmental scale established 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 (1.9-m-wide) hand- held boom with a CO_2 backpack sprayer calibrated to deliver a spray volume of 140 L/ha. TeeJet TTI 11002 spray tips were used to apply all treatments. 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 injury evaluations at 7, 14, 21, and 28 DAT. Visual evaluations were recorded as a percentage ranging from 0 (no injury) to 100 (plant death). Visual injury evaluations were collected at all locations except Rohwer.

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Table 2.1. Planting year, location, date, and seed variety information for 2,4-D application rate and timing effect on soybean growth and yield[a]

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

[a] All locations were used for first research objective, only 2013 locations were used for second research objective.
[b] Determinate varieties, all other locations were planted with indeterminate varieties.

RESULTS AND DISCUSSION

2,4-D application rate and timing effect on soybean growth and yield

The effect of 2,4-D application timing and rate on visual injury ratings at 7, 14, 21, and 28 DAT is shown in Table 2.2.

The 1X and 1/4X rates resulted in significant injury ratings that were above 15% at all rating periods.

At 7 DAT, the 1X rate resulted in 56% and 45% injury ratings for the V3 and R1 timings, respectively, and significant injury was observed at both application timings for all application rates that were 1/16X or higher. The 1/64X and 1/256X rates applied at both timings resulted in injury ratings that were below 10%.

At 14 DAT, injury ratings were significant at the 1X, 1/4X, 1/16X rates at both application timings, but declined to less than 10% at the lower rates.

At 21 and 28 DAT, ratings were generally low for the three lowest rates applied at both the V3 and R1 timings.

Table 2.2. Visual injury ratings at 7, 14, 21, and 28 days after treatment with 2,4-D.[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	56a	45b	62a	45b	63a	40b	58a	30b
1/4X	31c	31c	35c	25d	27c	20d	23c	16d
1/16X	10de	11d	15e	11efg	11ef	13e	5ef	8e
1/64X	9def	3efgh	6ghi	6fgh	8ef	8ef	7ef	7e
1/256X	2fgh	8defg	4hi	12ef	6fg	12ef	4ef	9e
0X[c]	0h	0gh	0hi	0i	0g	0g	0f	0f

[a] Mean separation within date of injury ratings.
[b] 1X application rate equivalent to 0.56kg ai/ha.
[c] Untreated check treatments.

Plant heights were recorded in the field and are shown in Table 2.3. Height reductions were calculated as a percentage based on the untreated check plots using the formula [(average check plot height – average plot height) / average check plot height x 100 = percent reduction].

Height reductions were greatest where the two higher rates of 2,4-D were applied at both the V3 and R1 timings. The 1X rate applied at the V3 and R1 stages resulted in 39 and 25% height reductions, respectively, and 13% height reduction where the 1/4X rate was applied at both timings. Height reduction resulting from application of the lowest three rates at both the V3 and R1 stages was below 10%.

Table 2.3. Height of soybean and height reductions following 2,4-D application at V3 and R1 stages.[a]

	Height		Height Reduction	
	Growth Stage		Growth Stage Rate[c]	
	V3[b]	R1	V3	R1
	-------------------cm------------------		------------------%-----------------	
1X	56f	72e	39a	25b
1/4X	92bcd	87d	13c	13c
1/16X	99ab	96abcd	5ef	7de
1/64X	96abcd	97abc	5efg	7de
1/256X	103a	87cd	3efg	10cd
0X[d]	103a	103a	0fg	0g

[a] Mean separation within columns of height and height reduction.
[b] Growth stage at application.
[c] 1X application rate equivalent to 0.56kg ai/ha.
[d] Untreated check treatments.

Yield was significantly reduced by the 1X and 1/4X rates applied at both the V3 and R1 timings (Table 2.4). The percentage reduction in yield from these two rates ranged from 65% for the 1X rate applied at the V3 stage to 12% for the 1/4X rate applied at the R1 stage.
Applications made at the V3 stage resulted in a greater yield reduction.

Yield reductions resulting from application rates below the 1/4X rate applied at both the V3 and R1 stages were below 10%. Yield of the untreated control averaged 3796 kg/ha.

Table 2.4. Yield of soybean and yield reductions following 2,4-D application at V3 and R1 stages.[a]

	Yield		Yield Reduction	
	Growth Stage[b]		Growth Stage	
Rate[c]	V3	R1	V3	R1
	------------------kg/ha----------------		----------------%---------------	
1X	1263f	2487e	65a	32b
1/4X	3023d	3289cd	20c	12d
1/16X	3507abc	3617abc	9de	6defg
1/64X	3431bc	3672ab	11d	5defg
1/256X	3858a	3620abc	2efg	8def
0X[d]	3751ab	3840a	0g	0g

[a] Mean separation within columns of yield and yield reduction.
[b] Growth stage at application.
[c] 1X application rate equivalent to 0.56kg ai/ha.
[d] Untreated check treatment.

These data indicate that normal and ¼ normal rates of 2,4-D will result in significant visual injury, and height and yield reduction in soybean.

The stage at which soybean is exposed to 2,4-D had an obvious effect on yield in these studies. Soybeans that were exposed to the labeled rate (1X) of 2,4-D at the V3 stage had a 65% yield reduction, while the same rate applied at the R1 stage resulted in a 32% yield reduction. When the 1/4X rate was applied at the same stages, the yield reductions were 20% and 12%, respectively.

These data also indicate that a greater yield reduction occurred where greater visual injury and height reduction occurred.

Based on the visual injury data, soybean injury was reduced over time; no new visual injury was observed in the new growth of the plants from the time the initial application was made.

Overall, 2,4-D applied at the lower rates used in this study (1/16X or less) has a low potential of causing a significant yield reduction regardless of when the soybeans come into contact with the herbicide.

Yield reductions from applications of 2,4-D were not as predictable as initially thought. Similar to the findings of Robinson et al (2013), this study indicates that soybean yield reductions were only affected by the higher application rates used in this study.

2,4-D application timing experiment using a single low-dose (1/4X) application rate

Visual injury data are in Table 2.5, and plant height and yield data are in Table 2.6.

At 7 DAT, visual injury was significant for applications made at the VE through R4 and at the R5.5 growth stage. Visual injury at 7 DAT was greatest at the V4 through R4 growth stages, with visual injury ratings ranging from 14% (VE) to 34% (R1).

At 14 DAT, visual injury ratings were significant when applications were made at the VE through R4 growth stages. Visual injury ratings were greatest when this rate was applied at the R1 and R2 stages.

At 21 DAT, visual injury ratings were significant at the VE through R4 growth stages, and were highest when this rate was applied at the R1 through R4 stages.

At 28 DAT, visual injury ratings were again significant at the VE through R4 stages, and did not decline greatly until applications were made at R5 and later stages.

Overall, significant visual injury following application of this 1/4X rate did not decline until applications were made at R5 and later stages. R5 is the growth stage at which pod fill begins (Fehr and Caviness 1977).

Height reductions resulting from the application of this low rate at all growth stages shown in Table 2.6 were below 10%.

Generally, yield was reduced when applications of the 1/4X rate were made from the VE through R4 stages. This yield reduction ranged from 7% (V1 stage) to 27% (V4 stage) of the 4175 kg/ha for the untreated check. An application made at the R5.5 stage resulted in a 10% reduction in yield.

These data indicate that soybeans that are exposed to a reduced rate of 2,4-D at anytime through stage R4 can show visual injury and height and yield reductions. Based on these data, all soybean growth stages through R4 are susceptible to injury and yield loss from accidental drift of or tank contamination with 2,4-D. Thus, producers should take caution when making applications of 2,4-D when susceptible crops are nearby.

Table 2.5. Visual injury ratings at 7, 14, 21, and 28 days after treatment with 2,4-D applied weekly.[a]

Growth Stage[b]	Days After Treatment			
	7	14	21	28
	-----------------	----------------- %	-----------------	-----------------
VE	14def	19cde	17abc	14bc
V1	18de	7efgh	8cde	7cd
V2	20cde	18cdef	17abcd	16abc
V3	24cd	22cd	14bcd	20ab
V4	25bcd	25abc	27a	23ab
V5	25abcd	12defg	19ab	24ab
R1	34a	33a	27a	26a
R2	32ab	31a	24a	20ab
R3	31abc	26abc	22ab	18ab
R4	20de	23bcd	25a	18ab
R5	3fg	6gh	2ef	1de
R5.5	20de	6fgh	5def	5cde
R6	6fg	2gh	5ef	3de
R6.5	8fg	2gh	6cde	-
Untreated[c]	0g	0h	0f	0e

[a] Means separated within each rating date column.
[b] All application timings received 0.14 kg ae/ha of 2,4-D.
[c] Untreated check treatments.

Table 2.6. Plant height, height reduction, yield, and yield reduction following weekly applications of 2,4-D.[a]

Growth Stage[c]	Height	Height Reduction[b]	Yield	Yield Reduction[b]
	-----cm-----	-------%-------	------kg/ha-----	---------%---------
VE	89bcde	8abcd	3387cde	16abc
V1	94ab	0.5ef	3901abc	7cd
V2	86cdef	6bcdef	3625abcde	9bcd
V3	85def	6bcde	3281de	21ab
V4	83def	10abc	2711f	27a
V5	89bcde	3cdef	3872abcd	4cd
R1	81f	13ab	3146ef	18ab
R2	82ef	9abc	3503cde	13bc
R3	88cde	8abc	3293de	17abc
R4	81ef	13a	3564bcde	12bc
R5	93ab	1ef	3986ab	6cd
R5.5	91bc	6bcde	3626abcd	10bc
R6	96a	2def	3977ab	5cd
R6.5	97a	2def	3816abcd	6cd
Untreated[c]	94ab	0f	4175a	0d

[a] Means separated within columns.
[b] Compared to untreated check.
[c] All application timings received 0.14 kg ae/ha of 2,4-D.

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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